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Vinod Kumar Yata
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Eric Lichtfouse *Editors*

Nano-pharmaceuticals: Principles and Applications Vol. 2

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Nanopharmaceuticals: Principles and Applications Vol. 2

Springer

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Preface to the Second Volume

The past decade has witnessed tremendous progress in the research of nanopharmaceuticals. While the first volume of the book covered the basic principles and some applications of the nanopharmaceuticals, the second volume covers topics on drug delivery and toxicity of the nanopharmaceuticals, primarily focusing on environment-friendly herbal and natural nanopharmaceuticals for health and environmental applications. We hope that this updated version of the volume will continue to be useful.

Chapter 1 describes the systemic toxicity and environmental effects of nanocarriers used in nanopharmaceuticals, focusing on in vivo biodistribution of nanopharmaceuticals which could help to understand the toxicity of conventional nanocarriers like metal nanoparticles and carbon nanoparticles. It also discusses future guidelines for the development of nanopharmaceuticals.

Chapter 2 consists of two parts: the first part deals with the phytochemicals and their targeting strategies for the treatment of various types of cancers, while the second part describes the applications of different types of herbal nanostructures for cancer treatment. This chapter provides recent research in herbal nanocarriers in cancer therapy which could be helpful in developing risk-free cancer treatment.

Chapter 3 is a worthy compilation of nanopharmaceuticals in drug delivery and targeting. It deals with the passive and active targeting of nanopharmaceuticals and focuses on principles and applications of current research on topical nanopharmaceuticals like carbon nanotubes, quantum dots, nano-shells, etc. This chapter discusses Food and Drug Administration (FDA)-approved nanopharmaceuticals.

Chapter 4 is a comprehensive summary of therapeutic natural products and their encapsulation in nanocarriers which are synthesized from natural products like chitosan, alginate, gelatin, etc. It presents a brief introduction about the different types of nanocarriers and a detailed note on natural products like paclitaxel, doxorubicin ,curcumin, etc. and explores the current research on natural products as drugs as well as drug carriers.

Chapter 5 deals with transdermal delivery of therapeutic agents by vesicular carriers and discusses brief introduction to skin anatomy and physiology which is helpful in understanding and developing novel carrier systems for skin delivery. It

thoroughly discusses methods, mechanisms, and applications of different types of vesicular nanocarriers.

Chapter 6 is an inclusive review on nano-delivery platforms for phytochemicals and applications of nano-phytochemicals. It lucidly explains the applications of nano-phytochemicals as anti-inflammatory and anticancer agents and covers a brief section on nanocosmeceuticals.

Chapter 7 describes the emerging applications of nanopharmaceuticals in drug delivery, cell imaging, and treatment of diseases like cancer and AIDS. It also focuses on potential health and environmental risks of nanopharmaceuticals. It concludes with a discussion on future research direction.

Chapter 8 reviews the mode of action and ecotoxicity of the nanopharmaceuticals in aquatic environment. It covers the topic on production of nanopharmaceuticals by using biotechnology methods and also describes the environmental risk assessment of nanopharmaceuticals.

Chapter 9 is a valuable summary on recent advances in nanopharmaceuticals for drug delivery. The first part of this chapter covers the types, composition, structure, and methods of preparation, while the second part covers the recent applications with challenges associated with the use of nanomaterials in pharmaceutical formulations.

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About the Editors

Dr. Vinod Kumar Yata is an Interdisciplinary Researcher working in the National Dairy Research Institute, Karnal, India. Previously, he worked as an Assistant Professor in the Department of Biotechnology, Dr. B.R. Ambedkar National Institute of Technology Jalandhar, Punjab, India. He received his PhD in Biotechnology from Indian Institute of Technology Guwahati. He specializes in interdisciplinary research which includes nanotechnology, microfluidics, biotechnology, cancer biology, and bioinformatics. He has developed a microfluidic device for the separation of live and motile spermatozoa from cattle semen samples. He opened up a new avenue to prodrug enzyme therapy by introducing the nanocarriers for the delivery of non-mammalian prodrug-activating enzymes. He elucidated the structural features and binding interactions of several biomolecules by *in silico* methods. He has published several research papers in peer-reviewed international journals and presented papers in several international conferences.

Dr. Shivendu Ranjan has completed his BTech and PhD in Biotechnology from VIT University, Vellore, India, and has expertise in Nano(bio)technology. He was elected as a Fellow (FLS) of the oldest active biological society started in 1778, The Linnean Society (London), and elected as Fellow of Bose Scientific Society (FBSS). In 2018 he was elected as Fellow of Indian Chemical Society (FICS) – a society founded in 1924. He has also been elected as Fellow (FIETA) of the Indian Engineering Teachers Association. Currently, he is Senior Research Associate at the Faculty of Engineering and Built Environment, University of Johannesburg, Johannesburg, South Africa. Recently he has accepted the role of Strategic Head, Research and Development at Ennoble IP, Noida, India. He is also Visiting Faculty at the National Institute of Pharmaceutical Education and Research-R (NIPER-R), Lucknow. He is Vice President, Indian Chemical Society North Branch. Earlier, he has worked as Scientist at DST-Centre for Policy Research, Lucknow, supported by Ministry of Science and Technology, Government of India. He was also Head, Research and Technology Development at E-Spin Nanotech Pvt. Ltd., SIDBI Incubation Center, Indian Institute of Technology, Kanpur, India. After joining E-Spin Nanotech, IIT Kanpur, he has successfully developed prototypes for many products, has applied one patent and has taken lead in the DSIR Certification for R&D Unit of E-Spin Nanotech Pvt Ltd. He is also Advisor for many companies, such as Eckovation Solutions Pvt Ltd. (IIT Delhi–based start-up), Chaperon Biotech Pvt Ltd (IIT Kanpur–based start up), Kyntox Biotech India Pvt Ltd., and Xcellogen Biotech Pvt Ltd. Dr. Shivendu is also reviewer of Iran National Science Foundation (INSF), Tehran, Iran, and jury at Venture Cup, Denmark, for the past 3 consecutive years. He had founded and drafted the concept for the first edition of the "VIT Bio Summit" in 2012, and the same has been continued till date by the university. He is Associate Editor of Environmental Chemistry Letters (Springer journal of 4.6 impact factor), Section Editor of Pharmaceutical Nanotechnology (Bentham Science, UAE), Editor of Journal of the Indian Chemical Society and editorial board member of Biotechnology and Biotechnological Equipment (Taylor and Francis, USA). He is Advisory Board Member in Elsevier to provide feedback on the new prototypes of Elsevier, Netherlands. He serves as Executive Editor of a journal in

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Dr. Nandita Dasgupta has completed her BTech and PhD from VIT University, Vellore, India, and is Elected Fellow (FBSS) of Bose Science Society. She has major working experience in micro-/nanoscience and currently works as Assistant Professor in the Department of Biotechnology, Institute of Engineering and Technology, Lucknow, India. Earlier at LV Prasad Eye Institute, Bhubaneswar, India, she has worked on mesenchymal stem cell-derived exosomes for the treatment of uveitis. She has exposure of working at university, research institutes, and industries including VIT University, Vellore, Tamil Nadu, India; CSIR-Central Food Technological Research Institute, Mysore, India; and Uttar Pradesh Drug & Pharmaceutical Co. Ltd., Lucknow, India; and Indian Institute of Food Processing Technology (IIFPT), Thanjavur, Ministry of Food Processing Industries, Government of India. At IIFPT, Thanjavur, she was involved in a project funded by a leading pharmaceutical company, Dr. Reddy's Laboratories, and has successfully engineered micro-vehicles for model drug molecules. Her areas of interest include micro-/nanomaterial fabrication and its applications in various fields – medicine, food, environment, and biomedical agriculture.

She has published 13 edited books and 1 authored book with Springer, Switzerland, and 2 with CRC Press, USA. She has finished a contract of three book volumes with Elsevier, one with Wiley, two book volumes with CRC Press, and one with RSC (UK). She has authored many chapters and also published many scientific articles in international peer-reviewed journals. She has received the Certificate for "Outstanding Contribution" in Reviewing from Elsevier, Netherlands. She has also been nominated for advisory panel for Elsevier Inc., Netherlands. She is the Associate Editor of *Environmental Chemistry Letters* – a Springer journal of 3.2

impact factor – and also serves as Editorial Board Member and Referee for reputed international peer-reviewed journals. She has received several awards and recognitions from different national and international organizations.

Dr. Eric Lichtfouse, PhD, born in 1960, is an Environmental Chemist working at the University of Aix-Marseille, France. He has invented carbon-13 dating, a method allowing to measure the relative age and turnover of molecular organic compounds occurring in different temporal pools of any complex media. He is teaching scientific writing and communication and has published the book *Scientific Writing for Impact Factor Journals*, which includes a new tool – the micro-article – to identify the novelty of research results. He is Founder and Chief Editor of scientific journals and series in environmental chemistry and agriculture. He has founded the European Association of Chemistry and the Environment. He received the Analytical Chemistry Prize by the French Chemical Society, the Grand Prize of the Universities of Nancy and Metz, and the Journal Citation Award by the Essential Indicators.

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Chapter 1
Environmental and Toxicological Implications of Nanopharmaceuticals: An Overview

Priyanshu Verma and Jatinder Kumar Ratan

Contents

Abstract Over the last couple of years, several studies have been communicated to endorse the tremendous potential of nanopharmaceuticals for the welfare of human beings and other living organisms. However, very limited concepts have been realized in the form of final products. There are various factors which are responsible for the inadequate development of nanopharmaceuticals such as inherent faults of nanocarrier; implausible absorption, distribution, metabolism, excretion/elimina-

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tion, and toxicity (ADMET); and other associated toxicity and confined biocompatibility. Here we review various nanocarriers used in nanopharmaceuticals with respect to their environmental and toxicological implications, including their detection and contamination routes. In addition, we have put forward various concerns and guidelines for the future development of nanopharmaceuticals.

Keywords Nanopharmaceuticals · Nanocarriers · Bioaccumulation · ADMET studies · Ecotoxicity

1.1 Introduction

Over the past few years, pharmaceutical industries have grown with a tremendous potential as a huge investment has been done on the research and development of novel, safe, effective, and efficient pharmaceutical formulations. The development of novel pharmaceutical formulations is overstimulated and propelled due to the perpetual emergence of various disease outbreaks and human-/poultry-based apocalypses such as malaria, dengue, cholera, chikungunya, Zika, and Ebola virus infections, hepatitis, plague, yellow fever and many more. Besides this, cancer, diabetes, and cardiac disease based medications are the leading pharmaceutical revenue generator due to the widely affected population. The high demand of cancer, diabetes, and cardiac disease based medications results in huge and sustainable development in the advancement of respective medicaments to make them more efficacious with minimal side effects. During the advancement of respective medicaments, the existing nanotechnologies and their features have been materialized and consolidated in the last few years (Bawa et al. 2016; Cornier et al. 2017; Müller 2017).

The nanopharmaceuticals have various advantages over common medicaments such as an exceptional targeting ability with significant accuracy, followed by better stability and sustainability at the targeted sites (De Villiers et al. 2008; Jain 2008; Nagarajan 2012; Liang 2013; Ge et al. 2014). Hence, in the recent years, the field of nanopharmaceuticals or nanomedicaments or nanomedicines or nanodrugs has grown rapidly, mainly in the direction of nano-support-based drug delivery systems for the treatment as well as diagnostic applications (Kumar 2007; Peer et al. 2007; Claire du Toit et al. 2007; Bawarski et al. 2008; Weissig et al. 2014; Bassyouni et al. 2015; Bawa et al. 2016; Berkner et al. 2016). Integration of nanotechnology and pharmaceuticals provides some extraordinary features such as vast surface area and improved penetrability in comparison to their larger counterparts (Varadan et al. 2008).

Nanopharmaceuticals have quite peculiar physical, chemical, and biological properties due to their unique structures and functionalities (Kumar 2007; Mozafari 2007). These properties are closely related to the pharmacokinetics/pharmacodynamics that influence the biocompatibility and the ADMET (absorption, distribution, metabolism, excretion/elimination, and toxicity) behavior of nanodrug formulations inside the living body (Houdy et al. 2011). Basically, conventional

drug formulations show a huge variation and instability inside the living body due to their differential size and shape, chemical composition, solubility, surface charge, and other surface modifications. The combination of nanotechnology and pharmaceuticals provides improved biological adhesion, rapid or controlled dissolution, lower settling, and higher saturation solubility inside the living body. These features of nanopharmaceuticals established them as relatively more effective and proactive medicaments.

As per the current scenario, the nanopharmaceuticals cover a broad range of medicines and medical treatment related products for humans as well as animals. The major classes of those medicines and products are as follows:

- Medicines for diseases, in both solid and aqueous forms
- Vaccines
- Chemotherapeutic agents
- Anticancer or antitumor or antineoplastic drugs
- Lipid lowering agents
- Cardiac, pulmonary, and neuro medicines
- Ocular health products and instruments
- Dental care products
- Osteoporosis medicaments and bone implants
- Wound and burn healing drugs
- Medical diagnostics such as molecular diagnostics and diagnostic test cards
- Medical or surgical tools and devices
- Multivitamins, amino acids, proteins, and other nutritional products
- Medicinal personal care products such as soaps, shampoos, and hygiene products
- Over-the-counter and generic medicines

In the last few years, numerous studies have been dedicated for the exploration of nanocarrier-based medicaments that involves carbon nanotubes, nanofiber, nanoparticles, nanoshells, liposomes, dendrimers, quantum dots, nanoclay, and many other nanosized entities with exclusive shape, size, composition, surface chemistry, and targeting ligands (Claire du Toit et al. 2007; Narducci 2007; Gaur and Bhatia 2008; Menaa 2013; Hassanzadeh 2014; Chavda 2016; Loretz et al. 2016), as shown in Fig. 1.1. Nanocarrier-based medicaments are potentially being used for efficient drug delivery, amino acids or peptides delivery, chemotherapy, neurotherapy, diabetes and many other treatments (De Villiers et al. 2008; Jain 2008; Nagarajan 2012; Liang 2013). The nanocarrier-based hybrid medicaments are usually given by injection into the blood vessels or through the oral administration. Here, typically 50–200 nm-sized nanocarriers are incapable to cross the endothelial barrier except spleen and liver due to the availability of gaps (Juliano 2013).

In addition, the nanocarriers have regular tendencies to interact with each other and form agglomerates that also increase nanocarriers' effective size. Hence, the restricted biodistribution and increased bioaccumulation of nanopharmaceuticals imply various therapeutic limitations too. Therefore, the drawbacks of nanopharmaceutical technologies are also needed to be reviewed and scrutinized for the safer

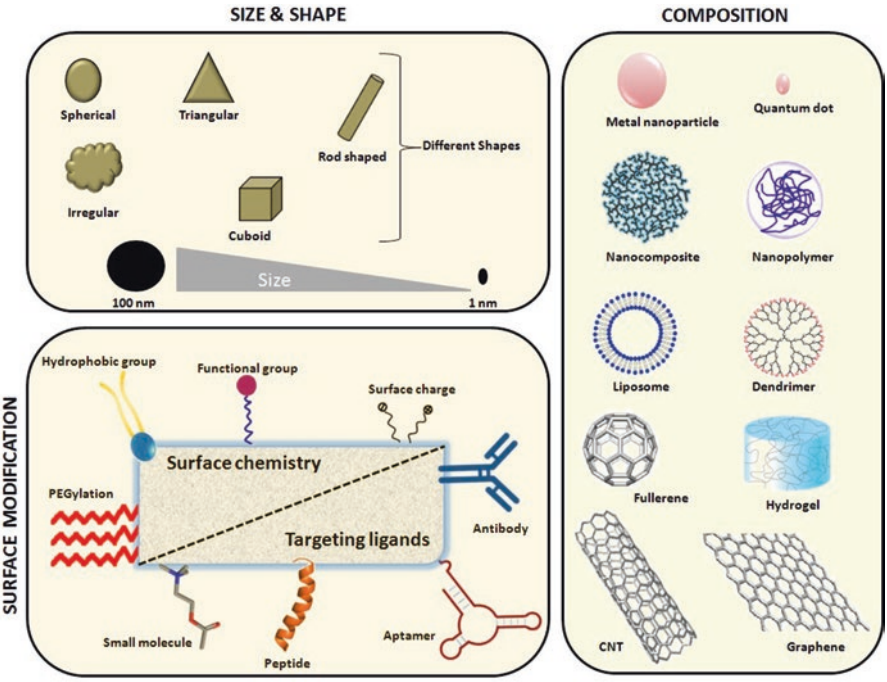

Fig. 1.1 Designing of nanocarrier-based drug delivery system: multifunctional nanocarrier medicament could be produced from the combination of various suitable materials with their specific composition and functional properties that can be further utilized for the therapeutic and/or diagnostic applications (Seleci et al. 2016; Hua et al. 2018)

and eco-friendly benefits of nanopharmaceuticals. However, the major limitations of the potential nanopharmaceuticals are the unavailability of sufficient number of ideal studies and data confirming the environmental and toxicological implications of nanomedicaments. In addition, the localized detection of nanocarriers inside the living body is quite difficult due to the nanosized characteristics. Hence, with the longer treatment duration, nanopharmaceuticals may start accumulating in the specific body regions (Buzea et al. 2007; Ray et al. 2009; Maurer-Jones et al. 2013). Long-term accumulation of nanopharmaceuticals inside the human body may lead to more disastrous health situations or hazards such as a cyst or tumor formation, activation of autoimmune system, and suppression of nearby blood vessels and nerve cell growth (Buzea et al. 2007; Ray et al. 2009; Maurer-Jones et al. 2013).

Furthermore, there are various ethical, scientific, and regulatory guidelines followed by social concerns and implications that are also posing a great restriction on the smooth evolution of nanopharmaceuticals (Koopaei and Abdollahi 2016). The major issues and health risks associated with nanomedicaments are environmental complications, cytotoxicity, translocation to undesired or nontargeted tissues or cells, unknown or unpredictable and undetermined safety norms, and bioaccumulation and non-biocompatibility inside the living body. However, in ethical

perspective, nanomedicaments may alter the genetic sequence of coding or noncoding genes that may result in an abnormal behavior of cellular system which may or may not be persistent in nature depending on the duration of exposure or treatment (Manickam et al. 2017; Sardoiwala et al. 2017). Consequently, a very few nanocarrier-based medicaments used to be approved by the Food and Drug Administration and reached in the open pharmaceutical market. The examples of such nanocarriers are liposome, nanopolymer-protein conjugate, nanopolymer-drug conjugate, and nanoparticle-monoclonal antibody complex-based medical formulations (Bawarski et al. 2008; Weissig et al. 2014; Berkner et al. 2016; Panda et al. 2017). Nevertheless, to extract the more potential benefits of nanomedicaments, it is very essential to work on the genuine guidelines and developments in the all aspects of nanopharmaceuticals under a proper coordination between various industrial, governmental, and academic research and development bodies.

Overall, there are various emerging issues related to ethical, social, and regulatory aspects of nanopharmaceuticals that affect the environment as well as public and/or animal health. In this regard, Maurer-Jones et al. (2013) summarized the toxicity of engineered nanoparticles with respect to various trophic levels such as bacteria, plants, and multicellular organisms including aquatic organisms. Maurer-Jones et al. (2013) also highlighted the important challenges within the field of eco-nanotoxicity and the kind of challenges that are being faced during engineered nanoparticles' analytical assessments.

Buzea et al. (2007) acknowledged that humans had been exposed to nanoparticles from very ancient eras via natural or anthropogenic sources. Buzea et al. (2007) heightened some concerns related to the development of nanotechnology due to the negative impacts of nanosubstances on the public health. For example, engineered nanosubstances could be a potential source of nanoparticle pollution if they are not safely manufactured, handled, and disposed of or recycled. Since, some nanoparticles are able to enter into the living bodies and rapidly migrate to the organs and tissues via the body's circulatory and lymphatic systems. The toxic effects of nanoparticles used to be more intensive in the cases of various pre-existing diseases such as asthma, diabetes, and allergies. Riehemann et al. (2009) discussed the biocompatibility and toxicity-related safety issues of nanomedicines that contain nanoparticles as carrier or active substance. Moreover, there are some associated risks with the nanocarrier coupled pharmaceutical compounds too that may also affect the human body and ecological health (Boxall et al. 2012). In this regard, Koopaei and Abdollahi (2016) recommended to perform well-established toxicology profiling through in vivo tests, since the in vitro tests mostly evaluate samples' toxicity in cell lines with different physiological properties rather than the realistic conditions of a host body.

Sardoiwala et al. (2017) reviewed cytotoxicity, genotoxicity, and immunogenicity-based aspects of various metal and metal oxide nanoparticles. These nanoparticles are widely being used as the nanocarrier for pharmaceutical drugs. Berkner et al. (2016) enlisted a variety of nanocarriers that fall under the European Medicines Agency's nanopharmaceutical definition. These nanocarriers basically include liposomes, polymer/copolymer particles or micelles, dendrimers, coated metal or metal

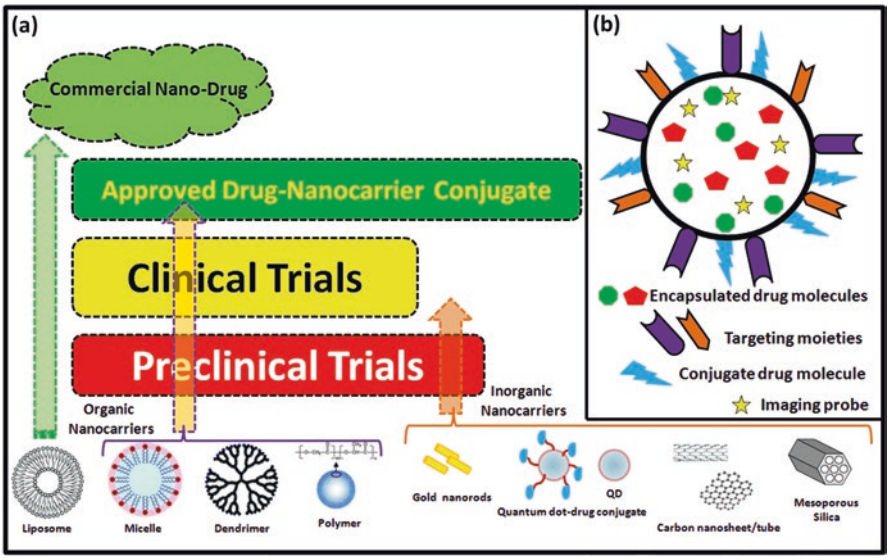

Fig. 1.2 (**a**) The schematic showing versatile conventional nanocarriers such as quantum dots (QD), carbon nanotubes, liposomes, micelles, dendrimers, metallic and polymeric nanocarriers primarily studied for the drug delivery applications with respect to the drug development stages (Mai and Meng 2013); (**b**) Schematic of a multifunctional nanocarrier (Cho et al. 2008)

oxide particles, nonmetal particles, and biological macromolecules such as proteins, peptides, and oligonucleotides (Fan et al. 2014).

As a whole, the aforementioned concerns may limit or restrict nanopharmaceuticals' future evolution, if they are not properly studied, reported, treated, or acknowledged in the literature. The associated hazards of nanopharmaceuticals may cause a negative impression and loss of faith on nanopharmaceuticals in the nonscientific communities, which represent a large section of end users for this oversold and overwhelming technology, although there are various well-characterized and developed nanomaterials, which are precisely studied and already being used as an effective and efficient carrier for medicaments (Cho et al. 2008; Mai and Meng 2013; Sachan and Gupta 2015; Seleci et al. 2016; Subramanian et al. 2016), as shown in Figs. 1.1, 1.2, and 1.3. However, in this review, a few selected ones with respect to their recognized and potential environmental cum toxicological implications have been addressed. For example, phytochemicals have been proven to be more soluble when delivered through the nanocarriers as they exhibit a notable absorption in cancerous cells in comparison to that with the direct phytochemical administered dose. Nevertheless, the half-maximal dose of phytochemicals is greatly reduced due to the nanocarrier-mediated delivery of phytochemicals (Subramanian et al. 2016). Therefore, Subramanian et al. (2016) termed nanocarriers as a crusader in advanced cancer chemotherapy attributed to their minimal side effects and site-specific delivery of drug molecules.

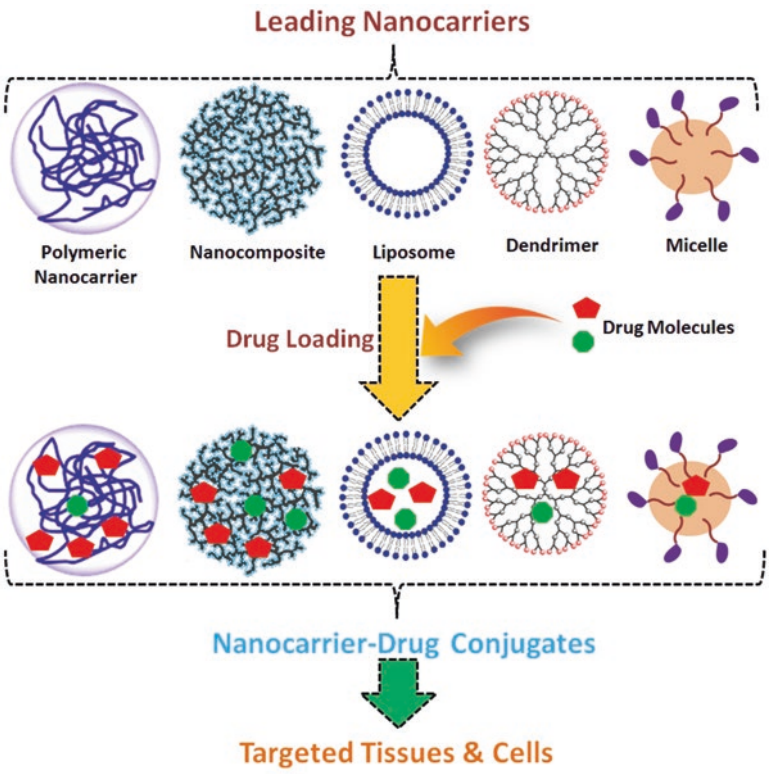

Fig. 1.3 Various leading nanocarriers as an efficient transporter of drug molecules for their better effectiveness on the targeted tissues and cells (He et al. 2016; Subramanian et al. 2016; Zhang et al. 2017a)

1.2 Conventional Nanocarriers Used in Nanopharmaceuticals

1.2.1 Pure Metal-Based Nanocarriers such as Silver, Gold, Iron, and Copper

Metallic nanoparticles are submicrometer (1–100 nm) particles of specific metals such as iron (Fe), gold (Au), copper (Cu), and silver (Ag). They are highly reactive due to their nanosized characteristics such as the large surface area to volume ratio, high surface energies, quantum confinement, and plasmon excitation and contain a large number of "dangling bonds" that provide exceptional chemical properties and additional electron storage capabilities. In addition, few metallic nanoparticles such as Cu and Ag have antimicrobial properties too. Hence, metallic nanoparticles have been widely used and studied for various pharmaceutical applications such as metallic nanoparticle-based biosensors and nanocarriers for disease diagnosis and

therapeutic implementation. However, in recent years, the metallic nanoparticle-based environmental and toxicological studies have been reported that suggest their negative aspects. Inevitably, the expeditious development and production of metallic nanoparticle-based pharmaceuticals have equal contribution in the implications that arise due to the metallic nanoparticle contamination. However, in open literature, it has also been reported that most of the environmental or toxicological implications are primarily because of hazardous chemicals (such as reagents, precursors, and capping agents) and the complex fabrication steps used in the production of metallic nanoparticles for various pharmaceutical-based applications. Furthermore, a few of metallic nanoparticles have shown toxic effects after chemical transition. For example, Ag nanoparticles show dissolution behavior by releasing silver ions which reportedly induce a potential toxic effect in cells (Pandiarajan and Krishnan 2017). However, this concluding remark is still open for intensive research and debate between the concerned researchers and observer field experts. Since, a large number of studies are in favor of the both silver nanoparticles and silver ion-induced cytotoxicity. The combined mechanism of Ag nanoparticle-based cytotoxicity follows a Trojan-horse type mechanism of action (Park et al. 2010). In this mechanism, Ag nanoparticle is supposed to facilitate the release of nontoxic Ag species followed by the entrance of these species into the cell matrices where they get ionized and become toxic in nature and ultimately kill the host cell.

Moreover, exposure to metallic nanoparticles has also been associated with other negative effects such as inflammation, oxidative stress, and genotoxic behavior. In addition, these metallic nanoparticles may accumulate in the living body parts, especially in the liver and/or spleen due to their noncompetitive endothelial barrier. However, metallic nanoparticles may also bioaccumulate in the specific sensitive organ tissues such as brain, spinal cord, and heart. In vitro and in vivo for both conditions, metallic nanoparticles may lead to the formation of reactive oxygen species (ROS) such as superoxide anions ($O_2^{\bullet -}$) and hydroxyl ($OH^{\bullet}$) free radicals, which are potential health hazard substance due to their rapid protein and cell destruction activity (Brohi et al. 2017; Jahan et al. 2017). In general, the oxidative stress in the affected tissues or cells may lead to the DNA, protein, and membrane damages followed by inflammation that ultimately results in the cell death, i.e., apoptosis or necrosis. To minimize the reactive oxygen species-mediated side effects, various antioxidants such as ascorbic acid, citric acid, quercetin, α-tocopherol, and lycopene are used in combination with the surface modification of nanocarriers (Khanna et al. 2015; Wang et al. 2016; Brohi et al. 2017).

Furthermore, metallic nanoparticles are also associated with the hypersensitivity of living organism that may result in the allergic and/or autoimmune response. There are reported studies in the existing literature that ascertained the role of metallic nanoparticles in allergic reactions (Dobrovolskaia and McNeil 2007; Syed et al. 2013; Yoshioka et al. 2017). In addition, a pictorial representation has also been shown for better comprehension on the mode/pathway of allergic reaction stimulated through the exposure of metallic nanoparticles or nanocarriers (Fig. 1.4). In an allergic response, cells of the immune system are activated. Here, the immune cells such as mast cell recognize the foreign substance and trigger the inflammatory response. This response also involves the secretion of cytokines or signaling

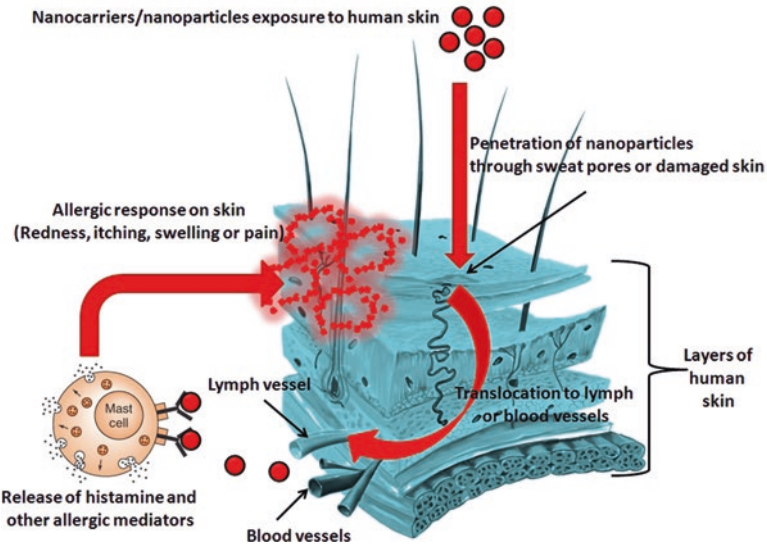

Fig. 1.4 Induction mechanism of metallic nanocarrier-associated allergic reactions in human body. It involves the penetration of human skin, followed by metallic nanocarriers' distribution to the nearby organs and tissues through the blood and lymph vessels. In vivo, these metallic nanocarriers activate the immune response by interacting with the antigen presenting cells that ultimately results in the release of signaling cytokines and histamines

molecules that attract more cells to destroy the foreign substance. In general, the immune cell recognizes nanoparticles by their surface properties and core composition and accordingly produces an inflammatory response. Therefore, Dobrovolskaia and McNeil (2007) recommended a systematic examination of different classes of engineered nanomaterials with their wide range of sizes and surface charges. It may deduce how the change in nanoparticle size and surface charge influence the immune response. In addition to this, the trace impurities present within the nanomaterial-based formulations may also potentially induce the immune response. For example, purified gold and iron oxide nanoparticles do not induce cytokine secretion (Dobrovolskaia and McNeil 2007). It confirms that the purity of metallic nanoparticles may also affect their toxicity. In general, metallic nanoparticles such as Zn, Au, Al, Ag, carbon-coated silver, and carbon black may lead to inflammation through the activation of tumor necrosis factor alpha (TNF-α). These metallic nanoparticles may also increase the levels of interleukin 6 (IL-6), tumor necrosis factor alpha (TNF-α), and nuclear translocation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB). The different metallic nanoparticles stimulate inflammation through nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) regulation followed by the release of pro-inflammatory cytokines (Syed et al. 2013). However, the penetration of metallic nanocarriers to the human skin varies from individual to individual. Here, after getting physical contact with the healthy skin, nanocarriers use to penetrate to the stratum corneum or epidermis. In case of damaged skin, nanocarriers may penetrate into the epidermis and dermis

layer too (Yoshioka et al. 2017). Therefore, in case of healthy skin, it is quite difficult to predict whether the topical use of nanomedicament may result in negative allergic immune response or not. Since, most of the nanocarriers are incompetent to penetrate the healthy skin.

1.2.2 Carbon-Based Nanocarriers

Fullerenes or C_{60}

Fullerenes are closed hollow shells of carbon atoms or giant carbon molecules consisting of perfect hexagons and pentagon defects. Fullerenes have been reported for their therapeutic actions, among them the major ones are antioxidant activity, antiviral and antimicrobial behavior, neuroprotective activity, enzyme inhibition, gene delivery, and so on (Jensen et al. 1996; Piotrovski 2006; Sheka 2011). In addition, fullerenes also act as an oxidative agent under the influence of photoexcitation in the presence of molecular oxygen. Hence, it is also used in the photodynamic therapy, where triplet oxygen ($^3O_2$) to highly active singlet oxygen ($^1O_2$) transformation occurs (Mroz et al. 2008; Sheka 2011). The overall scheme of fullerene-based photodynamic therapy is given below:

$$^1C_{60} + h\nu \rightarrow {}^1C_{60}^* \rightarrow {}^3C_{60}^* \xrightarrow{^3O_2 \rightarrow {}^1O_2} {}^1C_{60}$$

However, the in vivo delivery of fullerene-based drugs may present some serious implications. Therefore, various specific routes and modifications are being followed for effective administration of fullerene-based drugs, such as suspension of micron-sized fullerenes, use of stable colloidal fullerenes, solubilization of fullerenes, immobilization of fullerenes, and so on. Additionally, the incorporation of nano-sized silica to the fullerene-based drugs has greatly enhanced the therapeutic values and offers a synergistic behavior. This advancement is mainly due to the improved hydrophobicity of the nano-drug conjugates and increased sorption of receptor proteins followed by the enhancement of drug action (Sheka 2011). Apart from this, a few studies have also raised the safety concerns of fullerenes and its derivatives with respect to their possible cytotoxic effects (Gelderman et al. 2008; Kepley 2012), although the concluding remarks of similar studies are also believed to be conflicting, ambiguous, and critically debatable. Therefore, only those studies that represent a well-characterized single species as a lead candidate of fullerene-based pharmaceutical formulation should be recommended as a suitable reference for further argumentation. The characterization studies should include the information about the surface area, size distribution, purity, crystallinity, surface reactivity or affinity, surface coating, solubility, morphology or shape, and aggregation behavior of the nanostructures. This will provide more meaningful information regarding the potential environmental and toxicological characteristics of respective

formulations. Furthermore, fullerenes show stability in aqueous medium due to their net negative charge produced by the networks of charge polarization interaction in large aggregates (Choi et al. 2015). Therefore, aggregation of water-dispersed fullerenes is quite obvious and causes the larger particle formation that consequently reduces the effective surface area of fullerenes too.

Graphene and Graphene Oxide-Based Nanostructures

Graphene is one of the most studied allotropes of carbon. Graphene is a 2D material and consists of a single layer of hexagonically arranged carbon atoms. Graphene has various unique properties such as excellent heat and electric conductivity and good transparency and great strength that makes graphene a wonder material of the twenty-first century. The versatile intrinsic qualities make graphene ideal for utilization in various combinations with improved thermostable, mechanical, and biocompatible properties (Ioniţă et al. 2017). Pure graphene structure used to be hydrophobic in nature that's why it requires to be oxidized to improve its dispersibility in aqueous medium. The oxidized graphene-based formulations have been broadly explored for various biomedical applications such as bioimaging and sensing applications, drug and gene delivery, photothermal therapy, tissue engineering, stem cell technology, and so on, whereas the use of graphene is limited to sensing or diagnostic applications only (Lalwani et al. 2016; Ioniţă et al. 2017).

However, similar to other engineered nanocarriers, the graphene and graphene oxide-based nanocarriers also have the toxicological and environmental implications. For example the inhalation of graphene and graphene oxide may induce lower pulmonary toxicity. However, the bolus airway exposure to graphene and graphene oxide-based nanomaterials may cause acute and subacute pulmonary toxicity. More often, it has been observed that in comparison to small-sized graphene oxide materials, the large-sized ones are more toxic in nature. Moreover, the accumulation of administered graphene oxide in the liver, lungs, and spleen has also been reported in the open literature (Ema et al. 2017). However, the studies showing the negative sides of graphene or graphene oxide-based engineered nanocarriers are very limited in number. Besides this, a few studies show oxidative stress and inflammation as a prominent factor behind the toxicity of pure and oxidized graphene-based nanopharmaceutical formulations (Fahmi et al. 2017). In addition, it is also believed that the surface reactivity, size, and dispersion level of graphene or graphene oxide-based formulations are very crucial factors in the induction of toxicity and undesired biodistribution inside the living body.

Zhao et al. (2014) have reviewed the behavior of graphene-based nanomaterials in the aquatic environment with respect to the adsorption, dispersion, transformation, and toxicity. Notably, graphene or reduced graphene oxides adsorb hydrophobic and aromatic molecules, whereas the graphene oxides adsorb metal ions and positively charged organic molecules, preferably. Since, the studies based on the environmental behavior of graphene are still in the lag phase and confine the understanding of environmental exposure, fate, and risk of graphene-based nanopharmaceuticals.

Carbon Nanotubes

Carbon nanotubes (CNTs) are cylindrical nanostructure usually formed by rolling a single layer or multiple layers of graphene sheet and termed as single-walled carbon nanotubes or multi-walled carbon nanotubes, respectively. The cylindrical carbon nanostructures used to have dimensions in the ratio of up to 1:132000000 (diameter/length). Carbon nanotubes have various extraordinary features such as high conductivity, exceptional stiffness, excellent tensile strength, high surface area, good adsorption properties, and so on. These notable properties are mainly responsible for the emergence and development of carbon nanotube-based drug nanocarriers. Here, the hollow monolithic structure of carbon nanotubes is very advantageous for the incorporation of drug molecules that offers a controlled and targeted delivery of medicaments. In addition, the outer surface of both single-walled and multi-walled carbon nanotubes could also be functionalized through the addition of various specific functional groups that may enhance the biocompatibility and biodegradability of nanoformulations.

However, a few toxicological studies also indicated the carbon nanotubes' negative outcomes after the oral administration (Araújo et al. 2015). Interestingly, the reported toxicological studies are quite contradictory to each other as few of them report genotoxicity or toxicity with carbon nanotubes (Muller et al. 2005; Bottini et al. 2006; Smith et al. 2007; Folkmann et al. 2008; Jos et al. 2009; Cicchetti et al. 2011), whereas a few of them affirm no toxic effect with the application of carbon nanotubes (Kolosnjaj-Tabi et al. 2010; Naya et al. 2011; Simonin and Richaume 2015). Furthermore, carbon nanotubes are also believed to be capable of induction of immune responses. It further clarified that the immune responses are stimulated from the metallic impurities and contaminants inherently present in the carbon nanotubes (Pulskamp et al. 2007). In addition, the insoluble characteristics of carbon nanotubes induce their bioaccumulation that may cause health and environment-related complications.

Jackson et al. (2013) have identified carbon nanotubes as very hazardous substance for aquatic life, where single-walled carbon nanotube used to be more toxic than multi-walled carbon nanotube. In the same study, the invertebrates were reportedly found to be more sensitive than vertebrates. Since most of the observations are based on the higher exposure concentrations of carbon nanotubes than their routine possible availability in the ecosystem, therefore the negative inferences of carbon nanotubes are quite uncertain and have low impact. Therefore, more rigorous studies considering the production volume and actual contamination level of carbon nanotubes are needed for their better estimation and understanding over the therapeutic and environmental implications.

Carbon Dots

Carbon dots are one of the carbon element-based nanoparticles with the size of less than 10 nm. Carbon dots are water soluble or perfectly dispersible, biocompatible, and cheaper to produce. In addition, carbon dots might also have specific

fluorescence nature and photostability (Lim et al. 2015; Singh et al. 2017). In general, carbon dots used to be a prime choice as a suitable nanocarrier due to their nontoxic nature. Carbon dots have shown a tremendous biocompatibility with blood at lower concentrations (Li et al. 2015a). Carbon dots are widely being used and studied for their potential application in drug and gene delivery, primarily as a photosensitizer and for other sensing applications (Hu et al. 2014; Lim et al. 2015). Here, the functional groups attached on the surface of carbon dots are primarily responsible for their water solubility and suitable conjugation with the chemical entities such as polymers and inorganic compounds including DNA for carbon dots wider theranostic applications (Hu et al. 2014; Hassan et al. 2017; Singh et al. 2017). Moreover, carbon dots also provide a significant flexibility over the surface functionality that could be wisely utilized or selected to hold a desired medicament through various surface interactions. This modification results in an improved control over the release of the conjugated drug molecules. Therefore, carbon dots are widely being used as a potential carrier for pharmaceutical compounds.

Besides this, intrinsically, carbon is not considered as a toxic element; however the specific material morphologies or structures of carbon dots have shown few potential hazards related to human health and to the environment (Wang et al. 2013). Havrdova et al. (2016) suggested that neutral carbon dots have most promising biological applicability as neutral carbon does not induce any impairment in the cell morphology, intracellular transportation, and cell cycle, up to a certain level, whereas negatively charged carbon dots may seize the G2/M phase of the cell cycle and may also increase the oxidative stress. Moreover, negatively charged carbon dots are incapable of cell nucleus penetration. Interestingly, positively charged carbon dots are found to be more cytotoxic in nature, due to their nucleus penetration capability that induce a significant change in G0/G1 phase of cell cycle, even at very low concentration. However, Li et al. (2015b) shown that pristine carbon dots have very low cytotoxicity and recommended their application in the pharmaceuticals, especially for the processing and formulation of insulin due to carbon dots inhibitory effect on human insulin fibrillation.

Furthermore, Xiao et al. (2016) have investigated the toxic effects of carbon dots on different developmental stages of rare minnow's embryo. That study reported a significant developmental toxicity on rare minnow embryos or larvae that may result due to the induced oxidative stress followed by abnormal development-related gene expression led by the carbon dots exposure. Therefore, it is also very essential to re-investigate and ascertain the suitability of carbon dots as a potential nanocarrier for various medical applications.

1.2.3 Quantum Dots

Quantum dots are ultrafine nanoparticles, usually of less than 10 nm, composed of pure elements such as Au, Ag, and C and semiconductor materials such as ZnS, ZnSe, CdS, CdSe, CdTe, and InP. In comparison to nanoparticles, quantum dots' short size causes a shift in the electronic excitation, i.e., towards higher energy, and

concentrates oscillations to a few transitions that results in a unique electronic and photonic behavior of respective quantum dots. Due to the size tunable properties, quantum dots are widely being used to obtain a broad adsorption profile, narrow size, and symmetric photoluminescence spectra depending on the material composition of quantum dots. Quantum dots have also shown a tremendous stability or resistance towards photo and/or chemical degradation followed by their higher quantum yield (Conde et al. 2014). However, in nanopharmaceuticals, quantum dots are widely being used for protein or drug-based sensing applications including the role as drug carrier to cross versatile cell barriers due to quantum dots characteristic smaller size. Very recently, Ranjbar-Navazi et al. (2017) have reported the possible application of doxorubicin-conjugated D-glucosamine- and folate- bi-functionalized InP/ZnS quantum dots for cancer cell imaging and therapy, where this nanocarrier complex is acting as theranostics for simultaneous imaging and cancer treatment. Lai et al. (2017) have synthesized Ag and Mn co-doped In_2S_3/ZnS quantum dots conjugated to hyaluronic acid for selective and efficient internalization in CD44-expressing tumor cells. The study confirms that the resultant quantum dots could be used as dual-mode imaging probes for more accurate and rapid diagnosis.

Since quantum dots exhibit unique luminescence and electronic properties like broad and continuous absorption spectra, narrow emission spectra, and high light stability (Valizadeh et al. 2012), they are highly used for tracking studies of nanopharmaceuticals and quantum dots biodistribution. Hardman (2006) revealed that the assessment of quantum dots exposure routes and related toxicity of the same are not very straightforward, because all the quantum dots are not similar and their toxicity depends on multiple physicochemical and environmental factors. Tsoi et al. (2013) stated that the in vitro and in vivo quantum dot studies have improved our knowledge regarding quantum dots cellular transport kinetics, mechanisms of toxicity, and biodistribution. The cell culture-based experiments have shown that quantum dots encounter design-dependent intracellular localization and cause cytotoxicity, probably by releasing free metals into the matrix and also by generating reactive oxygen species (ROS), whereas in case of tissues and organs the quantum dots primarily enter the liver and spleen. However, there are some apparent discrepancies in the in vitro and in vivo toxicity of quantum dots, since the available dose of quantum dots may vary significantly and quite uncertain in case of in vivo model studies due to their absorption, distribution, metabolism, and excretion-/elimination-based mechanisms. Consequently, the organ-/tissue-specific dose of quantum dots could not be sufficient to induce a perceptible toxic effect, although quantum dots may retain within the tissues or organs and are also susceptible to induce a long-term toxic effect due to their progressive bioaccumulation. Hence, the quantum dot-induced toxicity studies need to be more standardized and systematized to overcome the existing difficulties. Tsoi et al. (2013) also recommended some steps to obtain a consistent and comparable toxicology data, which are as follows: (1) standardize dose metrics; (2) characterize quantum dot uptake concentration; (3) identify in vitro models that replicate cells and quantum dots interactions similar to in vivo; and (4) use multiple assays to determine sublethal dose and biocompatibility of quantum dots.

1.2.4 Metal Oxide-Based Nanocarriers

Metal oxides such as titanium dioxide (TiO_2), zinc oxide (ZnO), aluminum oxide, cerium oxide, and silicon dioxide (SiO_2) are comparatively more stable than pure metal-based nanocarriers and are less prone to dissolution and/or ionization. The metal oxide-based nanocarriers have gained significant interest as an effective and targeted transporter of pharmaceutical compounds. It is mainly due to their unique characteristics like unusual shape, size, and other morphological and structural properties (Jahan et al. 2017; Huang et al. 2017). Vinardell and Mitjans (2015) reviewed the role of various metal oxide-based nanocarriers for antitumor activity. The reported studies have shown that metal oxide nanocarrier-drug conjugates can selectively kill the cancerous cell without any negative impact on the normal cells.

However, metal oxide-based nanocarriers have also shown substantial toxicity towards the human body and the environment as well (Chen et al. 2008; Vega-Villa et al. 2008). It is primarily due to the bio-persistence and nondegradable nature of metal oxide-based nanocarriers that make them a potential source of sustainable chronic hazards, although a few of them are often reported as less toxic than many others, which is quite insignificant to judge the comparative toxicity of metal oxide-based nanocarriers. Since, the protocol, condition, and host used for the toxicity assessment of respective materials are very different from each other (Jahan et al. 2017). Therefore, in spite of the large number of studies related to the assessment of metal oxide-based nanocarriers' toxicity, a limited information is available about the toxicity expressiveness, evaluation routes, influencing factors, accurate reasons, and mode of actions (Ding et al. 2015; Simonin and Richaume 2015). Therefore, it is an incessant necessity to acquire a better understanding of metal oxide-based nanocarriers for their safe and effective utilization in therapeutic and diagnostic applications.

1.2.5 Nanoclays

Clay is a natural material composed of different minerals such as phyllosilicate mineral that show polymeric behavior and have specific water activity. It has tetrahedral and octahedral structural symmetries. There are various classes of clay; however the major ones are kaolinite, montmorillonite, illite, and chlorite. The difference between the nanoclay and normal clay is that nanoclays have high aspect ratios with at least one dimension of nanometer range (Nazir et al. 2016). Nanoclays have better surface integrity with comprehensible thermal and mechanical characteristics. Nanoclay has a great potential as compared to carbon nanotubes and polymers for controlled release of drug compounds (Ward et al. 2012). Conventional immediate release of the drug compounds causes a sudden raise or imbalance in the plasmatic level of the human body that may induce a negative impact or side effect too. Therefore, the use of nanocarriers like nanoclay is quite significant that can

supervise the release of drug compounds, help in the reduction of the undesirable plasmatic level, and reduce the therapeutic side effects with improved performance and treatment outcomes (Jafarbeglou et al. 2016; Jayrajsinh et al. 2017). In clay-based nanomedicaments, the drug molecules are encapsulated in clay minerals to modify the drug release rate or time and also to target the selected site of drug release. Here, clay minerals not only are used as inert fillers but also offer the functionalities of targeted release, prevention or reduction of side effects, and increase in the formulations' shelf life (Lazzara et al. 2017; Zhang et al. 2016, 2017b). The affinity of drug molecules on nanoclay is governed by the functional groups present in the drug molecules, which can generate different interactions such as hydrogen bonding, hydrophilic/hydrophobic interaction, and ion exchange (Lazzara et al. 2017). There are reported studies that concern with the application of various clay minerals such as montmorillonite, sepiolite, and halloysite nanotubes as the suitable nanocarrier for medical applications (Lee et al. 2005; Saha et al. 2014; Lvov et al. 2016; Jafarbeglou et al. 2016; Jayrajsinh et al. 2017; Zhang et al. 2016, 2017b).

Similar to the other nanocarriers, the clay-based nanocarriers also have therapeutic and environmental implications that have been reported in the existing literature (Lee et al. 2005; Ellenbecker and Tsai 2011; Lordan et al. 2011; Verma et al. 2012; Isoda et al. 2017 and many more). The referred studies have indicated about the probable cytotoxic, hepatotoxic, and nephrotoxic nature of nanoclay-based nanocarriers. Therefore, it is necessarily required to fully elucidate the toxicological profiles of nanoclay-based pharmaceutical compounds for their safer applications.

1.2.6 Dendrimers

Due to the inherent limitation of monofunctional nanocarriers, the need of various multifunctional nanocarriers such as dendrimers has been significantly increased (Bai et al. 2006; Menjoge et al. 2010). Dendrimers are basically multibranched macromolecules that have a specific molecular architecture which makes them more advantageous for being used as a multifunctional nanocarrier (Hsu et al. 2017). Dendrimers have precise control over their size, shape, number of branches, and attached functional groups or drug conjugates. Therefore, dendrimers offer exceptional potential in terms of enhanced solubility, stability, stimuli responsiveness, targeted biodistribution, parallel monitoring, and many other characteristics. Hence, dendrimer-based multifunctional nanocarriers present an unmatched capability for various applications in the continuously expanding nanopharmaceutical field. In this way, it may also reduce the possible therapeutic and toxicological implications in human as well as in animals (Sharma and Kakkar 2015). Very recently, Nierengarten (2017) has reported the use of hexa-substituted fullerene-based scaffolds for the faster and bigger construction of globular dendrimers. He has used this method to prepare giant glycoclusters with the medicinal values of antiviral activity and multivalent glycosidase inhibition properties. This example is basically a combination of two different categories of nanocarriers where one

nanocarrier such as fullerene is acting as a support for another nanocarrier like dendrimer. Similarly, different combinations could also be prepared and studied for dendrimers' safer and effective implementation in nanocarrier-based drug delivery and sensing applications.

Despite the substantial lab-scale applicability of dendrimers in nanopharmaceuticals, the full-scale application of dendrimers is quite limited due to the inherent toxicity of associated submolecules or building blocks (Duncan and Izzo 2005; Jain et al. 2010). The toxicity is mainly attributed to the interaction of the positively charged branch of dendrimers with the negatively charged biological membranes in vivo. These interactions may result in the membrane disruption via nano-hole formation, membrane thinning, and erosion (Jain et al. 2010). Dendrimer-based toxicity could be defined as hemolytic toxicity, cytotoxicity, and hematological toxicity, depending on the mode of action. Designing of biocompatible dendrimers and masking of their peripheral charge are the major ways to reduce their toxicity; for example, acetylation, PEGylation, carbohydrate and peptide conjugation, and introduction of negative charge or charge neutralization could be used (Jain et al. 2010).

1.2.7 Polymeric Nanocarriers

In the last few years, polymeric nanocarriers such as micelles, capsules, vesicles, polymersomes, hydro- or nanogels, nanospheres, nanofibers, and polyplexes have gained tremendous attention in the field of nanopharmaceuticals (Park et al. 2008; Ding et al. 2016). Development of advanced and smart polymeric nanocarriers could offer personalized and on-demand treatment possibilities. In general, polymer-based nanopharmaceuticals represent a very heterogeneous form of nanosized drugs where polymer core provides biodegradable and biocompatible features and polymer shell or surface used to have hydrophilic nature (Kadajji and Betageri 2011). Basically, the pharmaceutically active compounds are incorporated or attached to the polymer-based nanosized substances. These modifications offer a significant change in pharmacokinetics, in passive or active targeting via enhanced permeability and retention followed by sustained release of drug compounds (Weissig et al. 2014). The availability of a wide variety of monomers for assembly of polymeric nanocarriers offers a large versatility due to the variation in the structural and physiochemical properties of monomers. As a result, there are significant examples of application of synthetic polymers such as polyethylene glycol (PEG), polylactide–co-glycolide) and polylactide (Shroff and Vidyasagar 2013). The conventional examples of polymers used as gene carrier are polyethyleneimine (PEI), poly(L-lysine) (PLL), synthetic biodegradable polycations, polyacrylamide, chitosan, and cyclodextrins. The examples of poorly water-soluble and amphiphilic drug-based polymeric carriers are PEG–poly(amino acid), PEG–polyester, PEG–lipid, and polysaccharides.

Unlike the other nanocarriers, the polymeric or lipid-based nanocarriers are quite safe and less toxic in nature due to polymers organic composition. However, any associated toxicity of polymeric nanocarriers might be attributed to the polymers' inherent toxic nature, such that monomer of polyacrylamide has neurotoxic behavior. On the other context, Voigt et al. (2014) reported that polybutylcyanoacrylate-based polymeric nanocarrier could be used as a potential drug delivery candidate for the central nervous system. Polybutylcyanoacrylate-based polymeric nanocarriers can cross the blood–brain barrier and are nontoxic in nature with reference to the reported in vivo and in vitro studies (Voigt et al. 2014).

1.3 Quantitative Techniques for Nanopharmaceuticals

Transportation and delivery of xenobiotics, peptides, antibodies, and gene-based medicament by the means of nanocarriers have tremendous potential to reduce drug resistance and ineffectiveness during their therapeutic application. Hence, to compare the performance of transported drugs, the in vitro and in vivo quantification of nanocarriers are very essential. There are various techniques which are widely being used for the similar objective. The most conventional techniques for the quantification of nanocarriers are ICP-MS (inductively coupled plasma mass spectrometry) and ICP-AES (inductively coupled plasma atomic emission spectroscopy) that can quantify the nanocarriers' uptake with respect to their elemental composition. These techniques have the advantage of very low concentration-based detection with a great precision and accuracy.

Paya-Perez et al. (1993) compared the performances of ICP-AES and ICP-MS for the analysis of trace elements present in soil extracts such as Cr, Ni, Cu, Zn, Cd, and Pb. Overall, Paya-Perez et al. (1993) found that the reproducibility of ICP-AES measurements were relatively better than ICP-MS measurements, possibly due to the less involvement of various reagents. However, for Pb and Ni, the ICP-AES sensitivity was not reportedly up to the mark. Hence, ICP-MS was recommended for the samples with very less concentration of some elements. Altogether, the ICP-MS provides a fast estimation of the concentration of various trace metals with good precision and higher sensitivity.

Recently, Legat et al. (2017) reported a capillary electrophoresis-combined ICP-MS technique to study the behavior of different gold nanoparticles during the interaction with the serum proteins and their mixtures. This technique reportedly provided a somewhat real-time measurement of bare nanoparticles and different protein conjugates, followed by their conversion into the protein-attached forms with respect to their reaction time. The capillary electrophoresis-combined ICP-MS technique looks quite suitable for bioanalysis of metallic nanoparticles under more realistic physiological conditions.

It should also be noted that the ICP-MS (inductively coupled plasma mass spectrometry) and ICP-AES (inductively coupled plasma atomic emission spectroscopy) are limited to quantify element-based nanocarriers such as pure metal, metal

oxide, or carbon-based nanostructures only. Moreover, there are other targeted techniques too, such as "mass barcoding" in which specific nanoparticles are tagged with specific functional group and their transportation is monitored by LDI-MS (laser desorption/ionization mass spectrometry) (Shi and Deng 2016). Unfortunately, LDI-MS (laser desorption/ionization mass spectrometry) technique is not widely adopted due to its inherent complexities, uncertainties, and time-intensive behavior.

Furthermore, a new technique was also reported by Lin et al. (2010) that employs cell mass spectrometry (CMS) for the quantitative measurement of micro- and/or nanocarriers uptake in cells. It has a unique ability to rapidly detect the elements from different nanomaterials, simultaneously. This technique exclusively helps in the evaluation of drug targeting efficiency of nanocarriers and their cellular uptake and associated cytotoxicity emerging due to the differential size and surface properties. Therefore, it is believed that the cell mass spectrometry (CMS)-based technology could be efficiently utilized for the rapid and accurate tracking of therapeutic nanocarriers. More interestingly, cell mass spectrometry (CMS) could be used to determine the exact number of nanocarrier uptake in each cell, whereas ICP-MS (inductively coupled plasma mass spectrometry) can only provide an average uptake of nanocarriers for all cells. In addition, cell mass spectrometry (CMS)-based technique could also be used to measure the cellular uptake of nonmetal-based nanotherapeutic agents (Peng et al. 2010).

Moore et al. (2013) reviewed noninvasive measurement-based techniques for the assessment of the release of nanomedicine. Mostly, the pharmaceutical nanoparticles have been studied in laboratory scale for noninvasive measurement of in situ drug release. However, there are various approaches such as optical upconversion, fluorescence, luminescence, radioluminescence, and magnetic resonance imaging-based techniques that could be utilized for nanopharmaceuticals noninvasive measurements in full scale. These approaches involve the complementation of pharmaceutical nanocarriers with some probes like MRI (magnetic resonance imaging) contrast agents and optically or thermally active species. Besides this, there are some obstacles in the development of noninvasive techniques too, such as the physical limitations of optical techniques, imaging sensitivity and resolution-based limitations, and toxicity of complemented species. In addition to this, for further details about the available techniques that could be effectively utilized for the physicochemical characterization of nanopharmaceuticals, it is suggested to go through the referred literature (Lin et al. 2014; Moore et al. 2013).

Moreover, Summers et al. (2013) shown a promising route to assess the dose of nanocarriers in the form of nanoparticle–cell interactions that used to be very difficult due to the complex multiplicity of possible mechanisms and metrics controlling nanocarriers' uptake. Here, the dose basically signifies the number of nanocarriers internalized per cell. Through the use of limited cell sampling using high-resolution electron microscopy, a calibration can be made relating large population and cytometric measurements of fluorescence to the exact nanocarrier dose taken through the endocytosis. Then, through a probabilistic approach, one can easily quantify the level of nanocarriers per cell.

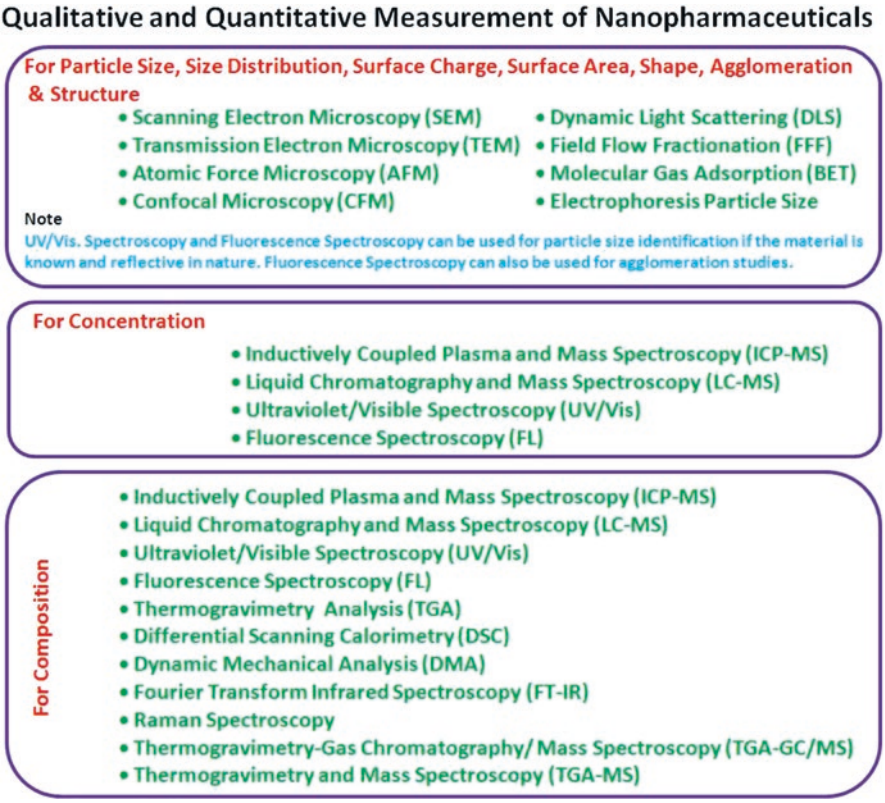

Fig. 1.5 Different techniques used for the qualitative and quantitative characterization of nanopharmaceuticals

In addition, there are various characterization techniques which are widely being used to study the qualitative and quantitative properties of versatile nanocarriers used in nanopharmaceuticals, both in discrete and conjugate forms. A detailed chart of those suitable and advanced techniques has also been represented in Fig. 1.5. Moreover, the quantification of nanocarriers used in nanopharmaceuticals has also become quite essential for the environmental risk assessment. This quantification gives information about the environmental availability of nanocarriers and also helps in the estimation of minimum concentration or limit that may induce any toxic or unfavorable effect. This information could be further used for the estimation of the environmental risk quotient (ERQ). To estimate the environmental risk quotient (ERQ), we need to have the information about the following two parameters:

- Environmental concentration of nanocarriers (X)
- Nanocarriers' minimal dose for negative outcomes (Y)

Environmental risk quotient (ERQ) could be easily calculated from Eq. 1.1.

$$\text{Environmental risk quotient}(\text{ERQ}) = \frac{\text{Environmental concentration of nanocarriers}\,(X)}{\text{Nanocarriers' minimal dose for negative outcomes}\,(Y)} \quad (1.1)$$

If the value of the environmental risk quotient (ERQ) is less than one, then it is non-hazardous for the environment. However, if the obtained value is greater than or equal to one, then it is considered to be hazardous for the open environment; and the extent of hazards could be assessed by its magnitude.

1.4 Absorption, Distribution, Metabolism, Excretion/ Elimination, and Toxicity-Based Studies of Nanopharmaceuticals

Nanocarriers used to have a prominent control over the nanodrugs' pharmacokinetics and pharmacodynamics. The pharmacokinetics and pharmacodynamics of nanodrugs are significantly different from the bare or pure drug molecules (Moss and Siccardi 2014; Griffin et al. 2016; Li et al. 2017). Thus, the better understanding and estimation of the physiological pharmacokinetic parameters of nanopharmaceuticals are very crucial for their development and pharmacodynamic cum biodistribution-based studies.

Therefore, a thorough study of nanocarrier-mediated absorption, distribution, metabolism, excretion/elimination, and toxicity (ADMET) routes, as represented in Fig. 1.6, is always recommended for nanomedicaments to ascertain their

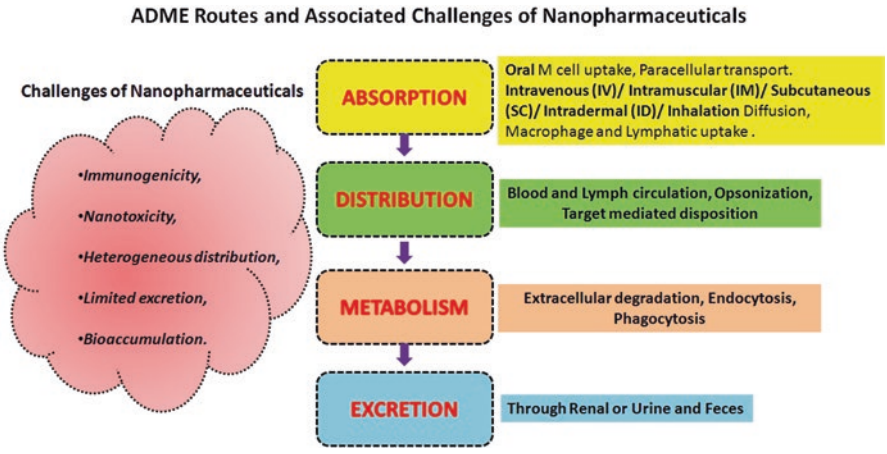

Fig. 1.6 Absorption, distribution, metabolism, excretion/elimination, and toxicity routes of nanomedicaments

effectiveness, biodistribution, and toxicity in a living organism. It will greatly improve the commercial applicability of nanopharmaceuticals, specifically in terms of their enhanced delivery, facilitated targeting, and reduced immunogenicity or nanotoxicity followed by low bioaccumulation.

In general, the environmental safety and toxicological data of nanocarriers used to be considered as a prime requirement for any associated risks or benefit assessment. In the available literature, there is a significant lack of thorough and systematically defined nanocarrier-based environmental and toxicological studies. Hence, the absorption, distribution, metabolism, excretion/elimination, and toxicity (ADMET) studies are highly desirable to ascertain the suitability or efficacy of nanopharmaceuticals. Moreover, it has been also observed that the impact of nanocarriers' toxicity from different administration routes is greatly influenced by some specific factors such as size, shape, composition, surface chemistry, and presence of the targeting ligands (Moss and Siccardi 2014; Griffin et al. 2016; Brohi et al. 2017; Li et al. 2017).

Almeida et al. (2011) summerized the in vivo biodistribution of various nanoparticles such as iron oxide nanoparticles, gold nanoparticles and quantum dots, and their associated immune response. Here, small nanoparticles with less than 50 nm size show enhanced distribution to the lymph nodes and also have long circulation time, whereas large nanoparticles are preferably captured by the liver and spleen and exhibit short circulation time. Since the metallic nanoparticles have tendency to be quickly removed from the bloodstreams by the reticuloendothelial system and they may remain in the liver and spleen for a longer duration, raised various biodistribution and toxicity related concerns. Although the renal excretion based removal of nanocarriers is also feasible and highly size dependent, the need of the additional coatings and surface modifications of the nanosubstances has reduced their renal excretion. Besides this, gold nanoparticles have shown both inflammatory and anti-inflammatory behavior in vitro studies. The in vivo studies have ascertained that the nanocarriers could induce the activation of macrophages and engagement of leukocytes that may result in immunogenic toxicity. However, there is a further scope of understanding of in vivo immunotoxicity.

Furthermore, due to the inherent variations in the biodistribution, biocompatibility, and biodegradability of nanocarriers, the toxicity of nanocarriers may also vary from micro- to macro-sized carriers made up of similar materials. Therefore, it is always recommended to improve the existing toxicological methods through the employment of novel and accurate bio-relevant tools. The follow-up of the recommendation may provide a more comprehensive information about the risk of nanopharmaceutical-based toxicity. However, the frequent changes in absorption, distribution, metabolism, and excretion/elimination profiles of nanomedicines are due to their complex nature and presence of various excipients and need new or revised regulatory frameworks to assess the quality, safety, and efficacy of complex nanodrug formulations.

Figure 1.7 illustrates various rate constants (k) influencing the absorption, distribution, metabolism, and excretion of nanomedicaments administered through oral intravenous (IV) or intramuscular (IM) or subcutaneous (SC) or intradermal (ID) or inhalation routes. Table 1.1 summarizes the salient features of versatile nanocarriers affecting the rate constants of ADMET stages and the intensity of the impact on pharmacokinetics rate constants in terms of less, moderate, and high order.

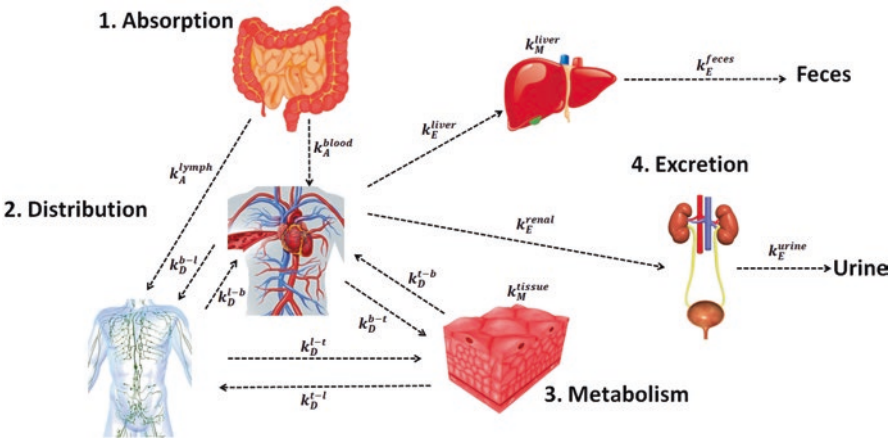

Fig. 1.7 Kinetic factors affecting or controlling the activity of nanomedicines inside the human body (Griffin et al. 2016)

Table 1.1 Impact of nanocarriers' characteristics on pharmacokinetics rate constants

	Salient features of nanocarriers used in pharmaceuticals			
Factors (rate constant)	Size and shape	Composition	Surface chemistry	Targeting ligands
Absorption (k_A)	***	***	***	*
Distribution (k_D)	***	***	***	***
Metabolism (k_M)	***	**	**	***
Excretion (k_E)	**	*	**	**
Toxicity (k_T)	*	***	***	***

*Less impact; **Moderate impact; ***High impact

The detailed summary of the specific nanopharmaceuticals with respect to their absorption, distribution, metabolism, excretion/elimination, and toxicity (ADMET)-based studies have not been discussed here to maintain the brevity of this review article. However, the interested ones are requested to go through the referred scientific literature (van de Waterbeemd and Gifford 2003; Li et al. 2010, 2013, 2017; Hamidi et al. 2013; Moss and Siccardi 2014; Griffin et al. 2016).

1.5 Environmental Contamination of Nanopharmaceuticals

Due to a rapid and sustainable development, production, and commercialization of nanopharmaceuticals or nanoparticle-based drug carriers in the current era (Buzea et al. 2007; Wagner et al. 2014), the ecosystem has become a major victim of nanocarrier-induced pollution or toxicity, especially the aquatic environment (Klaine et al. 2008; Navarro et al. 2008; Brar et al. 2010; Gottschalk et al. 2011; Miralles et al. 2012; Maurer-Jones et al. 2013; Wang et al. 2016; Brohi et al. 2017; Jahan et al. 2017). The nanopharmaceuticals are coming into the environment through

different exposure routes starting from the initial manufacturing and production of nanopharmaceuticals to their consumption and use followed by their excretion and repudiation. A flowchart of major contamination routes of nanopharmaceuticals is also presented in Fig. 1.8 that indicates soil, ground water, and surface water as the end sufferer of nanopharmaceutical-based contamination. The exposure of the nanocarriers which are substantially used in nanopharmaceuticals to the human body through contaminated soil, ground water, and surface water or through the direct administration as a medicament may induce related diseases depending on the regions of human body that get affected (see Table 1.2). Besides this, it should also be noted that the environmental contamination of nanoparticles is also proliferating due to the extended usage of various nanoparticles in the wastewater treatment-related applications such as photocatalysis, adsorption, and advanced oxidation processes (Ghasemzadeh et al. 2014; Ma et al. 2016; Verma and Samanta 2017, 2018a, b, c). Metal oxides such as TiO_2, ZnO, and carbon nanomaterials including graphene and graphene oxide-based nanostructures and quantum dots are the very established and overused candidates for the water and wastewater treatment-based applications (Ghasemzadeh et al. 2014; Lu et al. 2016; Verma and Samanta 2017, 2018b).

Parthasarathi (2011) and Dev et al. (2017) reviewed the effect of various nanosubstance-based toxicity in the plants and food crops which are also widely being used as the nanocarrier for the pharmaceutical compounds. They included latest studies based on phytotoxicity of different nanosubstances such as TiO_2, ZnO, CeO_2, NiO, CuO, Ag, Au, SiO_2, nano zerovalent iron, fullerenes, graphene, graphene oxide, carbon dots, and carbon nanotubes and elaborated individual nanoparticle-based toxic effects observed in plants. The studies show a clear negative impact on the plant growth, root and shoot lengths, biomass accumulation, and seed germination. In addition to this, the oxidative stress and cytotoxic and genotoxic effects of nanoparticles have also been observed in plants (Sardoiwala et al. 2017). Hence, nanopharmaceuticals-mediated phytotoxicity in plants could also be emerged as a major concern for the environment.

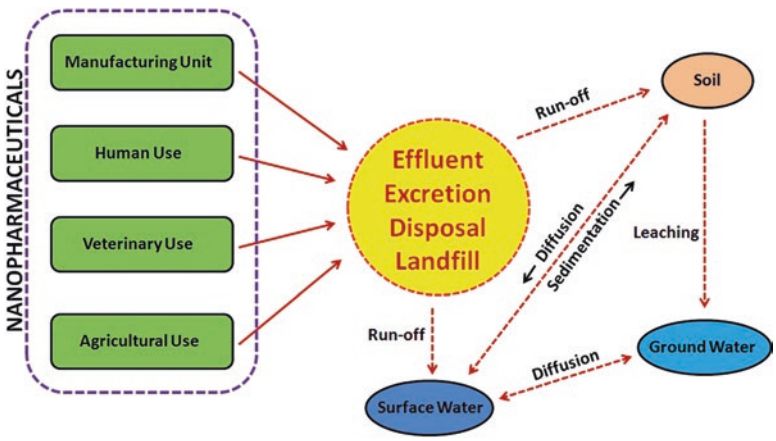

Fig. 1.8 Environmental contamination routes of nanopharmaceuticals

Table 1.2 Various human diseases related to the exposure of nanocarriers (Buzea et al. 2007; Manickam et al. 2017; Sardoiwala et al. 2017)

Route of administration	Primarily affected body part(s)	Associated diseases
Ingestion or oral administration	Gastrointestinal system	Crohn's disease
		Colon cancer
		Ulcer
Inhalation	Brain	Neurological diseases:
		Alzheimer
		Parkinson
	Lungs	Asthma
		Bronchitis
		Emphysema
		Tumor
		Cancer
Injection (IV/IM/SC)	Circulatory system	Atherosclerosis
		Vasoconstriction
		Thrombus
		High blood pressure
	Lymphatic system	Podoconiosis
		Kaposi's sarcoma
	Heart	Arrhythmia
		Heart failure
	Other organs	Unknown impairments of kidneys, liver, and spleen
Topical application	Skin	Allergy
		Autoimmune diseases
		Dermatitis
Orthopedic implant residues	Around implant region	Autoimmune diseases
		Dermatitis
		Urticaria
		Vasculitis
Cellular absorption	Cells and tissues	Bioaccumulation of nanocarriers in cell organelles such as mitochondrion, vacuoles, nucleolus, cell membrane, and cytosol
		Apoptosis
		Necrosis

Jahan et al. (2017) reviewed the silver, graphene oxide, zinc oxide, titanium dioxide nanoparticles, and single-walled or multi-walled carbon nanotube-induced toxicity in aquatic plant and microbial and vertebrate models. They have also enlightened the double-edged sword nature of versatile nanocarriers due to the nanoparticles toxic effects on aquatic ecosystem. They also summarized that both the physicochemical properties such as shape, size, and surface charge and environmental factors such as pH, temperature, type of irradiation, dissolved natural organic matter, ionic strength or presence of electrolytes, and other contaminants primarily control the transportation,

transformation, and toxicological behavior of respective nanoparticles. Since the nanoparticles also have the preeminent potential to cause oxidative response, cellular toxicity, and inflammatory responses, they have become an impetuous source of damage to the aquatic ecosystem. Therefore, it is always essential to know about the physicochemical properties of nanopharmaceuticals followed by their effective concentration prediction in the open environment to calculate the environmental risk quotient (ERQ), nanocarrier transportation, and transformational nature. Subsequently, the evaluation of benefits and risks associated with the use of nanopharmaceuticals has been always crucial and required to be discussed in a specific manner. Hence, more detailed case-by-case toxicity analyses of versatile nanopharmaceuticals are recommended to obtain a more trustworthy predictive model that could estimate and quantify the possible short-term and long-term outcomes of nanopharmaceuticals in the open environment.

Furthermore, with the similar opinion of Jahan et al. (2017), it is recommended that the toxicity, fate, and behavior of engineered nanomaterials or nanopharmaceuticals from a large-scale synthesis to industrial application and disposal should be the prime focus of concern and necessary steps should be taken in the direction of the following: (1) nanocarriers' synthesis and modification parameters; (2) determination of nanocarriers' source, point of entry, and end point; and (3) safety regulations, which are highly essential in case of nanopharmaceuticals. Hence, ecotoxicological tests for nanopharmaceuticals are always required and recommended with the suitable or desired adaptations depending on nanopharmaceutical usage and applications. The potential risks of nanocarriers used in nanopharmaceuticals have been tabulated in Table 1.3, where oxidative stress, cytotoxicity, phytotoxicity, genotoxicity, and immunogenicity are the ultimate toxic response emerging from the biological interaction of these nanosubstances.

Table 1.3 Various associated potential risks of nanocarriers used in nanopharmaceuticals (Ray et al. 2009; Sardoiwala et al. 2017)

S. no.	Nanocarriers	Potential risks
1.	Carbon nanomaterials and silica nanoparticles	May induce pulmonary inflammation, granulomas, and fibrosis
2.	Silver and gold nanoparticles	Widespread biodistribution to different organs and possible passage through the blood–brain barrier
3.	Iron oxide nanoparticles	Significant distribution in reticuloendothelial system based organs
4.	Quantum dots, carbon dots, and titanium dioxide nanoparticles	Skin penetration followed by immunogenic responses
5.	Manganese dioxide, titanium dioxide, and carbon nanoparticles	May enter in the brain through the nasal olfactory epithelium
6.	Titanium dioxide, aluminum oxide, carbon black, cobalt, and nickel nanoparticles	Usually more toxic than micron-sized particles

Note: Oxidative stress, cytotoxicity, phytotoxicity, genotoxicity, and immunogenicity are the ultimate toxic effects associated with these nanosubstances

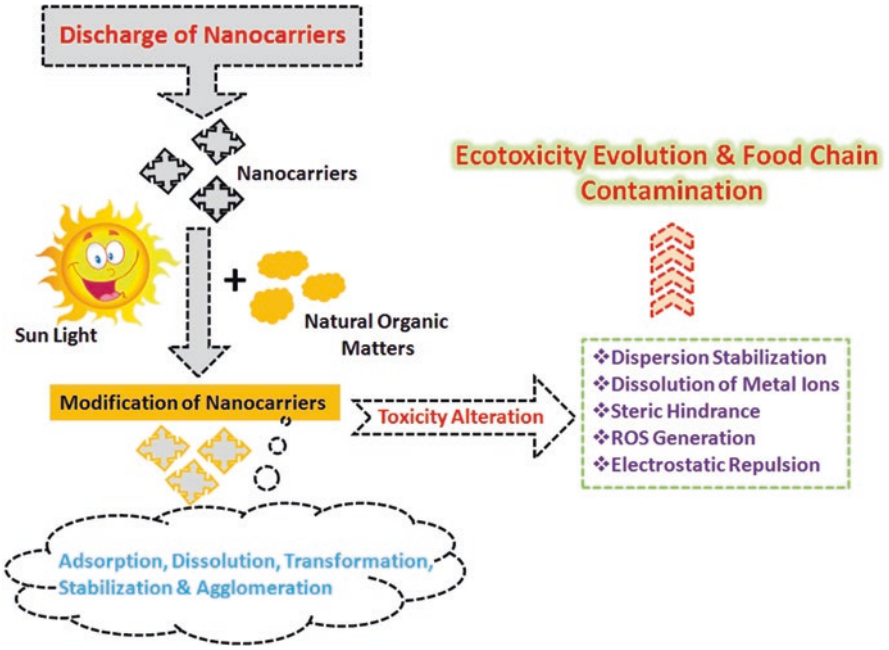

Fig. 1.9 Mechanism of alteration or evolution in the ecotoxicity of nanocarriers discarded or exposed to the natural aquatic system containing natural organic matters, under the influence of solar irradiation (*ROS* reactive oxygen species)

A summarized mechanism of alteration or evolution in the ecotoxicity of nanocarriers which are potentially being used in nanopharmaceuticals is shown in Fig. 1.9. Here, the discarded or runoff nano-contaminants are exposed to the natural aquatic system containing various types of natural organic materials such as humic acid and sulfides in the presence of solar irradiation. These conditions cause an unpredicted change or transformation in the bare nanocarriers that may result in either a positive or negative way depending on the characteristics of transforming or modified nanocarriers (Wang et al. 2016; Jahan et al. 2017). In addition, these nanocarriers may also enter into the food chain due to their bioaccumulating behavior and may cause various diseases in the consuming human as mentioned in Table 1.2. Therefore, it is very essential to study and obtain the data of aquatic life exposure test of versatile nanopharmaceuticals. It should also include the tests with and without drug molecules using nanocarrier–drug conjugates and bare nanocarriers, respectively. Here, activated sludge microbes, algae, daphnia, fishes, earthworms, and various sediment organisms should be considered as the test microorganism for the evaluation of respective nanopharmaceutical-mediated toxicity in aquatic life. In addition, aquatic plants should also be considered for the estimation of bioaccumulation of nanopharmaceuticals or associated nanocarriers using suitable qualitative and quantitative measurement techniques as presented in Fig. 1.5.

There are various categories of tests which are widely being used for the assessment of nanopharmaceuticals. The examples of such major categories have been presented in Table 1.4 with respect to their specific objectives and limitations that

Table 1.4 The categories of tests with their objectives and limitations that are commonly used for the toxicity evaluation of nanomaterials

S. no.	Test	Objectives	Limitations	References
1.	Cytotoxicity	Study of nanosubstance-induced cytotoxicity including cellular metabolic activity, oxidative stress, apoptosis or necrosis, cell membrane damage, and impedance-based analysis	These tests are very time-consuming, labor-intensive, complex in nature Most often, they are unreliable and non-reproducible owing to the nanomaterial and environmental interferences	Parthasarathi (2011), Sardoiwala et al. (2017) and Accomasso et al. (2018)
2.	Phytotoxicity	Study of change or inhibition in the seed germination, root, and shoot growth. Change in biomass of whole plant or specific parts	These tests are highly time-consuming Used to be very inconsistent in nature. Generally not recommended for comparisons with separately reported or conducted studies	Lin and Xing (2007), Parthasarathi (2011) and Dev et al. (2017)
3.	Genotoxicity	Rapid measurement of DNA and/or chromosomal damage Detection of unregulated DNA damage signaling pathways	Various other factors may substantially influence the assay results such as variation in the same material properties and environmental conditions	Parthasarathi (2011), Manickam et al. (2017), Sardoiwala et al. (2017) and Accomasso et al. (2018)
4.	Band gap analysis	Prediction of toxicity level via conduction band energy level of metal oxide or semiconductor-based nanocarriers Study of in vitro toxic effects related to energy of conduction band and metal dissolution	Suitable for metal/metal oxide or semiconductor-based nanocarriers	Accomasso et al. (2018)
5.	Quantitative structure activity relationships (QSAR)	Prediction of nanocarriers or nanopharmaceuticals exposure-dose-response that includes data assembling, structure characterization, model construction, model evaluation, interpretation, and review of mechanisms	Availability of small number of data sets	Accomasso et al. (2018)

are commonly used for the toxicity evaluation of engineered nanomaterials. In contrast, Berkner et al. (2016) enlisted various protocols concerning about the physicochemical properties, fate, and ecotoxicity behavior of nanopharmaceuticals that could be further used for the environmental risk assessment (see Table 1.5). In this regard, Maurer-Jones et al. (2013) enlisted various bacterial monoculture models which are reportedly used for the toxicity assessment of various nanoparticles. In addition, Wang et al. (2016) exclusively reviewed about the alteration of various metallic nanocarriers' toxicity in the presence of natural organic matters with the possible mechanisms. Reviewed studies include bacteria, algae, plant, vertebrates, and invertebrates as the test organism. Overall, the assessment of the fate of an active pharmaceutical ingredient in the open ecosystem as conducted for small drug molecules and bare nanoparticles is mostly found to be missing or not suitable for nanopharmaceuticals. Therefore, for nanopharmaceuticals and nano-bio drug conjugates, the biodegradability test is also recommended for more informative and

Table 1.5 Type of studies related to the physical and chemical properties, fate, and ecotoxicological effects of nanopharmaceuticals which are primarily recommended for the environmental risk assessment (European Medicines Agency 2006; Berkner et al. 2016)

S. no.	Study type	Recommended protocols
1.	Water solubility	OECD 105
2.	Dissociation constants in water	OECD 112
3.	Vapor pressure	OECD 104
4.	*n*-Octanol–water partition coefficient	OECD 107/123
5.	Adsorption–desorption using a batch equilibrium method: a study using two types of sludge and three types of soil is preferred	OECD 106
6.	Ready biodegradability	OECD 301
7.	Aerobic and anaerobic transformation in aquatic sediment systems	OECD 308
8.	Freshwater alga and cyanobacteria, growth inhibition test	OECD 201
9.	*Daphnia magna* reproduction test	OECD 211
10.	Fish, early life stage toxicity test	OECD 210
11.	Fish full life cycle test	ENV/JM/MONO(2008)22[a]
12.	Activated sludge, respiration inhibition test	OECD 209
13.	Bioaccumulation in fish: aqueous and dietary exposure	OECD 305
14.	Sediment–water Chironomid toxicity test using spiked sediment	OECD 219
15.	Sediment–water *Lumbriculus* toxicity test using spiked sediment	OECD 225
16.	Aerobic and anaerobic transformation in soil	OECD 307
17.	Soil microorganisms: nitrogen transformation test	OECD 216
18.	Terrestrial plants, growth test	OECD 208
19.	Earthworm, acute toxicity tests	OECD 207
20.	*Collembola*, reproduction test	OECD 232

OECD Organisation for Economic Co-operation and Development
[a]OECD Series on testing and assessment: Number 95

factual details. Moreover, a few ideal study protocols should also require to be developed to characterize the transformation and alteration in the nanopharmaceuticals inside the human and/or animal body or in other suitable media during the metabolism.

1.6 Future Research Directions

In the last few decades, nanotechnology has evolved in different directions, such as catalysis, electronics, sensing, biomedical applications, and many others. However, various human-, animal-, and plant-related concerns have hindered the comprehensive utilization of this promising technology due to the associated environmental and toxicological implications. Interestingly, humans are routinely exposed to airborne nanosized dust particles from a very early age; however the exposure to such particles has dramatically increased in the last few years due to various human activities in the field of nanotechnology and related applications (Doong et al. 2013; Brohi et al. 2017). Therefore, the large-scale application of nanomaterials in industries, food products, and medicines has alarmed major concerns about the humans as well as animals and plants (Navarro et al. 2008; Houdy et al. 2011; Miralles et al. 2012; Dev et al. 2017; Kaphle et al. 2017; Manickam et al. 2017; Sardoiwala et al. 2017). It is mainly due to the significant number of reported and validated studies claiming the potential toxic hazards of various nanomaterials. The reported hazards are primarily associated with nanocarriers' composition, concentration, administration routes, modification, and the exposed species. Therefore, a proper understanding of the impacts of nanocarriers on human, animal, or plant growth and reproductive system is very essential, so that the minimization of adverse effects of various nanocarriers could also be planned and implemented on the vulnerable population of humans, animals, and plants.

For the same objective, some novel study protocols are needed to be developed and standardized to study the contamination level, bioaccumulation limit, environmental risk quotient (ERQ) measurement, chemical and physical transformation in nanocarriers, and resulting alteration in the toxicity of associated nanopharmaceuticals. Inevitably, the more comprehensive absorption, distribution, metabolism, excretion/elimination, and toxicity (ADMET) studies in addition to the routine pharmacokinetics and pharmacodynamics are recommended for the newly developed nanocarriers which are being planned to be utilized in the nanopharmaceuticals for effective transportation and targeting of medicaments. Moreover, the inherent modification in the nanocarriers such as surface modification, coating, and co-doping of other nontoxic or toxicity retardants are also recommended for safer application of nanopharmaceuticals.

Furthermore, the researchers need to consider and scrutinize at least few prominent concerns before going for any conclusive solid remarks related to the usage and development of nanopharmaceuticals. The concerns were mainly proposed by Boxall et al. (2012) for pharmaceuticals and personal care products (PPCPs) presence in the open environment; and the most important concerns were selected

through the experts voting at an international expert workshop. However, the similar and most important concerns with more than 30% votes are rephrased here with respect to the nanopharmaceuticals. These prominent concerns are as follows: (1) importance of nanopharmaceuticals relative to other chemicals and non-chemical stressors in terms of biological impacts in the natural environment; (2) approaches to prioritize nanopharmaceuticals for research on the environmental and human health exposure-based effects; (3) environmental exposure to the nanopharmaceutical residues that results in the selection of antimicrobial-resistant microorganisms and affects human health outcomes; (4) observation of ecotoxicological responses such as histological and molecular-level responses for nanopharmaceuticals, and their translation into traditional cum ecologically important end points such as survival, growth, and reproduction of a species; (5) usage of pharmaceuticals preclinical and clinical information to assess the potential of adverse environmental impacts of nanopharmaceuticals; (6) evolutionary conservation of nanopharmaceutical targets across species and life stages in the context of potential adverse outcomes and effects; and (7) effects from long-term exposure to low concentrations of nanopharmaceuticals mixtures on the nontargeted organisms.

On the similar note, Ågerstrand et al. (2015) recommended ten directions for improving the European Medicines Agency's guideline on environmental risk assessment of human pharmaceutical products. The recommendations were based on the up-to-date available scientific information in combination to the experiences from other chemical endorsement entities. Those recommendations are as follows: "(**1**) *Expanding the scope of the current guideline*; (**2**) *Requirements to assess the risk for development of antibiotic resistance*; (**3**) *Jointly performed assessments*; (**4**) *Refinement of the test proposal*; (**5**) *Mixture toxicity assessments on active pharmaceutical ingredients with similar modes of action*; (**6**) *Use of all available ecotoxicity studies*; (**7**) *Mandatory reviews*; (**8**) *Increased transparency*; (**9**) *Inclusion of emission data from production*; and (**10**) *A risk management option*". The implementation of the aforementioned recommendations with respect to nanopharmaceuticals is equally rational and crucial for the protection of the environment, human, and other living organisms.

1.7 Conclusions

Overall, nanopharmaceuticals have a tremendous potential to have a significant impact on the human beings and other living organisms. However, the proper risk evaluation either related to the environment or related to the health of the living organism is still a major challenge in the development of nanopharmaceuticals. The concerns related to the nanopharmaceuticals and their associated nanocarriers are quite indispensable for the ethical and legal acceptance of nanomedicaments. Here, the presented study is a sincere attempt to emphasize the environmental and toxicological implications of nanocarriers used in various nanopharmaceuticals. The discussion includes the key issues related to the nanopharmaceutical types, exposure,

effects, quantification, absorption, distribution, metabolism, excretion/elimination, and toxicity (ADMET) behavior and potential hazards of nanopharmaceuticals.

The inferences made in this review article suggested that the understanding of the in vivo biodistribution of nanocarriers is significant but still inadequate for the critical evaluation of the efficacy and safety related to the nanopharmaceuticals. Hence, for the development of nanopharmaceuticals with improved efficacy and safety, various nanocarriers' assessment techniques and toxicity measurement protocols have been pointed out for the long-term safety and sustainability of nanopharmaceuticals. Moreover, there are various experts' recommendations and concerns too that have been positively put forward for the further development of nanopharmaceuticals. More precisely, the factors and processes affecting nanopharmaceuticals and their associated nanocarriers' biodistribution, such as physicochemical properties of nanosubstances, interaction with membranes and proteins, extravasation or transportation to the tissues and specific cells via lymph and blood vessels, uptake by the reticuloendothelial system, and clearance through the liver and kidneys, need to be scrutinized very carefully. Hence, more advanced and systematic in vitro and in vivo approaches are needed to be developed and recommended for the better correlation of nanopharmaceuticals properties with their biological effects.

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Chapter 2
Herbal Nanocarriers for Cancer Therapy

Mrityunjoy Mahato, Sanjukta Patra, and Manashjit Gogoi

Contents

Abstract Cancer is a group of around 100 diseases that has been tormenting mankind since ancient time. Due to cancer, estimated 8.2 million people died globally in 2012, and the toll is expected to reach 13 million in 2030. Despite the improvement of conventional therapeutic modalities, the outcome of cancer patients has not improved significantly. So, alternative therapeutic modalities and new effective anticancer drugs are highly sought for. Different parts of plants and their extracts have been used to cure many diseases and relive from physical agony since ancient

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times. In the traditional system of medicine, herbal products have been used for treating different types of diseases and alignments globally. Active compounds from herbal medicine, such as curcumin, are found to be effective against cancer. Despite their excellent therapeutic ability, the potential of these herbal compounds or phytochemicals is limited due to their low water solubility and poor bioavailability.

Advances in nanomedicines are revolutionizing the healthcare sector. Significant progresses have been made in development of nanocarriers in recent decades. Therapeutic efficacies of conventional drugs are reported to enhance by many folds using these novel nanocarriers through the intervention of nanotechnology. Application of nanotechnology may be effective in overcoming limitations of herbal drugs such as low water solubility, poor bioavailability, toxicity, and poor therapeutic efficacy of the drugs. It greatly helps in achieving higher efficiency of the drugs compared to its molecular form. Development of herbal-based nanocarriers like polymeric nanoparticles, dendrimers, liposomes, and micelles is reported to be more effective in treatment and managements of cancer. Loading of herbal compounds within these nanodrug delivery systems changes their pharmacokinetics profile and increases their bioavailability and therapeutic efficacy.

In this review, a comprehensive effort has been made on discovery of herbal drugs, herbal nanocarriers, and their application for cancer therapy. The coverage of this review will also extend to its current status and future prospects with elaborative and graphical examples.

Keywords Cancer · Chemotherapy · Herbal compounds · Nanocarriers · Nanomedicine

2.1 Introduction

2.1.1 Cancer Overview

Cancer represents a diverse group of life-threatening diseases that causes abnormal and uncontrolled growth of malignant cells. These malignant cells are highly unorganized, irregular in shape and size, and capable of invading neighboring healthy tissues and organs. The characteristics of cancer cells are: ability of tissue invasion and metastasis, sustain angiogenesis, self-sufficiency in growth signals, limitless replicative potential, evasion of apoptosis, and insensitivity to anti-growth signals (Hanahan and Weinberg 2000; Gogoi et al. 2016). Cancer has been affecting mankind since ancient times. There are more than 100 types of cancers reported till date, and their subtypes are found within specific organs (Gogoi et al. 2016). With time, the tumor cells disintegrate from the primary tumor and migrate through the blood vessels and lymphatic streams to form their colonies at different sites of the patient's body. This process is called metastasis and it leads to the death of the host.

The exact causes and the ways of initiation and spreading of cancer are still not well understood, but both external factors (e.g., tobacco smoking, infections, exposure to retroviruses, chemicals, and radiations) and internal factors (e.g., inherited

metabolism mutations, hormones, and immune conditions) are believed to be the reasons for cancer formation and growth (Feng and Chien 2003). These factors may act together or in sequential manners to initiate and promote cancer. Till date, no complete curing procedure for cancer is available, only remission or palliation is possible with the current treatment procedures. A cancer is said to be in remission state when all clinical evidence of cancer has been disappeared and the microscopic foci of cancer cells may still remain (Feng and Chien 2003).

2.1.2 Limitation of Conventional Therapeutic Modalities

The most common and effective cancer treatment modalities are surgery, radiotherapy, chemotherapy, hormone therapy, and immunotherapy. All these modalities have their own advantages as well as disadvantages and usually combination of two or more modalities gives the best result (Feng and Chien 2003). Surgery is one of major treatment procedures for treating tumor; but, erroneous or inadequately margined resection of tumor cells may lead to faster metastasis (Feng and Chien 2003; Gogoi et al. 2017). Moreover, tumors at metastasis cannot be treated with either surgery or radiotherapy. Radiotherapy is not selective to cancer cells, and it kills both malignant and healthy cells. Success of chemotherapeutic agents in treating cancer is limited by their severe side effects and development of multidrug resistance by the cancer cells. A schematic representation of how chemotherapy kills cancer cells is shown in Fig. 2.1. Chemotherapeutic drugs which are effective against rapidly dividing cells cannot kill large portion of dormant tumor cells. Thus the chemotherapy is compromised (Gogoi et al. 2017; Paszek et al. 2005; Tannock 2001). Hormone therapy is applicable to only hormone-sensitive cancers like breast cancer, prostate cancer, ovarian cancer, etc. Hormone therapy inhibits the growth of cancer cell by blocking the action of hormones such as estrogen receptor-α responsible for the tumor growth (Hayashi and Kimura 2015). But, almost all patients with metastatic breast and prostate cancer that initially respond to hormone therapy develop resistance to hormone therapy, and it leads to progression of the disease (Abraham and Staffurth 2016). Despite having number of therapeutic options, cancer is posing as a big menace to mankind. In 2008, 7.6 million people died of cancer, and toll is

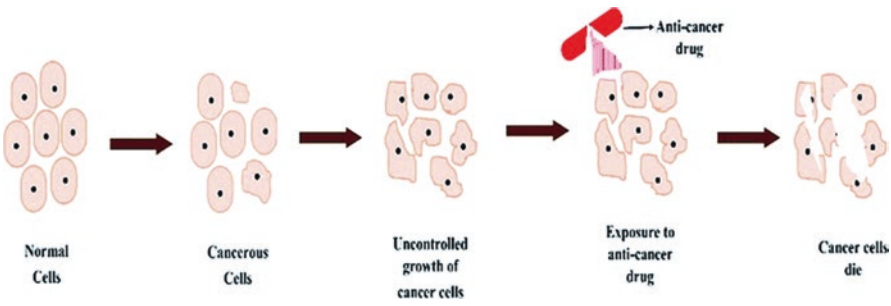


Fig. 2.1 Outline of chemotherapy (Subramanian et al. 2016)

expected to reach 13.2 million by 2030 (Global cancer facts and figures, 2015, 2nd ed.). So, search for new effective and developed therapy is still going on.

2.1.3 Importance of Nanomedicine

Application of nanotechnology especially nanomedicines opens up a window of opportunities to enhance the efficacy of anticancer drugs. According to the National Institute of Health, nanomedicine is referred as application of nanotechnology for treatment diagnosis, monitoring, and control of biological systems. Research into nanodrug delivery systems and diagnostic agents come within the preview of nanomedicine (Moghimi et al. 2005). The size of nanocarriers is generally in the range of 1 to 100 nm (Subramanian et al. 2016). But, for the purposes of this chapter, we are considering all the drug delivery systems below size 1000 nm as "nanodrug delivery systems" or "nanocarriers." Nowadays a lot of herbal nanocarrier-based nanomedicines are being investigated globally and showing promising results for the holistic treatment of cancer disease. Herbal compound-loaded nanocarriers can overcome the problems like aqueous solubility and permeability through biological membrane due to their size and modified surface properties as faced by herbal bioactive compounds. Encapsulation of herbal drugs in nanocarriers improves the pharmacological activity and biodistribution of drugs, ensures their solubility and stability, and helps in maintaining sustained delivery (Jain et al. 2011). Moreover, application of nanodrug delivery systems may help in (i) achieving enhanced and targeted delivery of phytochemicals; (ii) crossing the tight epithelial and endothelial barriers and delivering large molecules to intracellular sites of action; and (iii) co-delivering of two or more phytomedicines or therapeutic modalities for combined therapy and imaging the site of drug action (Lambert 2010; Liong et al. 2008; Gunasekaran et al. 2014). Targeted delivery herbal bioactive molecules to the tumor site(s) reduces the side effects caused by off targeted delivery and increases the therapeutic efficacy of the nanoformulations.

Herein, we are reviewing the different types of herb-based nanocomposite existing in the literatures along with illustrative figures and explanation, which have been specially applied for the cancer treatment. The current development and future prospects in this direction have also been discussed.

2.2 Bioactive Herbal Compounds: History and Discovery Strategies

Historically, plants and their products have been playing an important role in curing many diseases and reliving from different physical agonies. Plants are important sources of traditional medicines (Bhattacharjya and Borah 2008; Newman et al. 2000; Buss et al. 2003). Herbal medicines were reported to use in different

civilizations around the world since ancient times. In Mesopotamia, approximately 1000 plant-derived substances were reported to use as medicine in around 2600 BCE. Egyptians had been using herbal medicines since 2900 BCE; but, the *Ebers Papyrus* only properly reported use of over 700 drugs of mostly plant origin in 1550 BCE. The Indian Ayurvedic system is dated prior to 1000 BCE. Charaka Samhita and Sushruta Samhita documented use of 341 and 516 drugs, respectively (Kapoor 1990; Dev 1999).The Chinese materia medica also documented use of large number of herbal medicine for treating different diseases. The Greco–Roman knowledge on their traditional herbal medicine was dated back to the first century AD, and large amount of this knowledge bases were preserved by the Arabs during the dark and middle ages during the fifth to twelfth centuries (Cragg and Newman 2013). Much later, numbers of German books on herbal medicines were compiled during the period of the fourteenth to seventeenth century (Atanasov et al. 2015).

During all those periods, herbal medicines were used to treat different diseases or alignments without the in-depth knowledge of pharmacological activity or active components of the herbs (Atanasov et al. 2015). But, rational clinical investigation on medicinal herbs was laid down in the eighteenth century, when Anton von Störck had studied the properties of poisonous herbs like aconite and colchicum and William Withering had studied foxglove for the treatment of edema (Sneader 2005).

At the beginning of the nineteenth century, rational drug discovery from plants started when the German apothecary assistant Friedrich Sertürner had successfully isolated analgesic and sleep-inducing agent from opium named morphium (morphine). Later numbers of papers were published based on this discovery. This led to successful isolation and study of numerous natural drugs from herbs and followed by chemical synthesis of these drugs (Kaiser 2008). As per the world health organization (WHO) report, 80% of rural people of world's population especially in developing countries depend on the herbal medicine (World Health Organization Guideline 2001). Till today, substantial portion of therapeutic agents are comprised of natural products and their derivatives; e.g., 61% of anticancer compounds and 49% anti-infectives approved during the period of 1981 to 2010 are derived from nature (Newman and Cragg 2012). However, the pharmaceutical companies have been avoiding investigation on natural product discovery processes since 1990, due to difficulties in supply, screening, characterization, and increase in rate of rediscovering the known compounds (Li and Vederas 2009). Still, research on fast, inexpensive next-generation genome sequence technology and the discovery of natural product is flourishing at academic level (Luo et al. 2014).

The natural product discovery processes are broadly classified into two categories, namely, top-down and bottom-up approaches (Fig. 2.2). In top-down approaches, system level information are utilized to generate the new natural products without having prior knowledge of genes and enzymes involved in the biosynthesis. These approaches don't require complicated genome sequencing and sophisticated genetic manipulation.

In these approaches, biological samples are collected from diverse environments either for extraction or laboratory cultivation. The extracts are then screened for a desired bioactivity, and the "hits" are isolated for structural characterization. New

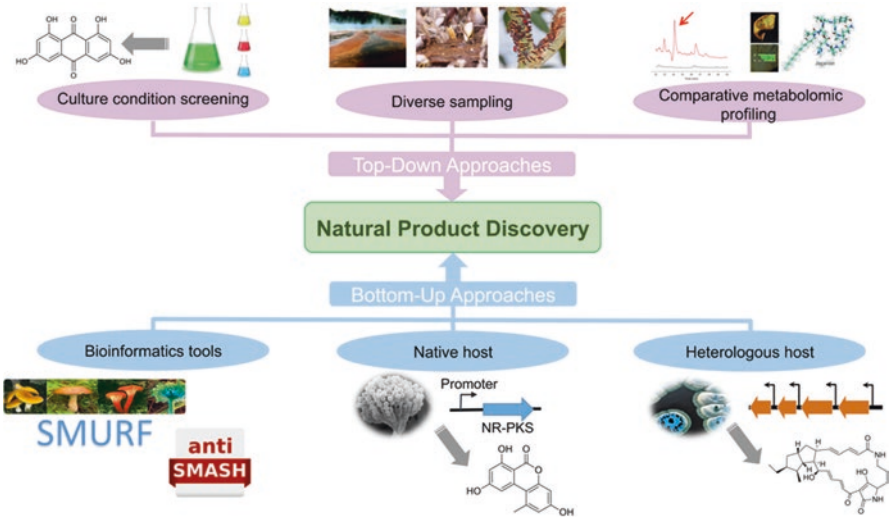


Fig. 2.2 Overview of the recent strategies applied for the discovery of natural products (Luo et al. 2014)

innovation in sampling and screening has mitigated the risk of rediscovering new chemical entities and allowing this approach to remain a viable means of natural product discovery (Luo et al. 2014). On the other hand, in the bottom-up approaches, the gene cluster of interest is identified, manipulated using transcription and translation processes, and then the corresponding natural product is synthesized (Luo et al. 2014). Plant-derived natural products are generally nontoxic to the normal cells and well tolerated by our body (Singh et al. 2016).

The plant-derived marketed anticancer compounds can be divided into four important classes, the vinca alkaloids (vinblastine, vincristine, and vindesine), the epipodophyllotoxins (etoposide and teniposide), the taxanes (paclitaxel and docetaxel), and the camptothecin derivatives (camptothecin and irinotecan) (Desai et al. 2008). Apart from these, the plants have tremendous potential to provide newer drugs, and search for new medicinal plants with potential anticancer compounds is going on.

Vinca alkaloids are herbal compounds extracted from Madagascar periwinkle plant, *Catharanthus roseus G. Don*., and they have the potential to treat diabetes and cancer (Moudi et al. 2013). Vinca alkaloids inhibit microtubule assembly and hence disrupt the cellular division process of tumor cells (Duflos et al. 2002). Moreover, disruption of microtubules function affects the cellular functions like intracellular organelle transport, cell migration, cell signaling, and mitosis (Perez 2009). Herbal compounds derived from vinca alkaloid are used to treat breast cancer, Hodgkin's lymphoma and Kaposi's sarcoma, severe lymphoblastic leukemia, non-Hodgkin leukemia, William's tumor, and non-small cell lung cancer (Safarzadeh et al. 2014).

Epipodophyllotoxins or podophyllotoxins are extracted from the root of the Indian podophyllum plant (*Podophyllum peltatum*). Etoposide and teniposide are

two active and semisynthetic compounds belonging to this family. These compounds arrest the proliferation of tumor cells by inhibiting topoisomerase II, which causes breakdown of DNA double strands (Damayanthi and Lown 1998; Safarzadeh et al. 2014).

Taxanes such as paclitaxel, docetaxel, and other taxane homologs are considered as the most effective antitumor agents and effective against wide range of cancers such as breast, ovary, lung, and other metastatic cancers. Paclitaxel is derived from Pacific yew bark (*Taxus brevifolia*). These taxanes inhibit the polymerization of microtubules and thereby prevent proliferation of tumor cells (Hagiwara and Sunada 2004).

Camptothecins are natural cytotoxic drugs isolated from *Camptotheca accuminata* of the Nyssaceae family. These are strong inhibitor of nucleic acid in mammalian cells and induce strand breaks in chromosomal DNA topoisomerase I (Hsiang et al. 1985).

Apart from these four groups of drugs, a large number of herbal drugs have been tried/investigated for their anticancer properties. These drugs from herbs or spices reveal their anticancer properties either by direct cytotoxic effects or modulating the immune system (Kitagishi et al. 2012). There are at least 2,50,000 species of plants out of which more than 1000 plants have been found to possess significant anticancer properties (Mukherjee et al. 2001). Active phytochemicals and their derivatives are found in leaf, root, flower, stem, and bark, and they perform number of pharmacological activities in human body (Singh et al. 2016). The search of novel bioactive compounds from natural sources continues with botanists, marine biologists, and microbiologists teaming up with chemists, pharmacologists, toxicologists, and clinicians. A comprehensive list of phytochemicals investigated for treatment of different cancers is shown in Table 2.1.

2.2.1 Structures of Important Herbal Compounds

Though a large number of plant-derived chemicals are investigated for their anticancer activity, only a few chemical entities were able to get approved for clinical applications due to stringent evaluation processes of pharmaceutical agents. The plant-derived anticancer agents approved for therapeutic use in the last 30 years (1984–2014) are summarized in Table 2.2.

2.3 Cancer Targeting Strategies and Herbal Nanostructures

Despite discovery of large numbers of plant-derived drugs, success in treating solid tumor is limited due to the severe side effects of chemotherapeutic agents and the development of multidrug resistance. Moreover, highly acidic and oxygen-deprived hypoxic environments within the tumor mass reduce the effectiveness of drugs that

Table 2.1 Phytochemicals found to be effective in different types of cancers (Singh et al. 2016)

Phytochemical(s)	Cancer models suppressed	References
Alexin B, Emodin (*Aloe vera*)	Leukemia, stomach cancer, neuroectodermal tumors	Elshamy et al. (2010)
Allylmercaptocysteine, allicin (*Allium sativum*)	Colon cancer, bladder carcinoma	Ranjani and Ayya (2012)
Amooranin (*Aphanamixis polystachya*)	Breast, cervical, and pancreatic cancer	Chan et al. (2011)
Andrographolide (*Andrographis paniculata*)	Cancers of the breast, ovary, stomach, prostate, and kidney, nasopharynx malignant melanoma, leukemia	Geethangili et al. (2008)
Ashwagandhanolide (*Withania somnifera*)	Cancers of the breast, stomach, colon, lung, and central nervous system	Yadav et al. (2010)
Bavachinin, corylfolinin, psoralen (*Psoralea corylifolia*)	Cancers of the lung and liver, osteosarcoma, malignant ascites, fibrosarcoma, and leukemia	Wang et al. (2011b)
Berberine, cannabisin-G (*Berberis vulgaris*)	Cancers of the breast, prostate, liver, and leukemia	Elisa et al. (2015)
Betulinic acid (*Betula utilis*)	Melanomas	Król et al. (2015)
Boswellic acid (*Boswellia serrata*)	Prostate cancer	Garg and Deep (2015)
Costunolide, Cynaropicrine, Mokkolactone (*Saussurea lappa*)	Intestinal cancer, malignant lymphoma, and leukemia	Lin et al. (2015)
Curcumin (*Curcuma longa*)	Cancers of the breast, lung, esophagus, liver, colon, prostate, skin, and stomach	Perrone et al. (2015)
Daidzein and genistein (*Glycine max*)	Cancers of the breast, uterus, cervix, lung, stomach, colon, pancreas, liver, kidney, urinary bladder, prostate, testis, oral cavity, larynx, and thyroid	Li et al. (2012)
Damnacanthal (*Morinda citrifolia*)	Lung cancer, sarcomas	Aziz et al. (2014)
β-Dimethyl acryl shikonin, arnebin (*Arnebia nobilis*)	Rat walker carcinosarcoma	Thangapazham et al. (2016)
Emblicanin A & B (*Emblica officinalis*)	Cancers of breast, uterus, pancreas, stomach, liver, and malignant ascites	Dasaroju and Gottumukkala (2014)
Eugenol, orientin, vicenin (*Ocimum sanctum*)	Cancers of the breast and liver and fibrosarcoma	Preethi and Padma (2016)
Galangin, pinocembrine, acetoxychavicol acetate (*Alpinia galangal*)	Cancers of lung, breast, digestive systems, and prostate and leukemia	Sulaiman (2016)
Gingerol (*Zingiber officinale*)	Cancers of ovary, cervix, colon, rectum, liver, urinary bladder, oral cavity, neuroblastoma, and leukemia	Rastogi et al. (2015)
Ginkgetin, ginkgolide A & B (*Ginkgo biloba*)	Glioblastoma multiforme, ovary, colon, hepatocarcinoma, prostate, and liver	Xiong et al. (2016)

(continued)

Table 2.1 (continued)

Phytochemical(s)	Cancer models suppressed	References
Glycyrrhizin (*Glycyrrhiza glabra*)	Lung cancer, fibrosarcomas	Huang et al. (2014)
Gossypol (*Gossypium hirsutum*)	Cancers of the breast, esophagus, stomach, liver, colon, pancreas, adrenal gland, prostate, and urinary bladder, malignant lymphoma and myeloma, brain tumor, and leukemia	Zhan et al. (2009)
Kaempferol galactoside (*Bauhinia variegata*)	Cancers of the breast, lung, liver, oral cavity, and larynx and malignant ascites	Tu et al. (2016)
Licochalcone A, licoagrochalcone (*Glycyrrhiza glabra*)	Cancers of the prostate, breast, lung, stomach, colon, liver, and kidney and leukemia	Zhang et al. (2016)
Lupeol (*Aegle marmelos*)	Breast cancer, lymphoma, melanoma, and leukemia	Wal et al. (2015)
Nimbolide (*Azadirachta indica*)	Colon cancer, lymphoma, melanoma, leukemia, and prostate cancer	Wang et al. (2016)
Panaxadiol, panaxatriol (*Panax ginseng*)	Cancers of the breast, ovary, lung, prostate, and colon, renal cell carcinoma, leukemia, malignant lymphoma, and melanoma	Du et al. (2013)
Plumbagin (*Plumbago zeylanica*)	Cancers of the breast and liver, fibrosarcoma, leukemia, and malignant ascites	Yan et al. (2015)
Podophyllin and podophyllotoxin (*Podophyllum hexandrum*)	Cancers of the breast, ovary, lung, liver, urinary bladder, testis, and brain, neuroblastoma, and Hodgkin's disease	Liu et al. (2015)
Psoralidin (*Psoralea corylifolia*)	Stomach and prostate cancer	Pahari et al. (2016)
Sesquiterpenes and diterpenes (*Tinospora cordifolia*)	Lung, cervix, throat, and malignant ascites	Gach et al. (2015)
6-Shogaol (*Zingiber officinale*)	Ovary cancer	Ghasemzadeh et al. (2015)
Skimmianine (*Aegle marmelos*)	Liver tumors	Mukhija et al. (2015)
Solamargine, solasonine (*Solanum nigrum*)	Cancers of the breast, liver, lung, and skin	Al Sinani et al. (2016)
Thymoquinone (*Nigella sativa*)	Cancers of the colon, breast, prostate, pancreas, and uterus, malignant lymphoma, ascites, melanoma, and leukemia	Fakhoury et al. (2016)
Ursolic acid and oleanolic acid (*Prunella vulgaris*)	Cancers of the breast, cervix, lung, oral cavity, esophagus, stomach, colon, and thyroid, malignant lymphoma, intracranial tumors, and leukemia	Wozniak et al. (2015)

(continued)

Table 2.1 (continued)

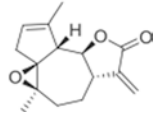
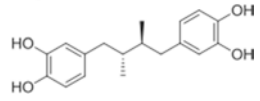
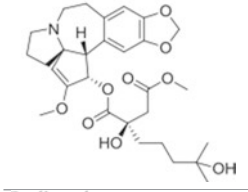
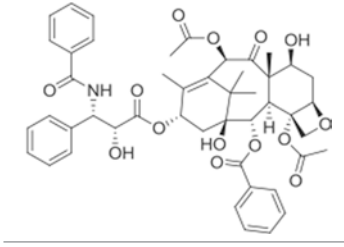
Phytochemical(s)	Cancer models suppressed	References
Ursolic acid (*Oldenlandia diffusa*)	Cancers of the lung, ovary, uterus, stomach, liver, colon, rectum, and brain, lymphosarcoma, and leukemia	Al Sinani et al. (2016) and Wozniak et al. (2015)
Vinblastine, vincristine (*Catharanthus roseus*)	Cancers of the breast, ovary, cervix, lung, colon, rectum, and testis, neuroblastoma, leukemia, rhabdomyosarcoma, malignant lymphoma, and Hodgkin's disease	Keglevich et al. (2012)
Viscumin, digallic acid (*Viscum album*)	Cancers of the breast, cervix, ovary, lung, stomach, colon, rectum, kidney, urinary bladder, and testis, fibrosarcoma, melanoma	Bhouri et al. (2012)
Withaferin A, D (*Withania somnifera*)	Cancers of the breast, cervix, prostate, colon, nasopharynx, and larynx and malignant melanoma	Lee and Choi (2016)

are basic in nature and/or utilize oxygen-free radicals for anticancer action (Kellen 1993). In solid tumor, a substantial portion of tumor cells present in dormant state, and they do not divide in the early stage of tumor formation (Rockwell and Hughes 1994). Therefore, chemotherapeutic agents effective against rapidly dividing cells could not kill them (Slingerland and Tannock 1998; Gogoi et al. 2017). Under this circumstances, the intervention of nanotechnology into the herbal drugs start playing an enhancing factor of its therapeutic efficacy towards the targeted diseases. Herbal drug-loaded nanoformulations can be prepared using methods such as high pressure homogenization, complex coacervation, co-precipitation, salting out, nanoprecipitation or solvent displacement, solvent emulsification–diffusion, supercritical fluid method and self-assembly method, etc. (Gunasekaran et al. 2014). Some of the common herbal nanodrug delivery systems are liposomes, emulsions, solid lipid nanoparticles, micelles, polymeric nanoparticles, dendrimers, carbon nanotube, inorganic nanoparticles (silica, ZnO), etc. These nanoparticles deliver drug to the cancer site(s) by two strategies, i.e., active and passive targeting.

2.3.1 Active Targeting

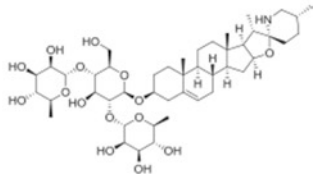
In active targeting, nanocarriers are channeled to tumor sites with the help of targeting ligands specific against receptors overexpressed on tumor cells or tumor vasculature, which are not expressed by normal cells. In this process, chemotherapeutic agent-loaded nanocarriers are conjugated with targeting ligands or moieties such as folic acid, monoclonal antibody, integrin, etc. which can target (i) receptors preferentially expressed on endothelial cells of tumor blood vessels (e.g., integrin-α_v β_3 and negatively charged phospholipids) (Li et al. 2004; Nisato et al. 2003); (ii) receptors overexpressed on tumor cells, e.g., HER2 and folate receptor (Chen et al. 2008; Pradhan et al. 2010); and (iii) lineage-specific targets that are expressed at the same

Table 2.2 Plant-derived anticancer agents approved for therapeutic use in the last 30 years (1984–2014) (Atanasov et al. 2015; Newman and Cragg 2012)

Generic name and chemical structures	Plant species (literature reference)	Trade name (year of introduction)	Indication (mechanism of action)
Arglabin 	*Artemisia glabella* Kar. et Kir. replaced by *Artemisia obtusiloba* var. *glabraLedeb.* (Adekenov et al. 1982)	Arglabin (1999)	Cancer chemotherapy (farnesyl transferase inhibition)
Masoprocol 	*Larrea tridentata* (Sessé & Moc. ex DC.) Coville (Luo et al. 1998)	Actinex (1992)	Cancer chemotherapy (lipoxygenase inhibitor)
Omacetaxine mepesuccinate (Homoharringtonine) 	*Cephalotaxus harringtonia* (Knight ex Forbes) K. Koch (Powell et al. 1974)	Synribo (2012)	Oncology (protein translation inhibitor)
Paclitaxel 	*Taxus brevifolia Nutt.* (Wani et al. 1971)	Taxol (1993), Abraxane (2005) and Nanoxel (2007)	Cancer chemotherapy (mitotic inhibitor)

(continued)

Table 2.2 (continued)

Generic name and chemical structures	Plant species (literature reference)	Trade name (year of introduction)	Indication (mechanism of action)
Solamargine 	*Solanum* spp. (Hsu and Tien 1974; Liljegren 1971)	Curaderm (1989)	Cancer chemotherapy (apoptosis triggering)

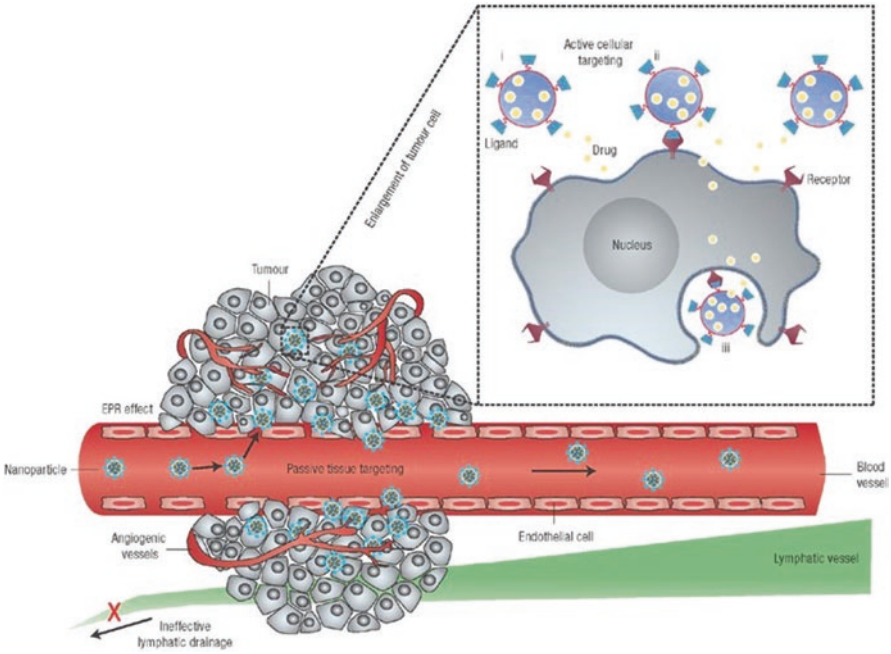


Fig. 2.3 Schematic representation of different mechanisms through which nanocarriers can deliver drugs at tumor sites. Polymeric nanoparticles are shown as representative nanocarriers (circles). Passive tissue targeting is achieved by extravasation of nanoparticles through enhanced permeability and retention (EPR) effect. Active cellular targeting (inset) can be achieved by functionalizing the surface of nanoparticles with ligands/moieties specific to the receptors/biomolecules expressed on the surface of the cancer cells (Peer et al. 2007)

level on both tumor and normal cells (e.g., CD19) (Cheng and Allen 2008) and kill tumor cells. These targeting ligands or moieties tagged effectively internalized by the tumor cells through receptor-mediated endocytosis. For effective deployment of active targeting strategy, the following issues need to be addressed: (i) liposome prepared for active targeting extravasated and bound to the first line of targeted tumor cells in the interstitial compartment and reported to obstruct the way for more liposomes to accumulate (Barenholz 2001), (ii) immunoliposomes prepared for active targeting were found to be cleared rapidly (Koning et al. 2002), and (iii) nanocarriers prepared for active targeting were reported to internalize via endocytosis process and end up with degradation in endosomes/lysosomes. Moreover, drug loading methods need to be devised properly so that the encapsulated drug does not form aggregate and degrade instantly for effective cancer treatment (Barenholz 2001). A schematic representation of active and passive targeting strategies of nanocarriers is demonstrated in Fig. 2.3.

2.3.2 *Passive Targeting: Enhanced Permeability and Retention (EPR) Effect*

In passive targeting process, nanocarriers/molecules are guided into the tumor interstitium or tissue through leaky tumor vasculature with the help of molecular movement within fluids (i.e., convection) or passive diffusion (Haley and Frenkel 2008). Conventional force mostly transports the larger molecules, whereas diffusion helps in transportation of low molecular weight compounds. It is well-known that tumor vasculatures are highly chaotic and complex structures, and they have the ability of extensive angiogenesis or forming hyperbranched defective vasculatures, impaired lymphatic drainage systems, and ability to generate number of vasculature permeability factors such as bradykinin, nitric oxide (NO) (Maeda et al. 1988; Matsumura et al. 1988; Maeda et al. 1994), and peroxynitrite ($ONOO^-$) (Maeda et al. 2000); and hence, tumor vasculatures are highly porous. The pore size in the tumor vasculature is in the range of 100–780 nm (Yuan et al. 1995) which is much larger than normal tissue junctions, i.e., less than 6 nm (Drummond et al. 1999). So, nanocarriers circulating in the blood selectively enter into the interstitial spaces of tumor tissues and get accumulated there due to impaired lymphatic drainage system. This effect is called enhanced permeability and retention (EPR) effect. But, the pore size of endothelium tissues of kidney glomerulus is in the range of 40–60 nm size; sinusoidal endothelium of liver and spleen have pores of size up to 150 nm (Seymour 1992). Nanocarriers like liposomes can avoid accumulation in the kidney due to their bigger size, but macrophages present in the liver and spleen can remove them from blood circulation. PEG coating onto surface of nanocarriers prevents their clearance by macrophages due to steric hindrance offered by PEG coating, increases their blood circulation time, and hence helps in selective accumulation of the nanocarriers in tumor through passive diffusion (Andresen et al. 2005).

A large numbers of nanocarriers have been investigated for treatment of cancer exploiting the active and passive targeting strategies. Surface functionalization of nanoparticles using PEG or similar molecules has been reported to improve the bioavailability of drugs at tumor sites in different preclinical animal models. However, clinical translation of the nanocarriers from bench to bedside is a huge challenge due to stochastic nature of ligand–receptor interactions and difficulties in controlling release of drug at diseased sites (Gogoi et al. 2017). In order to improve the therapeutic index of drugs, drug release at tumor sites is essential as it prevents their rapid metabolization and clearance from the patient's body. Drug release from nanocarriers can be triggered using either exogenous stimuli such as temperature, ultrasound, light, and electric fields or endogenous stimuli like change in pH, enzyme, redox potential, etc.

2.3.3 Herbal Nanostructures for Cancer Treatment

A large number of nanocarriers with herbal compounds have been investigated for treatment of various types of cancer. These nanocarriers target cancer cells either by active targeting or passive targeting strategy. A lot of herbal compounds are poorly soluble in aqueous solubility and resulting in poor bioavailability following oral administration (Bansal et al. 2011). Delivery of these poorly aqueous soluble drugs through nanocarriers reduces their systemic toxicity, improves pharmacokinetic properties, enhances their delivery at tumor sites, and hence improves the therapeutic indices of the drugs (Aqil et al. 2013).The following section discusses the application of herbal nanocarriers in treatment of cancer.

Liposomes and Other Lipid Carriers

Liposomes

Liposomes are spherical vesicles made up of phospholipids which have a hydrophilic head and a hydrophobic tail. These phospholipids self-assemble under given conditions to form a bilayered structure called liposome. These liposomes have the ability to carry both hydrophobic and hydrophilic payload together. They have the advantages of high biocompatibility, biodegradability, ease of preparation, chemical versatility, and the ability to modulate the pharmacokinetic properties by changing the chemical composition and the components of the bilayers (Terreno et al. 2008). Dhule et al. (2014) investigated the combined antitumor effect of curcumin and C6 ceramide (C6) against osteosarcoma (OS) cell lines. They prepared three liposomal formulations, i.e., curcumin liposomes, C6 liposomes, and C6-curcumin liposomes. Curcumin in combination with C6 was found to be effective against MG-63 and KHOS OS cell lines, in comparison with curcumin liposomes alone. The therapeutic efficacy of the preparations was tested in vivo using a human osteosarcoma xenograft assay. PEGylated and folate tagged liposomes prepared for targeted delivery of curcumin and C6 significantly reduce the tumor volume in vivo. Recently, Gogoi et al. (2017) investigated the therapeutic efficacy of paclitaxel-loaded magnetic liposomes in vitro and in vivo under self-controlled hyperthermic condition. Results showed that the combined thermochemotherapy was effective in treating cancer in comparison to the drug and heat alone. Similar results were demonstrated by Gharib et al. (2015) who treated breast cancer using artemisinin and transferrin-loaded magnetic liposomes under AC magnetic field. In another study, berberine derivatives and doxorubicin-loaded long-circulating liposomes were studied for their ability to target mitochondria of drug-resistant cancer cells. Results demonstrated the superiority of these liposomes over regular doxorubicin-loaded liposomes and free doxorubicin (Tuo et al. 2016).

Solid Lipid Nanoparticles (SLNs)

Solid lipid nanoparticles have generated tremendous attention in last few decades due to their good release profile and targeted drug delivery with excellent physical

stability. Good deals of studies on SLNs have been done for improvising the delivery of phytochemicals with anticancer properties in recent decades. Phytochemicals like berberine (Xue et al. 2015), resveratrol (Teskac and Kristl 2010), and paclitaxel (Pooja et al. 2016) were encapsulated in SLNs and studied their therapeutic properties. Teskac and Kristl (2010) demonstrated that encapsulation of resveratrol within SLNs enhances the bioavailability of drug and hence increases the therapeutic efficacy of the drug.

Micelles

Polymeric micelles have been drawing attention due to their ability of site-specific delivery of therapeutic agents, reducing off-target toxicity, and improving pharmacokinetics (Biswas et al. 2016). Tea epigallocatechin gallate and Herceptin loaded polymeric micelles were reported to use for cancer therapy. These nanomicelles demonstrated better tumor selectivity and growth reduction, as well as longer blood half-life, than free Herceptin (Chung et al. 2014). Micelles have been used for delivery of poorly water-soluble anticancer agent quercetin. Tan et al. (2012) reported development of quercetin-loaded micelles for treatment of lung cancer. Nanomicelles made from the diblock copolymer and polyethylene glycol (PEG)-derivatized phosphatidylethanolamine (PE) were found to enhance peroral anticancer activity and no apparent toxicity to the intestinal epithelium.

Polymeric Nanoparticles

Polymeric nanoparticles are drawing huge attention in cancer drug delivery due to their stability, ease of conjugating functional moieties, and ease of surface modification. Yallapu et al. (2012b) developed curcumin-loaded cellulose nanoparticles for targeting prostate cancer. They investigated and compared cellular uptake and cytotoxicity of these curcumin-loaded cellulose nanoparticles with β-cyclodextrin (CD), hydroxypropyl methylcellulose (cellulose), poly(lactic-co-glycolic acid) (PLGA), magnetic nanoparticles (MNP), and dendrimer-based curcumin nanoformulations in prostate cancer cells. Results demonstrated the superiority of curcumin-loaded cellulose nanoparticles in comparison to the other nanoformulations in inducing apoptosis in cancer cells. Recently, paclitaxel-loaded polymeric nanoparticles combined with chronomodulated chemotherapy were evaluated in lung cancer both in vitro and in vivo. Results suggested that these paclitaxel-loaded nanoparticles exhibit greater anti-tumor activity against A549 cells, in comparison with paclitaxel. The anti-tumor effect at 15 h after light onset (HALO) administration was reported to be the best in all groups (Hu et al. 2017). Curcumin-loaded PLGA nanoparticles were reported to enhance the aqueous solubility of curcumin and increase the antitumor potential of curcumin (Nair et al. 2012).

Nanoemulsions

Nanoemulsions are colloidal nanoparticles known for their stability and high loading efficiency. These carriers are solid spheres, and their surface is amorphous and lipophilic with a negative charge. Recently, a good deal of works has been done on herbal agent-loaded nanoemulsions for cancer therapy. Anuchapreeda et al. (2012) studied therapeutic efficacy of curcumin-loaded nanoemulsion in number of different cancer cell lines. Results showed high encapsulation of curcumin, physical stability of these nanocarriers, and their preserved toxicity. In another study, Pool et al. (2013) studied the feasibility of encapsulating hydrophobic quercetin in nanoemulsion. In a recent study, camptothecin-loaded polymer stabilized nanoemulsion was investigated for the in vitro cytotoxicity as well as their potential to target breast cancer in vivo. Results showed the possibility of targeting breast cancer using these nanocarriers (Sugumaran et al. 2017) (Fig. 2.4).

Nanocapsules

Nanocapsules consist of a liquid/solid core in which the drug is placed into a cavity, which is surrounded by a distinctive polymer membrane made up of natural or synthetic polymers. They have been drawing huge attention due to the protective coating which can be tuned to achieve sustain and controlled release of active ingredients (Kothamasu et al. 2012). Artemisinin crystals were encapsulated using nanocapsules composed of chitosan, gelatin, and alginate. This investigation showed the

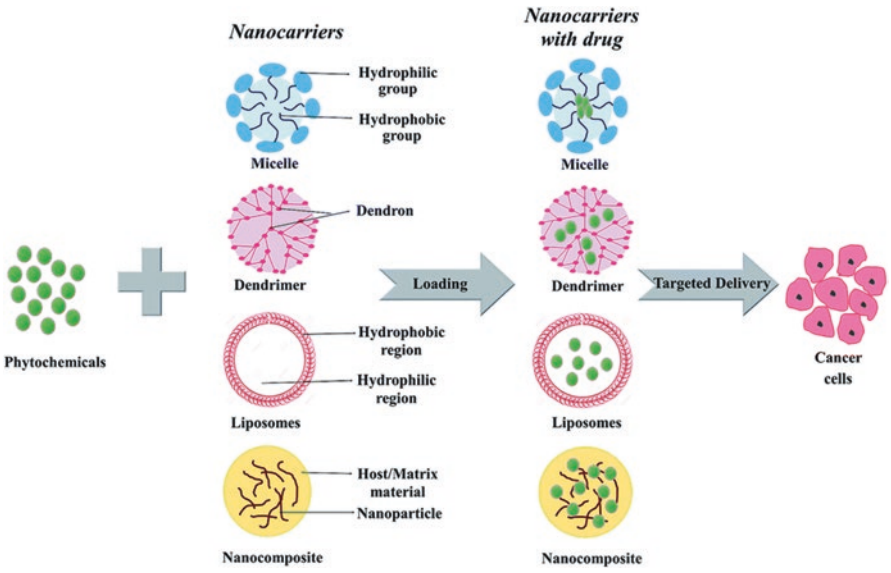


Fig. 2.4 Phytochemical loaded different types of nanocarriers (Subramanian et al. 2016)

possibility of achieving prolonged drug release through self-assembly of polyelectrolytes on natural drug crystals (Chen et al. 2009). In another study, anticancer drug quercetin was encapsulated in nanocapsules prepared for passive and active targeting to tumors. The investigators prepared nanocapsules from folic acid conjugated to poly(lactide-co-glycolide) (PLGA) polymer to facilitate active targeting to cancer cells and PEGylated PLGA for passive targeting. Comparative in vitro studies on the cytotoxicity and cellular uptake of the different formulations were carried out using MTT assay and confocal laser scanning microscopy, respectively. Results confirmed the selective uptake and cytotoxicity of the folic acid targeted nanocapsules to the folate enriched cancer cells in a folate-dependent manner. Finally, in vivo experiments were done to evaluate the passive tumor accumulation and the active targeting of the nanocapsules to folate-expressing cells in HeLa or IGROV-1 tumor-bearing mice. The developed nanocapsules provide a system for targeted delivery of a range of hydrophobic anticancer drugs in vivo (El-Gogary et al. 2014). Recently, Boissenot et al. (2016) developed a paclitaxel-loaded nanocapsule formulation composed of poly(lactide-co-glycolide)-polyethylene glycol shell and perfluorooctyl bromide (PFOB) core for cancer theranostic application. PFOB was used as imaging agent. This nanocapsule formulation was tested in vitro and in vivo. Results demonstrated that the formulation could be applied as a cancer theranostic agent.

Dendrimers

Dendrimers are hyperbranched polymeric architectures widely investigated these days due to their versatility in drug delivery and high functionality. These nanostructured macromolecules have the abilities to entrap and/or conjugate the high molecular weight hydrophilic/hydrophobic entities by host–guest interactions and covalent bonding (prodrug approach), respectively. Moreover, due to high ratio of surface groups to molecular volume, they are extensively studied for gene delivery (Madaan et al. 2014). Fox et al. (2009) prepared a PEGylated poly(l-lysine) (PLL) dendrimer formulation by covalently binding polymer conjugates of camptothecin to improve solubility, increase blood circulation time, enhance tumor uptake, and hence significantly improve efficacy of the drug. The reported formulation was found to be effective in treating HT-29 tumor-bearing mice. Therapeutic efficacy of hydrophilic paclitaxel-conjugated polyamidoamine (PAMAM) dendrimers was studied cancer cells. Combination of ensemble and single microtubule imaging techniques were used to determine the mechanism of action of these dendrimers in vitro. Results provided mechanistic insights into the cytotoxicity of paclitaxel-conjugated PAMAM dendrimers and uncovered unexpected risks of using such conjugates therapeutically (Cline et al. 2013). Anticancer agent berberine (BBR) was attempted to deliver using G4-PAMAM dendrimers by conjugation (BPC) as well as encapsulation (BPE) approach. The entrapment efficiency in BPE was found to be 29.9%, whereas the percentage conjugation in BPC was found to be 37.49% indicating high drug payload in conjugation. In vitro results showed significantly higher anticancer activity for the PAMAM-BBR ($p < 0.01$) against MCF-7 and

MDA-MB-468 breast cancer cells. In vivo results showed that the formulation was safer and biocompatible with very least but insignificant ($p > 0.05$) effects. The study demonstrated that conjugated formulation (BPC) was found to be more prominent than the encapsulated one (BPE) (Gupta et al. 2017).

Inorganic Nanoparticles

Inorganic nanoparticles including gold, oxides of iron, zinc, silicon, etc. were extensively investigated in both preclinical and clinical setting for delivering different anticancer phytochemicals. Poorly water-soluble curcumin was encapsulated in PMMA-PEG/ZnO bionanocomposite, and therapeutic potential and cellular uptake were studied in gastric cancer cell line (Dhivya et al. 2017). Results showed that curcumin-loaded PMMA-PEG/ZnO can induce the apoptosis of cancer cells through a cell cycle-mediated apoptosis corridor. In another study, cellular uptake and phototoxic potential of curcumin organically modified silica nanoparticle complexes and free curcumin were reported to investigate in multicellular spheroids of human oral cancer cells. Results showed accumulation of nanoformulated curcumin was higher in cancer cells, and hence cell death in the spheroids was more following irradiation of blue light in comparison to free curcumin. Results suggested that nanoformulated curcumin was able to improve the phototoxic effects of curcumin in spheroids in comparison to free curcumin (Singh et al. 2015). In another study, Janus magnetic mesoporous silica (Fe_3O_4-$mSiO_2$) nanoparticles consisting of a Fe_3O_4 head for magnetic targeting and a mesoporous SiO_2 body was reported to develop for berberine delivery. This pH responsive nanoformulation was designed for magnetic targeting of berberine to hepatocellular carcinoma. Results suggested that Janus nanocarriers driven by the magnetic field might be use for effective and safe delivery of berberine to against hepatocellular carcinoma (Wang et al. 2016).

Apart from these studies, a host of nanoparticles with different shape, size, architecture, materials, and inherent properties were studied for improvising delivery of anticancer agent in recent decades. These studies were tried to summarize with the help of Table 2.3.

In recent years a wide range of herbal compound-loaded nanocarriers with heterogeneous structures are developed and investigated their efficacy in various cancer cell lines. These nanocarriers are internalized by the cancer cells via phagocytosis or endocytosis processes depending upon their size, shape, and surface treatment (Zhang et al. 2015). These bioactive natural compounds inhibit the growth of cancer cells by inducing apoptosis or programmed cell death. Initifvtion of cell death indicated by the significant changes in DNA structure (Wei et al. 2009); ROS generation (Wei et al. 2009; Das et al. 2013); cytochrome C release (Guo et al. 2010; Mulik et al. 2010); activation of caspases 3/7 (Zheng et al. 2011; Guo et al. 2010; Zhang et al. 2013a); cell cycle arrest (Kumar et al. 2014); activation of NF-κB (Bisht et al. 2007); and downregulation of MMP, BaX, Cyclin D, and VEGF (Subramanian et al. 2016) along with visible morphological changes (Merlina et al. 2012). The different targets of bioactive compounds inside the cancer cell are demonstrated in Fig. 2.5.

Table 2.3 Nanoparticles used to deliver different phytochemicals with anticancer property and the statuses of these studies were summarized

Sl. no.	Nanocarriers	Drug	Status	References
1.	Magnetic liposomes	Paclitaxel	In vitro	Gogoi et al. (2014) and Kulshrestha et al. (2012)
2.	Magnetic liposomes	Paclitaxel	In vitro and in vivo	Gogoi et al. (2017)
3.	Solid lipid nanoparticles	Paclitaxel	In vivo	Banerjee et al. (2016)
4.	Polymeric micelles	Paclitaxel	Phase II, clinical trial	Saif et al. (2010)
5.	Nanohydrogel	Paclitaxel and cisplatin	In vivo	Wu et al. (2014)
6.	Nanoemusion	Paclitaxel	In vitro and in vivo	Kim and Park (2017)
7.	Polymeric nanoparticles	Paclitaxel	In vitro and in vivo	Hu et al. (2017)
8.	Dendrimers	Paclitaxel	In vitro	Cline et al. (2013)
9	Nanocapsules	Paclitaxel	In vitro and in vivo	Boissenot et al. (2016)
10.	Solid lipid nanoparticles	Paclitaxel	In vivo	Pooja et al. (2016)
11.	Liposomes	Curcumin	In vitro and in vivo	Chen et al. (2012)
12.	Polymeric nanoparticles	Curcumin	In vitro	Yallapu et al. (2012b)
13.	Silica nanoparticles	Curcumin	In vitro	Singh et al. (2015)
14.	ZnO nanoparticles	Curcumin	In vitro	Dhivya et al. (2017)
15.	Nanoemulsion	Curcumin	In vitro	Anuchapreeda et al. (2012)
16.	Nanohydrogel	Curcumin	In vitro	Teong et al. (2015)
17.	Magnetic nanoparticles	Curcumin	In vitro	Yallapu et al. (2012a)
18.	Phytosome	Curcumin	In vivo	Maiti et al. (2007)
19.	Nanospheres	Curcumin	In vitro	Mukerjee and Vishwanatha (2009)
20.	Polymeric nanoparticles	Curcumin	In vitro	Bisht et al. (2007)
21.	Polymeric nanoparticles	Curcumin	In vitro	Punfa et al. (2012)

(continued)

Table 2.3 (continued)

Sl. no.	Nanocarriers	Drug	Status	References
22.	Polymeric nanoparticles	Curcumin	In vitro	Nair et al. (2012)
23.	Protein nanoparticles	Curcumin	In vivo	Kim et al. (2011)
24.	Lipid carriers	Curcumin and genistein	In vitro	Aditya et al. (2013)
25.	Nanocapsule	Artemisinin	In vitro	Chen et al. (2009)
26.	Magnetic liposomes	Artemisinin	In vitro and in vivo	Gharib et al. (2015)
27.	Lipid nanoparticles	Artemisinin derivatives	In vitro	Zhang et al. (2013b)
28.	Solid lipid nanoparticles	Artemisinin derivatives artemisone	In vitro	Dwivedi et al. (2015)
29.	Polymeric magnetic nanoparticles	Artemisinin	In vitro	Natesan et al. (2017)
30.	Solid lipid nanoparticles	Berberine	In vivo	Xue et al. (2015)
31.	Liposomes	Berberine derivatives and doxorubicin	In vitro and in vivo	Tuo et al. (2016)
32.	Hybrid nanoparticle	Berberine	In vitro and in vivo	Yu et al. (2017)
33.	Dendrimer	Berberine	Ex vivo and in vivo	Gupta et al. (2017)
34.	Magnetic mesoporous silica nanoparticles	Berberine	In vitro	Wang et al. (2017)
35.	Polymeric nanoparticles	Camptothecin	In vitro and in vivo	Min et al. (2008)
36.	Magnetic cyclodextrin nanovehicles	Camptothecin	In vitro	Rajan et al. (2017)
37.	Polymeric nanoparticles	Camptothecin	In vivo	Householder et al. (2015)
38.	Dendrimer	Camptothecin	In vivo	Fox et al. (2009)
39.	Mesoporous silica nanoparticles	Camptothecin	In vivo	Lu et al. (2010)
40.	Nanoemulsion	Camptothecin	In vitro and in vivo	Sugumaran et al. (2017)
41.	Polymer nanoparticles	Epigallocatechin gallate	In vitro	Rocha et al. (2011)
42.	Polymeric nanoparticles	Epigallocatechin-3-gallate	In vitro and in vivo	Siddiqui et al. (2009)
43.	Polymeric nanoparticles	Epigallocatechin 3-gallate	In vitro	Sanna et al. (2011)
44.	Polymeric nanoparticles	Green tea	In vivo	Khan et al. (2013)
		Polyphenol EGCG		
45.	Micelle	Green tea catechin derivatives and protein drugs	In vitro and in vivo	Chung et al. (2014)

(continued)

Table 2.3 (continued)

Sl. no.	Nanocarriers	Drug	Status	References
46.	Liposomes	Epigallocatechin-3-gallate	In vitro	de Pace et al. (2013)
47.	Polymeric nanoparticles	Root extract of *Phytolacca decandra* Phytolaccaceae	In vitro and in vivo	Das et al. (2012)
48.	Polymeric NP	Ethanolic extract of *Polygala senega* Polygalaceae	In vitro	Paul et al. (2011)
49.	Liposomes	Vincristine, vinblastine and vinorelbine	In vitro and in vivo	Zhigaltsev et al. (2005)
50.	Liposomes	Vincristine	In vivo	Tokudome et al. (1996)
51.	Nanoemulsion	Quercetin	In vitro	Pool et al. (2013)
52.	Polymeric nanocapsules	Quercetin	In vitro and in vivo	El-Gogary et al. (2014)
53.	Liposomes	Quercetin	In vitro	Wang et al. (2012)
54.	Micelle	Quercetin	In vitro and in vivo	Tan et al. (2012)
55.	Liposomes	Resveratrol	In vitro and in vivo	Wang et al. (2011a)
56.	Polymeric nanoparticles	Resveratrol	In vitro and in vivo	Karthikeyan et al. (2013)
57.	Polymeric nanoparticles	Resveratrol	In vitro	Karthikeyan et al. (2015)
58.	Protein nanoparticles	Resveratrol	In vivo	Guo et al. (2010)
59.	Clay nanotube	Resveratrol	In vitro	Vergaro (2012)
60.	Polymer nanoparticles	Resveratrol	In vitro	Sanna et al. (2013)
61.	Liposomes, polymeric lipid-core nanocapsules and nanospheres and solid lipid Nanoparticles	E-resveratrol	Ex vivo	Detoni et al. (2012)
63.	Solid lipid nanoparticles	Resveratrol	In vitro	Teskac and Kristl (2010)
64.	Liposomes	Oleanolic acid	In vitro and in vivo	Tang et al. (2013)
65.	Solid lipid nanoparticles	Baicalein	In vivo	Tsai et al. (2012)
66.	Self-assembled polymer nanoparticles	Baicalein	In vitro and in vivo	Wang et al. (2015)
67.	Liposomes	Baicalein	In vitro and in vivo	Li et al. (2016a)

(continued)

Table 2.3 (continued)

Sl. no.	Nanocarriers	Drug	Status	References
68.	Magnetic nanoparticles	Baicalein	In vitro	Kavithaa et al. (2017)
69.	Liposomes	Combretastatin A4	In vitro	Nallamothu et al. (2006)
70.	Magnetic polymer nanoparticles	Noscapine	Synthesis and characterization	Abdalla et al. (2010)
71.	Human serum albumin nanoparticles	Noscapine	In vitro	Sebak et al. (2010)
72.	Polymeric nanoparticles	Noscapine	In vitro	Madan et al. (2011)
73.	Liposomes	Betulinic acid	In vitro and in vivo	Mullauera et al. (2011)
74.	Polymeric nanoparticles	Betulinic acid	In vitro and in vivo	Das et al. (2016)
75.	Polymeric nanoparticles	Curcumin and 5-fluorouracil	In vitro	Anitha et al. (2014)
76.	Liposomes	Paclitaxel/ epigallocatechin gallate	In vitro	Ramadass et al. (2015)
77.	Nanoemulsion	Paclitaxel and curcumin	In vitro	Ganta and Amiji (2009)
78.	Liposomes	Curcumin and resveratrol	In vivo	Narayanan et al. (2009)
79.	Mesoporous silica nanoparticles	Combretastatin A4 and doxorubicin	In vitro and in vivo	Li et al. (2016b)
80.	Nano cell	Combretastatin A4 and doxorubicin	In vitro and in vivo	Sengupta et al. (2005)
81.	Liposomes	Combretastatin A4 and doxorubicin	In vitro and in vivo	Mitrus et al. (2009)
82.	Nanocapsule	Combretastatin A4 and paclitaxel	In vitro	Wang and Ho (2010)
83.	Self-assembled polymeric nanoparticles	Betulinic acid and hydroxycamptothecin	In vitro and in vivo	Dai et al. (2015)

2.4 Challenges and Future Prospects

Though a large number of nanomedicines are investigated for treatment of different types of cancers, only few nanoformulations reached the market today. A nanocarrier formulation has to go through a host of evaluation processes before it reaches the market. Though most of the nanocarriers are developed based on EPR effect, the EPR effect is unlikely to be present and equal in all the tumors nor the sole driver for efficacy of nanocarriers. Moreover, the pathological heterogeneity among different types of tumors and within the same type of tumor possesses a big challenge in the nanomedicine development process (Hare et al. 2017).The success rate of nanomedicine can be improved by adopting a specific decision-making framework, such

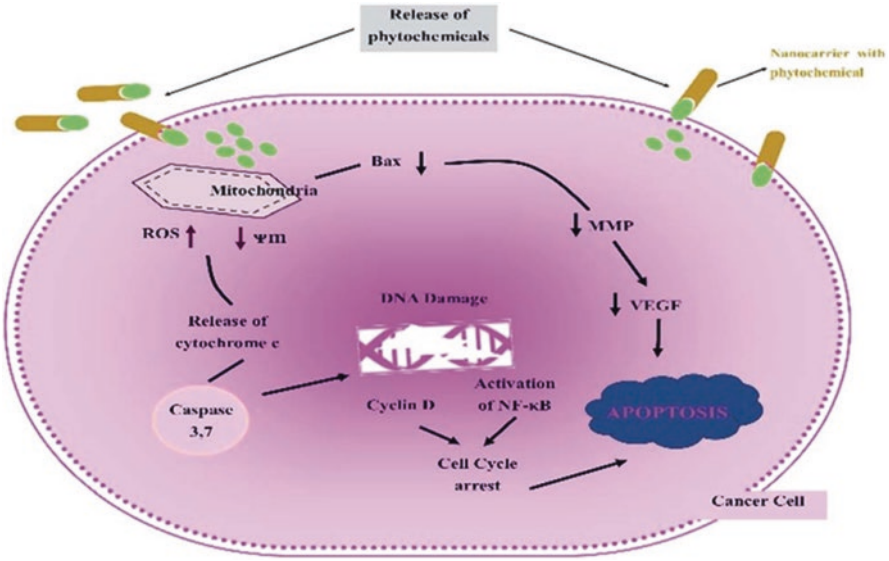


Fig. 2.5 Molecular targets of herbal compounds loaded nanocarriers against cancer cell (Subramanian et al. 2016)

as AstraZeneca's 5Rs principle: right target/efficacy, right tissue/exposure, right safety, right patient, and right commercial potential. The following points need to be addressed for development of cost-effective superior therapies for the patients, i.e., (i) should have a clear cut understanding about the heterogeneity of clinical cancers and the biological factors influencing the behavior of nanomedicines in patients' tumors; (ii) transition from formulation-driven research to disease-driven development; (iii) adaptation of more relevant animal models and testing protocols; and (iv) preselection of the patients most likely to respond to nanomedicine therapies.

Nanocarriers offer novel efficient strategies to treat cancer; nanotoxicity is a major area of concern as potentially high reactivity arising from the large surface-to-volume ratio of nanoparticles compared to bulk systems. Besides these, biodegradability of nanoparticles, side effects from by-products and bioaccumulation, and change in physicochemical characteristics of material at nanoscale are few apprehensions related to the nanomedicine. Moreover, distribution of nanocarriers in the body following systemic administration; development of mathematical and computer models to predict risk and benefits of nanoparticles; safe processes of nanoparticle manufacturing; and disposal and detrimental effects of nanoparticles to environment are few issues related to the nanomedicine to be addressed. Limited work has been done in scaling up laboratory or pilot technologies of nanodrug delivery for commercialization due to high cost of materials and challenges associated to maintain size and composition of nanomaterials at large scale.

2.5 Conclusions

Cancer has been tormenting the mankind from ancient times. Despite improvement in different therapeutic modalities, the number of deaths due to cancer is on rise. Therefore, a large number of herbs and their parts or extracts have been used to treat cancer. Nowadays, bioactive compounds from herbs have been extracted for effective treatment of different types of cancer. Due to the side effects of conventional therapies, herbal compounds or their derivatives have been loaded in different nanocarriers and investigated. Herbal compound-loaded nanocarriers have been able to effectively deliver drugs to the tumor site(s), reduce the side effects associated with the therapy, and kill the tumor cells more effectively. These nanocarriers can target tumor either by passive targeting or active targeting strategy. Though a host of nanocarriers have been investigated for cancer therapy, due to stringent preclinical evaluation and regulatory processes, only few nanoformulations have reached the market. The success rate of the nanocarriers in reaching market can be improved by adapting efficient decision-making strategies like AstraZeneca's 5Rs framework, implementing new validation method and preselection of patients, etc. Moreover, issues like nanotoxicity, prior prediction of nanoparticles distribution in the body, and risk–benefit analysis are to be addressed.

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Chapter 3
Nanopharmaceuticals: In Relevance to Drug Delivery and Targeting

Md Akhlaquer Rahman, Mohammad Yusuf, Thamir Alshammari, and Md Faiyazuddin

Contents

Abstract A budding concern in nanopharmaceuticals has generated a number of advancements throughout recent years with a focus on commercialization and engineering novel products. The integration of nanotechnology into medical field has given birth to some new interdisciplinary areas of nanomedicine including nanopharmaceuticals. This is relatively a new class of therapeutic-containing nanomaterials that often have unique nanoproperties including small particle size, high

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surface-to-volume ratio, ability to improve solubility, multi-functionality, and the possibility of modulating their properties. Nanopharmaceuticals in delivery systems provide new opportunities for solving issues associated with problematic drugs; those were previously unsuitable for conventional oral or injectable formulations could now be formulated and designed to interact with the body at subcellular (i.e., molecular) scales with a high degree of specificity. Due to superior pharmacokinetics/pharmacodynamics and/or active intracellular delivery with reduced toxicity and enhanced bioavailability, this created great expectations in the field of drug delivery. With these advantages, nanopharmaceuticals have the ability to extend the economic life of proprietary drugs, thereby creating additional revenue streams. This chapter focuses on the potential application of nanopharmaceuticals including carbon nanotubes, quantum dots, dendrimers, nanoshells, niosomes, magnetic nanoparticles, polymeric NPs, and lipid NPs in drug delivery and drug targeting. This chapter also includes some of the FDA-approved nanopharmaceuticals meant for various routes of administration.

Keywords Nanopharmaceuticals · Nanomedicine · Nanoparticulate · Drug delivery · Drug targeting · Carbon nanotubes · Dendrimers · Quantum dots

3.1 Introduction

Nanotechnology can be defined as the science and engineering that involves the design, creation, synthesis, manipulation, and application of functional materials, devices, and systems through control of matter at the nanometer scale (Emerich and Thanos 2003; Sahoo and Labhasetwar 2003). Nanotechnology involves utilization of man-made products not larger than 1000 nm. In the past few years, nanotechnology has grown by leaps and bounds, and this multidisciplinary scientific field is undergoing explosive development in the field of molecular biology, chemistry, genomics, physics, material science, and medicine (Cheng et al. 2006; Chan 2006). It can prove to be a boon for human healthcare, because nanoscience and nanotechnologies have a huge potential to bring benefits in areas as diverse as drug development, water decontamination, information and communication technologies, and the production of stronger, lighter materials. Human healthcare nanotechnology research can definitely result in immense health benefits. The genesis of nanotechnology can be traced to the promise of revolutionary advances across medicine, communications, genomics, and robotics. A complete list of the potential applications of nanotechnology is too vast and diverse to discuss in detail, but without doubt, one of the greatest values of nanotechnology will be in the development of new and effective medical treatments (Shaffer 2005; Emerich 2005).

A detailed evaluation of each formulation is essential to expand our current nanopharmaceutical repertoire. Nanopharmaceuticals have received a lot of attention due to their potential to revolutionize drug delivery systems. It has the potential

to revolutionize medical treatment by permitting the design of more potent, less toxic smart therapeutics. It has been extensively described in numerous literatures, which have discussed the rationales, challenges, efficacy, safety, and regulatory issues related to the development of nanoscale drug delivery systems (Desai 2012; Duncan and Gaspar 2011; Riehemann et al. 2009).

Nanopharmaceuticals cum nanotechnology are nowadays broadly regarded as the enabling technology of the present century where the sizes of the drug particle or delivery system work at the nano level may be utilized to detect diseases at considerable earlier stages. Successful design of delivery system to provide the right dose of particular drug to specific site of disease still remains challenging for the industry. In this situation, nanopharmaceuticals have huge potential to tackle this disappointment of old-style therapeutics which deals site-specific targeting of drugs. Nanomaterials that bring unique shapes and functionalities and nanodevices show a strategic role in pharmaceutical nanotechnology and have enormous promise for healthcare (Torchilin 2006; Otsuka et al. 2003). Nanopharmaceuticals have the ability to improve the pharmacokinetics and increase biodistribution of therapeutic agents to target organs, which will result in improved efficacy (Fetterly and Straubinger 2003; Hoarau et al. 2004). Second, drug toxicity is reduced as a consequence of preferential accumulation at target sites and lower concentration in healthy tissues. Therefore, pharmaceutical corporations are focusing their vision towards nanotechnology-based pharmaceuticals to augment the formulation and drug target discovery.

Besides larger surface area and nanoscale properties, they have distinctive physical, chemical, and biological properties as compared to their large counterparts, therefore used as a hopeful tool for drug and gene delivery advancement. Properties of nanopharmaceuticals such as charge, chemical composition, peculiar size and shape, surface structure, and solubility can impressively influence their interactions with biological molecules and cells. Furthermore, nanopharmaceuticals are resistant to settling and may possess higher saturation solubility, fast dissolution, and improved adhesion to biological surfaces. These features render them therapeutically effective and more bioavailable.

Nanopharmaceuticals have wide scope that includes smart materials for tissue engineering, intelligent tools for drug delivery, diagnostics, theranostics, and many more (Davis 2006; Pene et al. 2009). Existing claims of nanotechnology in pharmaceutical field are development of advanced diagnostic, bioactive surfaces, biomarker, biosensor, image enhancement device, implant technology, nanocarrier, nanomedicine, nanorobots, tissue engineering, etc. An enormous number of nanosystems that have been explored in pharmacy to date are nanocrystals and nanostructures like carbon nanotubes, quantum dots, dendrimers, nanoshells, niosomes, magnetic nanoparticles, polymeric NPs, lipid NPs, etc. (Fig. 3.1).

Many drugs exhibit such a low solubility that micronization does not lead sufficiently to high bioavailability and so the next step was taken to move from micronization to nanocrystals. Drug nanocrystals are nanoparticles composed of 100% drug without any matrix material that act as its own carrier, in contrast to that nanostructures composed of matrix system incorporated with drug. Nanopharmaceutical

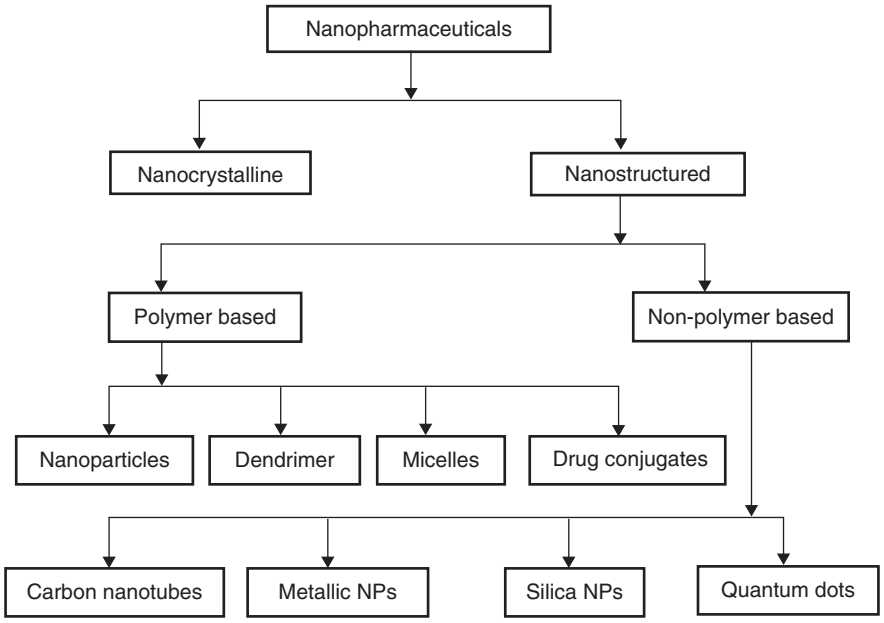

Fig. 3.1 Schematic diagram of various types of pharmaceuticals

applications are growing exponentially and are presently used in various applications including drug delivery, proteins and peptide delivery, cancer treatment, treatment of neurodegenerative disorders, etc. (Panyam and Labhasetwar 2003; Calvo et al. 1997).

The ideal features of nanopharmaceuticals include enhancing drug accumulation in the target site, providing protection of drugs against potential enzymatic or hydrolytic degradation in the body, providing biocompatibility and biodegradability, offering a high drug-loading capacity, extended circulation or residence time, controlled drug release profiles, providing long-term physical and chemical stability, and the ability to efficiently carry poorly soluble pharmaceuticals (Torchilin 2005). These features can be engineered into the delivery system along with effective delivery to target sites; hence, this area is gaining attraction (Marcato and Duran 2008).

The size and surface properties of nanopharmaceuticals (including the presence of targeting moieties) largely dictate their in vivo behavior. Specifically, these properties permit systemic circulation and determine their biodistribution within the human body. Therefore, an understanding of these properties can aid in designing nanopharmaceuticals that can be localized to specific tissue/body sites. The small size of nanopharmaceuticals imparts them with unique properties in contrast to larger particles; it is this small size that allows them access to places in the human body where larger particles cannot reach. It is generally accepted that for systemic applications, the diameter of nanopharmaceuticals should be in the range of

10–100 nm, with minimum surface charge (Davis 2006). Nanopharmaceuticals have a high surface-to-volume ratio when compared to their larger counterparts. Therefore, their surface properties are critical to their in vivo performance. In fact, their interaction with the local environment (which, again, is the end result of a combination of size and surface properties) determines if they will be lost to undesired locations within the body. A large number of approaches focus on minimizing nonspecific binding of nanopharmaceuticals to undesired tissue surfaces as well as reducing interactions with each other. The endothelial surfaces, as well as cell membranes, are typically negatively charged, which repel negatively charged nanopharmaceuticals. Also, as the surface charge on the nanopharmaceuticals becomes larger (either positive or negative), a greater clearance by the macrophage-mediated RES is generally observed. In this context, synthesis of sterically stabilized nanopharmaceuticals is the subject of active R&D. For example, incorporation of polyethylene glycol (PEG) polymers on the surface of nanopharmaceuticals (i.e., PEGylation) provides a means to increase solubility, reduce immunogenicity, prolong half-life, and prevent a rapid renal clearance via the RES (due to larger particle size resulting from PEGylation) (Harris and Chess 2003).

In addition to this, it may also be necessary to design nanopharmaceuticals that can undergo efficient intracellular uptake and arrival at specific organelles (Breunig et al. 2008). Nanopharmaceuticals are better suited than their microparticle counterparts for intravenous (IV) delivery because the tiniest capillaries are in the 5–6 micron range, a size that impedes most microparticles (or aggregations thereof) from distributing into the bloodstream.

The blood–brain barrier (BBB) and the blood–retinal barrier (BRB) protect the brain and eyes, respectively, due to their unique anatomical features, including the presence of tight junctions that seal adjacent cells. The BBB has strict size and surface property limitations for entrance. For gene delivery, both viral vectors and nonviral vectors have been generally unsuccessful; the former are unable to penetrate the BBB or the BRB, while the latter lack sufficient efficiency. On the other hand, nanopharmaceuticals have been shown to cross biological barriers and may be able to cross both the intact BBB (Tosi et al. 2008) as well as the BRB (Zhang et al. 2003). Often, nanopharmaceuticals can be delivered directly to the nervous system (NS) without prior need for drug modification or functionalization. Moreover, both hydrophilic and hydrophobic therapeutics can be delivered without first opening the BBB. However, in this context systemic delivery for non-NS diseases is of general concern because these agents may cross the BBB and cause brain damage or psychoactive effects. Nanopharmaceuticals can also permeate the tight epithelial junctions of the skin that normally impede delivery of active agents to the desired target (Emerich and Thanos 2006). Topical emulsion systems incorporating nanoparticles are being developed which rapidly permeate tissue to delivery actives or remove lethal toxins from the bloodstream. Nanopharmaceuticals of specific size (generally greater than 10 nm) can be designed that are able to penetrate tumors due to the "leaky" nature of their microvasculature. This classic effect, referred to as the "enhanced permeability and retention (EPR) effect," results in prolonged circulation and accumulation within the tumor (Matsumura and Maeda 1986). It is

generally accepted that nanoparticles in the 10–100 nm size range and with a slightly positive or slightly negative surface charge should be able to disseminate within tumors when delivered to the circulatory system.

By controlling the particle size and architecture of nanopharmaceuticals, a particular pharmacokinetic release profile of the drug may be generated. Often, a near zero-order kinetic drug release profile is desired since it maintains a steadier therapeutic concentration at the site of delivery. Such a profile is more likely to be achieved by nanopharmaceuticals where a drug has been functionalized onto or encapsulated within a polymeric carrier matrix. For oral applications,

research has focused on lymphatic uptake of nanopharmaceuticals by the Peyer's patches of the gut-associated lymphoid tissue (GALT). It has been shown that during oral delivery, nanopharmaceuticals are disseminated systemically, while their microparticle counterparts remain in the Peyer's patches (Blanco and Alonso 1997).

The objective of the chapter is to focus on the potential application of nanopharmaceuticals in drug delivery and a short snap on some of the FDA-approved nanopharmaceutical products developed for various routes of administration.

However, many other products such as product that have multifunctional property are still in pipeline due to their safety, efficacy, and product development issues. This chapter will provide a thorough discussion of the major nanopharmaceutical formulations as well as the impact of nanotechnology into the future.

3.2 Nanopharmaceuticals in Drug Delivery and Targeting

One of the most important long-standing issues in the pharmaceutical industry is the proper distribution of drugs and other therapeutic agents to a specific disease site within the patient's body (Jain 2003; Labhasetwar 2005). Since this is generally unachievable, active agents have to be administered in excessively high doses, thereby increasing the odds of toxic side effects. The concept of site-specific delivery of a therapeutic arises from this classic drawback of traditional therapeutics. Nanopharmaceuticals have enormous potential in addressing this failure of traditional therapeutics – they offer site-specific targeting of active agents (Moghimi and Szebeni 2003).

Nanoparticles, because of their small size, can extravasate through the endothelium in inflammatory sites, epithelium (e.g., intestinal tract and liver), tumors, or penetrate microcapillaries. In general, the nanosize of these particles allows for efficient uptake by a variety of cell types and selective drug accumulation at target sites. Such precision targeting via nanopharmaceuticals will reduce toxic systemic side effects, resulting in better patient compliance. This can be achieved either through passive targeting of drugs to the site of action or by active targeting of the drug (Fig. 3.2). As a result, nanopharmaceuticals present novel opportunities for reformulation of active agents whose previous versions were unsuitable for traditional delivery.

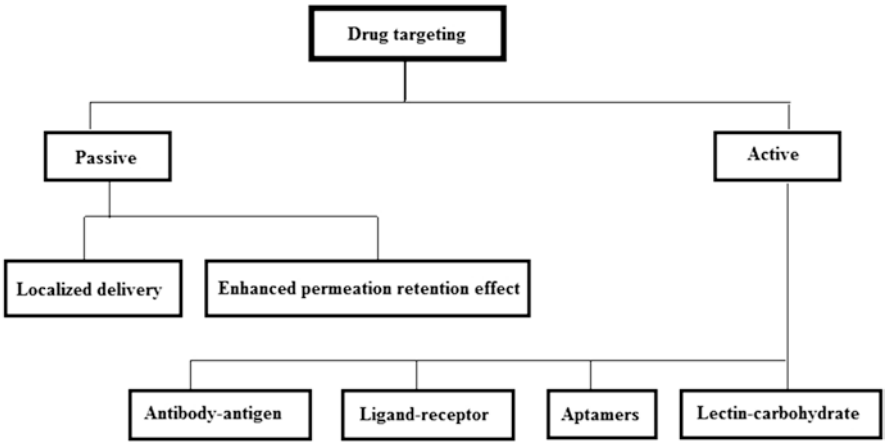

Fig. 3.2 Schematic representation of different drug-targeting approaches

3.2.1 Passive Targeting

Passive targeting occurs due to extravasation of the nanoparticles at the diseased site where the microvasculature is leaky. Examples of such diseases where passive targeting of nanocarriers can be achieved are tumor and inflamed tissues. Tumor vascular leakiness is the result of increased angiogenesis and the presence of cytokines and other vasoactive factors that enhance permeability. Tumor angiogenesis is characterized by vessels with irregular diameters and branching, and tumors lacking defining structures of vasculature such as arterioles, capillaries, or venules (Oeffinger and Wheatley 2004). Vascular endothelial growth factor (VEGF) and the angiopoietins are critical in regulating the balance between the leakiness associated with the defective endothelial linings of tumor vessels and vascular growth, maturation, and regression (Holash et al. 1999; Brown et al. 1997). Elevated levels of bradykinin result in vasodilatation and enhance the extravasation of large molecules and their retention in tumors (Matsumura et al. 1988). The increase in vascular permeability by VEGF and bradykinin is mediated by nitric oxide generation (Wu et al. 1998). The majority of solid tumors exhibit a vascular pore cutoff size between 380 and 780 nm (Hobbs et al. 1998), although tumor vasculature organization may differ depending on the tumor type, its growth rate, and microenvironment (Jain 1998).

Therefore, particles need to be of a size much smaller than the cutoff pore diameter to reach to the target tumor sites. By contrast, normal vasculature is impermeable to drug-associated carriers larger than 2–4 nm compared to free, unassociated drug molecules (Fu et al. 1998; Firth 2002). This nanosize window offers the opportunity to increase drug accumulation and local concentration in target sites such as tumor or inflamed sites by extravasation and significantly to reduce drug distribution and toxicity to normal tissues. For passive targeting to be successful, the nanocarriers need to circulate in the blood for extended times so that there will be

multiple possibilities for the nanocarriers to pass by the target site. Nanoparticulates usually have short circulation half-lives due to natural defense mechanisms of the body to eliminate them after opsonization by the mononuclear phagocytic system (MPS, also known as reticuloendothelial system). Therefore, the particle surfaces need to be modified to be invisible Q to opsonization. A hydrophilic polymer such as polyethylene glycol (PEG) is commonly used for this purpose because it has desirable attributes such as low degree of immunogenicity and antigenicity, chemical inertness of the polymer backbone, and availability of the terminal primary hydroxyl groups for derivatization (Moghimi et al. 2001). PEG-grafted liposomes, in the size range of 70–200 nm, containing 3–7 mol% methoxy-PEG-2000 grafted to distearoyl phosphatidylethanolamine (DSPE) or dipalmitoyl phosphatidylethanolamine, showed extended circulation half-lives of 15–24 h in rodents and up to 45 h in humans (Klibanov et al. 1990; Allen et al. 1990; Woodle 1993), whereas non-PEGylated liposomes had half-lives of 2 h (Allen and Everest 1983).

3.2.2 Active Targeting

Localized diseases such as cancer or inflammation not only have leaky vasculature but also overexpressed some epitopes or receptors that can be used as targets. Therefore, nanomedicines can also be actively targeted to these sites. Ligands that specifically bind to surface epitopes or receptors, preferentially overexpressed at target sites, have been coupled to the surface of long-circulating nanocarriers (Medina et al. 2005; Mitra et al. 2005; Missailidis et al. 2005). Ligand-mediated active binding to sites and cellular uptake are particularly valuable to therapeutics that are not taken up easily by cells and require facilitation by fusion, endocytosis, or other processes to access their cellular active sites (Willis and Forssen 1998).

Passive targeting facilitates the efficient localization of nanoparticles in the tumor interstitium but cannot further promote their uptake by cancer cells (Sapra and Allen 2003). This second step in uptake can be achieved by actively targeting nanoparticles to receptors or other surface membrane proteins overexpressed on target cells. The addition of targeting ligands allows the delivery of drug-encapsulated nanoparticles to uniquely identifiable cells or even subcellular sites, thereby reducing the unwanted systemic exposure of cytotoxic drug. Specific interactions between the ligands on the surface of nanocarriers and receptors expressed on the tumor cells may facilitate nanoparticle internalization by triggering receptor-mediated endocytosis. Furthermore, active targeting of nanocarriers with small molecule therapeutic cargo has shown the potential to suppress multidrug resistance (MDR) via bypassing of P-glycoprotein-mediated drug efflux (Yu et al. 2010a; Talekar et al. 2011). Recognizing that receptor-based active targeting of nanoparticles has the potential to be the optimal delivery strategy, there has been tremendous interest in developing novel-targeted nanoparticles for diagnostic and therapeutic applications (Wang et al. 2008). Numerous targeting ligands have been employed to actively target nanoparticles including antibodies, antibody fragments, aptamers, peptides and whole proteins (e.g., transferrin), and different receptor ligands (e.g., folic acid).

An important consideration when selecting the type of targeting ligand is its immunogenicity. For example, whole antibodies that expose their constant regions on the liposomal surface are more susceptible to Fc-receptor-mediated phagocytosis by the MPS (Harding et al. 1997; Metselaar et al. 2002). Various methods have been employed to couple ligands to the surface of the nanocarriers with reactive groups. These can be divided into covalent and noncovalent couplings. Common covalent coupling methods involve formation of a disulfide bond, cross-linking between 2 primary amines, reaction between a carboxylic acid and primary amine, reaction between maleimide and thiol, reaction between hydrazide and aldehyde, and reaction between a primary amine and free aldehyde (Nobs et al. 2004). Noncovalent binding by physical association of targeting ligands to the nanocarrier surface has the advantage of eliminating the use of rigorous, destructive reaction agents. However, there are potential problems, such as low and weak binding and poor control of the reactions, and the ligands may not be in the desired orientation after binding.

Active targeting nanocarriers have a number of advantages over targeting ligand–drug conjugates. First, high concentrations of drug within the carrier can be delivered to the target cell when a ligand interacts with its receptor and large payloads of therapeutic agent relative to number of ligand binding sites can be achieved. This is especially advantageous in increasing tumor to background ratio in imaging. Second, the ligand is associated with the carrier, and the drug is not modified with the coupling of ligands. Drug activity may be compromised as the ligand–drug conjugate or inactivated by the potentially aggressive coupling reaction. Third, numerous ligand molecules can be attached to the nanocarrier to increase probability of binding to target cells, particularly for those of lower binding affinities. Fourth, active targeting enables more efficient distribution of the carriers in the tumor interstitium and reduces return of drug back to the circulation due to high intratumoral pressure. Last, but also a very important point, is that when ligand is only attached to the carrier due to the small size of the conjugate, it can only extravasate at the disease site but not normal vasculature; therefore, the ligand cannot interact with the target epitopes of normal tissues and show side effects.

3.3 Nanopharmaceutical Types

Various types of nanopharmaceuticals in relevance to drug delivery and targeting potential are given below.

3.3.1 Carbon-Based Nanotubes

Carbon nanotubes (CNTs) are cylindrical tubular structures that were discovered in 1991 (Iijima 1991). These structures are set in mode like a graphite sheet rolled up into a cylinder and capped at one or both ends by a bucky ball. These are hexagonal

networks of carbon atoms having diameter of 1 nm and length from 1 to 100 nm. These carbon networks are arranged layer of graphite rolled up into a cylinder. There are two carbon-based designs that have received much interest recently: single-walled nanotubes (SWNTs) and multi-walled nanotubes (MWNTs). In addition to these types, C60 fullerenes are also a part of common configurations. As far as their structural design is concerned fullerenes and carbon nanotubes are classically fabricated using laser ablation, chemical vapor deposition, electric arc discharge, or combustion processes. Characterization of these concentric forms is based on their strength and stability so that they can be used as stable drug carriers. Cellular entry of nanotubes may be mediated by endocytosis or by inclusion through the cell membrane. Fullerenes have also shown drug targeting capability. Tissue-selective targeting and intracellular targeting of mitochondria have been shown with use of fullerene structures. Furthermore, experiments with fullerenes have also shown that they exhibit antioxidant and antimicrobial behavior.

The search for new and effective drug delivery systems is a fundamental issue of continuous interest (Allen and Cullis 2004). A drug delivery system is generally designed to improve the pharmacological and therapeutic profile of a drug molecule (Kostarelos 2003). Carbon nanotubes have been used for developing next-generation drug delivery system, which enable the delivery of drugs and biomolecules with a very high efficiency due to their large surface area, unique structural properties, and well-defined physicochemical properties using functionalized carbon nanotubes (f-CNTs).

The ability of f-CNTs to penetrate into the cells offers the potential of using f-CNTs as vehicles for the delivery of small drug molecules (Shi Kam et al. 2004). However, the use of f-CNTs for the delivery of anticancer, antibacterial, or antiviral agents has not yet been fully ascertained. The development of delivery systems able to carry one or more therapeutic agents with recognition capacity, optical signals for imaging, and/or specific targeting is of fundamental advantage, for example, in the treatment of cancer and different types of infectious diseases (Ferrari 2005). In a study, fluorescent probe for tracking the cellular uptake of the material and an antibiotic moiety as the active molecule were covalently linked to CNTs. MWNTs were functionalized with amphotericin B and fluorescein. The antibiotic linked to the nanotubes was easily internalized into mammalian cells without toxic effects in comparison with the antibiotic incubated alone. In addition, amphotericin B bound to CNTs preserved its high antifungal activity against a broad range of pathogens, including *Candida albicans*, *Cryptococcus neoformans*, and *Candida parapsilosis*.

In an alternative approach by a different group, SWNTs have been functionalized with substituted carborane cages to develop a new delivery system for an efficient boron neutron capture therapy (Yinghuai et al. 2005). These types of water-soluble CNTs were aimed at the treatment of cancer cells. Indeed, the studies showed that some specific tissues contained carborane following intravenous administration of the CNTs conjugate and, more interestingly, that carborane was concentrated mainly at the tumor site. Another class of carbon nanomaterials similar to CNTs has also been used for drug delivery (Murakami et al. 2004). Single-walled carbon nanohorns are nanostructured spherical aggregates of graphitic tubes. Murakami et al.

Table 3.1 Carbon nanotubes and their respective applications

Drug specimen	Modification/ functionalization	Type of CNTs	Advantage
Doxorubicin	PEG conjugation	SWCNT	Reduced toxicity (Allen and Cullis 2004)
Doxorubicin	Conjugated with folate	MWCNT	Active targeting (Allen and Cullis 2004; Kostarelos 2003)
Mitoxantrone	PEG conjugation	SWCNT	Reduced toxicity (Shi Kam et al. 2004)
Methotrexate	PEG conjugation	MWCNT	Controlled toxicity (Ferrari 2005)
Paclitaxel	PEG conjugation	SWCNT	Increased circulation period (Yinghuai et al. 2005)
Paclitaxel	Folate conjugate	MWCNT	Increased circulation period(Yinghuai et al. 2005)
Cisplatin	Nonfunctionalized	SWCNT	Decreased toxicity (Allen and Cullis 2004; Kostarelos 2003; Shi Kam et al. 2004; Ferrari 2005)
Carboplatin	Nonfunctionalized	SWCNT	Decreased toxicity (Kostarelos 2003; Shi Kam et al. 2004; Ferrari 2005)
Quercetin	PEG conjugation	SWCNT	Reduced side effect (Yinghuai et al. 2005)
Folic acid	–	MWCNT	Active targeting, longer circulation period (Murakami et al. 2004)

loaded these tubes with dexamethasone and studied the binding and release of the drug. They found that dexamethasone could be adsorbed in large amounts onto oxidized nanohorns and maintains its biological integrity after being liberated. This was confirmed by activation of glucocorticoid response in mouse bone marrow cells and induction of alkaline phosphatase in mouse osteoblast.

In view of these results, f-CNTs represent a new, emerging class of delivery systems for the transport and translocation of drug molecules into different types of mammalian cells. Although these CNTs conjugates displayed no cytotoxicity in vitro, for further development, it will be important to assess their metabolism, biodistribution, and clearance from the body. Some of the lead examples of carbon nanotubes and their respective applications are highlighted in Table 3.1.

3.3.2 Quantum Dots (QDs)

The integration concern of light with nanotechnology and biological sciences has given rise to exciting new developments in nanobiophotonics (Yinghuai et al. 2005; Iga et al. 2007). Nanoscale fluorescent materials are particularly important in nanobiophotonics as they produce intense and stable responses to incident light, providing unique tools such as optical emission, charge/electron transfer, and in situ heat generation for diverse photomediated bioapplications (Iga et al. 2007). Quantum dots (QDs) are nanocrystals made of semiconducting materials overlaid with a coating of ZnS to improve optical properties and can be made to fluorescence when

stimulated by light. Quantum dots bear a cap which enables them in improving their solubility in aqueous buffers. They are neither atomic nor bulk semiconductors. Core of the quantum dots determines the color emitted, and outer aqueous shell is available for conjugation with biomolecules. Biomolecular conjugation of the quantum dots can be modified according to target various biomarkers (Iga et al. 2007). Their properties originate from their physical size, which ranges from 2 to 10 nm in radius (smaller than its exciton Bohr radius can luminesce when the quantum confinement effects dominate). Owing to their narrow emission, bright fluorescence, high photostability, and broad UV excitation QDs have been adopted for tracking of intracellular process for longer time, for in vitro bioimaging, and for real-time monitoring. Within a carbon QD, the heterogeneous size distribution of sp^2 π-conjugated islands in an sp^3 non-conjugated matrix creates distinct energy levels, leading to the observation of radically different optical, electrical, and chemical properties compared to other nanoparticles.

In recent years, scientists have developed several types of luminescent nanomaterials, including semiconductor quantum dots (QDs), dye-doped nanoparticles, upconverting lanthanide-doped nanoparticles, polymer dots, QDs derived from 2D materials, and carbon nanodots (Iga et al. 2007; Jayagopal et al. 2007). The colloidal synthesis of QDs creates more biocompatible products and lends itself best to translational biomedical applications. PEG coating enhances biocompatibility of QDs and enables vascular targeting through ligand-functionalized PEG (Jayagopal et al. 2007). In addition to PEG, amphiphilic polymers, proteins, and polyethyleneimine have been used to coat QDs in order to improve biocompatibility and convert the immiscible, hydrophobic surface of QDs to hydrophilic particles without affecting the fluorescence quantum yield (Smith et al. 2006).

QDs cover medical areas as a diagnostic as well as therapeutic tool for in vitro and in vivo detection and analysis of biomolecules, immunoassays, DNA hybridization, diagnostic tools (magnetic resonance imaging, MRI), time-graded fluorescence imaging of tissue, development of nonviral vectors for gene therapy, and labeling of cells as therapeutic tools for cancer treatment and transport vehicles for DNA, protein, drugs, or cells (Bailey et al. 2004). In addition they can be also tagged with biomolecules and used as highly sensitive probes. Quantum dots and their therapeutic applications are highlighted in Table 3.2.

3.3.3 Dendrimers

Honorable Prof. Donald A. Tomalia coined the term “dendrimer” an integration of two Greek words *δέντρο* (dendro), which translates to “tree,” and *μέρος* (meros), which translates to “part,” and synthesized the famous PAMAM (PolyAMidoAMine) dendrimers (Moghimi et al. 2005). Therefore, dendrimers are a unique class of polymers; are hyperbranched, tree-like structures, whose size and shape can be precisely controlled and have compartmentalized chemical polymer (Bai et al. 2007). Dendrimers are fabricated from monomers using either convergent or divergent step

Table 3.2 Quantum dots and their therapeutic applications

Drug specimen	Target cells/ diseases	Type of QDs	Advantage
5-Fluorouracil	Breast cancer	ZnS QDs	Targeting and controlled drug delivery to cancer cells (Iga et al. 2007)
Daunorubicin	Leukemia	CdTe QDs	Enhanced drug uptake (Jayagopal et al. 2007)
Daunorubicin	Leukemia K562 cells	CdS QDs	Inhibit multidrug resistance (Jayagopal et al. 2007)
Doxorubicin	Ovarian cancer	Mucin1-aptamer QD	Higher accumulation on target (Jayagopal et al. 2007; Smith et al. 2006)
Saquinavir	HIV-1	Carboxyl-terminated QDs	High site-specificity and can cross BBB (Bailey et al. 2004)

growth polymerization. Size of these regular branching polymeric nanostructures is dependent on the number of branching which can be controlled. These nanostructures arise several branches from the core in shape of a spherical structure by means of polymerization, resulting in formation of cavities within the dendrimer molecule which can be used for drug transport. Free ends of dendrimer can be utilized for conjugation or attachment to other molecule. These end groups can be tailored according to requirements. Such interconnecting networks transport the attached molecules at desirable site and give dendrimers various functional applications (Bailey et al. 2004; Moghimi et al. 2005). Nanoparticles that are polycation–nucleic acid composites or normal cationic liposomes generally are unstable in biological fluids (Bai et al. 2007). These well-defined nanostructures are equipped with surface functionalization capability, monodispersity of size, and stability properties that make them attractive drug carrier candidates. Incorporation of drug molecule can be easily achieved via either complexation or encapsulation. As far as the construction is concerned, it contains three different basic regions: core, branches, and surface. Branches or end groups can be tailored or modified into biocompatible compounds with low cytotoxicity and high biopermeability. Such branches or networks assist in delivery of bioactive ranging from vaccines, drugs, genes, and metal to desired sites. Hollow networks present in dendrimers presents space to incorporate drugs and other bioactive physically or by various interactions to act as drug delivery vehicles. Dendrimers covers distinct applications mainly; solubilization, gene therapy, immunoassay and as MRI contrast agent. The poly(ethylene glycol)-block-poly (D,L-lactic acid) (PEG-PLA) block copolymer is a widely used and reliable biodegradable polymer that has been approved by the Food and Drug Administration (FDA) for multiple drug delivery and biomedical device applications (Cheng 2008)**.** Dendrimers have been reported for pulmonary drug delivery of Enoxaparin. G2 and G3 generation positively charged PAMAM dendrimers were reported to increase the relative bioavailability of Enoxaparin by 40%. The positively charged dendrimer forms complex with enoxaparin, which was effective in deep vein thrombosis after pulmonary administration (Bai et al. 2007).

Dendrimers has also been found to improve solubility and plasma circulation time via transdermal formulations and to deliver drugs efficiently. PAMAM dendrimer complex with NSAIDs (e.g., ketoprofen, diflunisal) have been reported to improve the drug permeation through the skin as penetration enhancers. Ketoprofen and diflunisal were conjugated with G5 PAMAM dendrimer and showed 3.4 and 3.2 times higher permeation. Enhanced bioavailability of PAMAM dendrimers by using indomethacin as the model drug in transdermal drug application was reported to be effective (Cheng 2008; Chauhan and Jain 2003; Jevprasesphant et al. 2003).

Oral drug delivery studies using the human colon adenocarcinoma cell line, Caco2, have indicated that low-generation PAMAM dendrimers cross cell membranes, presumably through a combination of two processes, i.e., paracellular transport and adsorptive endocytosis. Remarkably, the P-glycoprotein efflux transporter does not appear to affect dendrimers; therefore drug dendrimer complexes are able to bypass the efflux transporter. PAMAM dendrimers conjugated with the folic acid and fluorescein isothiocyanate for targeting the tumor cells and imaging, respectively. DNA-assembled dendrimer conjugates may allow the combination of different drugs with different targeting and imaging agents (Barbara and Maria 2001). Dendrimers are especially ideal for synthesizing hydrogel cross-linked networks that increase in volume in aqueous solution and are more similar to living tissue than any other synthetic compound. By adding polyethylene glycol or PEG groups to the dendrimers, these hydrogels have applications including cartilage tissue production and for sealing ophthalmic injuries. Hydrogel composed of PEGylated dendrimers that contain ocular drug molecules attached to the dendrimers efficiently deliver the drugs to the eye (Yang and Kao 2006).

The anticancer drugs Adriamycin and methotrexate were encapsulated into PAMAM dendrimers (i.e., G = 3 and 4) which had been modified with PEG monomethyl ether chains (i.e., 550 and 2000 Da, respectively) attached to their surfaces. A similar construct involving PEG chains and PAMAM dendrimers was used to deliver the anticancer drug 5-fluorouracil. Encapsulation of 5-fluorouracil into G4 increases in the cytotoxicity and permeation of dendrimers. The earlier discussed dendrimer drug interaction techniques are used to control the drug delivery. A third-generation dendritic unimolecular micelle with indomethacin entrapped as model drug gives slow and sustained in vitro release, as compared to cellulose membrane control. Controlled release of the flurbiprofen could be achieved by formation of complex with amine-terminated generation 4 (G4) PAMAM dendrimers (Chen et al. 2004; Malik et al. 2012; Liu et al. 2000; Liu et al. 1999).

Dendrimers have ideal properties which are brought in application in targeted drug delivery system. One of the most effective cell-specific targeting agents delivered by dendrimers is folic acid PAMAM dendrimers modified with carboxymethyl PEG5000 surface chains possessed reasonable drug loading, a reduced release rate and reduced hemolytic toxicity compared with the non-PEGylated dendrimer (Kolhe et al. 2003; Mohammad and Antony 2006; Hawker 2006). The star polymers were reported to give the most promising results regarding cytotoxicity and systemic circulatory half-life (72 h). In addition to improving drug properties such as solubility and plasma circulation time, polymeric carriers can also facilitate the

passive targeting of drugs to solid tumors. Combined these factors lead to the selective accumulation of macromolecules in tumor tissue, a phenomenon termed the "enhanced permeability and retention" (EPR) effect. Therefore, the anticancer drug doxorubicin was reported to be covalently bound to this carrier via an acid-labile hydrazone linkage. The cytotoxicity of doxorubicin was significantly reduced (80–98%), and the drug was successfully taken up by several cancer cell lines (Medina and Mohamed 2009; Bharali et al. 2009; Sonke and Tomalia 2005).

However, negatively charged dendrimers (phosphorhydrazone dendrimers) are classically obtained by grafting carboxylic acids as terminal functions, from which sodium salts are easily obtained (Hawker 2006; Bharali et al. 2009). However, the negatively charged phosphorus dendrimer possessing the most important biological properties up to now has no carboxylates but AzaBisPhosphonate (ABP) salts as terminal functions. Indeed, with strictly identical terminal functions, the dendrimers containing heteroatoms (P or Si) in their structure have anti-inflammatory properties, whereas the "organic" dendrimers do not.

A list of the various drugs that can be delivered through dendrimers is highlighted in Table 3.3.

3.3.4 Nanoshells

Multimodal therapeutic agents based on novel nanomaterials for delivery impact have attracted increasing attention in the field of pharmaceutical sciences. Nanoshells are the new modified forms of targeted therapy, having core of silica and a metallic outer layer (West and Halas 2000). These thin-coated core particles of different materials have gained considerable attention now days. The properties of nanoshells can be altered by simply tuning the core to shell ratio. With the recent advancement in new techniques, it is now possible to synthesize these nanostructures in desired shape, size, and morphology (Shetty et al. 2008). The integration of multiple components into a nanocomposite with each material exhibiting its pharmacological activity in a coordinated way provides interesting and creative possibilities (Sershen et al. 2002). Nanoshells are synthesized to create novel structures with different morphologies, since not possible to synthesize all the materials in desired morphologies. For obtaining desirable morphology, core particles of different morphologies such as rods, wires, tubes, rings, cubes, etc. can be coated with thin shell in core shell structures. These shells are inexpensive as precious materials can be deposited on inexpensive cores. Therefore, while synthesizing nanoshells, expensive material is required in lesser amount than usual. Targeting of nanoshells can be achieved by using immunological methods. Nanoshells occupies variety of applications in diverse areas such as providing chemical stability to colloids, enhancing luminescence properties, and engineering band structures, biosensors, drug delivery, etc. Nanoshells have long shown promise for increasing drug delivery to tumors. Shetty et al. have demonstrated enhanced tumor perfusion in mice with xenografted prostate tumors, the perfusion being increased by nanoshell-mediated heating

Table 3.3 List of the various drugs that can be delivered through dendrimers

Drug specimen	Target cells/ indications/ functions	Type of dendrimers/ conjugates	Advantages/features
Boron	Neuron capture Technology	EGF-carrying PAMAM dendrimers	Intratumoral injection (Bai et al. 2007)
Efavirenz	HIV	Tuftsin-conjugated PPE dendrimers	Targeted delivery to macrophages (Cheng 2008)
EGFR siRNA	Knockdown EGFR Expression	Dendriworms	IV or CED (Chauhan and Jain 2003)
Lamivudine	HIV	Mannose-capped PPE dendrimers	Increased cellular uptake (Jevprasesphant et al. 2003), reduced toxicity (Barbara and Maria 2001)
siRNA	Lymphocytes	Amino-terminated carbosilane dendrimers	Reduced HIV infection (Yang and Kao 2006; Chen et al. 2004; Malik et al. 2012)
Doxorubicin	Colon carcinoma cells of Rat	2,2 Bis(hydroxymethyl) propanoic acid-based dendrimers	Dendrimer product Less toxic (Chen et al. 2004)
Galactosylceramide analogues	HIV-1	Multivalent phosphorus containing cationic dendrimers	Antiviral property, lower toxicity (Liu et al. 2000; Liu et al. 1999)
Plasmid pEGFP-N2	Encode green fluorescence protein	Angiopep-carrying PEGylated PAMAM dendrimer G5.0	IV (Kolhe et al. 2003)
SN38	Hepatic colorectal cancer cells	G3.5 PAMAM Dendrimers	Increase oral bioavailability and decrease GI toxicity (Mohammad and Antony 2006; Hawker 2006)
Sulfated oligosaccharides	HIV	Polylysine Dendrimers	Higher activity due to dendrimer product (Medina and Mohamed 2009; Bharali et al. 2009)

PAMAM poly(amido amine), *PPE* poly(propyleneimine)

(Shetty et al. 2008; Sershen et al. 2002). Mice were injected with nanoshells at 24 h before laser treatment, and perfusion was monitored using MRI. Whereas heating with low (0.8 W cm^{-2}) and high (4 W cm^{-2}) laser intensities decreased contrast uptake, heating with an intensity of 2 W cm^{-2} almost doubled the uptake, thus highlighting the potential of nanoshells for improving drug delivery.

Nanoshells have also been demonstrated to modulate drug delivery. For example, Sershen et al. incorporated nanoshells with an 832 nm resonance into a thermally responsive polymer, *N*- isopropylacrylamide-*co*-acrylamide (NIPAAm), to create a photomediated drug delivery hydrogel composite (Sershen et al. 2002; Sershen et al. 2001). Hydrogels based on NIPAAm exhibit a lower critical solution

temperature above which the hydrogel undergoes a reversible volume phase change transition. The nanoshells used in the experiment were engineered to have a core radius of 50 nm and shell thickness of 7 nm, in order to maximize absorption. When the composite is illuminated with a diode laser at 832 nm, the nanoshells convert light into heat, inducing a reversible and repeatable light-driven collapse of the composite hydrogel matrix. After 40 min of irradiation at 1.8 W cm^{-2}, the hydrogel composite had shrunk to 10% of its initial weight.

Recently, Bikram et al. demonstrated the potential value of nanoshell composites as drug delivery vehicles in specific applications (Bikram et al. 2007). In this case, hydrogels containing 10^9 nanoshells ml^{-1} were swollen in solutions containing 10 mg ml^{-1} insulin, lysozyme, and methylene blue, which were used as a model drug. When the release of each compound was monitored before and after laser irradiation, the release profiles of the embedded drugs upon irradiation were found to depend on their molecular weights. The release of methylene blue (14.1 mg g^{-1} polymer) and insulin (12.9 mg g^{-1}) occurred spontaneously, but the release of lysozyme occurred only upon laser irradiation. Moreover, the amounts of insulin and methylene blue released were approximately doubled on irradiation. Nanoshells are currently studied for micro metastasis of tumors and also for treatment of diabetes (Kherlopian et al. 2008). Taken together, these results indicate that nanoshell-composite hydrogels have great potential for future drug delivery applications.

Nanoshells may represent a rapid means of treating lacerations in an emergency room setting. As an example, Gobin et al. have used nanoshells as an exogenous NIR absorber for welding deep tissue wounds (Gobin et al. 2005). In this study, a nanoshell-based solder (nanoshells + bovine serum albumin (BSA)) was applied to full-thickness incisions made on rats, after which the incisions were irradiated with NIR laser light for several minutes to initiate tissue welding. Notably, the healing results were similar to the suture-treat control group until day 5, after which healing was shown to be better in the suture group.

Because of their unique features and vast potential for a variety of biomedical applications, nanoshells represent a major achievement in nanotechnology. The synergy of ideal chemical, physical, and optical properties in a single particle is a resounding affirmation of the promise of nanotechnology in general. Gold nanoshells have opened new frontiers in medicine. Because they are biocompatible, optically tunable, and strongly photoluminescent and bind to antibodies, nanoshells are highly suitable for in vivo imaging studies. Likewise, because they accumulate within tumors due to passive and active mechanisms, they hold great promise for revolutionizing cancer detection. Their success in multiple animal studies has confirmed a great potential as agents for photothermal cancer therapy, with the added benefit of serving as contrast agents for cancer detection. Clinical trials, which are currently under way, will most likely establish their efficacy for the treatment of human forms of cancer. The feasibility of novel metals is presented in Table 3.4, towards synthesis of the metallic nanoparticle and their concomitant delivery.

Table 3.4 Feasibility of novel metals in the synthesis of metallic nanoparticle and their concomitant delivery

Metal specimen	Redox potential (V)	Reducing agent	Condition
Cu^{2+}, Ru^{3+}, Re^{3+}	< 0.7 and > 0	$NaBH_4$,	Ambient (Fast) (Bai et al. 2007)
		Hydrazine, hydrogen	
		Aldehydes, sugars	
		Polyols	< 70 °C (Moderate)
			70–100 °C (Slow)
			> 120 °C (Slow)
Rh^{3+}, Pd^{2+}, Ag^{+} , Ir^{3+}, Pt^{4+}, Au^{3+}, Hg^{2+}	> 0.7	Hydrazine, H_2SO_4, H_3BO_3, $NaBH_4$, boranes	Ambient (Very fast) (Bai et al. 2007)
		Aldehydes, sugars	
		Polyols	Ambient (Fast)
		Organic acids, alcohols	<50 °C (Moderate)
			> 70 °C (Slow)
Cr^{3+}, Mn^{2+}, Re^{3+}	< 0.7 and > 0	$NaBH_4$,	Ambient (Fast) (Bai et al. 2007)
		Hydrazine, hydrogen	
		Aldehydes, sugars	
		Polyols	< 70 °C (Moderate)
			70–100 °C (Slow)
			> 120 °C (Slow)
Cu^{2+}, Ru^{3+}, Re^{3+}	< −0.6	Hydrated e^{-}, radical	Ambient (Fast) (Bai et al. 2007)
		$NaBH_4$, boranes	Temperature & Pressure (Slow) (Bai et al. 2007)
Fe^{2+}, Co^{2+}, Ni^{2+}, Mo^{3+} Cd^{2+}, In^{3+}, Sn^{2+}, W^{6+}	< 0 and > 0–0.5	Hydrated e^{-}, radical	Ambient (Very fast) (Bai et al. 2007)
		$NaBH_4$, Boranes	
		Hydrazine, hydroxylamine	
		Polyols	Ambient (Fast)
			70–100 °C (Slow)
			> 180 °C (Slow)

3.3.5 Niosome

Many drug nanocarriers have emerged to achieve controlled delivery of drugs, genes, or gene expression-modifying compounds, or vaccine antigens to a specific target site. Lipid-based systems including niosomes are non-toxic self-assembly vesicles with a unilamellar or multilamellar structure, which can encapsulate hydrophobic/hydrophilic therapeutic agents (Hood et al. 2007). Niosome is a class of molecular cluster formed by self-association of non-ionic surfactants in an aqueous phase. The unique structure of niosome presents an effective novel drug delivery system (NDDS) with ability of loading both hydrophilic and lipophilic drugs (Hood et al. 2007; Kong et al. 2013). Niosomes are vesicles composed of non-ionic

surfactants, which are biodegradable, relatively nontoxic, more stable, and inexpensive, an alternative to liposomes (Kong et al. 2013). Characteristics such as loading capacity, drug release rate, stability (physical and chemical), and vesicle size are highly reliant on experimental situation and type of material and method at the time of manufacturing (Hood et al. 2007; Widder et al. 1979). Niosomes behave in vivo like liposomes, prolonging the circulation of entrapped drug and altering its organ distribution and metabolic stability. As with liposomes, the properties of niosomes depend on the composition of the bilayer as well as method of their production. It is reported that the intercalation of cholesterol in the bilayer decreases the entrapment volume during formulation and thus entrapment efficiency. However, differences in characteristics exist between liposomes and niosomes, especially since niosomes are prepared from uncharged single-chain surfactant and cholesterol, whereas liposomes are prepared from double-chain phospholipids (neutral or charged) (Tavano et al. 2013). The concentration of cholesterol in liposomes is much more than that in niosomes. As a result, drug entrapment efficiency of liposomes becomes lesser than niosomes. Besides, liposomes are expensive, and its ingredients, such as phospholipids, are chemically unstable because of their predisposition to oxidative degradation; moreover, these require special storage and handling, and purity of natural phospholipids is variable. Current opinions for the utilization of niosomes in the delivery of biomolecules can be unsubstantiated with a wide scope in encapsulating toxic drugs such as anti-AIDS drugs, anticancer drugs, and antiviral drugs (Widder et al. 1979; Tavano et al. 2013). Niosomes offers a promising carrier system in comparison with ionic drug carriers, which are relatively toxic and unsuitable. However, the technology utilized in niosomes is still in pipeline. Therefore researches are going on to develop a suitable technology for large production because it is a promising targeted drug delivery system.

Niosomes have been applied in various fields such as medicine, diagnostics, and cosmetics; it seems that drug delivery application is the best well-studied area. Niosomes can be used in a wide range of pharmaceutical applications due to their inherent advantages.

Niosomes can be conjugated to antibodies on their surface to form immune-niosomes. Conjugation of the monoclonal IgG antibodies to the vesicle surfaces was carried out through incorporation of a cyanuric chloride derivatized Tween 61 in the niosome formulation formed using thin film hydration techniques followed by sonication (Hood et al. 2007; Widder et al. 1979). The presence of cyanuric chloride in the structure of Tween 61 provides the linkage of IgG antibody to vesicle surface. Conjugation of the monoclonal antibody to the specific cell receptors (CD44) was demonstrated using cultured fixed synovial lining cells expressing CD44 and showed the capability of immune-niosome binding to target antigens which might provide an effective method for targeted drug delivery (Kong et al. 2013; Tavano et al. 2013).

Niosomes show potential in combination of drug delivery and magnetic targeting in various applications particularly in cancer therapy (Kong et al. 2013; Widder et al. 1979; Tavano et al. 2013). The basic concept of using magnetic materials in cancer therapy is to direct drug-loaded magneto-niosomes to specific organ or tissue

in the body by applying extracorporeal magnets (Widder et al. 1979). Formulation of niosome in magnetically controlled drug targeting of doxorubicin is a good example to prove this ability of niosomal systems. Doxorubicin-loaded magneto-niosomal formulations were developed by encapsulating both antitumoral model drug and magnetic material (EMG 707 ferrofluid) into the niosome aqueous core. In addition, these formulations exhibited a controlled drug release without any additional toxicity due to incorporation of magnetic material into the niosomes (Tavano et al. 2013).

Although niosomes have been used in pharmaceutics since the 1980s, to date a few studies have focused on the application of niosomes for gene delivery. Since niosomes are biodegradable, biocompatible, and nontoxic, they have potential to be safely used in gene therapy (Huang et al. 2008). Niosomes have been used as cutaneous gene delivery system especially for the treatment of a variety of skin diseases (Geusens et al. 2011). Huang et al. reported an effective delivery of antisense oligonucleotides (OND) via cationic niosomes of spans in a COS-7 cell line with positive results on cellular uptake of OND (Huang et al. 2005). Further studies by incorporation of polyethylene glycol into OND/niosome complexes showed a higher efficiency of OND cellular uptake in serum which demonstrates positive results for gene delivery through niosomal formulations (Huang et al. 2005).

Niosomes showed great potential in the targeted delivery of some anticancer drugs. Niosomes composed of a non-ionic surfactant, cholesterol, and dicetyl phosphate encapsulating methotrexate (MTX) showed improvement in absorption of the drug from the gastrointestinal tract following oral ingestion and a higher uptake of MTX into the liver following the intravenously administration of the niosomes as compared to methotrexate solution, administered either orally or intravenously (Azmin et al. 1985). Jain and Vyas (Jain and Vyas 1995) reported that high levels of MTX were found in the thoracic lymph following niosomal administration by this route as compared to administration through the intravenous route and the administration of the free drug via the peritoneal route. Doxorubicin niosomes composed of span 60 showed improvement in the doxorubicin pharmacokinetics and tumoricidal activity after a single intravenous dose in the mouse adenocarcinoma as compare to the drug in solution. Improvement in anticancer activity or reduced toxicity of niosomal formulations of other anticancer agents such as vincristine (Parthasarathi et al. 1994), bleomycin (Raja et al. 1996), and paclitaxel (Bayindir and Yuksel 2010) showed that niosomes can be used as efficient drug carriers for anticancer drugs. Some of the applications of niosomes are detailed in Table 3.5.

3.3.6 Magnetic Nanoparticles

Magnetic drug targeting is conceptualized with an objective to target magnetic drug carrier particles at a specific site in the body using an externally applied magnetic field. Magnetic nanoparticles (MNPs) are a class of particulate materials of less than 100 nm size that can be manipulated under the magnetic field (Cuenca et al. 2006).

Table 3.5 Drug delivery through niosomes

Drug specimen	Biological activity	Applications	Route of administration
Gentamicin sulfate	Antibiotic	Prolongation of drug release	In vivo (Hood et al. 2007)
Hemoglobin		Stabilizing and protection of structure behaviors of Hb	In vitro (Kong et al. 2013)
Ampicillin	Antimicrobial	Increase antimicrobial activity	In vivo (Hood et al. 2007)
Colchicine-5-fluorouracil	Treat rheumatic complaints	Prolonged release profile	In vitro (Kong et al. 2013)
	Treatment of cancer		
Indomethacin	Antiplatelet activity	Enhanced inhibition of platelet aggregation	In vitro (Widder et al. 1979)
Hyaluronic acid	Tumor therapy	Improve endocytosis	In vitro/in vivo (Widder et al. 1979)
Silymarin	Treat liver and gallbladder disorders	Increase drug bioavailability	In vivo (Tavano et al. 2013)
Zidovudine	Treat AIDS	Enhance entrapment and sustainability of release	In vivo (Huang et al. 2008)
Beclomethasone dipropionate	Treatment of inflammatory lung diseases	Improve inflammatory activity	In vivo (Huang et al. 2008)
Ammonium glycyrrhizinate	Treatment of various inflammatory based diseases	Improve the drug anti-inflammatory activity	In vivo/in vivo (Geusens et al. 2011)
Miconazole	Treatment of candida infections, fungal infections	Increase residence time of drug in the stratum corneum	In vivo (Huang et al. 2005)
Acyclovir	Treatment of herpes simplex virus	Prolonged activity improve the oral bioavailability	In vivo (Azmin et al. 1985)
Insulin	Blood glucose lowering agent	Sustained release and increase absorption	In vivo (Jain and Vyas 1995)
Tyloxapol	Anti-tuberculosis	Improve the drug bioavailability	In vivo (Parthasarathi et al. 1994)
Nimesulide	Anti-inflammatory activity	Prolongation of drug release	In vivo (Raja et al. 1996)
Acetazolamide	Treatment of glaucoma	Improve the low corneal penetration and bioavailability promote absorption	In vivo/in vivo (Bayindir and Yuksel 2010)

These particles are composed of magnetic elements such as cobalt, nickel, iron, and their respective oxides such as magnetite, cobalt ferrite, and chromium dioxide. The classification of these particles is based on their magnetic susceptibility which is defined as ratio of induced magnetization to the applied field. Paramagnetic nanoparticles have a greater magnetic susceptibility than conventional contrast agents.

They are investigated for both diagnostic and therapeutic purposes. For diagnostic purpose, paramagnetic iron oxide nanoparticles are used as contrast agents in magnetic resonance imaging. Targeting with paramagnetic nanoparticles enables identification of specific organs and tissues (Cuenca et al. 2006).

The primary shortcoming of most chemotherapeutic agents is their relative nonspecificity and thus potential side effects to healthy tissues. To overcome this problem, magnetic drug targeting (MDT) utilizes the attraction of MNP carriers to an external magnetic field to increase site-specific delivery of therapeutic agents (Pankhurst et al. 2003; Dobson 2006). In general, this process involves the attachment of a cytotoxic drug to a biocompatible MNP carrier (a.k.a. magnetic targeted carrier or MTC), intravenous injection of these MTCs in the form of a colloidal suspension, application of a magnetic field gradient to direct the MTC to the pathological site, and release of the therapeutic agent from the MTC. Although seemingly straightforward, there are many variables that complicate the execution of this technique. Parameters such as the physicochemical properties of the drug-loaded MNP, field strength and geometry, depth of the target tissue, rate of blood flow, and vascular supply all play a role in determining the effectiveness of this method of drug delivery (Neuberger et al. 2005).

Early clinical trials of colloidal iron oxide MTCs loaded with epirubicin and directed towards solid tumors have demonstrated successful accumulation in the target site in about half the patients in this study (Lubbe et al. 2001). These MTCs were also shown to be well tolerated by patients. Unfortunately, several problems have been identified with this technique including the possibility of embolization of the blood vessels, difficulty in scaling up from animal models due to limited field penetration of commercial magnets, control of drug diffusion after release from the MTC, and toxic responses to the MTCs. To address some of these issues and develop a theoretical basis for this technique, Grief and Richardson created a mathematical model incorporating the effects of hydrodynamics within blood vessels, particle volumes, magnetic field strength, and even the effects of cells within the plasma (Grief and Richardson 2005). In this study it has been concluded that MDT could only be used effectively for targets close to the surface of the body.

Given this limitation, Alexiou et al. recently demonstrated the successful in vivo delivery of MCT composed of starch-coated USPIO loaded with mitoxantrone into VX2-squamous cell carcinomas on the hind limbs of New Zealand White Rabbits (Alexiou et al. 2006). The group demonstrated the effectiveness of these MCTs to completely eliminate tumors after approximately 35 days of treatment.

The attachment of targeting agents to MNPs can be used to increase the specific accumulation of nanoparticles within diseased tissue. By integrating therapeutic agents, these multifunctional MNPs can serve strictly as a vehicle for drug delivery (Kohler et al. 2006). One advantage of these MNPs, as well as other nanoparticle carriers, is their high surface area-to-volume ratios allowing for a large number of therapeutic molecules to be attached to individual nanoparticles. Additionally, while utilizing an active targeting strategy for specific delivery, the magnetic properties of

the nanoparticle may be used to provide imaging modality for monitoring of drug delivery through MRI (Kohler et al. 2006) or an alternative source of treatment through magnetic fluid hyperthermia (MFH) therapy (Mornet et al. 2004). MNPs have been evaluated as drug carries for a variety of chemotherapeutic agents. Traditional drugs such as etoposide, doxorubicin, and methotrexate have been attached or encapsulated in MNPs for potential treatment of diseases ranging from rheumatoid arthritis to highly malignant prostate and breast tumors (Schulze et al. 2005; Jain et al. 2005). With the wide variety of nanostructures described in the previous sections, carriers can be designed with specific characteristics to enhance the efficacy of these therapeutic agents over that achieved by typical systemic delivery. Characteristics such as loading capacities and drug release profiles can now be tailored by controlling structural features and chemical bonding within the MNP conjugate.

Yang et al. investigated the synthesis and release characteristics of poly(ethyl-2-cyanoacrylate) (PECA)-coated magnetite nanoparticles containing anticancer agents cisplatin and gemcitabine (Yang et al. 2006). In this study, cisplatin was shown to exhibit a sustained release behavior due to its hydrophobicity in comparison to the more rapid release of the hydrophilic gemcitabine. *Kohler* et al., demonstrated a sustained release of methotrexate (MTX) in breast and brain tumor cells delivered by iron oxide nanoparticles. In this study, the authors covalently attached MTX to amine functionalized nanoparticles through amide bonds to ensure stability of the drug conjugate under intravenous conditions. Cleavage of the MTX from the MNPs was evaluated over a range of pH values and in the presence of lysozymes to mimic conditions present in the lysosomal compartments. Through the use the covalent linkage, the group demonstrated the controlled release of MTX to the cellular cytosol and the subsequent cytotoxicity to these cancer cells (Yang et al. 2006). Different particles are designed as drug delivery vehicles, and a summary of these particles is given in Table 3.6.

3.3.7 Polymeric Nanoparticle

Most polymeric nanoparticles (PNPs) are biodegradable and biocompatible, and over the past few decades, researchers have had considerable interest in developing biodegradable NPs as a drug-delivery system (Panyam and Labhasetwar 2003). Moreover, they also exhibit a good potential for surface modification and functionalization with different ligands, provide excellent pharmacokinetic control, and are suitable to encapsulate and deliver a plethora of therapeutic agents. Depending on the process used for their preparation, these can be NPs, nanospheres, or nanocapsules. Nanospheres have a matrix-like structure, where active compounds can be firmly adsorbed at their surface and entrapped or dissolved in the matrix. Nanocapsules have a polymeric shell and an inner core. In that case, an active

Table 3.6 Different magnetic particles are designed as vehicles for the drug delivery

Drug specimen	Type of MNPs	Coating agent	Design matrix
Cefradine	Fe_3O_4	Chitosan/PAA multilayer	Drug molecules were entrapped inside the hollow spheres through diffusion process (Cuenca et al. 2006)
Cefradine	Fe_3O_4	Chitosan	Cross-linking the particles with glutaraldehyde and the drug is embedded in the polymer matrix (Cuenca et al. 2006)
Insulin	Fe_3O_4	Alginate/chitosan	Insulin encapsulation in alginate/chitosan beads prepared in triplicate by extrusion method (Pankhurst et al. 2003)
Doxorubicin	Fe_3O_4	Multi-walled carbon nanotubes (MWCNTs)	MWNT-hybrid nanocomposites provided an efficient way for the extraction and enrichment of doxorubicin via π–π stacking DOX molecules onto the polyaromatic surface of MWNTs (Dobson 2006)
Methylene blue	y-Fe_2O_3	CNT	Monodisperse, inherently open ended, multi-walled CNTs loaded with magnetic iron-based nanoparticles that are encapsulated within the tube graphitic walls (Neuberger et al. 2005)
Gemcitabine	Fe_3O_4	MWCNTs and magnetic activated carbon particles	Fe_3O_4 nanoparticles are on the outer surface of the PAA functionalized MWNTs and the drug is adsorbed on the surface (Lubbe et al. 2001)
Doxorubicin	$CoFe_2O_4$ Nanoparticles	MWCNT/cobalt ferrite ($CoFe_2O_4$) hybrids	Cobalt ferrite is on the outer surface of the MWCNT (Dobson 2006)
Fluorescein	y-Fe_20_3	DNA	*ss*DNA was immobilized onto the silica network, and the magnetic particles are loaded onto the network. Complementary DNA sequence was then attached to MNPs (Grief and Richardson 2005)
Doxorubicin	Fe_3O_4	PEG-functionalized porous silica shell	DOX conjugated magnetite particles are coated with silica to obtain core/shell nanoparticles, and the whole composite is coated with PEG (Alexiou et al. 2006)
Curcumin	Fe_3O_4	β-Cyclodextrin and pluronic polymer (F-127)	Multilayer polymer coating around the magnetic particle and the drug is encapsulated via diffusion into polymer matrix (Kohler et al. 2006)
Ketoprofen	Fe_3O_4	2-Hydroxypropyl cyclodextrin (HCD)-Gum arabic modified MPs (GAMNPs)	Drug molecules are rapidly released from HCD-GAMNPs, whereas some remains associated with degradation of HCD-GAMNPs (Mornet et al. 2004)

(continued)

Table 3.6 (continued)

Drug specimen	Type of MNPs	Coating agent	Design matrix
Doxorubicin	y-Fe_20_3	PNIPAM	MNP cluster is coated with PNIPAM, and the nanoparticle is dehydrated. Core shell morphology is achieved with dispersion free-radical polymerization (Schulze et al. 2005)
Doxorubicin	Fe_3O_4	PNIPAM	Core shell morphology by dispersion polymerization where drug-loaded PNIPAM shell contains magnetite clusters (Jain et al. 2005)
Doxorubicin	y-Fe_20_3	Carbon	Drug is released from the surface of on-coated or partially coated magnetic particles (Yang et al. 2006)

substance is usually dissolved in the core but can also be adsorbed at their surface (Sahoo and Labhasetwar 2003). The main advantage of using NPs for drug-delivery applications is their small size when taken up by cells, which could allow efficient drug accumulation at the target sites (Panyam et al. 2003). Biodegradable materials used for the formulation of NPs allow sustained drug release within the target site over a period of days or even weeks. Biodegradable NPs formulated from poly D,L-lactide co-glycolide (PLGA) and polylactide (PLA) have been investigated for sustained drug delivery (Panyam et al. 2002). The main interest of researchers is to study their intracellular trafficking and to determine the parameters that are critical to their efficient cellular uptake and retention. Recently, studies have demonstrated rapid escape of NPs from the endolysosomal compartment to the cytoplasmic compartment (Panyam et al. 2002). Greater and sustained antiproliferative activity of paclitaxel-loaded PLGA NPs in HeLa cells was observed by the research group of Yang et al. Enhanced apoptosis of HeLa cells was observed, which may be due to the sustained release of paclitaxel from the PLGA NPs, which in turn showed that PLGA NP-encapsulated paclitaxel is promising as a controlled drug-delivery system in future clinic application (Yang et al. 2009). Recently, NPs formulated from PLGA were investigated as a drug-delivery system to enhance tissue uptake and permeation and targeting of zinc (II) phthalocyanine (ZnPc) for photodynamic therapy. Tumor-bearing mice injected with ZnPc NPs exhibited significantly smaller mean tumor volume, increased tumor growth delay, and longer survival in comparison with the control group and the group injected with free ZnPc during the time course of the experiment. Histopathological examination of tumor from animals treated with PLGA ZnPc showed regression of tumor cells, in contrast to those obtained from animals treated with free ZnPc. The results indicate that ZnPc encapsulated in PLGA NPs is a successful delivery system to improve photodynamic activity in the target tissue (Fadel et al. 2010).

Multidrug resistance (MDR) is one of the major causes of treatment failure in cancer therapy, which may be attributed to the decreased accumulation of drug in the tumor site in addition to the possibility of membrane glycoprotein (P-gp)-dependent accelerated drug efflux (Brigger et al. 2002; Vauthier et al. 2003). To overcome the problem of efflux action of P-gp and to sustain drug effect, various drug-delivery systems have been developed. PLGA NP formulations capable of delivering a cytotoxic drug, vincristine, a chemosensitizer, verapamil, or their combination were prepared by the research group of Song et al. The results showed that PLGA NPs simultaneously loaded with anticancer drug and chemosensitizer might be the one of the potential formulations in the treatment of drug-resistant cancers in vivo as the simultaneous administration of vincristine and verapamil could achieve the highest reversal efficacy on MCF-7/ADR cells resistant to vincristine (Song et al. 2009). In other studies, *Wang* et al. developed an efficient and targeted delivery of antisense oligodeoxynucleotides (asODNs), using folic acid (FA)-conjugated hydroxypropyl-chitosan (HPCS) NPs to reduce production of P-gp to overcome tumor drug resistance. The FAHPCS-asODNs NPs demonstrated significant inhibition of the MDR 1 gene levels and P-gp levels in vitro and in vivo, respectively, in comparison with asODNs and HPCS-asODNs alone. Thus, these results suggest that the use of targeted, antisense agent NPs would be a potential approach to overcome tumor drug resistance (Wang et al. 2010).

Another characteristic function of NPs is their ability to deliver drugs to the target sites across biological barriers such as the blood–brain barrier (BBB) (Fisher and Ho 2002; Lockman et al. 2002). The brain delivery of a wide variety of drugs, such as antineoplastic and anti-HIV drugs, is markedly hindered because they have great difficulty in crossing the BBB (Sun et al. 2003). Thus, by using the nanotechnological approaches, researchers have tried to improve the pharmacokinetics of drugs for the treatment of central nervous system (CNS) diseases. The application of NPs to brain delivery is a promising way to overcome this barrier. Kreuter and colleagues demonstrated that poly-(butylcyanoacrylate) NPs coated with polysorbate-80 are effective in carrying different drugs to the brain (Kreuter et al. 2003). Although not fully elucidated, the most likely transport mechanism for these particles is via endocytosis across the endothelial cell lining of the BBB. Moreover, by packaging therapeutic molecules inside a liposome and decorating the surface of the liposome using molecular "Trojan horse" technology, researchers have obtained promising results.

Recently many surface-modified NPs are being used to treat various diseases. Surface modification of PLGA NPs with polyethyleneimine (PEI) utilizing a cetyl derivative was used to improve surface functionalization and aid siRNA delivery. Specific reduction in the anti-apoptotic oncogene BCL-w in U2OS cells was achieved with particles containing cetylated-PEI with no apparent cellular toxicity. In addition, particles containing cetylated-PEI achieved 64% silencing of TNF alpha in J774.1 cells (Andersen et al. 2010).

3.3.8 Solid Lipid Nanoparticle

Solid lipid NPs (SLNs) were developed at the beginning of the 1990s as an alternative carrier system to emulsions, liposomes, and polymeric NPs as a colloidal carrier system for controlled drug delivery (Kayser et al. 2005). These particles are made from solid lipids (i.e., lipids that are solid at room temperature and also at body temperature) and stabilized by surfactant(s). SLN can be formulated by using highly purified triglycerides, complex glyceride mixtures, or even waxes. In comparison with other particulate carriers, SLN has many advantages for drug delivery, such as good tolerability, biodegradability (Sahoo et al. 2007), a high bioavailability by ocular administration (Sahoo and Labhasetwar 2003), and a targeting effect on the brain (Yang et al. 1999). In recent years, the study of SLN has markedly increased, especially with the method of high pressure homogenization. SLN have been developed and investigated for parenteral, pulmonary, and dermal application routes (Wissing et al. 2004).

Because of their small size, SLN may be injected intravenously and used to target drugs to particular organs. The particles together with all intravenously injected and colloidal particulates are cleared from the circulation by the liver and spleen. In tumor tissues, drugs can be targeted by PEG-coated polymeric NPs (known as stealth property) that help in escaping from reticuloendothelial system (RES) (Uner et al. 2004). This may be achieved using block polyoxyethylene polypropylene copolymers like Pluronic F188 in which the hydrophobic portion of the molecule forms the NP matrix, while the water-soluble polyoxyethylene block forms a hydrophilic coating on the particle. Stealth SLN increases the tumor accumulation and antibacterial activity of antiparasitic and antifungal drugs and allows brain delivery of anticancer drugs not capable of crossing the BBB (Zara et al. 2002). Recently SLNs have also been used for the targeted delivery of therapeutics to the alveolar macrophages by Yu et al. In their study, a mannan-based PE-grafted ligand was synthesized and used for the surface modification of DNA-loaded cationic SLN to prepare Man-SLN-DNA. Their results showed that in comparison with non-modified SLN-DNA and Lipofectamine 2000-DNA, Man-SLN-DNA produced the highest gene expressions, especially in vivo. Thus, these modified SLNs may have great potential for targeted gene delivery (Yu et al. 2010b).

3.4 FDA-Approved Nanopharmaceuticals

Many nanopharmaceutical products got approval from the Food and Drug Administration (FDA). It has been noted that more than 1000 nanopharmaceutical-based patents is issued by the US Patent and Trademark Office (US PTO) during the last decade (Qadir et al. 2016)**.** There are a number of FDA-approved, marketed nanopharmaceuticals for the intravenous administration route as well as the non-intravenous route (Table 3.7). However, numerous nanopharmaceuticals are still at the development or clinical trial phase due to the extremely complex nature of human medicinal applications.

Table 3.7 FDA-approved and commercial nanoproducts (Faiyazuddin et al. 2013)

Drug specimen	Nanotechnology	Brand	Company
1. Route of administration: intravenous			
Doxorubicin	PEGylated liposomes	Doxil® (US)	OrthoBiotech
		Caelyx (others)	Schering-Plough
Doxorubicin	Liposomes	Myocet®	Zeneus Pharma
Paclitaxel	Albumin-bound nanoparticles	Abraxane®	Abraxis
			BioScience
			AstraZeneca
Paclitaxel	Polymeric micelles	Genesol-PM®	Samyang
Amphotericin B	Liposomes	AmBisome®	NeXstar
			Pharmaceuticals Inc.
Amphotericin B	Phospholipid Complex	Abelcet®	Enzon
Propofol	Lipid emulsion	Diprivan®	AstraZeneca
Cytarabine	Liposomes	DepoCyt®	SkyePharma
			Enzon
Daunorubicin citrate	Liposomes	DaunoXome®	Gilead Sciences
Adenosine deaminase	PEGylation	Adagen®	Enzon
Iron oxide	Iron oxide nanoparticles	Feridex I.V.®	AMAG
			Pharmaceuticals, Inc.
2. Route of administration: oral			
Sirolimus	NanoCrystal®	Rapamune®	Wyeth, Elan
Fenofibrate	NanoCrystal®	TriCor®	Abbott
Fenofibrate	NanoCrystal®	Triglide®	SkyePharma
Aprepitant	NanoCrystal®	Emend®	Merck, Elan
Megestrol acetate	NanoCrystal®	Megace ES	Par Pharma, Elan
Morphine sulfate	NanoCrystal®	Avinza®	King Pharma, Elan
Dexmethylphenidate HCl	NanoCrystal®	Focalin® XR	Novartis, Elan
Methylphenidate HCl	NanoCrystal®	Ritalin® LA	Novartis, Elan
Tizanidine HCl	NanoCrystal®	Zanaflex®	Acorda Inc., Elan
Cyclosporine A	Self-microemulsifying drug delivery systems (SMEDDS)	Neoral®	Novartis
Saquinavir	SMEDDS	Forovase®	Roche
Ritonavir	SMEDDS	Norvir®	Abbott laboratories
3. Route of administration: pulmonary			
Artificial lung surfactant replacement	Synthetic recombinant Polypeptide liposomal lung surfactant	Surfaxin®	Drug discovery Lab

(continued)

Table 3.7 (continued)

Drug specimen	Nanotechnology	Brand	Company
Artificial lung surfactant replacement	Natural bovine lung extract	Survanta®	Abbott labs
Artificial lung surfactant replacement	Synthetic lung surfactant (protein-free)	Exosurf®	GlaxoSmithKline
Artificial lung surfactant replacement	Natural porcine lung extract	Curosurf®	Dey
Artificial lung surfactant replacement	Natural bovine lung extract	Alveofact®	Boehringer Ingelheim
Route of administration: subcutaneous			
Interferon alfa-2a	PEGylation	Pegasys®	Nektar Hoffmann-La Roche
hGH (human growth hormone)	PEGylation	Somavert®	Nektar Pfizer
Recombinant methionyl human G-CSF (granulocyte colony stimulating factor)	PEGylation	Neulasta®	Amgen
Glatiramer acetate	Copolymer of L-glutamic acid, L-alanine, L-lysine, and L-tyrosine	Copaxone®	Teva
Amphotericin B	Lipid colloidal dispersion	Amphotec®	Sequus
Interferon alfa-2b	PEGylation	PEGIntron®	Enzon Schering-Plough
Asparaginase	PEGylation	Oncaspar®	Enzon
Route of administration: transdermal			
Estradiol	Micellar nanoparticles	Estrasorb®	Novavax
Estradiol	Estradiol gel (0.06%) incorporating calcium phosphate nanoparticles	Elestrin®	BioSante
Lidocaine	Liposomes	LMX®-4	Ferndale laboratories
Cyclosporine A	Lipid emulsion	Restasis®	Allergan

3.5 Futuristic Scenario of Nanopharmaceuticals

Due to the rapid increase in the applications of nanotechnology in different spheres of our life especially in biomedicine, the conventional therapies face a large number of challenges including poor bioavailability and intrinsic toxicity. These have seriously compromised the therapeutic efficacy of many otherwise beneficial drugs. The market for nanopharmaceuticals has made a rapid tread from $406 million in 2004 to $3 billion in 2009 and $16.6 billion in 2015 (Wissing et al. 2004). Currently, nanopharmaceuticals have become prerequisite to sustain the growth of the pharmaceutical industry. According to the latest report published by Visiongain forecasts, the world market for nanopharmaceuticals will reach $130 billion in 2017. There is

ongoing vigorous research and increasing demand of nanopharmaceuticals resulting in better positions in the pharmaceutical and other healthcare industries (Wissing et al. 2004; Qadir et al. 2016; Ahmad et al. 2015a). Henceforth, nanopharmaceuticals will greatly influence medical practice and healthcare because of their ability, in many cases, to shorten the time market for active agents, extend the economic life of proprietary drugs, and create additional revenue streams.

3.6 Conclusion

Drug molecules that failed previously because of unacceptable toxicity profiles, poor bioavailability, solubility issues, or the inability to be delivered via conventional forms/routes may be reformulated as nanopharmaceuticals. From a business point-of view, nanopharmaceuticals offer the ability to extend the economic life of proprietary drugs and create additional revenue streams, thereby significantly affecting the drug commercialization landscape (Qadir et al. 2016; Faiyazuddin et al. 2013; Ahmad et al. 2015a).

Nanopharmaceuticals often go hand-in-hand with novel drug delivery methods and technologies. This in turn may result in more efficacious treatments that generate new niche markets to provide greater patent protection to already existing drug formulations (Ahmad et al. 2015a; Ahmad et al. 2015b). As discussed earlier, nanopharmaceuticals will provide faster drug absorption, controlled dosage releases, and effective shielding from the body's immune system enhancing the effectiveness of pre-existing drugs (Faiyazuddin et al. 2013; Ahmad et al. 2015a; Ahmad et al. 2015b).

As nanopharmaceuticals move out of the laboratory and into the clinic, federal agencies like the FDA and the PTO will continue to struggle to encourage their development while imposing some sort of order. At present, both these critical agencies are in flux, and their credibility has sunk to an all-time low. It is hoped that desperately needed reforms to overhaul the PTO and the decades-old US patent system along with clearer regulatory/safety guidelines from the FDA regarding nanopharmaceuticals will be forthcoming. As nanotechnology begins to appear in a wide variety of products, safety and effectiveness of these nanoscale products will warrant a careful review because size changes within the nanoscale are likely to add additional complexity to the FDA product review process (Faiyazuddin et al. 2010).

In future, novel "multifunctional" nanopharmaceuticals will be designed and delivered to the human body via a variety of routes (Ahmad et al. 2015a; Faiyazuddin et al. 2010). It will be imperative that each of these be evaluated and characterized on a case-by-case basis in an effort to correlate nanopharmaceuticals physiochemical property with in vivo biological behavior and therapeutic outcome. In this regard, any research strategy must involve adsorption, distribution, metabolism, and excretion (ADME) testing, toxicology tests, and physiochemical characterization (Faiyazuddin et al. 2012). Eventually, all these undertakings will certainly expand the burgeoning field of nanopharmaceuticals. Big pharma and biotech will further

embrace nanopharmaceuticals, and this pace of adoption will enhance as they offer novel properties that address unmet medical needs with low development costs and risks.

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Chapter 4
Natural Products and Nanopharmaceuticals

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Abstract Drug discovery has faced many challenges, and the diversity of natural products offers a huge number of opportunities for new drug findings. Most of the potential candidates result from plants since plants have several and interesting biological activities. However, the *in vivo* efficacy of such candidates is frequently limited due to their low absorption. Thus, enhancing the bioavailability of natural products through the improvement of their pharmacokinetic and biodistribution features, as well as their targeting efficacy, is a crucial step in the development of new therapeutic strategies.

Here, we reviewed nanotechnology as a rising approach for drug delivery, presenting smart nanocarriers that can selectively deliver appropriate levels of a therapeutic agent. Moreover, in order to deliver the therapeutic agent to target cells, nanocarriers can also be efficient targeting systems. Another benefit here discussed in the use of nanocarriers to deliver natural products is the controlled drug release.

This review describes many types of nanocarriers with structural and functional differences between them which can be chosen accordingly to the encapsulated drug characteristics, to the specific target, or even to the desired release rate. With regard to natural products, we highlight several natural products that are already being commercialized or in clinical study phase with impressive therapeutic improvements using these nanocarriers. On the other hand, there are also a large number of natural products that are being used as encapsulant material in pioneering nanocarriers. This review aims to summarize the development in several key areas relevant to natural products in nanopharmaceuticals. Besides the potential beneficial use, also attention is drawn to the question how we should proceed with the safety and efficacy evaluation of the nanopharmaceuticals for natural product delivery. Nonetheless, research into sophisticated, science-driven solutions is still continuing; expectations related to therapeutic efficacy are high to meet clinical needs, but the progress made has been noticeable.

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Keywords Natural products · Plants · Therapeutic activity · Adverse side effects · Encapsulation · Polymers · Nanopharmaceuticals · Bioavailability · Targeting · Controlled release

4.1 Introduction

The pharmacological and biological activities of some natural compounds have provided therapeutic benefits in human disease treatment (Watkins et al. 2015). The diversity of products from plants, animals, and minerals offers a huge number of opportunities for new drug discoveries due to the incomparable availability of chemical range (Fig. 4.1). It is known that more than 50% of all drugs in clinical use came from natural products (Bilia et al. 2017; Madhuri and Pandey 2009; Sasidharan et al. 2011). In the past decades, regulatory agencies have approved ~61% of the developed natural products to treat cancer and 49% of them to treat infections (Watkins et al. 2015). Moreover, according to the World Health Organization, more than 80% of people in developing countries use traditional medicine for their primary health essentials (Madhuri and Pandey 2009). Currently, the global market of natural products is mainly derived from the plant origin (Bilia et al. 2017). Herbal medicines have been universally accepted, over the past decade, and they have a huge impact on world health as in international trade (Madhuri and Pandey 2009).

Drug discovery has undergone many challenges, and the innumerous phytochemicals from plants, with apparently no direct contribution to their growth and development, namely, secondary metabolites, were found to be safe and broadly

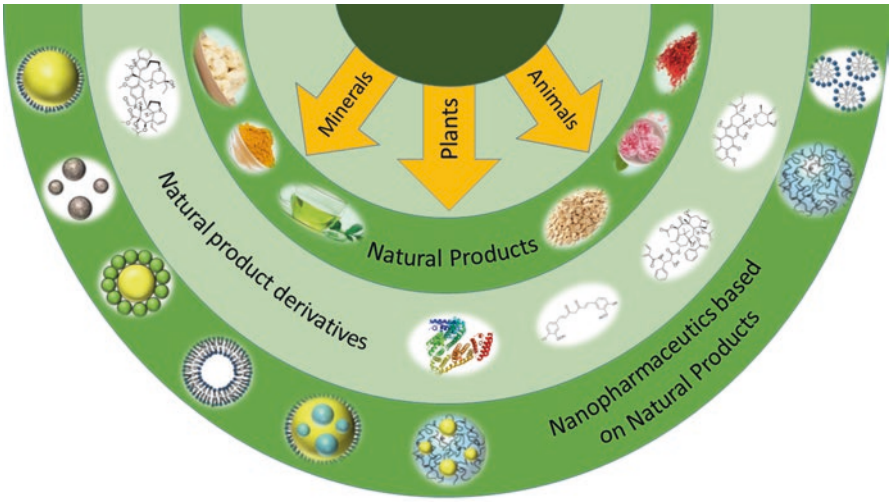

Fig. 4.1 It is possible to obtain natural products from raw materials, such as animals, plants, and minerals. Through them, their derivatives are extracted, which can be used not only as encapsulating material but also to be encapsulated so that they can achieve greater targeting and therapeutic effect

effective alternatives with less adverse effects. Those secondary metabolites have shown to have several biological activities such as anticancer, antimicrobial, antioxidant, analgesic, and wound-healing (Bilia et al. 2017; Sasidharan et al. 2011; Watkins et al. 2015). The use of plants in cancer treatment has a long history, and over the last 70 years, 49% of small-molecule anticancer agents are either natural products or directly derived from these (Bahmani et al. 2016; Burmistrova et al. 2013, 2015). Thus, natural products can modulate multiple targets activating various signaling or functional pathways (Asadi-Samani et al. 2015; Bilia et al. 2017). Although almost all have promising therapeutic potentials, the *in vivo* efficacy of the natural products is frequently limited because of their low absorption; they are unable to cross lipid membranes due to their hydrophilicity, intrinsic dissolution rate and physical/chemical instability, resulting in loss of bioavailability and efficacy. In addition, they can have limited biodistribution, extensive first-pass metabolism, poor penetration and accumulation in the non-target organs, and insignificant targeting efficacy (Bilia et al. 2017; Bonifácio et al. 2014).

Some isolated compounds are highly sensitive to the acidic pH of the stomach which promotes their degradation (Ansari et al. 2012; Bonifácio et al. 2014). The susceptibility to liver metabolism can also lead to low blood levels and, consequently, to less or no therapeutic result. Some isolated compounds and extracts are not used clinically because of those obstacles (Ansari et al. 2012; Bonifácio et al. 2014; Goyal et al. 2011). Therefore, they commonly need repeated administrations or higher doses. Enhancing the bioavailability of natural products, such as improving their pharmacokinetic and biodistribution features, and improving their targeting efficacy are crucial steps in the development of new therapeutic strategies (Sannaa et al. 2012).

The effectiveness of natural products depends on their transportation to a specific site to deliver them in a controlled manner, increasing patient compliance, and avoid repeated administration. New systems should deliver the active compound in a therapeutic concentration during the entire treatment period and direct it towards the desired targets (Ansari et al. 2012; Bonifácio et al. 2014; Goyal et al. 2011). Here, nanotechnology is a rising approach for drug delivery, presenting smart nanodrug delivery systems that can selectively deliver appropriate levels of a therapeutic agent, such as natural products, in a specific region. Therefore, nanotechnology-based solutions can be a breakthrough in solving natural product efficacy problems (Rebelo et al. 2017).

This review aims to summarize recent progress in several key areas relevant to natural products in nanoparticle delivery systems for medical applications.

4.2 Nanopharmaceuticals

Nanopharmaceuticals are increasingly becoming attractive to researchers due to its numerous benefits in the treatment of several diseases, particularly in oncological pathologies (Pinto Reis et al. 2013). Those systems strongly increase drug

bioavailability due to the high relative surface area of nanoparticles (Van Eerdenbrugh et al. 2008). In the nanoengineering of nanopharmaceuticals, there are main principles that should be respected, such as the nanomaterial used must be indispensable to the therapeutic activity, or it should add supplementary and unique properties to the active substance (Rivera Gil et al. 2010). Particle size is also a keystone; a study demonstrated that the liposomes around 150–200 nm diameter remain longer in the bloodstream, comparing with liposomes which diameter is larger than 300 nm or smaller than 70 nm, once they accumulate in the spleen and liver, respectively (Litzinger et al. 1994).

On the other hand, systems based on nanotechnology, such as liposomes, solid lipid nanoparticles, polymeric nanoparticles, metallic nanoparticles, nanocrystals, and nanoemulsions, among others, allow that compounds with different properties can be used in the same formulation. Systems based on nanotechnology can combine active substances with different degrees of hydrophilicity or lipophilicity and even manage to change these same properties, such as the behavior of these substances in a biological environment, especially targeting a tissue or organ, allowing a specific site effect (Chen et al. 2009; Gasco et al. 1989; Pestana et al. 2008; Sintov and Shapiro 2004). These new drug delivery systems have the ability to increase the effectiveness of active substances and, in addition, the ability to increase selectivity, reducing the side effects and controlling the release of the active substances (Chorilli et al. 2007; Mainardes et al. 2006).

4.2.1 Bioavailability

Nanoparticles can improve the effectiveness of natural products in the treatment and prevention of diseases through increasing their bioavailability (Watkins et al. 2015; Yu and Huang 2010). This parameter is closely related to low water solubility of drugs which can be classified through the Biopharmaceutics Classification System in class II and class IV or limited permeability like classes III and IV drugs (Liu and Feng 2015). For example, tannins and terpenoids are highly hydrophilic and have low bioavailability because of their inability to cross biological membranes. Incorporating them into nanoparticles can improve the bioavailability and lower the dose needed to obtain the therapeutic effect (Watkins et al. 2015). In the case of Biopharmaceutics Classification System class II drugs (high permeability and low solubility), nanoparticles can enhance transport through biological membranes due to increased solubility and improved permeation via transcellular and paracellular routes, as well as carrier-mediated route, when compared with the free drug (Liu and Feng 2015).

A large number of known natural compounds, such as curcumin, resveratrol, or epigallocatechin-3-gallate, are highly lipophilic, and low water solubility induces low bioavailability. In addition, higher doses must be administered in order to achieve the desired therapeutic effects. However, higher doses can induce toxicity and low patient compliance. Encapsulating these products, their water solubility

and efficiency can be improved, as well as their bioavailability (Kumar and Gupta 2015; Watkins et al. 2015).

4.2.2 Targeting

The main obstacle of the current treatments is the inefficient drug delivery to target cells, which can lead to nonspecific interactions that can cause many adverse side effects in patients. Therefore, this gap must be filled, for example, with the use of nanoparticles, since one of the major advantages of those systems is, indeed, the target delivery (Havel et al. 2016; Kuen et al. 2017). This advantage can be used in drug delivery of natural products to target specific tissues or organs (Kumar and Gupta 2015; Namdari et al. 2017; Watkins et al. 2015).

In this way, we can define two types of targeting approaches: active and passive; the active targeting involves a ligand attachment to the nanoparticle's surface. Many different molecules can be attached on the surface to specifically target different cells. As example, the targets are usually overexpressed receptors on tumor or endothelial cells. Thus, nanoparticle system with the targeting agent may deliver the drug specifically into the tumor to further decrease drug systemic toxicity. Some studies also show that active targeting can enhance nanoparticle internalization into the tumor (Wang et al. 2016; Watkins et al. 2015). In passive targeting, nanoparticles reach the targeted area without specific chemical interactions, depending upon physical transport due to size, shape, and surface charge. Using tumor cells as an example again, it is due to its fast and uncontrolled growing nature that there is a different lymphatic drainage system and the space or fenestration between the endothelial cells that line the blood vessel wall of the tumor vasculature is much larger than usual. This phenomenon, called the enhanced permeability and retention effect of the tumor vasculature, is the basis for passive targeting. Nanoparticles of approximately 20–150 nm can cross the blood vessel walls and preferentially accumulate in the interstitial space of the tumor. On the other hand, chemotherapeutic drugs are usually small molecules with less than 10 nm and can cross the blood vessels of both tumor and normal tissues, leading to systemic toxicity. Many nanoparticle systems have been developed to encapsulate anticancer drugs with demonstrated capability of reducing the systemic toxicity of chemotherapy, including both organic nanoparticle systems (e.g., liposome and polymeric nanoparticles) and inorganic nanoparticle systems (e.g., silica and gold nanoparticles) (Kumar and Gupta 2015; Nakamura et al. 2015; Wang et al. 2016; Watkins et al. 2015).

Target delivery of natural compounds using nanoparticles with small molecules is still in development. Thus, more research is required to improve those methods in order to take advantages of what has already been found about this type of product potential (Havel et al. 2016; Watkins et al. 2015).

4.2.3 Sustained Release

Another benefit of using nanoparticles to deliver natural products is that the drug release can be controlled. The drug amount and rate at which it is released from a nanoparticle depends on many factors such as size, amount of the active compound used, and the microenvironment (Havel et al. 2016; Kumar and Gupta 2015). The type and nature of the nanoparticle used is the major factor to consider. In case of polymeric nanoparticles, for example, the polymers used to produce the nanoparticles can also be adjusted to optimize the drug release. The polymer must be as more biocompatible as possible, so that the nanoparticles can stay longer in the body without being excreted or early detected by the immune system. One strategy that can be applied is the use of polyethylene glycol (PEG) as second coating, improving the nanoparticles' circulation time (Watkins et al. 2015).

4.2.4 Types of Nanocarriers

Liposomes

Liposomes can be described as spherical vesicles composed of amphoteric phospholipids and cholesterol, and these are combined in bilayers to encapsulate an aqueous medium. This bilayer of phospholipids has the shape of a sphere, protecting their hydrophobic groups from the aqueous phase and allowing their hydrophilic heads to contact with the aqueous environment (Fig. 4.2). Liposomes are used as drug delivery systems, and the drugs can be encapsulated in liposomes in different locations in the phospholipid bilayer, depending on the lipophilicity of the drug in the aqueous core (hydrophilic drugs) or at the bilayer interface (lipophilic drugs) (Bawarski et al. 2008; Islan et al. 2017; Jurj et al. 2017; Mota et al. 2017; Watkins et al. 2015; Weissig et al. 2014). Liposomes can be classified in different categories,

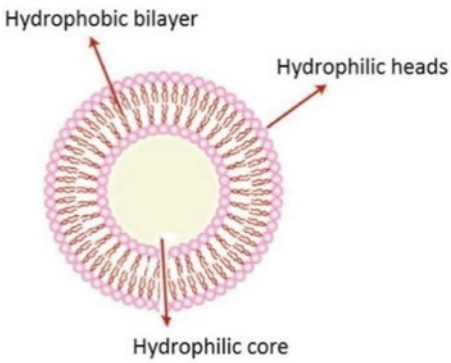

Fig. 4.2 Liposome with a spherical shape, composed of amphoteric phospholipids combined in a bilayer, protecting their hydrophobic groups from the aqueous phase, allowing a hydrophilic core. (Modified after (Honda et al. 2013))

depending on the number of bilayers, surface charge (neutral, cationic, or anionic), size, and preparation method. The size can be greatly diversified, 25 and 1000 nm; however, the typical size ranges between 50 and 200 nm (Bawarski et al. 2008; Bonifácio et al. 2014; Mota et al. 2017; Weissig et al. 2014). Liposome technology was discovered in the decade of 1960, and there has been a progress since then in terms of modulating their composition, size, and charge of the vesicle. It is also possible to functionalize liposomes with molecules, like sialic acid, PEG, or glycolipids, promoting targeting to particular cells or receptors (Gregoriadis 2016; Islan et al. 2017; Watkins et al. 2015).

The method of producing liposomes is generally very simple, since they spontaneously form after hydration of the dry phospholipids. However, this process of phospholipid aggregation into bilayer membranes may take a long period of time and the size distribution can be very diverse (Mota et al. 2017; Weissig et al. 2014). The preparation methods include thin film evaporation, freezing-thawing, diethyl ether injection, ethanol injection, and reverse phase evaporation. All methods have specific preparations, but all include solubilization of the lipids in an organic solvent, drying of the lipids, hydration of the lipids, and purification of the resultant liposomes (Mota et al. 2017).

One potential disadvantage of liposome as a carrier is that after an intravenous injection, it is rapidly intercepted by the fixed macrophages of the liver and spleen. However, the basis of the mechanism of action of several of the licensed liposome-based products is precisely the involvement of the reticular endothelial system in vesicle uptake, extending liposomes' potential uses to cancer and antimicrobial therapy (Gregoriadis 2016). Liposomes were the first colloidal drug carriers used in gene therapy, and these have already been used for drug-targeted delivery of natural or synthetic chemotherapeutics (Jurj et al. 2017).

These types of nanocarriers have several advantages, namely, their biodegradability, the variety of drugs or active substances that can be encapsulated, their biocompatibility, their low toxicity, the passive targeting to the cells of the immune system, the sustained release system, and the ease of surface manipulation. Due to all of these advantages, liposomes have been approved for multiple clinical trials (Bawarski et al. 2008; Jurj et al. 2017; Mota et al. 2017; Wu et al. 2017). However, they have some disadvantages associated with poor storage stability and *in vivo* stability, mainly due to oxidation of phospholipids. Additionally, liposomes have shown short release time and, in some cases, low encapsulation efficiency when compared with polymeric carriers (Bawarski et al. 2008; Jurj et al. 2017; Mota et al. 2017; Pelaz et al. 2017; Weissig et al. 2014).

Yang *et al.* prepared ginsenoside compound K (GCK)-loaded liposomes modified with tocopheryl polyethylene glycol succinate (GCKT-liposomes) to enhance solubility and targeting capability. The *in vitro* release studies had demonstrated that the dissolution of GCK was highly improved through its encapsulation into liposomes. In addition, GCKT-liposomes exhibited a great hypersensitizing effect on A549 cells, and the cellular uptake was enhanced. Compared with free GCK, the half maximal inhibitory concentration (IC_{50}) of GCKT-liposomes was significantly reduced. *In vivo* antitumor assay also indicated that GCKT-liposomes achieved

higher antitumor efficacy. These GCKT-liposomes significantly improved the antitumor efficacy of GCK (Yang et al. 2016).

Another example is related to the pomaces from red grapes that are used as a source of phenolic antioxidants. This extract was encapsulated in polymer-associated liposomes, and the results showed that the encapsulation prevented its degradation in the gastric environment and played a key role in improving its performance. The polymer-associated liposomes were biocompatible and protected Caco-2 cells against oxidative stress. The achieved results suggest a potential application of the polymer-associated liposomes loaded with the grape pomace extract in the nutraceutical field (Manconi et al. 2016).

Solid Lipid Nanoparticles

Solid lipid nanoparticles are colloidal carrier systems, in size range of 50–1000 nm, prepared from a lipid matrix that is solid at room temperature, stabilized through suitable emulsifiers, and combine the advantages of other colloidal systems for drug delivery, such as emulsions, liposomes, and polymeric nanoparticles (Bonifácio et al. 2014; Islan et al. 2017; Sutaria et al. 2012). Thus, these nanoparticles are a good choice for hydrophobic drugs (Watkins et al. 2015).

Depending on the composition of these particles and on their production conditions, the drug can either be homogeneously dispersed in lipid matrix of solid lipid nanoparticles, incorporated into the shell surrounding the lipid core, namely, drug-enriched shell model (Fig. 4.3), or incorporated into the core surrounded by a lipid shell, namely, drug-enriched core model (Din et al. 2017).

Once the matrix of the lipid particle is solid, it can protect drug molecules against chemical degradation. However, when the system is produced, crystallization occurs, resulting in low encapsulation efficiency and drug release (Bonifácio et al. 2014).

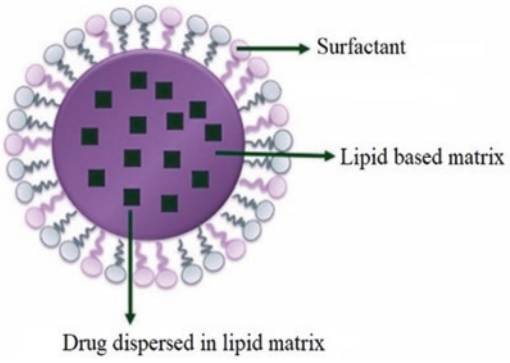

Fig. 4.3 Drug-enriched shell model – the drug is dispersed homogeneously in lipid matrix, incorporated into the shell surrounding the lipid core. (Modified after (Din et al. 2017))

On the other hand, Islan *et al.* showed that *in vitro* tolerability of solid lipid nanoparticles appears to be much higher than polymeric nanoparticles (Islan et al. 2017). There are substantial evidences that solid lipid nanoparticles can carry most of the drugs through the lymphatic system and in part through the general blood circulation, avoiding first-pass metabolism, which allows the administration of lower doses with less chances of toxic side effects (Grandhi et al. 2014; Sutaria et al. 2012).

Flavonoid extract from *Dracocephalum moldavica* L. (*Lamiaceae*) was encapsulated in solid lipid nanoparticles, and the drug release result *in vitro* exhibited that these nanoparticles had a 60% quicker release in the first 2 hours, which ensured a higher drug concentration for a long time. The amount of released drug was significantly higher than the free flavonoid extract and reached 95% after 48 h, which suggested an improvement of *in vitro* drug release. Compared to the flavonoid extract alone, solid lipid nanoparticles had a much better myocardial protective effect, which suggested that they can be used as safe and effective nanocarriers for the oral delivery of *Dracocephalum moldavica*'s flavonoid extract (Tan et al. 2017).

Marslin *et al.* studied the cytotoxicity of free albendazole and albendazole-loaded solid lipid nanoparticles in human glioma astrocytoma cell line, U-87 MG. *In vitro* cell line studies have shown that albendazole in the form of solid lipid nanoparticles was more cytotoxic (IC_{50} = 4.90 μg/mL) to U-87 MG cells compared to the free form (IC_{50} = 13.30 μg/mL) due to the efficient uptake of the solid lipid nanoparticles by these cells (Marslin et al. 2017).

Polymeric Nanoparticles

Polymeric nanoparticles are solid colloidal systems, usually with a size ranging between 100 and 500 nm, in which the therapeutic agent is dissolved, entrapped, encapsulated, or adsorbed onto the constituent polymer matrix (Lu et al. 2011; Prabhu et al. 2015). As Fig. 4.4 illustrates, polymeric nanoparticle platforms include not only solid polymeric nanoparticles but also polymeric micelles, dendrimers, polymer conjugates, polymersomes, polyplexes, and polymer hybrid systems, and they have unique physicochemical structures (Prabhu et al. 2015). The structure of these nanocarriers generally varies from nanospheres to nanocapsules, according to the methodology used for its formation. Nanospheres are matrix systems in which the drug is dispersed throughout the particles, while nanocapsules are vesicular systems, acting as a reservoir, where the entrapped substances are confined to a cavity composed of a liquid core, either oil or water, surrounded by a single polymeric membrane (Lu et al. 2011; Pinto Reis et al. 2006; Prabhu et al. 2015; Rao and Geckeler 2011).

The choosing of the appropriate synthesis method for preparation of these nanoparticles is based on a number of factors, such as application, size, material option, specificity, and morphology (Banik et al. 2016). Polymeric nanoparticles can be conveniently prepared from either dispersion of preformed polymers or classical polymerization, using direct polymerization of monomers (Banik et al. 2016;

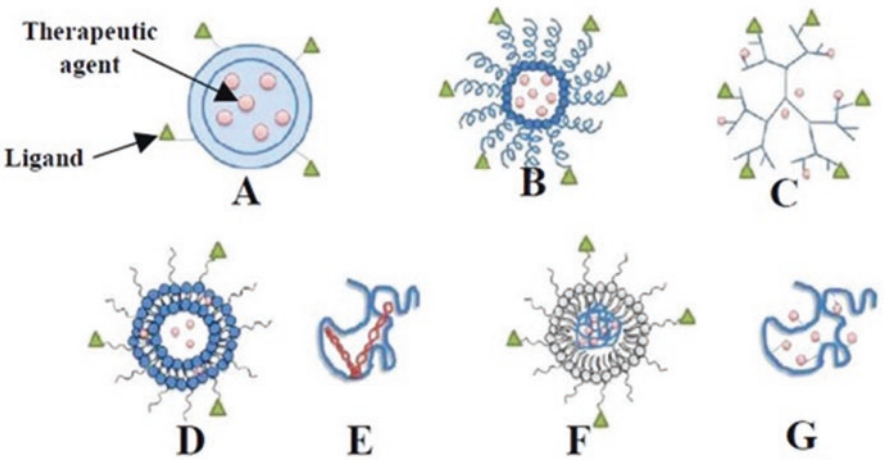

Fig. 4.4 Types of polymeric nanoparticle platforms: solid polymeric nanoparticles (**a**), polymeric micelles (**b**), dendrimers (**c**), polymer conjugates (**d**), polymersomes (**e**), polyplexes (**f**), and polymer hybrid systems (**g**). (Modified after (Prabhu et al. 2015))

Lu et al. 2011; Pinto Reis et al. 2006; Rao and Geckeler 2011). Another preparation procedure to be considered is Particle Replication in Nonwetting Templates (PRINT®), which has shown to have higher control over size and shape (Banik et al. 2016; Rolland et al. 2005).

Regarding the dispersion of preformed polymers, several methods have been proposed, namely, solvent evaporation method, in which the polymer is dissolved in an organic solvent and the drug dissolved in this solution. Then, they are emulsified using an emulsifier agent and under continuous stirring, and, eventually, an increase of temperature is needed to promote solvent evaporation. Emulsification-solvent diffusion method is a type of method in which the polymer is first dissolved in a water-soluble solvent saturated with water and then the solvent of the dispersed phase is diluted with an excess of water or with another organic solvent, leading lately to a solvent diffusion to the external phase and the formation of nanospheres or nanocapsules. The solvent displacement method, or nanoprecipitation, consists of an interfacial deposition of the polymer through an addition of an organic solvent or salts. A rapid diffusion of the solvent into a non-solvent phase results in a decrease of the interfacial tension between the two phases that can increase the surface area and lead to the formation of nanoparticles. The salting-out method differs from the emulsion process since it avoids emulsifiers and chlorinated solvents: a polymer solution normally totally miscible with water is used, and an emulsification is achieved, dissolving high concentration of salt or sucrose. In case of dialysis, the polymer is dissolved in an organic solvent and placed inside a dialysis tube with proper molecular weight cutoff. Another method to produce polymeric nanoparticles is based on supercritical fluid technology, avoiding the use of organic solvents. This last method produces polymeric nanoparticles with high purity (Lu et al. 2011; Rao and Geckeler 2011).

On the other hand, the polymerization of monomers allows the achievement of desired properties for a particular application, designing suitable polymeric nanoparticles. In the monomer polymerization method, a mixture of water with a monomer of low water solubility, a water-soluble initiator, and an emulsifier are stirred, at a certain temperature, for an extended period to proceed with the polymerization. Then, at the end of the reaction, polymeric nanoparticles are formed, with a particle size ranging between 10 nm and 150 nm and generally with some emulsifier agent trapped in the polymeric particles. The emulsifier agent removal is a hard and time-consuming process that increases the cost of production. Another strategy is interfacial polymerization that involves the polymerization of two reactive monomers or agents. The reaction takes place at the interface of the two liquids, with the elimination of the purification step. Usually, a high drug encapsulation efficiency is achieved (Lu et al. 2011; Rao and Geckeler 2011).

PRINT® is a top-down approach for preparation of nanomaterials that allows control over size, shape, composition, surface structure, and charge of synthesized particles (Rolland et al. 2005). Rolland *et al.*, in 2005, have developed the PRINT® process that improves the soft lithographic techniques, creating isolated particles instead of embossed films. These researchers and others demonstrated the utility of this method, producing particles of poly(ethylene glycol) diacrylate, triacrylate resin, poly(lactic acid), and poly(pyrrole) in different shapes and sizes with a range of drug agents, such as oligonucleotides and proteins.

The advantages of polymeric nanoparticles as drug delivery systems include their high drug encapsulation efficiency, higher intracellular uptake than other particulate drug delivery systems, and biocompatibility with tissue and cells. They are able to protect drugs from their rapid metabolism during systemic circulation and clearance by the liver, kidney, and reticuloendothelial system, which lead to improvements of the drug stability and its specificity. Even more, polymeric nanoparticles can be designed to effectively deliver the drug to a target site and, thus, increase therapeutic results, minimizing the adverse side effects (Lu et al. 2011; Prabhu et al. 2015).

Silva *et al.* have incorporated topical glucocorticosteroids into nanoparticles formulated with poly-Ɛ-caprolactone as the polymeric core to overcome side effects of conventional formulations and to achieve maximum skin deposition. They have proved that nanoparticles increase drug permeation into lipid membranes *in vitro*. Preliminary safety and permeation studies conducted on rats showed corticosteroids in serum after 48 h application of a gel containing nanoparticles with no skin reactions observed (Silva et al. 2015).

Dendrimers

Dendrimers are repeatedly branched polymeric macromolecules that have numerous extensions from central core, resulting in a nearly perfect three-dimensional structure (Bawarski et al. 2008; Prabhu et al. 2015). These particles usually have 10–100 nm of diameter with multiple functional groups on their surface, making

them ideal carriers for targeted drug delivery but being also a good choice for imaging (Bawarski et al. 2008; Prabhu et al. 2015). Dendrimers can be synthesized via divergent or convergent synthesis through a series of controlled polymerization reactions (Bawarski et al. 2008; Prabhu et al. 2015). In the different methods, dendrimer grows outward from a multifunctional core molecule. The core molecule reacts with monomer molecules, giving the first-generation dendrimer. Then, the new periphery of the molecule is activated for reactions with more monomers (Abbasi et al. 2014). With more than five generations, dendrimers are similar to spheres with countless cavities within their branches to contain therapeutic and diagnostic agents (Bawarski et al. 2008).

Khandare *et al.* prepared a poly(amidoamine) dendrimer-succinic acid-paclitaxel conjugate that showed a cytotoxicity ten times higher than the free unconjugated drug in A2780 human ovarian carcinoma cells. The conjugate was prepared through the condensation method, with paclitaxel covalently conjugated (Khandare et al. 2006). Furthermore, Majoros and his colleagues engineered a multifunctional poly(amidoamine) dendrimer that could also conjugate functional molecules like fluorescein isothiocyanate and folic acid, since the targets overexpressed folate receptors on specific cancer cells, acting both as targeted chemotherapeutic and imaging agents to cancer cells *in vitro* (Majoros et al. 2006). However, dendrimers may be toxic because of their ability to disrupt cell membranes, mainly due to the positive charge on their surface (Bawarski et al. 2008).

Polymeric Micelles

Polymeric micelles are spherical colloidal particles with a hydrophobic core and a hydrophilic shell in aqueous media that are regularly soluble in water and have an individual size lower than 50 nm. The copolymer hydrophobic fraction allows the encapsulation or the covalent linking of the drug or contrast agent, whereas the hydrophilic portion provides stealth property to the micellar system. This property prevents its uptake by reticuloendothelial system, and thereby, it enhances its circulation time in bloodstream and facilitates *in vivo* imaging (Banik et al. 2016; Bawarski et al. 2008; Prabhu et al. 2015).

For the formation of micelles, amphiphilic molecules must have both hydrophobic and hydrophilic segments. Hence, in the aqueous media, the core of the micelles can solubilize water-poor or non-soluble drugs, while the surface can adsorb polar molecules. Drugs with intermediate polarity can be distributed in intermediate positions. Therefore, polymeric micelles provide an alternative for parenteral administration of poorly water-soluble drugs (Bawarski et al. 2008). These nanoparticles can be modified using ligand molecules for targeted delivery to specific cells, for example, a pH-sensitive drug-binding linkers. Moreover, multifunctional polymeric micelles can be designed to facilitate simultaneous drug delivery and imaging, plus the possibility to encapsulate two or more drugs in one step (Banik et al. 2016; Bawarski et al. 2008). Polymeric micelles have been used for drug delivery applications due to their unique properties, namely, the increase of the solubility and

stability of anticancer agents, flexibility to choose from several hydrophobic regions, and nanoscale size (Banik et al. 2016).

Genexol® is an example of an anticancer drug delivery system using these nanoparticles to the treatment of metastatic breast and pancreatic cancer, and will be better discussed later on section Natural Products Encapsulated in Nanopharmaceuticals. Song and his collaborators, in 2011, reported the use of micelles of amphiphilic methoxy poly(ethylene glycol)-b-poly(Ɛ-caprolactone-co-p-dioxanone) loaded with curcumin. These micelles were prepared through a solid dispersion method and presented higher water solubility and faster hydrolytic degradation compared to mPEG–PCL micelles. They had a small size, around 30 nm, with a narrow size distribution, an entrapment efficiency higher than 95%, and a loading capacity of 12%. Moreover, the curcumin-loaded micelles were effective in inhibiting the growth of PC-3 human prostate cancer cells, appearing to be an attractive parenteral formulation for curcumin delivery (Song et al. 2011).

On the other hand, polymeric micelles can also be used on diagnosis as "smart imaging" approaches. Huang *et al.* developed a smart pH-activated 19F-probe consisting in micelles composed of fluorinated polymers with tertiary amines at different pKa values, which allowed the detection of specific and narrow pH transitions in biological systems. The protonation of such amines at pH lower than their pKa results in micelle disassembly and 19F-MRI/nuclear magnetic resonance spectroscopy signal activation (Huang et al. 2013; Pelaz et al. 2017).

Metallic Nanoparticles

Metallic nanoparticles include metal and metalloid elements, and they are a cluster of metal atoms that play an important role due to their unique optoelectronic and physicochemical properties, which depend strongly on their size, shape, crystallinity, and structure (Fig. 4.5) (Edmundson et al. 2014; Islan et al. 2017). Different physical and chemical methods have been used for the synthesis of metallic nanoparticles. Physical synthesis has a low production rate and a high consumption of

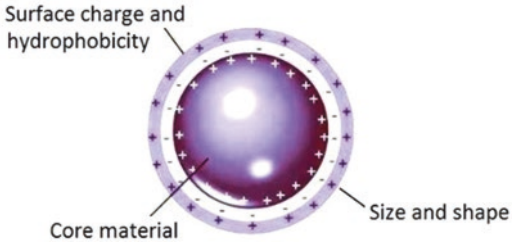

Fig. 4.5 Metal nanoparticle – metal and metalloid elements in a cluster of metal atoms with their optoelectronic and physicochemical properties (strongly dependent on size, shape, crystallinity, and structure). (Modified after (Marques Neto et al. 2017))

energy to maintain the high pressure and temperature, and, ultimately, it is very expensive. It includes methods such as attrition, where macro- or microscale particles are ground by a size-reducing mechanism, and pyrolysis method, which requires an organic precursor, either a liquid or a gas, that is forced through an orifice at high pressure and burned (Thakkar et al. 2010). On the other hand, chemical methods, like wet-chemical procedures, are low cost for high volume; however, they are associated with some contamination from chemical precursors and use of toxic solvents. A typical procedure involves producing nanoparticles in a liquid medium containing several reactants, in particular reducing agents, and a stabilizing agent to prevent the agglomeration of metallic nanoparticles (Thakkar et al. 2010).

The use of biological organisms in the synthesis and assembly of nanoparticles has received increasing attention due to the fact that they are clean, cost-effective, and efficient synthesis techniques, being some available biological resources like plants and plant products, algae, fungi, yeast, bacteria, and virus (Islan et al. 2017; Thakkar et al. 2010). Both unicellular and multicellular organisms have been known to produce intracellular or extracellular inorganic materials, and they are able to lower the toxicity of metal ions reducing them to elemental or less toxic or soluble forms (Edmundson et al. 2014; Thakkar et al. 2010). A eukaryotic nanoparticle producer is, for example, the fungus *Phoma* that is used to produce silver nanoparticles as antibacterial agents or *Magnetospirillum gryphiswaldense* that is used to produce magnetic nanoparticles. However, prokaryotes are the logical choice for developing nanoparticles due to their faster growth rates and easy manipulation (Edmundson et al. 2014).

Through the years, nanoparticles like magnetic, gold, and silver nanoparticles, nanoshells, and nanocages have been continuously used and modified to enable their use as diagnostic and therapeutic agents (Islan et al. 2017; Mody et al. 2010). Due to their ultra-small size, magnetic properties, and biocompatibility, superparamagnetic iron oxide nanoparticles have arisen as promising candidates for several biomedical applications, such as enhanced resolution contrast agents for MRI, targeted drug delivery and imaging, gene therapy, stem cell tracking, magnetic separation technologies (e.g., rapid DNA sequencing), and early detection of inflammatory diseases, cancer, diabetes, and atherosclerosis (Mody et al. 2010; Pelaz et al. 2017).

Gold nanoparticles due to their high atomic number and electron density have higher attenuation coefficients than the typical X-ray contrast agent. They can be used as contrast agents for X-ray imaging, computed tomography, and microcomputed tomography, but also in the immune gold labeling of samples to be observed using transmission electron microscopy (Edmundson et al. 2014; Pelaz et al. 2017). Moreover, gold nanoparticles are being used as drug delivery systems, and some nanoparticles have intrinsic healing properties (Edmundson et al. 2014; Pelaz et al. 2017). Mukherjee *et al.* reported in 2005, for the first time, anti-angiogenic properties of gold nanoparticles, making them a promising approach for tumor therapy, since intensive angiogenesis, i.e., the formation process of new blood vessels in organs or tissues, is considered one of the main tumor growth factors. These researchers showed through *in vitro* and *in vivo* tests that gold nanoparticles bind to heparin-binding growth factors, like VEGF165 and bFGF, inhibiting their activity.

It does not inhibit the activity of non-heparin-binding growth factors, like VEGF121 and endothelial growth factor (Dykman and Khlebtsov 2011; Mukherjee et al. 2005). Silva *et al.* have prepared gold nanoparticles encapsulating an aqueous plant extract, with gold as the reducing and capping agent, maximized in the near-infrared absorption (650–900 nm). Resultant nanoparticles were easily activated through controlled temperature with an ultrasonic water bath and application of a pulsed laser. Thus, the authors believe that those particles can be used in the future with adequate controlled optical properties for laser phototherapy of tumors and targeted drug delivery (Silva et al. 2016b). Wilson *et al.* have studied a dual-modality interventional magnetic resonance imaging/conventional angiography system for catheter-directed intra-arterial delivery of magnetic nanoparticles to target doxorubicin, a natural compound used as a chemotherapy agent, in the treatment of inoperable hepatocellular carcinoma. Their results have shown to be a promising method for targeting tumor therapies magnetically (Wilson et al. 2004).

Silver nanoparticles are being studied as new antimicrobial agents because of their significant activity against many types of pathogens, including multidrug-resistant organisms. Some anti-inflammatory properties have been attributed to these nanoparticles (Edmundson et al. 2014; Islan et al. 2017). The exact mechanism through which silver nanoparticles induce antimicrobial effect is not yet clearly known; however, there are some theories that try to explain it. One is due to their ability to anchor to the bacteria cell wall and thereafter penetrate it, causing structural changes, like enhancing permeability leading to cell death; another theory is the formation of free radicals which may cause damage in the cell membrane leading, ultimately, to cell death; the release of silver ions can also be an explanation, since these ions can interact with the thiol groups of many vital enzymes and inactivate them (Prabhu and Poulose 2012).

Nanocrystals

Nanocrystals are carrier-free colloidal delivery systems composed of 100% water-insoluble drug (Islan et al. 2017; Junghanns and Müller 2008; Weissig et al. 2014). Nanocrystals are characterized by a unique phenomenon, an increased dissolution pressure that can be associated with their nanometer size and that can be translated into the improvement of clinical efficacy (Weissig et al. 2014). Many different methods can be applied on the production of nanocrystals, such as disintegration processes, that include milling and high-pressure homogenization, precipitation, and combined methods (Gao et al. 2015; Islan et al. 2017; Junghanns and Müller 2008; Khan et al. 2013b). Depending on the chosen methodology, the process of drug microcrystals to drug nanoparticles can lead either to a crystalline or to an amorphous product, especially when precipitation is applied (Junghanns and Müller 2008).

On the milling process, the milling media, the dispersion medium, the stabilizer, and the drug are generally charged into the milling chamber, where shear impact forces contribute to particle size reduction; however, the erosion from the milling

material during the process is usually a disadvantage in this technology (Gao et al. 2015; Junghanns and Müller 2008). This method can work either with the stirring of the milling medium, or the complete container can move in a complex movement, leading consequently to the movement of the milling media (Junghanns and Müller 2008).

Homogenization methods consist of three important technologies, namely, Microfluidizer technology, which generates small particles due to a frontal collision of two fluid streams under pressures up to 1700 bars; piston gap homogenization in water and in water mixtures, where the drug powder is dispersed in an aqueous emulsifier solution and, subsequently, forced by a piston through the tiny homogenization gap; and nonaqueous media, which uses a dispersion media with low vapor pressure and optionally homogenization at low temperatures. In piston gap homogenization, particles become smaller due to the high shear forces, the turbulent flow, and the enormous power of shockwaves (Junghanns and Müller 2008).

Precipitation methods can reduce the mechanical energy input associated with milling and homogenization methodologies. In a classical precipitation process known as "via humida paratum", the drug is dissolved in a solvent, and then a nonsolvent is added, leading to the precipitation of finely dispersed drug nanocrystals. The major limitations of this method are considered to be uncontrolled particle growth, requiring stabilization in order not to grow to the micrometer scale and the need of a soluble drug in at least one solvent, which can be a problem for newly developed drugs that are insoluble in both aqueous and organic media (Junghanns and Müller 2008; Khan et al. 2013b).

One advantage from nanocrystals is their capability to provide smaller dose administration and, consequently, reduce the adverse side effects (Islan et al. 2017). Furthermore, those particles can be administrated through different routes, such as oral, parenteral, ocular, pulmonary, and dermal delivery. An effort to overcome the regulatory obstacles and to create high-quality standards must be applied (Islan et al. 2017; Müller and Junghanns 2006). The first nanocrystal-based product was approved by the FDA in 2000 under the name Rapamune®. Rapamune® from Wyeth Pharmaceuticals has in its composition sirolimus, a natural macrocyclic lactone produced by *Streptomyces hygroscopicus*, which acts as an immunosuppressive drug. Oral suspensions and nanocrystal tablets produced through pearl mill technology are the two existing formulations where tablets have shown an increased bioavailability (21%) compared to the oral solution (Junghanns and Müller 2008; Müller and Junghanns 2006).

Sahoo *et al.* studied the possibility to enhance the dissolution rate of the poorly water-soluble drug, quercetin, through nanocrystals. Using high-pressure homogenization method, they have obtained a size of about 483 nm after 20 cycles of homogenization at 1500 bar. These researchers found that the dissolution of the drug in nanocrystals was much higher when compared to the non-processed drug, as well as the antioxidant activity (Sahoo et al. 2011).

Nanoemulsions

Nanoemulsions are colloidal particulate systems, with sizes around 100 nm. They usually are amorphous and lipophilic solid spheres with a negative charge on their surface (Gupta et al. 2016; Jaiswal et al. 2015; Singh et al. 2017). A typical nanoemulsion system contains oil, water, and an emulsifier and can form an oil-in-water nanoemulsion, a water-in-oil nanoemulsion, or a bi-continuous nanoemulsion. This last one is a thermodynamically unstable system that can be stabilized through the addition of an emulsifier (Gupta et al. 2016; Jaiswal et al. 2015). Due to their ability to dissolve large amounts of hydrophobic compounds along with their mutual compatibility and ability to protect the drugs from hydrolysis and enzymatic degradation, nanoemulsions may act as ideal carriers for parenteral delivery (Islan et al. 2017). Moreover, as they enhance therapeutic efficacy of drug and minimize adverse side effects and toxic reactions, those particles are used in the treatment of infections, enzyme replacement therapy in the liver, treatment of cancer, and vaccination (Jaiswal et al. 2015).

Methodologies for nanoemulsion production can be divided into high-energy or low-energy emulsification or a combination of both (Gupta et al. 2016; Jaiswal et al. 2015; Koroleva and Yurtov 2012; Singh et al. 2017). High-energy emulsification methods require mechanical devices that allow the creation of powerful disruptive forces for size reduction and include high-energy stirring, ultrasonic emulsification, high-pressure homogenization, microfluidization, and membrane emulsification method (Jaiswal et al. 2015; Singh et al. 2017). Disadvantages associated with these methods are the high costs and generation of high operational temperatures, which are not applied to thermolabile drugs (Singh et al. 2017). On the other hand, in low-energy methods, droplets are formed when the system shows a phase inversion in response to changes in composition or temperature and goes through a state of low interfacial tension, taking advantages of the energy stored in the system to produce ultrafine droplets (Gupta et al. 2016; Singh et al. 2017). Phase inversion temperature, emulsion inversion point, and spontaneous emulsification method are the most common methods (Gupta et al. 2016; Jaiswal et al. 2015). A combined method that comprises the high-energy and low-energy emulsification is also possible in the preparation of a reverse nanoemulsion in a highly viscous system (Jaiswal et al. 2015; Koroleva and Yurtov 2012).

Camptothecin is an effective anticancer agent against a broad range of cancers. It is obtained from *Camptotheca acuminata* D. (*Cornaceae*), and its therapeutic use is hindered due to poor aqueous solubility and high lipophilicity. Thus, Natesan *et al.* formulated a camptothecin nanoemulsion stabilized by chitosan (CHI-CPT-NEs) to improve the cancer targeting efficiency of camptothecin. The new nanoemulsion showed uniform droplet size distribution, extended drug release (61.7% in 24 h), tolerable hemolytic potential, high cytotoxicity (178 ± 4.3 ng/mL) against MCF-7 cancer cells, and low DNA damage to lymphocytes. Moreover, it was seen an increase in targeting breast cancer by CHI-CPT-NEs when compared to the non-stabilized nanoemulsion in *in vivo* studies in four T1 breast tumor xenograft BALB/c mice (Natesan et al. 2017).

Phytosomes

Phytosome is also known as phyto-phospholipidic nanoparticles, planterosomes, and herbosomes (Khan et al. 2013a; Matias et al. 2016; Semalty et al. 2010). The name derived from the conjugation of the Greek words *phyto*, which means "from plant," and "some," which means "cell-like." A phytosome is an amphiphilic substance where individual components of an herbal extract are bound to phospholipids (Semalty et al. 2010). These type of nanoparticles can be considered to have as major advantages its high solubility and absorption rate, leading to the decrease in the drug dosage needed and, subsequent, they become safer to human organism (Khan et al. 2013a).

Briefly, for its preparation, the chosen proportions of phospholipids and phytocomponents must be dissolved in a suitable medium and react at an optimized temperature for the adequate time. Then, the complex must be obtained as dry powder or converted into a phytosomal suspension (Matias et al. 2016). There are essentially three methods applied to the preparation of phytosomes, namely, solvent evaporation, anti-solvent precipitation, and supercritical fluid technology. In the solvent evaporation method, a proportion of a natural product and phospholipids are mixed in a reaction vessel containing a suitable solvent system, such as tetrahydrofuran or ethanol, and the reaction is allowed to be carried for 2–6 h at room temperature or with moderate heating to get the maximum possible yield and drug entrapment (Khan et al. 2013a; Matias et al. 2016). Then, the solvent is evaporated, under reduced pressure in case of volatile solvents or using freeze-drying or spray-drying for non-volatile solvents, leading to the production of the dry complexes (Matias et al. 2016). When non-volatile solvents are used, the addition of a carbohydrate may be necessary regarding their cryoprotectant properties on the complex during the freeze-drying process. This recovery method can be an advantage since it does not require an additional drying step and it is a lower-temperature procedure being very useful for natural products (Matias et al. 2016).

The anti-solvent precipitation technique is similar to the solvent evaporation method but incorporates a polar solvent as the anti-solvent to stop the reaction and precipitate the drug–phospholipid complex from the organic solvent (Khan et al. 2013a; Matias et al. 2016). The phyto-phospholipidic complex is recovered after precipitation and eventual centrifugation, followed by solvent removal (Matias et al. 2016). It is suggested from some authors that due to the very weak interactions during the complex formation and/or the ability of the anti-solvent to dissolve the phospholipids leaving the crystalline drug precipitated, lower efficacy can be obtained with this technique (Matias et al. 2016). On the other hand, supercritical fluid technology uses mild temperatures and requires minimal solvent quantity (Matias et al. 2016). Different supercritical fluid techniques have been applied to improve solubility profiles of poorly soluble drugs, including compressed anti-solvent process or supercritical anti-solvent method, rapid expansion of supercritical solutions, solution-enhanced dispersion due to supercritical fluids, and gas anti-solvent technique (Khan et al. 2013a; Matias et al. 2016). In supercritical fluids, the equipment includes two concentric tubes leading supercritical carbon dioxide (SC-CO_2) and the solution of phytocomponents and phospholipids to a small premixing chamber

and then to a nozzle. After the entrance in the collection vessel, evaporation of the CO_2 occurs, leading to the removal of most of the remaining solvent, originating the precipitation of a solid powder which contains the phytosomes. On the other hand, gas anti-solvent technique uses a similar equipment but dispensing the inlet for the solution containing natural drug and phospholipids, being those components added into the vessel instead (Matias et al. 2016).

Mitomycin C is an antiproliferative and anticancer drug extensively used in clinical chemotherapy for the treatment of a variety of cancers including stomach, breast, pancreas, colon, and bladder cancer. This drug is rapidly absorbed into systemic circulation, increasing toxicity risk in systemic administration. Hou and his coworkers produced mitomycin C − soybean phosphatidylcholine complex through a solvent evaporation method combined with a nanoprecipitation technique. The mitomycin C-loaded phytosomes not only are able to reduce the drug degradation while encapsulated, but also have exhibited remarkably high cytotoxicity as well as higher inhibition effect compared to free mitomycin C. However, it does not demonstrated high selectivity for the tumor site (Hou et al. 2013; Matias et al. 2016). Matias *et al.* have developed an antibacterial phytosome formulation for topical application containing a bioactive extract of *Plectranthus madagascariensis* B. (*Lamiaceae*). The phytosomes were further encapsulated into chitosan microparticles with an average size of 1 μm and positive surface charge and presented a sustained release at physiologic pH, maintaining the antibacterial activity of the extract, making them a promising alternative to current topic antibacterial treatments (Matias et al. 2015).

4.3 Natural Products Encapsulated in Nanopharmaceuticals

The use of nanotechnology has shown major success in the field of drug delivery and brings multiple advantages to the delivery of natural products (Padmavathi 2013). The incorporation of these natural products in nanoparticles can increase their bioavailability (Alexander et al. 2016; Bonifácio et al. 2014; Borel and Sabliov 2014; Kumar and Gupta 2015; Watkins et al. 2015). These incorporation can also increase solubility and stability, protecting healthy cells from toxicity, enhancing pharmacological activity, allowing sustained delivery with protection from physical and chemical degradation, and consequently minimizing adverse side effects (Alexander et al. 2016; Ansari et al. 2012; Bonifácio et al. 2014; Goyal et al. 2011; Namdari et al. 2017; Watkins et al. 2015).

4.3.1 Examples of Natural Products Encapsulated into Nanocarriers

Paclitaxel

Paclitaxel, also named Taxol, is a natural compound isolated from *Taxus brevifolia* B. (*Taxaceae*), a slow-growing evergreen shrub or small tree (Bernabeu et al. 2017). It has proved to have anticancer properties, motivating an intense research effort over the years. Nowadays, it represents a first-line treatment of many types of solid cancers, and it is among the first clinically and US FDA-approved chemotherapy natural drugs (Bilia et al. 2017). This product is usually administered intravenously, but due to its low water solubility and toxicity, new formulations were developed. Many studies reported several investigations of different nanoformulations, from which some are now available in the market. There are mainly polymeric nanoparticles, lipid formulations, polymer conjugates, inorganic nanoparticles, nanocrystals, and cyclodextrin-based nanoformulations. As an example, albumin nanoparticles have increased the bioavailability of paclitaxel and led to higher intratumor concentrations of the drug (Bilia et al. 2017; Sannaa et al. 2012).

Genexol® has been approved by Korean regulatory agency as first-line treatment for ovarian cancer in combination with other chemotherapeutics agents in 2011. It is also administrated for metastatic breast cancer and pancreatic cancer. Genexol® consists of 20–50 nm micelles composed of block copolymer PEG-poly (D, L-lactide) loaded with placlitaxel (Ragelle et al. 2017). Preclinical studies in animal models showed enhanced efficacy and reduced toxicity (Bernabeu et al. 2017; Ragelle et al. 2017; Weissig et al. 2014). Another commercialized nanopharmaceutical to deliver paclitaxel is Opaxio®, a macromolecular polymer-drug conjugate of paclitaxel with α-poly(L-glutamic acid), that was developed in order to improve the safety profile of taxol (Bernabeu et al. 2017). Approved by the FDA in 2012 for glioblastoma, this nanopharmaceutical contains paclitaxel covalently linked to solid nanoparticles composed of polyglutamate in where the drug is released inside the solid tumor via enzymatic hydrolysis of polyglutamate (Bernabeu et al. 2017; Weissig et al. 2014).

Doxorubicin

Doxorubicin is one of the most potent and commonly used chemotherapeutic agents for the treatment of several types of cancer (Cagel et al. 2017; Shafei et al. 2017). It was isolated from *Streptomyces peucetius* var. *caesius* through mutagenic treatment of *S. peucetius* (Cagel et al. 2017). From the clinical point of view, doxorubicin is considered as one of the most effective chemotherapeutic agents against many types of cancers (Cagel et al. 2017). The mechanism for doxorubicin anticancer effect was recently discussed (Shafei et al. 2017). In 1995, the Oncologic Drugs Advisory Committee recommended FDA approval of Doxil® (Fig. 4.6), and 1 year later, it

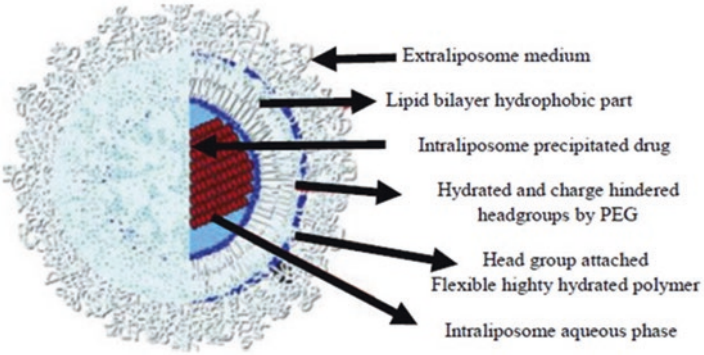

Fig. 4.6 Schematic representation of "stealth" liposome (Doxil®) (Barenholz 2012)

was first commercialized in the USA as Doxil® and in the European Union as Caelyx® (Cagel et al. 2017). It consists in a doxorubicin-loaded PEGylated liposomal bilayer with sizes ranging 80–90 nm, comprising hydrogenated soy phosphatidylcholine, cholesterol, and methyl-distearoyl phospho-ethanolamine PEG 2000 Q5 sodium salt (Cagel et al. 2017). Doxil® had shown higher clinical performance, compared to the free form of doxorubicin in a variety of neoplastic conditions, due to its unique pharmacokinetics and biodistribution, which reduces side effects when it comes to cardiac toxicity and improves overall patient compliance and quality of life (Barenholz 2012). Another example of doxorubicin nanoformulation is related to oleyl chitosan nanoparticles (Shafei et al. 2017). It was observed that stability increases with the increase of the hydrophobic chains and hydrophobic groups, which leads to higher drug protection (Barenholz 2012). These nanoparticles had demonstrated dual effect, inhibiting the promoter oncogene in breast cancer and increasing chemotherapeutic effectiveness. It was also demostrated that in pH of 3.8, doxorubicin was rapidly and completely released from the nanoparticles, whereas the pH 7.4 showed sustained release followed by burst release. Oleic acid and chitosan nanoparticles were also more efficient than free doxorubicin in terms of growth inhibitory rates and showed better inhibition of cancer cells, maintaining the pharmacological activity of doxorubicin (Tana et al. 2009).

Another nanoformulation with doxorubicin is the superparamagnetic iron oxide nanoparticles. These nanoparticles are guided by an external magnetic field to its target, and they are capable of targeting cancer cells while sparing healthy cells, which leads to a possible dose reduction (Shafei et al. 2017). Also, PEGylated superparamagnetic iron oxide nanoparticle complexes have also been synthesized, being firstly loaded with doxorubicin-Fe^{2+}.

Curcumin

Curcumin is the major bioactive compound of the rhizome of *Curcuma longa* L. (*Zingiberaceae*), an Indian herbal medicine. This compound has a high clinical interest mainly due to its properties that can be used in cancer treatment, inflammation, infection, angiogenesis, and amyloidosis, among others (Bilia et al. 2017; Mutoh et al. 2016; Watkins et al. 2015). Since curcumin is a lipophilic molecule, its characteristics limit its clinical use (Namdari et al. 2017). In the last decade, many strategies have been used to enhance curcumin efficiency, and recent formulations involve targeting strategies mediated by antibodies, peptides, and aptamers (Yu and Huang 2010).

Many studies led to new interesting findings, for example, curcumin-loaded lipid nanoparticles were shown to inhibit cellular proliferation, migration, and invasion along with a higher percentage of cell cycle inhibition (Bilia et al. 2017; Yu and Huang 2010). This fact led to a high apoptosis level when compared to free curcumin. Other study has demonstrated the increase of curcumin bioavailability using glyceryl monooleate nanoparticles, compared to free curcumin (Bilia et al. 2017). Also, Kakkar *et al.* have studied solid lipid nanoparticles which exhibit prolonged *in vitro* drug release of curcumin (Kakkar et al. 2011).

Besides anticancer properties, another application of curcumin is related to the antimicrobial activity of curcumin, which was enhanced using nanoparticles (Bilia et al. 2017). Moreover, a recent review reported the therapeutic application of different natural products in rheumatoid arthritis therapy, mainly polyphenols as curcumin (Bilia et al. 2017; Sannaa et al. 2012; Watkins et al. 2015).

Resveratrol

Resveratrol is a natural polyphenolic compound found in *Vitis vinífera* L. (*Vitaceae*). This compound has emerged as one of the most promising therapeutic agents in coronary disease prevention, as well as in neurodegenerative pathologies and in the inhibition of several types of cancer (Sannaa et al. 2012; Watkins et al. 2015). Nevertheless, resveratrol (as a free form) has some particular limitations. As a polyphenol, it possesses a rapid and extensive metabolism. In addition, the instability of resveratrol is also factor that affects its bioavailability.

Many nanoformulations have been tested and have proved to improve those mentioned characteristics (Watkins et al. 2015). As an example, a higher rate of glioma cell death was obtained with mPEG poly(ε-caprolactone)-based nanoparticles (Watkins et al. 2015). Also, solid lipid nanoparticles also showed to decrease cell proliferation in skin cancer using resveratrol as main therapeutic agent (Watkins et al. 2015). Besides the skin cancer, Sannaa *et al.* have shown an example of success when using nanoparticles as well as mitochondrial targeting liposomes that induced apoptosis in prostate cancer (Sannaa et al. 2012). Furthermore, previous studies showed that resveratrol stability was also improved through

nanoencapsulation, preventing structural degradation and enhancing its therapeutic effect (Sannaa et al. 2012; Watkins et al. 2015).

Silibinin

Silibinin is a major active constituent obtained from the seeds of *Silybum marianum* L. (*Asteraceae*), a polyphenolic flavonolignan with hepatoprotective and antioxidant activities (Kuen et al. 2017). It has been suggested to be a natural compound with therapeutic effect in diabetes and cancer (Ganesan et al. 2017; Kuen et al. 2017). Nonetheless, and similarly to all natural products already mentioned above, silibinin has low bioavailability mainly due to the low solubility in water. In order to overcome this disadvantage, several strategies such as solid lipid nanoparticles were developed (Kuen et al. 2017). Stearic acid was used with success to incorporate silibinin into the nanoparticles, but the rapid *in vivo* clearance through the reticuloendothelial system decreases the circulation time of silibinin (Kuen et al. 2017). Another example of success includes modified chitosan nanoparticles which proved to enhance the therapeutic efficacy of silibinin in lung cancer cells. Also, Ganesan *et al*. have produced PLGA nanoparticles loaded with silibinin for antidiabetic activities in streptozotocin-induced diabetes rat model (Ganesan et al. 2017). These silibinin-loaded nanoparticles with about 230 nm improved bioavailability of such compounds in the systemic circulation along with a higher restoration of the pancreatic cells (Ganesan et al. 2017).

Parvifloron D

Naturally occurring abietane diterpene, Parvifloron D is the main phytochemical constituent of *Plectranthus ecklonii* Benth. (*Lamiaceae*) (Gaspar-Marques et al. 2008). Abietane diterpenes have attracted much attention since they display a wide range of biological activities, including antitumor activities (Burmistrova et al. 2015). Parvifloron D has shown to have antioxidant, antibacterial, and antitumor activities. However, cytotoxicity towards human tumor cells is not selective (Burmistrova et al. 2015; Rosa et al. 2015; Simões et al. 2010). To develop a targeted anti-melanoma drug delivery system for Parvifloron D, Silva *et al.* have prepared hybrid nanoparticles with biopolymers and functionalized with α-melanocyte-stimulating hormone. Parvifloron D-loaded nanoparticles showed to be a promising approach for long-term drug release, presenting the desired structure and a robust performance for targeted anticancer therapy (Silva et al. 2016a).

Quercetin

Quercetin is a polyphenolic compound widely distributed in many plants, such as capers, lovage, dill, apple, and tea. Previous works stated that quercetin could suppress the growth of cancer cells through inducing apoptosis in several cancer cell

lines (Aras et al. 2014). Thus, many quercetin-loaded nanoparticles have been developed to increase the bioavailability of quercetin (Chuan et al. 2015). Quercetin-loaded liposomes have shown remarkable anticancer activity, and it was dose-dependent effect (Aras et al. 2014). Another report showed that quercetin-loaded liposomes also enhanced the cytotoxic effects on C6 glioma cells (Chuan et al. 2015). Besides liposomes, PLGA, polylactic acid-hyperbranched polyglycerol (HPG-PLA), and PEG (660)-12-hydroxystearate (PEG 660-stearate) are other examples of encapsulant material in which loaded quercetin has its activity improved. Here, quercetin encapsulated in PLGA has shown to strongly induce apoptosis in breast cancer cells, and nanoparticles prepared from HPG-PLA have proved to be new efficient carriers for quercetin, as well as PEG 660-stearate that has also revealed a considerable improvement in quercetin solubilization of up to five times (Aras et al. 2014).

Epigallocatechin Gallate

Epigallocatechin gallate is a potent phytochemical compound extracted from green tea. It is reported to be involved in regulation of apoptosis and carcinogenesis. However, its low bioavailability considerably reduces its biological effects *in vivo*. Thus, different approaches have been used to improve the bioavailability of epigallocatechin gallate such as chitosan-based nanocarriers (Tyagi et al. 2017). Those nanocarriers have become the ideal choice, due to the availability of amino groups for further functionalization, as well as a possible interaction with the mucus layer (Tyagi et al. 2017). In addition to chitosan, self-assembled 6-O-(3-hexadecyloxy-2-hydroxypropyl)-hyaluronic acid (HDHA) nanoparticles have recently been tested in swiss albino mice grafted with Ehrlich's ascites carcinoma cells. The results confirmed that epigallocatechin gallate-loaded HDHA nanoparticles were more effective in targeted drug delivery (Aras et al. 2014).

Vincristine

Vincristine is derived from the periwinkle plant *Vinca rosea* L. (*Apocynaceae*) with activity against many of the lymphoid malignancies, including aggressive non-Hodgkin's lymphoma (Boehlke and Winter 2006; Davis and Farag 2013). It is a cell cycle-specific agent that binds to tubulin, causing microtubule depolymerization, metaphase arrest, and apoptotic cell death of cells in mitosis (Boehlke and Winter 2006). The limitations of vincristine include low solubility in aqueous solutions at physiologic pH *in vitro* and rapid plasma clearance (Boehlke and Winter 2006; Davis and Farag 2013). These properties have led to the use of liposomes to deliver vincristine with an increased half-life while decreasing its toxicity in non-target tissues (Davis and Farag 2013; Douer 2016).

In addition, pharmacokinetic data from patients enrolled on a Phase I trial of vincristine-loaded liposomes have shown a significant increase of vincristine plasma levels over several hours. Liposomes protected vincristine from rapid elimination

observed in vincristine free form, and the systemic exposure of vincristine was significantly lower (Boehlke and Winter 2006).

Marqibo® is a sphingomyelin- and cholesterol-based liposome, with particle size around 100 nm, loaded with vincristine, designed to overcome the dosing and pharmacokinetic limitations of this compound (Silverman and Deitcher 2013). It was approved by the FDA in 2012 for acute lymphoid leukemia, either Philadelphia chromosome-negative, relapsed, or progressed (Weissig et al. 2014). The liposomal carrier was specifically designed to facilitate the loading and retention of vincristine, to prolong its circulation time, to increase extravasations into tumors, and to slowly release the drug in the tumor interstitium (Silverman and Deitcher 2013). Those characteristics resulted in high levels of encapsulated drug in target tissues and a long exposure duration of tumor cells to therapeutic drug concentrations as the drug was slowly released from the liposomes, leading to an enhanced activity (Silverman and Deitcher 2013).

4.4 Nanopharmaceuticals Based on Natural Products as Encapsulant Material

Polysaccharides, proteins, and glycosaminoglycans are the most used natural polymers not only in the biomedical sector but also in the industrial and food fields (Frantz et al. 2010). The use of these natural polymers is generally due to its completely biodegradability, safety, and non-immunogenic properties. Thus, they are preferred above synthetic, also because of their wide acceptance, their abundance, and especially their cost-effectiveness relation (Anwunobi and Emeje 2011).

4.4.1 Polysaccharides as Encapsulating Material

Chitosan

Chitosan is a polysaccharide obtained through partial deacetylation from chitin that is found in crustacean's shells, and it contains glucosamine and *N*-acetyl glucosamine subunits linked through β-(1–4) glycosidic bonds. Currently, these amino sugars are widely used for the synthesis of nanoparticles (Nair et al. 2009; Reis et al. 2008b). The presence of amine functional group in chitosan molecule plays a fundamental role in its use in nanoparticle preparation once it gives a positive charge and enables the interaction of negative polyelectrolytes, promoting the formation of a spontaneous nanocomplex. The most common techniques used in the production of chitosan nanoparticles are the cross-linking, the desolvation with cationic salts, and the complexation of polyelectrolytes/ionic gelation (Chen et al. 2003). Chitosan nanoparticles can also be produced with other polymers, and one of many examples are the poly(lactic acid)/chitosan nanoparticles, which were synthesized by Dev and

his coworkers, through the emulsion method for loading anti-HIV drugs (Dev et al. 2010). Also, chitosan–dextran sulfate nanoparticles, produced through the coacervation method, which were designed to study the encapsulation of an anti-angiogenic peptide, arginine-rich hexapeptide, are one of the examples of the application of chitosan in nanotechnology (Chen et al. 2007).

Zhi *et al.* have formulated chitosan nanoparticles with magnetic properties to potentially use as drug delivery carrier with a new method to *in situ* prepare magnetic chitosan/Fe_3O_4 composite nanoparticles. The resultant nanoparticles have shown high stability (Zhi et al. 2006). Other example is chitosan-PEC or *N*-acyl or *N*-alkyl derivatives that demonstrated to have improved solubility in water (Hawary et al. 2011; Wu et al. 2014). Several types of chitosan modified systems have been studied so far, among them are *N*-trimethyl chitosan nanoparticles, which have shown good results in brain target of anti-Alzheimer's drugs, as well as in the delivery of neuroprotective drugs (Sarvaiya and Agrawal 2015).

Some chitosan derivatives were synthesized by Ho *et al.*, aiming an efficient delivery of the basic fibroblast growth factor. Among all the tests made, only chitosan–sulfate nanoparticles, modified with thiol groups, had shown a greater efficacy due to its high affinity to basic fibroblast growth factor (Ho et al. 2010). Similarly, monophosphate conjugate nanoparticles of chitosan-O-isopropyl-5-O-d4T were synthesized in combination with tripolyphosphate through the ionotropic complexation method to *in vitro* deliver an anti-HIV drug. In this study, Yang *et al.* concluded that the reticulated conjugated nanoparticles can provide a controlled release of the drug (Yang et al. 2010).

Alginate

Alginate is a natural polymer with several biomedical applications and more recently a promising candidate in tissue engineering. Alginate is linearly disposed with β-D-mannuronic acid and α-L-guluronic acid residues, linked through bonds 1–4′. It can be extracted from several *Phaeophyceae* species, including *Laminaria japonica* J.E., *Laminaria digitata* H., *Laminaria hyperborea* G. (*Laminariaceae*), *Ascophyllum nodosum* L. (*Fucaceae*) and *Macrocystis pyrifera* L. (*Laminariaceae*) (Venkatesan et al. 2015).

The alginate has been widely used as a carrier for controlled and sustained delivery of drugs, small bioactive molecules, proteins, and cells (Raveendran et al. 2017). One example of alginate application in nanotechnology is the combination with calcium (Sinha and Kumria 2001). Reis *et al.* have studied nanoparticles composed of natural and biodegradable polymers to orally deliver insulin in the treatment of *diabetes mellitus* (Reis et al. 2008a, b, c). They have used a nanoparticulate system based on alginate–dextran sulfate core, complexed with a chitosan–polyethylene glycol-albumin shell. In this study, it was shown that those nanospheres preserved insulin's activity and demonstrated an antidiabetic effect after oral administration. These results were explained through a protective effect against proteolytic enzymes due to the albumin coating, but also through the mucoadhesive properties of chitosan–polyethylene glycol, and the possibility of chitosan reversibly altering tight

junctions, leading to an improved absorption of insulin. This formulation had revealed to be an interesting and promising approach in the treatment of diabetes with oral insulin (Reis et al. 2008c).

Starch

Starch is the major responsible for the polysaccharide storage in plants. It consists of two distinct polymers, amylose and amylopectin, and both of them involve repeated and linked D-glucose monomers units. Due to its physicochemical and biological features, this polysaccharide is used in the production of synthetic polymers such as polypropylene carbonate, in manufacturing of gels and granules, and in nanotechnology, among others (Cyprych et al. 2014).

Starch nanoparticles can be produced using several methods, such as precipitation, solvent evaporation, and spray-drying (Raveendran et al. 2017). Starch has also been widely used to produce metallic nanoparticles, reducing the stability of metallic oxides (Vigneshwaran et al. 2006). Vigneshwaran *et al.* have synthesized stable silver nanoparticles using soluble starch as a reducing and stabilizing agent. Iodometric titration confirmed the entrapment of silver nanoparticles inside the helical amylose chain. Also, the use of soluble starch offers innumerous benefits of eco-friendly and compatibility for pharmaceutical and biomedical environmental applications (Vigneshwaran et al. 2006).

A novel type of reduction-sensitive starch nanoparticles was prepared by J. Yang *et al.* (2014). The results have shown that the disulfide cross-linked starch nanoparticles exhibited an accelerated drug release behavior in the presence of dithiothreitol. *In vitro* methyl thiazolyl tetrazolium assays indicated that these nanoparticles had a good biocompatibility when co-cultured with human HeLa cancer cells. Another study showed that an *in situ* hydrogel preparation through the Schiff reaction and using cross-linked nanoparticles of starch and polyvinylamine was able to encapsulate doxorubicin (Li et al. 2014).

Pectin

At the structural level, pectin is one of the major compounds in plant cell walls. Although there are many plant tissues that contain pectin in their composition, citrus and apple peel are the main sources of this polysaccharide (Urias-Orona et al. 2010). The main structure of pectin is polygalacturonic acid which is composed of galacturonic acid residues linked linearly to each other through $\alpha(1 \rightarrow 4)$ bonds, and carboxyl groups thereof can be methyl esterified, amidated, or acetylated acid units (Urias-Orona et al. 2010). The pectin structure also contains galactose and arabinose. This polymer is not water soluble under suitable gelation conditions to other gel bases, and conditions of temperature, pectin concentration, pH, soluble solid content, and specific calcium ion concentration are required to a gel formation.

However, it is used as the gel base for oral gelatin prepared at the time of use (Kakino et al. 2017).

The application of pectin to nanotechnology is very common, mainly because of its biodegradability and nontoxicity. In the 1980s, pectin hydrogels were used in tablet formulations as binding agents. More recently, high-methoxyl pectins, with more than 50% of the carboxyl groups esterified, have been investigated due to their controlled release potential (Lofgren and Hermansson 2007).

Zhang *et al.* have prepared pectin nanoparticles for delivering the hydrophobic drug, honokiol, to HepG2 cells. Those particles demonstrated a specific active targeting ability to asialoglycoprotein receptor-positive HepG2 cells and could be used as a potential drug carrier for liver-related tumor treatment (Zhang et al. 2015). Moreover, zein and pectin composite gels can release a combination of several drugs for a given segment of the gastrointestinal tract at a specific period of time (Muruci de Paula and Lopez da Silva 2016). In addition to core material, pectin can act as coating material. Nguyen *et al.* investigated the surface coating of charged liposomes through three different types of pectin: low methoxy, high methoxy, and amidated pectin. The results have shown that pectin has been found to be mucoadhesive, improving therapeutic effect of drug-loaded liposomes (Muruci de Paula and Lopez da Silva 2016; Nguyen et al. 2011).

4.4.2 Glycosaminoglycans

Heparin

Despite the use of natural polymers mostly aiming to enhance drug bioavailability, there are some natural polymers that have by itself therapeutic activity, such as heparin. Heparin is a polymer categorized as a glycosaminoglycans, which is a class of compounds composed of long chains of branched polysaccharides with repeated disaccharide units. Heparin has a repetition of disaccharide units of D-glucuronic acid bound to (1,4)-D-glucuronic acid or L-iduronic acid and glucosamine residues (Nurunnabi et al. 2012). It is a highly sulfated polymer and, in addition to the sulfate group, heparin also contains a carboxylic group.

The most common clinical use of heparin is as an anticoagulant (Raveendran et al. 2013). Heparin sulfate has very promising biological properties, such as cell adhesion, cell growth, cell proliferation, inhibition of angiogenesis, and cancer growth, as well as viral infections, tumor invasion, and metastasis (Nurunnabi et al. 2012). For all these reasons, heparin and its derivatives are strong candidates for nanotechnological applications, such as for the delivery of several compounds, namely, small molecules, peptides, proteins, and siRNA. Particularly, it has been used for the synthesis of polymeric nanoparticles, nanogels, as well as in the stabilization or coating of nanocrystals and inorganic nanoparticles (Raveendran et al. 2017). In alternative, using heparin as a surface coating on nanoparticles, it can suppress the uptake via immune system and thus remain longer in the bloodstream

(Passirani et al. 1998). However, the type of heparin used is something to be considered; the low molecular weight is preferable to the non-fractionated, because the non-fractionated may cause hemorrhage or thrombocytopenia (Debergh et al. 2010). Hou *et al.* have tested paclitaxel–heparin nanoparticles in the treatment of cancer, and the results have demonstrated that some functions or modifications of heparin with specific ligands may increase cellular uptake in the target tissue (L. Hou et al. 2012; Park et al. 2010).

Dendrimers are also promising in nanotechnology due to their monodisperse size, water solubility, multivalence, and surface functionalization properties (She et al. 2013). She *et al.* have prepared and characterized a dendronized heparin–doxorubicin conjugate as pH-sensitive drug delivery vehicle through the combination of the features of dendrimer and heparin. These nanoparticles resulted in a strong antitumor activity and high anti-angiogenesis effects. Nanoparticles induced apoptosis on the 4T1 breast tumor model, with no significant toxicity to healthy organs of both tumor-bearing and healthy mice. The dendronized heparin–doxorubicin conjugate-based nanoparticles with high antitumor activity and low side effects may be a potential nanoscale drug delivery vehicle for breast cancer therapy (She et al. 2013).

Albumin

Albumin is an abundant protein in human plasma. It is biocompatible, biodegradable, nontoxic, and non-immunogenic. Its unique structure makes it easily conjugated to hydrophobic and hydrophilic compounds. This protein can also prolong circulation time, obtain a prolonged drug release, and accumulate at tumor sites, thereby maximizing therapeutic effects and minimizing toxicity (Kratz 2008).

Therefore, it is being increasingly studied in the field of nanotechnology for the treatment of oncological diseases, allowing the delivery of cytotoxic drugs only in the target tumor tissues, avoiding healthy tissues (Elzoghby et al. 2012; Xinzhe Yu et al. 2017; Xinzhe Yu and Jin 2016). Comparing conventional drug delivery systems, albumin-based nanoparticles offer several advantages, especially in relation to anticancer drugs (Li et al. 2013). Also, albumin is retained in the target tissue, thereby increasing the effectiveness of the treatment (Schnitzer 1992). Due to the large number of drug-binding sites present in the albumin molecule, it can encapsulate significant amounts of drug into nanoparticle matrix. Due to the functional carboxylic and amino groups on albumin surface, it also provides multiple prospects for surface functionalization. The conjugation between a highly compatible and specific ligand at the surface of albumin nanoparticles is essential for active targeting, allowing a selective bound to a particular receptor type on the target cells (Rebelo et al. 2017). Additionally, patients with advanced solid tumors usually present hypoalbuminemia. Thus, they may benefit if the delivery system of the antineoplastic drug is an albumin-based system (Schnitzerlt et al. 1992).

Regarding its application in nanotechnology, several studies have reported that doxorubicin-loaded albumin-based nanoparticles have demonstrated a strengthened

anticancer effect for mammary tumor cells (Abbasi et al. 2012). X. Yu *et al.* have studied gemcitabine-loaded albumin nanoparticles in pancreatic tumor cell lines (Yu et al. 2015). Data confirmed that the prepared nanoparticles could efficiently inhibit tumor growth in a pancreatic cancer cell line. However, further studies must be performed to optimize this therapy for clinical use.

In 2005, FDA approved Abraxane® for metastatic breast cancer, non-small cell lung cancer and recently for pancreatic ductal adenocarcinoma. Abraxane® consists of nanoparticles, with particle size around 130 nm, composed of albumin with conjugated paclitaxel. Dissociation into individual drug-bound albumin molecules may mediate endothelial transcytosis of paclitaxel via albumin receptor-mediated pathway (Ragelle et al. 2017; Weissig et al. 2014). This paclitaxel-protein-bound nanoparticle formulation was developed to reduce the toxicities associated with the emulsifier Cremophor EL® in the commercialized paclitaxel formulation, increasing the maximum tolerated dose in 50%. In addition, Abraxane® modifies the pharmacokinetic profile of the drug, resulting in faster clearance, increased distribution volume, and higher intratumoral concentrations (Ragelle et al. 2017). The enhanced tumor accumulation can be explained through the combination of an increased dosage due to better tolerability of the delivery system, enhanced permeability and retention effect, and receptor-ligand targeting via active albumin transport pathways. It is difficult to interpret the exact impact of each of these factors on the therapeutic performance of Abraxane®, but it is more likely that the clinical benefits explain both through the increased amount of drug available and the reduced toxicity of the delivery system (Ragelle et al. 2017).

Gelatin

Gelatin is a protein derived from collagen as a result of partial hydrolysis, which is extracted from animal by-products, mainly from the connective tissues. During the collagen hydrolysis process, the inter- and intramolecular covalent bonds are broken, leaving axes to give a more simplified form called tropocollagen. A later denaturation through the breakdown of hydrogen and hydrophobic bonds leads to a heterogeneous protein material, gelatin (Jahanshahi and Babaei 2008)**.** There are several methods of collagen hydrolysis, especially the acid and alkaline treatment which is applied essentially in the commercial production of gelatin. Nevertheless, there are also enzymatic and thermal degradation methods. There are two types of gelatin available: type A and type B. However, since it is a very heterogeneous molecule, it is difficult to prepare a highly homogeneous polymer. The presence of an amine and carboxylic group makes this protein very amphoteric and easily soluble in hot water. The viscosity of gelatin is affected by its type, concentration, and temperature. Gelatin is widely used in the food and pharmaceutical industry, and it has been increasingly used in nanotechnology (Djagny et al. 2001). Several techniques have been used in the preparation of gelatin nanoparticles, namely, emulsification, solvent extraction, and nanoprecipitation (Elzoghby 2013).

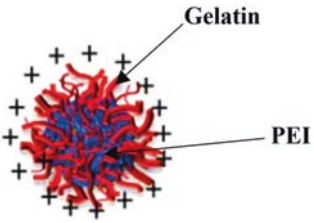

Fig. 4.7 Gelatin-polyethyleneimine (PEI) core-shell nanogel. (Modified after (Mimi et al. 2012))

Han *et al.* have used amphiphilic copolymer nanoparticles based on gelatin, poly(lactide), and 1,2- dipalmitoyl-sn-glycero-3-phosphoethanolamine. An anticancer drug model, doxorubicin hydrochloride salt, was incorporated into polymeric nanoparticles through nanoprecipitation method. These nanoparticles showed a comparable anticancer efficacy with the free drug *in vitro* and *in vivo* (Han et al. 2013).

Gelatin-based nanospheres have been developed for controlled and sustained release of drugs, peptides, and proteins. Mimi *et al.* have presented a novel type of polyethyleneimine-based nanogels, as Fig. 4.7 shows, with a biodegradable gelatin core. The gelatin–polyethyleneimine nanogels were able to completely condense small interfering ribonucleic acid (siRNA) and effectively protected siRNA against enzymatic degradation. Additionally, the nanogels were four times less toxic than native polyethyleneimine. Also, the nanogels were able to effectively deliver siRNA into HeLa cells. The results demonstrate that gelatin–polyethyleneimine core-shell nanogels have promising potential to act as an effective siRNA.

4.5 Challenges Involved in the Development of Nanopharmaceuticals

A growing number of nanopharmaceutical product submissions are being received by the drug approval authorities. There is an urgent need to establish specific regulatory guidelines to nanopharmaceuticals; however, an appropriate testing criteria needs to be established, and additional data need to be collected.

In the development of nanotherapeutics, the characterization of new nanomaterials in terms of safety and toxicity is considered a significant challenge. It is difficult to make a generalized statement about the safety of these systems. Regarding the natural products, this process is even more complicated since the therapeutic value of these products has been under evaluated (Alexander et al. 2016; Ansari et al. 2012; Bonifácio et al. 2014; Borel and Sabliov 2014; Goyal et al. 2011; Kumar and Gupta 2015; Namdari et al. 2017; Watkins et al. 2015). Thus, a sense of urgency for reforming current regulations is created, since the application of nanotechnology to medicine and to natural products may exacerbate some concerns about risk minimization (Ventola 2012).

Table 4.1 Nano-based products with natural active compounds or based on natural materials already approved by drug authorities. This table highlight the most representative (not all) commercialized nano-based systems, the year of their approval, and their therapeutic indication

Product name	Year of approval	Active compound/ based on	Nano system type	Indication
Genexol®	2011	Paclitaxel	Polymeric micelles	Metastatic breast and pancreatic cancer
Rapamune®	2000	Sirolimus	Nanocrystals	Immunosuppressive agent for the prophylaxis of organ rejection in renal transplants
Opaxio®	2012	Paclitaxel	Polymeric nanoparticles	Glioblastoma multiforme and malignant brain cancer
Doxil®	1995	Doxorubicin	Liposomes	Ovarian cancer, AIDS-related Kaposi's sarcoma and multiple myeloma
Caelyx®	1996	Doxorubicin	Liposomes	Ovarian cancer, AIDS-related Kaposi's sarcoma, multiple myeloma and breast neoplasms
Marqibo®	2012	Vincristine	Liposomes	Acute lymphoid leukemia
Abraxane®	2005	Paclitaxel/ albumin	Polymeric nanoparticles	Metastatic breast cancer, non-small cell lung cancer and pancreatic ductal adenocarcinoma

Financial issues are still a barrier in the development of these systems. Despite the success, it is not easy to demonstrate their efficacy and safety in order to be granted regulatory approval. The majority of currently approved nanopharmaceuticals are based on conventional drugs which have existing approval by the drug approval authorities and on a simple "reformulation." Concerning the large amount of nanopharmaceutics using natural products that are being studied, some of them which are in the early stages of development will hopefully receive regulatory approval. Table 4.1 describes the most representative examples (not all) of nanopharmaceutics which have been already approved, and they are already in the market.

4.6 Conclusion

Natural products have been widely used in medicine, and many top-selling in pharma are natural compound-based products or their derivatives. However, their success in clinical trials has been less impressive, partly due to the low water solubility and thus their low bioavailability. Nanoencapsulation of these natural products into nanocarriers would be a major advance in the efforts to increase their therapeutic effects.

Many of the described nanocarrier types in this review were found to have different properties, which make each one of them a unique delivery system with

remarkable results. However, even though these systems may provide unique solutions for clinical needs and significantly alter clinical practice, nanopharmaceutical development still faces many challenges. This is in part due to the difficulty in reproducing method of production of nanoparticles on a scale needed for commercialization (industrial scale-up), specific guidelines to evaluate their potential toxicity, lack of understanding regarding how nanocarriers will interact with cells, lack of technological platforms necessary to screen large quantities of nanoparticles, and insufficient knowledge about the metabolic and elimination mechanisms of the nanoparticles.

During the past three decades, conceptual and practical advancements have been made in the design and implementation of several nanopharmaceuticals. Progress has been noticeable and expectations related to therapeutic efficacy of natural products have really increased. Research is still increasingly focused on the development of new therapies to meet clinical expectations. Thus, many studies are required in the near future in order to improve the use of the complex relation between all the different types and characteristics either from natural products or nanopharmaceuticals.

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Chapter 5
Vesicular Nanocarriers: A Potential Platform for Dermal and Transdermal Drug Delivery

Ahmed Alaa Kassem and Sameh Hosam Abd El-Alim

Contents

Abstract The skin as a route of drug administration may offer numerous advantages despite its barrier nature which hinders most drugs to penetrate into and permeate across it. The main obstacle for drug permeation through the skin is the stratum corneum, the outermost layer of the skin. During the past decades, there has been a great interest in vesicular carriers as a tool to improve dermal and transdermal delivery of drugs. Vesicular carriers include liposomes, ultradeformable liposomes, ethosomes, and niosomes. These carrier systems are able to augment the skin drug permeation by enhancing drug solubilization in the formulation, controlling active drug release, improving drug partitioning into the skin, and fluidizing skin lipids. A wide variety of materials can be utilized to prepare vesicles, which are commonly composed of phospholipids (liposomes) or non-ionic surfactants (niosomes). Vesicle composition and method of preparation influence their physicochemical properties (size, charge, deformability) and therefore their efficacy as drug

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delivery systems. This chapter gives an overview of different vesicular carrier systems, with particular emphasis on the development of these delivery systems in light of comprehensive understanding of physicochemical properties of drug and delivery carriers, process and formulation variables, mechanism of skin delivery, recent technological improvements, and specific limitations.

Keywords Skin · Transdermal drug delivery · Penetration enhancement · Vesicular carriers · Liposomes · Transfersomes · Ethosomes · Niosomes

Abbreviations

CCPP	Cationic cell-penetrating peptide
CPP	Critical packing parameter
DDD	Dermal drug delivery
HLB	Hydrophilic–lipophilic balance
TDD	Transdermal drug delivery

5.1 Introduction

The largest organ in the human body is the skin, which offers large surface area for the application of drugs (El Maghraby and Williams 2009). The skin represents the organism's barrier from the environment, which protects against pathogen invasion, physical and chemical attacks in addition to the uncontrolled excretion of solutes and water (Sinico and Fadda 2009). Anatomically, it consists of a number of distinctive layers, namely, the stratum corneum, the viable epidermis, the dermis, and the subcutaneous "fat" layer. The majority of therapeutic agents fail to penetrate through or into the skin due to their impermeability, which is considered one of the major sites for noninvasive delivery of drugs (Foldvari 2000).

Skin delivery offers a promising substitute to oral drug delivery (Jain et al. 2017). Skin delivery may be generally distinguished into dermal (topical) and transdermal drug delivery (TDD). Dermal drug delivery (DDD) is the administration of therapeutic agent directly at the target organ (skin surface) where the action is required, leading to greater localized concentration of drug with minimized drug exposure to systemic circulation (Paudel et al. 2010). Conversely, TDD transfers the therapeutic agent through skin surface to the systemic circulation for attaining therapeutic levels. Both dermal and transdermal applications have effectively delivered various therapeutic agents (Basha et al. 2015; Kassem et al. 2017). The growing occurrence of chronic skin diseases, need for site-specific and patient-compliant delivery, extremely competitive oral drug delivery market, and increasing attention of

pharmaceutical companies in management plans of life cycle are the major driving force for the growing interest in skin drug delivery (Paudel et al. 2010; Jain et al. 2015).

TDD is a competent means for efficient delivery of various therapeutic agents. But the skin barrier represents a main difficulty towards fabrication of TDD systems where this natural transport barrier should be initially resolved (Singh et al. 2015). Highly organized crystalline lipid lamellae play a very significant role in the barrier characteristics of the stratum corneum (Prasanthi and Lakshmi 2012b). In order to weaken or break down the highly organized structure of intercellular lipids, various techniques have been adopted to improve drug permeation through the unbroken skin or to augment the delivery power for the penetration of therapeutic agents through this skin barrier (Singh et al. 2015). The initial main technique to circumvent the skin barrier is the utilization of chemical enhancers, e.g., glycols, azones, terpenes, ethanol, etc. (Jain et al. 2015; Pyatski et al. 2016). They aid drug transfer by partly fluidizing skin lipids and increasing drug partitioning. A second technique is to utilize physical enhancement methods, e.g., electroporation, sonophoresis (ultrasound), microneedles, magnetophoresis, thermal ablation, iontophoresis, and microdermabrasion (Barry 2002; Patel et al. 2015, 2016a, b; Kim et al. 2016). This technique circumvents the stratum corneum and transports the therapeutic agent directly to the desired skin layer. Both of the abovementioned techniques have revealed promoted delivery for various therapeutic agents (Kim et al. 2016; Mujica Ascencio et al. 2016). Nevertheless, physical techniques are frequently painful and costly, and lack patient compliance, whereas chemical permeation enhancers may result in skin irritation and lasting skin destruction (Jain et al. 2015). Lastly, the third technique is the utilization of drug delivery systems such as microparticles, nanoparticles, and vesicular delivery systems. These systems may improve skin permeation by enhancing drug solubilization in the formulation, drug partitioning into the skin, and fluidizing the skin lipids (Jain et al. 2015). Among the numerous investigated drug delivery systems, vesicular systems have revealed an increasing potential for both DDD and TDD, particularly in the previous few decades (Elsayed et al. 2007b). The initial commercial product employing vesicular delivery system was introduced in the market in 1988 for the antifungal agent econazole (Naeff 1996). After that, numerous research works were published demonstrating the potential of these delivery systems (Kitagawa and Kasamaki 2006; Elsayed et al. 2007b; Gershkovich et al. 2008; Hua 2015; Jain et al. 2015; Singla and Sachdeva 2015).

Vesicular carriers include liposomes, ultradeformable liposomes, and ethosomes, in addition to other specialized new vesicular carriers. The majority of the new studies are mainly concentrated on elastic liposomes such as ultradeformable liposomes and ethosomes as well as polymeric liposomes because of the inadequate achievement of conventional liposomes in skin delivery (Jain et al. 2017). Vesicular systems may be customized to target a variety of skin diseases/conditions relying on the chosen delivery system, manufacturing processes, formulation composition, and process variables. Nevertheless, development of vesicular systems needs understanding of formulation and process variables, knowledge of physicochemical

properties, mechanism of skin delivery, recent technological advancement, and specific limitations.

The present chapter provides a focused overview on vesicular delivery systems as a promising approach to circumvent the natural skin barrier for delivering therapeutic agents with emphasis on advancements, recent research, and challenges.

5.2 Skin Anatomy and Physiology

The skin represents the largest organ of the human body (Jain et al. 2017). The total surface area of the skin of an average male adult is about 2 m^2 (Chandrashekar and Shobha Rani 2008; Sala et al. 2018), which corresponds to 15% of the total body weight (Alexander et al. 2012). Anatomically, the skin is composed of three main distinguishable layers, namely, epidermis, dermis, and subcutaneous "fat" tissues (Erdő et al. 2016).

5.2.1 Epidermis

The epidermis is divided into two regions: the nonviable epidermis (the stratum corneum) and the viable epidermis. It consists of 70% water and keratinizing epithelial cells responsible for formation of the stratum corneum (Walters 2002). The epidermis lacks any blood vessels, and therefore molecules infusing through the epidermis must cross the dermal–epidermal layer to enter the systemic circulation of the body (Jain et al. 2017). The stratum corneum is the skin's outermost layer and is involved in skin protective and homeostatic functions. The stratum corneum is the end product of epidermal proliferation with about 10–20 μm thickness and is considered metabolically inactive (Walters 2002). It is comprised of 10–25 layers of elongated, dead, fully keratinized corneocytes, which are embedded in a matrix of the lipid bilayers. The viable epidermis is localized underneath the stratum corneum with thickness of around 50–100 μm (Feingold 2007). It differs from the stratum corneum because it physiologically resembles the other alive cellular tissues and comprises numerous metabolizing enzymes. The viable epidermis is concerned in the synthesis of stratum corneum and metabolism of foreign substances. Because of the presence of Langerhan cells, it is also concerned in the immune response of the skin (Klareskog et al. 1977). The viable epidermis is a superposition of a number of layers which are from outside to inside: the stratum granulosum, the stratum spinosum, and the stratum basale. The viable epidermis is fundamentally comprised of keratinocytes that represent approximately 95% of the epidermal cells and is in a steady cell self-renewal (Sala et al. 2018). Considering the complex composition of the stratum corneum, it is obvious to imagine that the cutaneous permeation of drugs across this layer is the limiting factor (Sala et al. 2018).

5.2.2 Dermis

The dermis represents the deeper and the thicker layer (1–4 mm) of the skin (Sala et al. 2018). The dermis protects the epidermis being compressible, supportive, and elastic connective tissue (Jain et al. 2017). It consists of fibrous proteins (elastin and collagen) and an interfibrillar gel of glycosaminoglycans, salts, and water. It also comprises lymphatic and blood vessels, hair follicles, nerve endings, sweat glands, and sebaceous glands. Widespread vascular network in the dermis is potentially responsible for skin repair, nutrition, thermal regulation, and immune responses (Walters 2002). The sweat ducts and hair follicles directly connect the dermis with the upper skin surface, crossing stratum corneum and hereafter offering appendageal route for skin penetration (Otberg et al. 2004).

5.2.3 Subcutaneous Tissue

The subcutaneous "fat" tissue is found underneath the dermis and consists of fat-rich cells, making the cytoplasm lipophilic in nature (Walters 2002). The collagen present between the fat cells affords the link of the epidermis and the dermis with the underlying structures of the skin. The chief function of subcutaneous tissue is to operate as a shock absorber and heat insulator (Jain et al. 2017).

5.2.4 Human Skin Functions

The human skin exhibits three major functions; each plays a role to the human body's homeostasis: temperature control, barrier function, and repair function (Sala et al. 2018). The skin is the body's first line of defense; it prevents the loss of body fluids and counteracts the passage of xenobiotics such as toxic compounds and microorganisms (Randhawa et al. 2015; Levy et al. 2016; Nawaz et al. 2016). Moreover, due to its great elasticity, the skin represents a protective barrier against the stress of mechanical forces. The skin is a crucial organ in the regulation of body temperature by means of two mechanisms: the blood flow and sweat (Hayden et al. 2005). The protective utility of the skin is because of both physical characteristics (desquamation, pH) and metabolic enzymes found in the interstitial spaces of the viable epidermis and in regions of dermal hair follicles (Guy et al. 1987; Oesch et al. 2007). This generates a harsh environment towards external agents. The defense means of the skin relies on the presence of immunocompetent cells (Dendritic cells, Langerhans cells), natural characteristics of the skin (peeling resident microflora, low pH), hair follicles and sebaceous glands (production of fatty acid and lysozyme), and melanocytes (protection against UV) (Sala et al. 2018).

5.2.5 *Pathways for Skin Penetration*

The process of percutaneous absorption may happen through two different routes: transepidermal (intracellular and intercellular) and transappendageal (sweat ducts, sebaceous glands, and hair follicles) pathways (Fig. 5.1) (Erdő et al. 2016; Jain et al. 2017). The stratum corneum represents the major physical barrier of the skin layer defending against foreign substances to penetrate the skin. The stratum corneum is considered as the rate-limiting step for the delivery of drugs through the skin. In fact, corneocyte differentiation, migration, and desquamation counteract drug passage across the skin. Noteworthy, a full renewal of the skin barrier takes place every 14 days in healthy individuals (Sala et al. 2018).

Transepidermal Pathway

Transepidermal pathway is comprised of intracellular and intercellular pathways (Jain et al. 2017). Intercellular pathway includes diffusion of solute across the intercellular lipid phases through winding pathway (through cornified cells of the stratum corneum, the viable epidermis, and the dermis) (Scheuplein and Blank 1971). Tracer studies have provided evidences that intercellular lipids, and not the corneocyte proteins, are the major epidermal permeability barrier (Elias and Friend 1975). Intercellular pathway was firstly revoked as a main skin permeation mechanism because of its small volume tenancy (Scheuplein and Blank 1971). However, afterwards the intercellular volume part was found to be much larger than originally anticipated (Berenson and Burch 1951; Nemanic and Elias 1980). These studies suggest that intercellular pathway provided a main resistance for skin permeation.

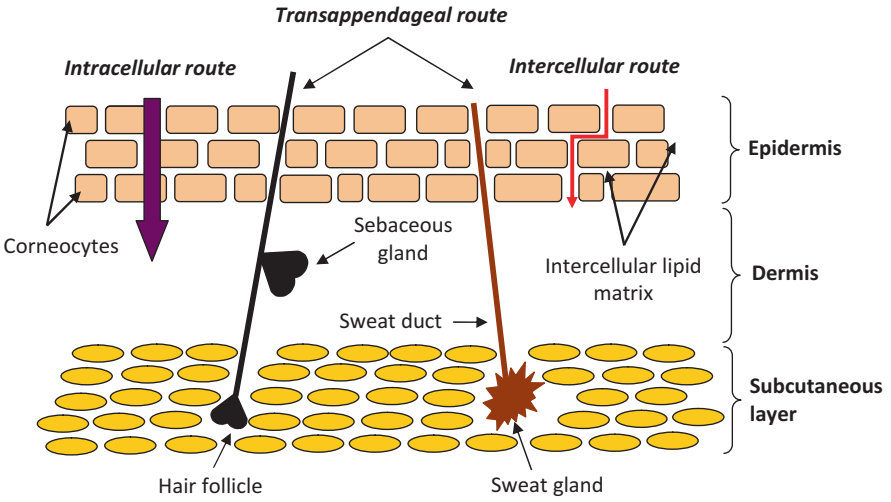

Fig. 5.1 The pathways for percutaneous absorption

However, it has been shown that polar molecules could barely permeate across the intercellular route, while nonpolar molecules possessing molecular weight <500 Da and Log P 1–4 could easily permeate (Cronin et al. 1999; Kang et al. 2007). It is notable to state that intercellular space width is likely to be 19 nm by van der Merwe et al. (2006) and 75 nm by Baroli et al. (2007) which hinders the diffusion of large molecules. In brief, drug molecular weight, solubility, and the ability to form hydrogen bonds are the physicochemical characteristics that influence the intercellular permeation across the skin (Potts and Guy 1995).

Intracellular (transcellular) pathway includes permeation across the corneocytes and the intercellular lipids, in the same order (Jain et al. 2017). Molecules diffusing by this route use the imperfections in the corneocytes that form gaps which consisted of water. This route is consequently believed to favor hydrophilic molecules for delivery. It is remarkable to note that the intracellular pathway needs partitioning not only into and permeation across corneocytes but into and through the intercellular lipids as well (El Maghraby et al. 2008).

Transappendageal Pathway

In transappendageal pathway, the penetrating compound crosses the stratum corneum through a "shunt" pathway afforded by sweat glands or hair follicles (Jain et al. 2017). Particularly, hair follicles play a main provider for this pathway because of higher follicular allocation. Although the accessible surface area for the follicular route is supposed to be restricted to about 0.1% of whole skin surface area, it has lately been proposed that follicular opening diameter, number, and follicular volume are significant considerations to identify the extent of delivery (Scheuplein 1965; Otberg et al. 2004). In addition, hair follicles expand deep into the dermis with potential increase in the real surface area accessible for permeation. Numerous research works have shown the significance of this pathway in skin penetration (Lademann et al. 2001; Essa et al. 2002; Otberg et al. 2008).

5.3 Dermal and Transdermal Drug Delivery

The skin is considered as a site for therapeutic agent application to treat various dermatological conditions/diseases and has been utilized for this purpose since Babylon and ancient Egypt (Perumal et al. 2013). For thousands of years, humans have applied compounds topically for pharmacological effects, and currently, various topical products have been developed to treat local dermatological conditions (Prausnitz and Langer 2008). Nevertheless, the dermal route was not recognized as an effective way for systemic drug delivery until the last years of the twentieth century. In 1924, Rein research works started to help the foundations of modern TDD to recognize the characteristics of the skin barrier (Perumal et al. 2013). The skin has been extensively employed for systemic drug delivery together with the

appearance of transdermal patches since the 1970s (Roy et al. 1996). The modern era of TDD started in 1979 with the marketing of transdermal scopolamine for motion sickness. Since then the industry has matured and grown, with various transdermal medications currently accessible for numerous indications (Giannos 2015).

DDD and TDD, normally illustrated as skin drug delivery, are a promising approach for the management of various dermatological conditions/diseases (Sala et al. 2018). DDD is the topical application of therapeutic agents to the skin in the management of dermatological diseases/conditions. This approach is advantageous for localizing high drug concentrations at the site of action, decreasing the systemic levels of drug and consequently decreasing the systemic adverse effects as well (Honeywell-Nguyen and Bouwstra 2005). Otherwise, TDD utilizes the skin as another route for the delivery of systemically acting therapeutic agents (Honeywell-Nguyen and Bouwstra 2005).

Nowadays, 74% of therapeutic agents which are administered by the oral route fail to show the desired efficacy. Aiming to enhance the efficacy, TDD has been introduced (Marwah et al. 2016). Topical application of drugs exhibits high potential in treatment of diseases due to the large surface area of the skin (Prausnitz et al. 2004). TDD system is an attractive option to avoid and reduce the side effects or limitations associated with parenteral and oral routes of drug administration (Alexander et al. 2012).

Initially, it avoids the gastrointestinal motility, pH, and food intake that might influence gastrointestinal absorption (Honeywell-Nguyen and Bouwstra 2005). TDD may allow therapeutic agents to avoid the first-pass metabolism, minimize adverse effects, and acquire a more effective and predictable pharmacological effect by circumventing fluctuations of the blood concentration and intra- and inter-patient variations (Sala et al. 2018). "Peak and valley" effect of oral injectable therapy might be avoided by TDD systems which delivers a steady drug flow into the circulation for a longer period of time (Singh et al. 2015). Furthermore, TDD enables controlled release of therapeutic agents across skin layers, due to its simple application and practical handling (Thomas and Finnin 2004). TDD is advantageous as well compared to hypodermic injections, which are painful, produce hazardous medical waste, and pose the danger of disease transmission by needle reuse, particularly in developing countries (Michaels et al. 1975). TDD is ideal for special people, e.g., children, elderly, and convalescent patients (Giannos 2015). In addition to these characteristics, the effortlessness of drug application and termination of treatment aid to augment patient compliance. Accordingly, transdermal route has a lot to praise (Perumal et al. 2013).

The aspects limiting the success of TDD approach comprise skin irritation at site of application and other side effects allied with definite therapeutic agents and formulations, restriction on the drug dose which might be delivered transdermally, a lag time related to the drug delivery through the skin which results in a delay in onset of action due to absorption rate variation depending on application site, skin condition (absorption might be delayed, particularly for water-soluble compounds), and diverse adhesive efficacy in different persons (Tanner and Marks 2008).

Maybe the biggest challenge facing the TDD is that only a small number of therapeutic agents are applicable by this route of administration (Prausnitz and Langer 2008). When designing TDD systems, the physicochemical properties of the therapeutic agents are of great significance and require to be considered in formulation of transdermal systems (Watkinson et al. 2016). Skin anatomy is the main barrier governing the limitations of TDD (Prausnitz et al. 2004). This is mostly ascribed to the substantial efficiency of the skin barrier (mainly its uppermost layer, the stratum corneum) in hindering drug permeation (Jepps et al. 2013). Guessing the permeability of a specified drug molecule across the skin is generally relatively hard, because of the extremely complex nature of the mechanisms and structures that comprise the delivery pathway (Jepps et al. 2013).

5.3.1 Strategies to Overcome the Skin Barrier

In order to circumvent the previously mentioned challenges for TDD, researchers started to investigate improvement approaches to increase the utilization of TDD for skin-impermeable drug molecules (Giannos 2015). Therefore, several methods have been employed to circumvent the skin barrier and may be classified into penetration enhancement by modifying stratum corneum and penetration enhancement via optimizing of drug and vehicle characteristics.

Penetration Enhancement by Modifying Stratum Corneum

Hydration

Using water is the safest and the most extensively employed technique to enhance skin permeation of both hydrophilic (Behl et al. 1980) and lipophilic penetrants (McKenzie and Stoughton 1962). Excess water inside the stratum corneum can modify penetrant solubility and thus alter partitioning from the vehicle into the membrane. Additionally, augmented hydration of skin can promote swelling and opening the stratum corneum structure which leads to an enhancement of permeation, even though this has yet to be confirmed experimentally (Benson 2005).

Chemical Penetration Enhancers

Penetration enhancers are defined as agents capable of modifying the barrier function acquired by the skin (Finnin and Morgan 1999). Preferably, these agents should be nontoxic, pharmacologically inert, nonallergenic, nonirritating, compatible with the therapeutic agent and excipients, tasteless, odorless, colorless, cheap and possess good solvent characteristics. The penetration enhancer shouldn't direct to the

loss of electrolytes, body fluids, or other endogenous compounds, and after its removal, skin should regain its barrier functions right away (Sinha and Kaur 2000).

There are a diversity of mechanisms for penetration enhancement by the penetration enhancers (Kalbitz et al. 1996). One option is the interaction of the penetration enhancers with the polar head groups of the lipophilic structure of the stratum corneum. Thus, the lipid–lipid head group interactions and the packing order of the lipids are disturbed. Consequently, the diffusion of hydrophilic therapeutic agents is facilitated (Walker and Smith 1996). Besides their influence on stratum corneum lipids, chemicals, e.g., dimethyl sulfoxide, surfactants, and urea, interact with keratin in the corneocytes as well (Walters et al. 1988). It has been proposed that entrance of a surfactant into the intracellular matrix of the stratum corneum, after interacting and binding with the keratin filaments, can result in disrupting the order inside the corneocyte (Benson 2005).

The enhancement of drug solubility as well as its partition coefficient (skin/vehicle) is an additional mechanism demonstrating the action of penetration enhancers (Hadgraft 2001). Numerous solvents (e.g., propylene glycol, ethanol, N-methyl pyrrolidone, and Transcutol) increase penetrant solubility and partitioning into the stratum corneum (Benson 2005).

Physical Penetration Enhancement

This approach comprises the application of different energy forms (such as sound, heat, light, magnetic, electrical, etc.), or weakening, reducing, or breaching the stratum corneum barrier by mechanical methods (Grice et al. 2012). Several techniques have been employed for physical enhancement of skin penetration (Table 5.1). For example, microneedles are synthesized by reactive ion-etching methods so as to produce microscopic arrays of needles (Giannos 2015). Following the insertion of these needles into the skin, they pierce the stratum corneum and generate micropores for drug transport through the stratum corneum (Giannos 2014). Another promising approach is iontophoresis which involves the assisted movement of ions through a membrane governed by a small externally applied electrical potential difference (0.5 mA/cm^2 or less) (Green 1996). The mechanisms of transdermal iontophoresis comprise electroporation (increasing the porosity of skin due to electric

Table 5.1 Physical penetration enhancement techniques

	Technique	Description
1	Microneedles	Reactive ion etching
2	Iontophoresis	Electrical potential assisted movement of ions
3	Phonophoresis or sonophoresis	Ultrasound assisted skin penetration enhancement
4	Microdermabrasion	Penetration enhancement via selective removal of the stratum corneum
5	Thermal ablation	Penetration enhancement via selective removal of the stratum corneum following focused heat application

field), electrophoresis (electric field–charge interaction), and electroosmosis (electric field-induced solvent flow) (Yan et al. 2005). On the other hand, phonophoresis (or sonophoresis) enhances the skin penetration of drugs via ultrasound energy (Mitragotri 2005). The ultrasound improves drug diffusion by temporary cavitation (Oberli et al. 2014). Selective removal of the stratum corneum, known as microdermabrasion, is also introduced as a way to enhance skin penetration of drugs. The deepness of cut relies on patient's condition. This method is beneficial to large molecular weight therapeutic agents such as peptides, insulin, and vaccines (Marwah et al. 2016). Thermal ablation is an analogous method in which micro-channels are formed by selectively removing the stratum corneum following focused heat application (Arora et al. 2008). This may be attained, for instance, with a microarray of heating elements (Badkar et al. 2007) or radiofrequency sources (Sintov et al. 2003) to produce temporary elevations in temperature above 100 °C.

Penetration Enhancement Through Optimization of Drug and Vehicle Properties

Optimization of Drug Properties

Drug permeation across the skin is mainly considered to be by diffusion: the "random walk" of therapeutic agents across the different layers of the skin. This walk can be mediated by active transport (whereby protein transporters facilitate drug transport in certain environments) and convective transport (whereby molecules are driven by local currents in the lymphatic or vascular systems or interstitial spaces). Uptake into the vascular or lymphatic systems impacts upon drug distribution, as well as facilitating drug clearance. Aside from clearance, drug can be efficiently removed through metabolism in the skin as well (Jepps et al. 2013).

At this time, drugs marketed as TDD products exhibit three common properties comprising low–moderate lipophilicity (log P 1–3), molecular weight (<500 Da), and good potency (typically, <10 mg/day) (Perumal et al. 2013). If a therapeutic agent acquires these ideal properties (such as nitroglycerin and nicotine), TDD is possible. Nevertheless, manipulation of the therapeutic agent or vehicle to improve permeation becomes essential if it does not exhibit ideal physicochemical characteristics (Benson 2005). Transdermal route is difficult to utilize for the delivery of hydrophilic drugs (Prausnitz and Langer 2008) and has posed particular challenges for macromolecules and peptides comprising new genetic engineering using DNA or small-interfering RNA (Foldvari et al. 2006). For instance, methods utilized to manipulate the therapeutic agent for improved permeation encompass techniques as prodrug, supersaturation, ion pairing, and eutectic mixtures.

Transdermal delivery of therapeutic agents that possess unfavorable solubility or partition coefficient might be enhanced by prodrug approach (Sloan and Wasdo 2003). A pro-moiety is principally incorporated to improve the permeation of therapeutic agent through the stratum corneum. Afterwards, parent drug is released by hydrolysis in the viable epidermis (Barry 2001).

Supersaturation is another reliable approach to improve drug penetration across the skin layers. This takes place by raising the concentration of the dissolved drug that doesn't harm the stratum corneum integrity at all (Alexander et al. 2012). At this point exists a correlation between drug concentration and drug permeation potential that eventually leads to an augmented drug flux with improved thermodynamic drug activity (Latsch et al. 2003). Elevation of drug flux of five- to tenfold has been revealed from supersaturated solutions of several therapeutic agents (Kemken et al. 1992; Pellett et al. 1994, 1997; Iervolino et al. 2001; Moser et al. 2001a, b; Dias et al. 2003). These systems are intrinsically unstable and need the addition of anti-nucleating agents to increase stability (Benson 2005).

Ion pairing can be employed in case of charged therapeutic agents which don't easily permeate across or partition into the human skin (Benson 2005). The ion pair subsequently detach in the aqueous viable epidermis releasing the parent charged therapeutic agents that can permeate inside the epidermal and dermal tissues (Megwa et al. 2000a, b; Valenta et al. 2000).

Melting point of a therapeutic agent is inversely proportional to its solubility and lipophilicity. Consequently, reducing the melting point acquires enhanced transdermal delivery (Alexander et al. 2012). For this approach, eutectic mixtures are feasible (a mixture of two components which, at a certain ratio, its crystalline phase is inhibited) so that melting point of two components is less than the single component (Fiala et al. 2010).

Optimization of Vehicle Properties

Numerous methods aimed to interrupt intercellular lipids in a challenge to improve drug permeation through the healthy skin. One of the most suitable techniques is the employment of vesicular carriers as TDD systems (Marwah et al. 2016). Employing vesicular carrier systems in DDD and TDD could offer many advantages. First of all, they act as drug carriers into or through the skin (Garg and Goyal 2014). They also function as penetration enhancers for the permeation by changing the intercellular lipids in the skin layer (Garg et al. 2012b). Moreover, vesicular carriers serve as a depot for sustained release (Garg et al. 2012a). Additionally, they serve as a rate-limiting membrane barrier for the modulation of systemic absorption, thus affording a controlled TDD (Garg et al. 2011).

5.4 Vesicular Carriers

Vesicles can be defined as water-filled colloidal particles with walls consisting of amphiphilic molecules arranged in a bilayered structure (Honeywell-Nguyen and Bouwstra 2005). In the presence of excess water, these amphiphilic molecules have the ability to form one (unilamellar) or more (multilamellar) concentric bilayers (Gregoriadis and Florence 1993). The internal aqueous compartment can entrap

hydrophilic drugs, while amphiphilic, lipophilic, and charged hydrophilic drugs can be associated with the vesicle bilayer by hydrophobic and/or electrostatic interactions (Martin and Lloyd 1992).

Various types of lipids employed in the fabrication of lipid-based vesicular carriers possess structural similarities with those comprising epidermis and especially the stratum corneum (Sala et al. 2018). They exhibit good biocompatibility, and assist in enhancing skin permeation through different mechanisms as proposed by Zhai and Zhai. (Zhai and Zhai 2014) (Fig. 5.1). Subsequent to sticking to the skin surface, lipid-based vesicular carriers are able to disturb the stratum corneum via different mechanisms, e.g., looseness of the structure or lipid exchanges, stratum corneum fluidization, and polarity alteration after an increased hydration (Zhai and Zhai 2014).

A broad range of lipids and surfactants can be utilized for vesicle preparation. Most frequently, the vesicles are made of phospholipids or non-ionic surfactants (Bouwstra and Hofland 1994; Crommelin and Schreier 1994). Accordingly, vesicular carriers can be divided into two major classes: liposomes, which are made up of phospholipids, discovered by Bangham in 1960 (Bangham 1995), and niosomes, non-ionic surfactant vesicles (discovered by L'Oreal in the 1970s) (Vanlerberghe and Handjani-Vila 1975).

5.4.1 Vesicular Carriers for Skin Delivery

Vesicular carriers have usually been used for dermal and transdermal delivery of drugs. They are typically composed of biocompatible lipids besides an aqueous phase which may be water, a buffer solution, or a cosolvent. Vesicles, due to their lipophilic nature, can theoretically partition into the skin layers and transport the entrapped drug across stratum corneum (Jain et al. 2017).

A variety of vesicular carriers with unique structural and functional characteristics have been introduced and modified in the last four decades in order to increase skin permeation potential. The first-generation lipid-based vesicular carrier (liposomes) was first reported in the field of skin delivery by Mezei and Gulasekharam in 1980 (Mezei and Gulasekharam 1980, 1982). However, the success of liposomal delivery was restricted by its vesicle size, ranging from 200 to 800 nm, and rigidity, which can hinder skin permeation (Verma et al. 2003; Jain et al. 2015). In 1992, Cevc and Blume introduced the second-generation vesicular carrier named ultradeformable liposomes or transfersomes®, characterized by higher elasticity (5–8 times more elastic than conventional liposomes) and smaller vesicle size (<300 nm) (Cevc and Blume 1992; Song et al. 2012). Because vesicles are mainly in nanosize range, they can further boost skin delivery of their drug load. In general, it is suggested that vesicles larger than 600 nm are unable to penetrate the deeper skin layers and stay in or on the stratum corneum. On the other hand, vesicles smaller than 300 nm can penetrate more deeply, while vesicles ≤70 nm can reach both epidermal and dermal layers (Verma et al. 2003). A third-generation vesicular carrier called ethosomes

(ethanol-based elastic lipid vesicles) was developed in 2000 by Touitou et al. (2000a). The unique physicochemical properties of ethosomes responsible for improved skin permeation are smaller vesicle size (<300 nm) and higher elasticity (10–30 times higher than conventional liposomes) besides the permeation enhancement effect of ethanol (Song et al. 2012; Jain et al. 2015).

More recently, various modifications of these vesicular carriers are also explored to provide specific structural or functional attribute for skin delivery. Each of these vesicles has its specific features, mechanism of drug delivery, advantages, and challenges. The following section discusses the vesicular carriers in detail.

Conventional Liposomes

Conventional liposomes are typically composed of synthetic or natural phospholipids which are the major component of most biological membranes (Sala et al. 2018). Phospholipids have the ability of spontaneous self-assembly in aqueous media and to form one or several bilayers (Fig. 5.2). They are widely used as drug carriers for hydrophilic and lipophilic molecules (Yoshida et al. 2010). Both natural and synthetic phospholipids are used for liposome preparation, but naturally occurring phosphatidylcholines (e.g., from soy or egg) are most commonly used due to toxicological considerations and relatively low cost (Kulkarni et al. 1995).

Cholesterol is often included to improve bilayer stability in the presence of biological fluids. Hence, cholesterol reduces permeability and prevents leakage of the entrapped drug (Sala et al. 2018) by increasing the gel (stable) to liquid crystalline (metastable) state transition temperature of the lipid bilayer (Bennett et al. 2009; Jain et al. 2017). Neutral pH buffers and antioxidants such as sodium ascorbate could also be incorporated to limit oxidation of the phospholipids (Yatvin and Lelkes 1982). Liposomes could be classified based on their size and lamellarity into multilamellar vesicles having a size >0.5 μm, small unilamellar vesicles with a size range of 20–100 nm, and large unilamellar vesicles with a size >100 nm (Sherry et al. 2013).

Methods of Liposome Preparation

The most frequently used conventional techniques for liposome preparation include thin-film hydration (Jain et al. 2015), reverse-phase evaporation (Parnami et al. 2014), and solvent injection techniques (Jaafar-Maalej et al. 2010). Thin-film hydration is most frequently used in studies involving skin delivery. In this method, lipids are dissolved in the organic solvent which is then removed by means of evaporation (using a rotary evaporator under reduced pressure) leaving behind a dry, thin lipid film on the wall of the flask. Finally, the dry lipid film is hydrated by aqueous phase (while vortexing the content) to obtain liposomes. Processing parameters such as hydration time, hydration temperature, and vortexing speed may affect vesicle size

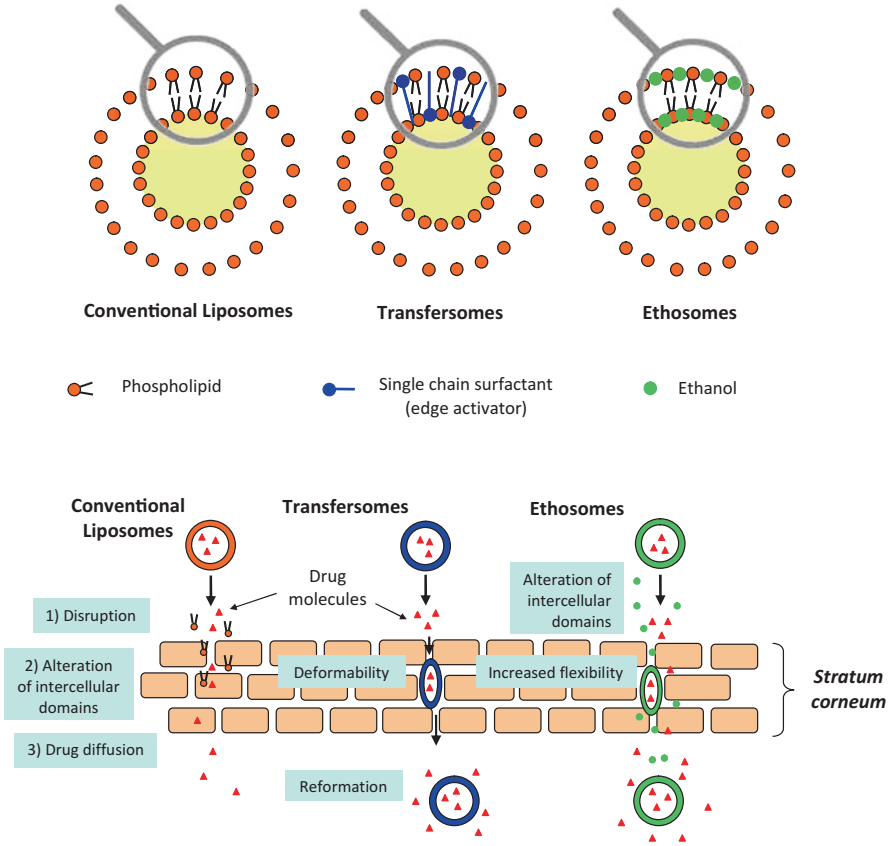

Fig. 5.2 Schematic illustration of conventional liposomes, transfersomes, and ethosomes and their main permeation mechanisms through the stratum corneum

and entrapment efficiency of the produced vesicles and change its skin permeation (Patel 2013).

The reverse-phase evaporation technique is comprised of two steps. Firstly, a water-in-oil emulsion of phospholipids and buffer in excess organic phase is prepared. This is followed by removal of the organic phase under reduced pressure. The two phases (phospholipids and water) are usually emulsified by mechanical methods or by sonication. Removal of the organic solvent under vacuum causes the phospholipid-coated water droplets to get together leading to the formation of a gel-like matrix (Vemuri and Rhodes 1995). Further removal of organic solvent will cause the gel-like matrix to form into a paste of smooth consistency. This paste is a suspension of large unilamellar vesicles (Szoka and Papahadjopoulos 1980).

The solvent injection methods involve the dissolution of the lipid into an organic phase (ethanol or ether), followed by the injection of the lipid solution into aqueous media, forming liposomes (Laouini et al. 2012). In ether injection method, a

solution of lipids (usually dissolved in diethyl ether or in ether–methanol mixture) is slowly injected to an aqueous solution of the drug at 55–65 °C under reduced pressure. The resulting removal of ether under vacuum leads to the vesicle formation (Akbarzadeh et al. 2013). The ethanol injection method was first described by Batzri and Korn (1973). In this method, the lipids dissolved in ethanol are rapidly injected into an excess amount of aqueous media resulting in a spontaneous formation of liposomal vesicles (Vemuri and Rhodes 1995).

Most of conventional technologies have major drawbacks. Besides problems of scale-up, thin-film hydration technique utilizes organic solvent and renders larger vesicle size liposomes (Jain et al. 2015). The main drawback of the reverse-phase evaporation technique is the contact of the materials to be encapsulated to organic solvents and to periods of sonication (Akbarzadeh et al. 2013). The ether injection technique has the main disadvantages of producing a heterogeneous vesicle population and the exposure of the drugs to be encapsulated to organic solvents at high temperature (Deamer and Bangham 1976; Schieren et al. 1978). The ethanol injection method results in a very dilute liposomal dispersion with heterogeneous population (Akbarzadeh et al. 2013). Also, it is difficult to remove all of the ethanol which may affect various biologically active macromolecules that tend to inactivate in the presence of even low amounts of ethanol (Batzri and Korn 1973). Recently, more sophisticated techniques have been employed in liposome preparation for skin delivery application. Examples include supercritical fluid (Xia et al. 2012; Xu et al. 2015), dual asymmetric centrifugation (Hirsch et al. 2009), and microfluidic channels (Hood et al. 2014; Zhou et al. 2014).

Supercritical fluid technology provides a green, nontoxic, cheap, and scalable substitute to conventional liposome preparation methods (Lesoin et al. 2011a). In this technique, phospholipids and cholesterol are dissolved in supercritical CO_2 and then allowed to precipitate in the form of ultrafine lipid particles. The aqueous medium is then added to consequently form liposomal vesicles. Several researchers have reported promising results using supercritical fluid technology (Otake et al. 2006; Lesoin et al. 2011b).

Dual asymmetric centrifugation is another novel technology for liposome preparation (Massing et al. 2008). In this unique advanced centrifugation technique, conventional centrifugation rotational force moves the sample outward, while additional rotational force is provided to move the sample towards the center of the centrifuge. This exceptional combination of two contrarotational movements causes shearing of the sample, thus resulting in liposome formation (Massing et al. 2008).

In microfluidic channels, a recent widely employed technique, liposomes are formed when a stream of the lipid alcoholic solution is passed through two aqueous streams in a microfluidic channel (Jahn et al. 2007; Sugiura et al. 2008; van Swaay and deMello 2013; Hood et al. 2014). The laminar flow in the channels makes possible to control the size and size distribution of the prepared liposomes. It was demonstrated that liposome vesicle size could be modified from 50 to 150 nm by adjusting alcohol-to-aqueous volumetric flow rate (Jahn et al. 2007).

Effect of Formulation Variables on Skin Penetration

Various studies have focused on the effect of formulation variables (e.g., lipid composition, type of lipid, drug–lipid ratio, concentration and type of surface charge imparting compound, etc.) on the physicochemical properties and skin permeation behavior of liposomes (Bhatia et al. 2004; Puglia et al. 2010; Ruozi et al. 2010). Jain et al. investigated the effect of lipid composition on entrapment efficiency, vesicle size, elasticity, and skin permeation of diclofenac-loaded liposomes (Jain et al. 2015). It was found that the change in phosphatidylcholine/cholesterol ratio from 1:1 to 9:1 (w/w) led to a decrease in vesicle size, whereas entrapment efficiency, vesicle elasticity, and drug permeation increased. Cholesterol is embedded in the lipid bilayered structure leading to a reduction in free volume available for drug entrapment, a reduction in motion of the lipid tails which decreases elasticity, and consequently, drug permeation through skin is decreased.

The type of lipid selected for liposome preparation also needs to be carefully evaluated. For instance, better oxidative stability is obtained in case of soy-based phosphatidylcholine compared to egg-based phosphatidylcholine (which is more saturated) (Li et al. 2015a). In another study, skin permeation behavior of natural lipid (soy phosphatidylcholine and egg phosphatidylcholine) and synthetic lipid (hydrogenated soy phosphatidylcholine) was compared in curcumin-loaded liposomes (Chen et al. 2012). It was found that although vesicle size and drug entrapment were similar, natural lipid-based liposomal formulations exhibited 1.5 times higher skin permeation and 1.7 times higher skin retention compared to synthetic lipid-based formulations. This observation was attributed to the low phase transition temperature of the natural lipids which causes an increase in liposomal fluidity and consequently enhances skin permeation (Jain et al. 2017).

Another important factor that should be considered is the vesicle surface charge. Compared to neutral and negatively charged liposomes, positively charged liposomes have shown enhanced skin permeation apparently due to interaction with negatively charged skin membrane (Katahira et al. 1999; Kitagawa and Kasamaki 2006; Hasanovic et al. 2010). More recently, drug-loaded liposomes are conjugated with cationic cell-penetrating peptide (CCPP) to improve skin membrane penetration of the liposomes (Kwon et al. 2015). In a study, the extract of *Polygonum aviculare* L., having antioxidative and cellular membrane protective activity, was loaded into CCPP-conjugated liposomes for transdermal delivery. In vivo studies showed more efficacy of CCPP-conjugated liposomes in depigmentation and anti-wrinkle potential than conventional liposomes. These results were attributed to the ability of cationic peptide-conjugated liposomes to effectively interact with the intercellular lipid lamellae of the stratum corneum (Kwon et al. 2015). Similar results were observed in case of CCPP-conjugated polymeric liposomes intended for topical delivery of lidocaine (Wang et al. 2013).

Mechanisms of Skin Penetration

Conventional liposomes may interact with the stratum corneum at the surface and with deeper layers under different assumptions described in the literature (Sinico et al. 2005) (Fig. 5.2). The assumption that vesicles could penetrate the skin and reach the dermis while maintaining their integrity was suggested by Foldvari et al. (1990). Yet, observed differences in size and structure of liposomes, before and after skin penetration, made this hypothesized passage of liposomes in their intact form doubtful (du Plessis et al. 1994; Korting et al. 1995; Zellmer et al. 1995). Presence of liposomes in the dermis was rather attributed to a passage via the follicular pathway (Betz et al. 2001). Another theory is that the vesicular structure would break at the stratum corneum surface which will enable penetration of the phospholipids within the stratum corneum, thus promoting skin permeation of the active drug. Phospholipids are well recognized as promoters for skin passage; this action takes place through disturbing the lipid matrix in the stratum corneum (Kato et al. 1987). Several researchers have verified this phospholipid property (Zellmer et al. 1995; Yokomizo and Sagitani 1996a, b); however, it was found to be strongly dependent on phospholipid type with a direct impact on the stiffness or the elasticity of the liposomes (Kirjavainen et al. 1996). A third hypothesis is that the vesicles get adsorbed on the surface of the skin followed by fusion with lipid matrix in the stratum corneum, thus allowing the active molecule to diffuse through skin layers. This "adsorption–fusion" interaction with the stratum corneum leads to the formation of intercellular lipid lamellae and increasing mobility of lipophilic drugs in the stratum corneum (Bouwstra et al. 1992). This was also suggested by Keith and Snipes (Keith and Snipes 1982) who described the melting of the vesicles with the stratum corneum lipids as a kind of extension of the lipid matrix which allows lipophilic active molecules "to flow" more easily. This was also confirmed in studies on tretinoin-loaded liposomes by Sinico et al. (Sinico et al. 2005). The authors have reported the formation of a liposomal lipid film on the skin surface. This proximity allowed exchange of vesicular material with the stratum corneum lipid matrix leading to a change in the phase transition of the new matrix. Furthermore, the aqueous content of the liposomal vesicles led to hydration of the stratum corneum as well as swelling of the fibrous proteins and the intercellular lipids. All of these changes caused destabilization of the stratum corneum structure, leading to increased permeability (Sala et al. 2018).

The last two theories, which are now more widely acceptable, put a limitation on the potential of liposomes as a suitable carrier for drug delivery through deep skin layers. Based on recent studies, it is generally acknowledged that conventional liposomes are not efficient for TDD (Sala et al. 2018). They do not exhibit an efficient permeation into deep skin layers and are mostly confined to upper layers of the epidermis in the intercellular pathways where vesicle rupture takes place due to lack of deformability. Such conduct was expressed by a relatively low percutaneous flux along with high skin retention of the loaded drug compared to flexible vesicles and other lipid-based carriers (Raza et al. 2013). Nevertheless, nanoliposomes with vesicle sizes ranging from 31 to 41 nm showed significant penetration enhancement. Imaging techniques revealed that smaller liposomes pass quickly through the

stratum corneum without vesicle rupture (Hood et al. 2014). This indicates that vesicle size may have the most critical role in liposomal drug delivery through the skin. In addition to vesicle size, several factors could change the skin permeation, such as zeta potential (Gillet et al. 2011a), lipid composition (Gillet et al. 2011a, b), entrapment efficiency of the drug as well as the type of skin disorders (Raza et al. 2013), occlusion condition, and integrity of skin barrier (Trauer et al. 2014).

In summary, studies on skin passage demonstrate the poor benefits of conventional liposomes in the treatment of skin diseases. Nevertheless, it is essential to take into account that these studies seldom considered the pathophysiological aspects when conducting in vitro/in vivo experiments. It has been suggested that liposomes have the ability to penetrate the skin if the skin barrier is impaired like in skin cancers or psoriasis (Korting et al. 1995; Fresta and Puglisi 1996). Yet, studies on the application of conventional liposomes on damaged human skin are still limited. Korting et al. conducted a randomized, double-blind trial comparing the efficacy of a betamethasone liposomal formulation compared to simple gel in patients with atopic dermatitis or psoriasis vulgaris (Korting et al. 1990). Their results revealed that betamethasone-loaded liposomes showed higher efficacy in atopic dermatitis than in psoriasis. The authors attributed this behavior to diseases pathophysiological differences. Hyperkeratosis is a characteristic of psoriasis vulgaris that would slow the passage of liposomes, whereas in atopic dermatitis, the stratum corneum becomes impaired and would enhance their passage (Melnik et al. 1988). Based on this study, conventional liposomes could have a potential in the topical treatment of atopic dermatitis.

Generally, liposomes are now considered to have a low potential as carriers for TDD as they are unable, at normal conditions, to penetrate deep skin layers. Most recent work on liposomes for TDD is essentially for means of comparison with newer vesicular carriers. However, liposomes may still have benefit in treatment of local skin conditions where deeper penetration could be expected in case of damaged or diseased skin.

Transfersomes

Thorough research took place over the last 20 years because conventional liposomes lacked the ability to deliver drugs across the skin. This resulted in the introduction and development of new classes of lipid vesicles (Sinico and Fadda 2009). Thus, a new category of ultraflexible, i.e., highly deformable, liposomes was developed and launched (Elsayed et al. 2007b). In the early 1990s, transfersomes, the first generation of elastic vesicles, were first introduced by Cevc and Blume (Cevc and Blume 1992). The name is derived from the Latin word “transferre” meaning “to carry across” and the Greek word “soma” means “body.” Conclusively it means “carrying body” (Singh et al. 2015).

The structure and method of preparation of these new elastic liposomes are similar to conventional liposomes; however, functionally they are adequately deformed to go through pores much smaller than their own size (i.e., skin pores). Additionally,

unlike conventional liposomes, ultradeformable liposomes are made up of phospholipids and aqueous medium, in addition to edge activators (Jain et al. 2017).

An edge activator is typically a single-chain surfactant, characterized by a high radius of curvature which causes destabilization in lipid bilayers and increases vesicle deformability (Cevc 1996; Cevc et al. 1996; Honeywell-Nguyen and Bouwstra 2005). These surfactant chains are not distributed randomly within the bilayers; they tend to gather at the high pressure points when vesicles are subjected to anisotropic stresses. The vesicle structure (Fig. 5.2) is made less stiff because of the discontinuities generated by the incorporation of the edge activator in the phospholipid bilayer (Kumar et al. 2012). The edge activator has affinities to special regions of phospholipids based on its physicochemical properties, thus leading to the formation of a destabilized bilayer (Sala et al. 2017).

Formulation Considerations

In transfersome-based drug delivery systems, phospholipid is an essential formulation ingredient (Kumar et al. 2012). The most commonly edge activators employed in transfersomes are Spans (Span 60, Span 65, and Span 80), Tweens (Tween 20, Tween 60, and Tween 80), and cholates (sodium cholate and sodium deoxycholate), in addition to dipotassium glycyrrhizinate (Cevc et al. 1998; El Maghraby et al. 1999, 2000a, b; Trotta et al. 2004; Garg et al. 2006; Oh et al. 2006). In a study to evaluate the effect of edge activator type on physicochemical properties of ultradeformable liposomes, sodium cholate and sodium deoxycholate were found to produce vesicle with more positive zeta potential and smaller vesicle size compared to Tween 80 (Lee et al. 2005). Ultradeformable liposomes prepared with 95%:5% (w/w) (phosphatidylcholine/edge activator) ratio showed entrapment efficiency in the following order: Span 85 > Span 80 > Na cholate > Na deoxycholate > Tween 80. The authors attributed these results to the hydrophilic–lipophilic balance (HLB) values of the respective edge activators (El Zaafarany et al. 2010).

The edge activator effect is generally produced with an edge activator/phospholipid ratio not exceeding 25% (Jain et al. 2003; El Zaafarany et al. 2010). The concentration of edge activator also plays a critical role in vesicle formation and properties. Ultradeformable liposomes prepared at different molar fractions of sodium cholate revealed that increasing sodium cholate content above the molar fraction of 0.2 may cause formation of aggregates of sodium cholate and phospholipid (e.g., mixed vesicles, mixed micelles, opened vesicles, and rodlike mixed micelles). This can therefore lead to a decrease in the drug's entrapment efficiency (Paolino et al. 2012). In another study concerning diclofenac-loaded ultradeformable liposomes, an increase in concentration of Span 80 from 2% to 5% resulted in an increase in the entrapment efficiency from 50.73% to 55.19%, respectively (Honeywell-Nguyen and Bouwstra 2005). However, with further increase in edge activator concentration up to 25%, a decrease in entrapment efficiency was observed. This decrease in entrapment efficiency at higher concentration of edge activators was attributed to the formation of micelle aggregates (El Zaafarany et al. 2010).

Mechanism of Skin Penetration

Depending on the structure of transfersomes and the hydrophilicity of the entrapped drug, recent studies show that the transport of transfersomes across the skin involves the combination of two key factors (Pirvu et al. 2010). These factors are the presence of an osmotic gradient across the skin and high elasticity of the transfersomal bilayers (Rai et al. 2017).

Cevc et al. suggested that the vesicles enter the stratum corneum intact, carrying drug molecules into the skin (Cevc and Blume 1992). The driving force for vesicles to enter the skin was thought to be xerophobia (i.e., the tendency to avoid dry surroundings) (Cevc and Blume 1992). Owing to their deformable nature, these vesicles are thought to be able to squeeze through the stratum corneum and into the deeper skin layers intact, under the influence of transcutaneous hydration gradient which is naturally present (Jain et al. 2017). Upon application of the elastic vesicles on the skin surface that is partially dehydrated, they move towards the deeper skin layers (e.g., viable epidermis and dermis) that are relatively hydrated. The deformable character of the vesicles acts to alleviate the stress induced during movement to deeper skin layers (Jain et al. 2017).

This ability of transfersomes to pass through skin is highly dependent on their membrane flexibility, which can be achieved using an appropriate ratio of edge activators. In this process, components of the transfersomes responsible for its deformability accumulate at the site of stress, whereas the less flexible components undergo dilution. This leads to a significant reduction of the active rate of membrane deformation and allows the highly flexible particles to pass through the pores (Kumar et al. 2012). Accordingly, transfersomes can deform and easily pass through the intercellular spaces of the stratum corneum. In fact, it is observed that the penetration of transfersomes was diminished in case of disturbance of osmotic gradient due to skin wetting (Morrow et al. 2007).

Merits of Transfersomes

Transfersomes have been employed as carriers for various therapeutic agents and have been confirmed to greatly enhance drug permeation through the skin (Cevc and Blume 1992). Examples of recent research involving transfersomes for skin delivery are presented in Table 5.2. On the other hand, difficulty of loading lipophilic drugs into the vesicles without negatively affecting their deformability and elastic properties is considered a major drawback of transfersomes (Chen et al. 2013). Transfersomes can be easily applied to the skin. They require no sophisticated procedure, and they can be applied by a non-occluded method, where hydration or osmotic force within the skin causes them to pass through the multilayered lipid matrix of the stratum corneum (Cevc 1996).

On the other hand, special care should be given to the amount of edge activator incorporated in transfersomes to avoid aggregation or micelle formation. Another shortcoming of transfersomes is that they are not suitable for use in occlusive

Table 5.2 Recent studies considering transfersomes as skin delivery system

	Drug	Indication	References
1	Apigenin	Treatment of skin cancer	Jangdey et al. (2017)
2	Asenapine maleate	Antipsychotic	Shreya et al. (2016)
3	Clindamycin phosphate	Antibacterial	Abdellatif and Tawfeek (2016)
4	Epigallocatechin-3-gallate / hyaluronic acid	Antioxidant/antiaging	Avadhani et al. (2017)
5	Eprosartan mesylate	Antihypertensive	Ahad et al. (2017)
6	Paclitaxel	Anticancer	Pathak et al. (2016)
7	Paeonol	Antiallergic/ antiinflammatory	Chen et al. (2017)
8	Pentoxifylline	Peripheral artery disease	Al Shuwaili et al. (2016)
9	Piroxicam	Antiinflammatory	Garg et al. (2017)
10	Sildenafil	Erectile dysfunction	Ahmed (2015)
11	Sinomenine hydrochloride	Antirheumatic	Wang et al. (2017)
12	Timolol maleate	Antihypertensive	Morsi et al. (2016)

conditions. This is because non-occlusive conditions are crucial for the creation of a transepidermal osmotic gradient, which is the main force driving the transport of these elastic vesicles through the skin.

Ethosomes

Ethosomes are a new generation of elastic lipid carriers that have exhibited enhanced delivery for both hydrophilic and lipophilic drugs through the skin (Jain et al. 2017). Although the concept of ethosomes is rather sophisticated, these carriers are suitable for skin delivery due to their improved efficacy and safety along with the simplicity of their preparation (Godin and Touitou 2003). Ethosomes are lipid vesicles composed of phospholipids and a large amount of ethanol (Sala et al. 2017). They have been described for the first time by Touitou et al. (Touitou et al. 2000a). Their name was selected to stress the presence of high concentrations of ethanol (usually ranging from 20% to 45%) (Sinico and Fadda 2009). It was generally considered that high amounts of ethanol have a detrimental effect on structure of liposomes, owing to the interdigitation effect of alcohol on the lipid bilayers (Fig. 5.2). However, the existence of vesicles as well as the ethosome structure was proven by several techniques such as phosphorus nuclear magnetic resonance and transmission and scanning electron microscopy (Müller et al. 2004). Foldvari et al. described liposomal formulations that contained up to 10% ethanol and up to 15% propylene glycol (Foldvari et al. 1993). However, Touitou et al. were the first to report the use of high concentration of ethanol (Touitou et al. 2000a).

Ethosomes are considered a modification of conventional liposomes that consist of phospholipids, water, and a high concentration of ethanol (Abdulbaqi et al. 2016). The superiority of ethosomes over conventional liposomes for TDD was reported as

they were smaller and having higher entrapment efficiency and negative zeta potential. Moreover, compared to conventional liposomes, ethosomes showed better skin permeation and stability profiles (Touitou et al. 2000a; Sarwa et al. 2014; Jain et al. 2015).

Formulation Considerations

Ethosomal systems are given their unique identity due to the vital role played by alcohol (Pandey et al. 2015). Ethanol is an efficient penetration enhancer (Finnin and Morgan 1999). Alcohol provides the ethosomal vesicles with distinct attributes concerning their entrapment efficiency, vesicle size, zeta potential, stability, and enhanced skin permeability (Abdulbaqi et al. 2016). Ethosomal systems having an amount of alcohol ranging from 10% to 50% have been reported (Touitou 1996; Puri and Jain 2012). It is fairly important to optimize the amount of ethanol in the system as it affects the entrapment efficiency, vesicle size, stability, and safety of the produced vesicles (Pandey et al. 2015). Ethanol concentration in the range of 30–40% was reported by several investigators as the optimal choice for the successful production of stable ethosomal vesicles (Limsuwan and Amnuaikit 2012; Chiu et al. 2013; Zhao et al. 2013).

Ethanol has a considerable effect on the capacity of ethosomal system to encapsulate drugs; generally an increase in entrapment efficiency is observed with increasing ethanol concentration (Jain et al. 2004). This effect is applicable in case of drugs with varying lipophilicities, where the increase in drug loading is attributed to increased solubility of lipophilic and amphiphilic drugs in the presence of ethanol. This behavior was found to be linear in case of ethanol amounts ranging from 20% to 40% (Prasanthi and Lakshmi 2012a). However, entrapment efficiency is decreased with further increase in ethanol concentration, probably due to excessive vesicular fluidization causing leakage of the drug (Dubey et al. 2010; Jain et al. 2015). Though ethanol is the alcohol usually incorporated in ethosomes, isopropyl alcohol has been shown to cause better entrapment efficiency than ethanol (Touitou et al. 2000a).

Ethanol also has a governing role on the size of the ethosomal vesicles through giving the vesicular surface a net negative charge, leading to a decrease in vesicle size (Pandey et al. 2015). A shift in the vesicular charge from positive to negative is reported with high concentrations of ethanol (Touitou et al. 2000a; Rao et al. 2008). Considering empty ethosomes, negative charge was found to increase by the increase in ethanol concentration as reported by Dayan and Touitou (Dayan and Touitou 2000). Similar findings were also reported by other researchers (Touitou et al. 2000a; Lopez-Pinto et al. 2005; Zhaowu et al. 2009; Liu et al. 2011; Li et al. 2012; Patel et al. 2012; Rakesh and Anoop 2012; Ahad et al. 2013). However, an increase in the amount of ethanol beyond the optimum level might lead to a minor increment in vesicle size and cause the bilayer to be leaky, and by further increasing in ethanol concentration, it would solubilize the vesicles (Abdulbaqi et al. 2016).

Mechanism of Skin Penetration

The suggested means of permeation enhancement by ethosomes are based on the dual effect of ethanol on lipid bilayers in the stratum corneum and in the vesicles (Touitou et al. 2000a, b; Godin et al. 2005; Touitou and Godin 2005; Ainbinder et al. 2010). This effect has been suggested as follows (Touitou et al. 2000a): ethanol, being a well-known penetration enhancer, disturbs the organization of stratum corneum lipids by both enhancing the lipid fluidity and decreasing the density of the intercellular lipid domains. The ethanol-containing vesicles would alter the intercellular lipid lamella, hence creating their own pathways across the disturbed stratum corneum to deeper skin layers. Ethanol acts to increase the vesicle fluidity and flexibility by increasing the mobility of polar lipid heads of lipid molecules. Such increased elasticity facilitates vesicle crossing in the disturbed intercellular narrow pathways.

In conclusion, presence of ethanol in ethosomal vesicle composition leads to fluidization of the vesicular bilayers along with changes in the arrangement of stratum corneum lipids. Ethosomes are then able to penetrate the altered stratum corneum barrier, releasing the drug in deeper skin layers (Ainbinder et al. 2016). This theory is supported by the fact that a great enhancement of permeation was observed from ethosomes when compared to conventional liposomes or any of the individual system components (Sala et al. 2017).

Merits of Ethosomes

When prepared employing normal preparation methods, ethosomes tend to have vesicle sizes smaller than other vesicular systems (when size reduction steps are excluded). The acquired negative charge to the system causes the size of vesicles to decrease, eventually enhancing bioavailability of drugs (Lopez-Pinto et al. 2005; Elsayed et al. 2007a, b). Ethosomes have the ability to efficiently entrap molecules with a wide range of physicochemical characteristics, including lipophilic, hydrophilic, and high molecular weight entities (Touitou et al. 2000a; Godin and Touitou 2003, 2004). They have the ability to enhance skin delivery of drugs both under occlusive (Dayan and Touitou 2000; Ainbinder and Touitou 2005; Lopez-Pinto et al. 2005; Paolino et al. 2005) and non-occlusive conditions (Dayan and Touitou 2000; Elsayed et al. 2007a), in contrast to deformable liposomes. Examples of recent research concerning ethosomes for skin delivery are found in Table 5.3.

Ethosomal system bears no outsized scale drug development risk since the toxicological profiling of ethosome components is finely acknowledged in scientific literature (Touitou et al. 2001).

Table 5.3 Recent studies considering ethosomes as skin delivery system

	Drug	Indication	References
1	5-Fluorouracil	Treatment of skin cancer	Khan and Wong (2016)
2	Crocin	Antioxidant	Esposito et al. (2016)
3	Cryptotanshinone	Treatment of acne	Yu et al. (2016)
4	Finestride	Treatment of androgenic alopecia	Wilson et al. (2017)
5	Glimepiride	Antidiabetic	Ahmed et al. (2016)
6	Griseofulvin	Antifungal	Marto et al. (2016)
7	Lidocaine	Local anesthetic	Babaie et al. (2015)
8	Lornoxicam	Analgesic/Antiinflammatory	Li et al. (2017)
9	Methoxsalen	Treatment of vitiligo	Garg et al. (2016)
10	Mitoxantrone	Anti-melanoma	Yu et al. (2015)
11	Phenylethyl resorcinol	Lightening agent in skin care products	Limsuwan et al. (2017)
12	Sertaconazole	Antifungal	Abdellatif et al. (2017)
13	Terbinafine hydrochloride	Antifungal	Iizhar et al. (2016)
14	Tropisetron hydrochloride	Antiemetic	Abdel Messih et al. (2017)
15	Vancomycin hydrochloride	Antibacterial	Mohammed et al. (2016)
16	Voriconazole	Antifungal	Faisal et al. (2016)

Niosomes

Niosomes are vesicles comprised essentially of non-ionic surfactants subjected to hydration. Cholesterol or its derivatives could also be incorporated. Niosome could be described as non-ionic surfactant-based liposome (Singh et al. 2015). The unique structure of niosomes makes them able of entrapping hydrophilic as well as lipophilic materials. Hydrophilic therapeutic agents could be entrapped in aqueous core or adsorbed on the bilayer surfaces, whereas lipophilic ones could be partitioned inside the bilayer's lipophilic domain (Moghassemi and Hadjizadeh 2014). After the formation of a thin film, hydration takes place and the liquid crystalline bilayers transform to fluid and swell, and hence niosomes are formed. Mechanical agitation attempts the detachment of the hydrated sheets and the self-assembly to form vesicles as well as avoids the interaction of hydrocarbon core of the bilayer with water at the edges (Pardakhty and Moazeni 2013). The first production line of niosomes was initiated from cosmetic industry; afterwards, the approach of niosomes in the field of drug delivery was investigated (Pardakhty and Moazeni 2013).

Since then, niosomes have been one of the most prominent vesicular systems and have recently been the center of great interest for their potential as drug delivery systems for different routes of administration. Examples of recent research involving niosomes for skin delivery are presented in Table 5.4. Niosomes avoid many of the shortcomings of other vesicular carriers which makes them a promising drug

Table 5.4 Recent studies considering niosomes as skin delivery system

	Drug	Indication	References
1	5-aminolevulinic acid	Treatment of skin malignancies	Bragagni et al. (2015)
2	8-methoxypsoralen	Treatment of psoriasis	Kassem et al. (2017)
3	Acyclovir	Antiviral	Jacob et al. (2017)
4	Caffeine	Anticellulite	Teaima et al. (2018)
5	Diclofenac	Antiinflammatory	Ioele et al. (2015)
6	Lacidipine	Antihypertensive	Qumbar et al. (2017)
7	Lornoxicam	Analgesic/antiinflammatory	El-Ridy et al. (2017)
8	Luteolin	Antiarthritic	Abidin et al. (2016)
9	Methotrexate	Treatment of psoriasis	Zidan et al. (2017)
10	Methotrexate	Treatment of psoriasis	Abdelbary and AbouGhaly (2015)
11	Pregabalin	Antiepileptic	Arafa and Ayoub (2017)
12	Salidroside	Antidepressant	Zhang et al. (2015)
13	Simvastatin	Antihyperlipidemic	Zidan et al. (2016)
14	Ursolic acid	Antiarthritic	Jamal et al. (2015)

delivery system with numerous applications. They also are capable of entrapping a variety of drugs, genes, proteins, and vaccines (Moghassemi and Hadjizadeh 2014).

According to niosome size, they may be divided into three categories depending on their method of preparation (Moghassemi and Hadjizadeh 2014; Singh et al. 2015), small unilamellar vesicles (10–100 nm), large unilamellar vesicles (100–3000 nm), and multilamellar vesicles where more than one bilayer is present (Kaur et al. 2004). The most common methods of preparation include thin-film hydration technique, ether injection method, bubble method, and reverse-phase evaporation technique (Mujoriya and Bodla 2011).

Effect of Formulation Variables

Niosome properties are potentially affected by several formulation variables. Generally, niosomes are formulated by suitable available raw components. Non-ionic surfactants are the basic components of niosomes (Moghassemi and Hadjizadeh 2014).

Non-ionic Surfactants

Surfactants are considered a distinctive class of materials characterized by being amphiphilic in nature. A surfactant molecule has two distinctive regions: a hydrophilic, water-soluble part and a lipophilic, organic-soluble part. The hydrophilic head group involves functionalities such as sulfonates, phosphonates, carboxylates, and ammonium derivatives. On the other hand, the lipophilic region usually consists of chains made up of fluorocarbons, alkanes, aromatic, or other nonpolar groups.

Surfactants can be classified according to their hydrophilic head group into anionic (e.g., sulfonate head group), cationic (e.g., quaternary ammonium salt head group), amphoteric (e.g., zwitterionic butane head group), and non-ionic (e.g., fatty acid head group) (Steed et al. 2007).

Non-ionic surfactants are characterized by having no charged groups in their hydrophilic heads. In solutions, they self-assemble to a characteristic structure in which hydrophilic heads are facing the aqueous solutions and hydrophilic tails are facing organic solutions. Due to this characteristic behavior, niosomes are formed by the self-assembly of non-ionic surfactants in aqueous dispersions (Moghassemi and Hadjizadeh 2014). The usually employed non-ionic amphiphiles, which are used in niosome preparation, have four categories: alkyl amides, alkyl esters, alkyl ethers, and ethers of fatty acids (Kumar and Rajeshwarrao 2011). The selection of surfactant relies on the HLB and critical packing parameter (CPP) values which are elucidated below.

Hydrophilic–Lipophilic Balance (HLB)

HLB is a dimensionless parameter which saves time and guides for proper selection of surfactants. For non-ionic surfactants, the HLB range is from 0 to 20 where a low HLB refers to a lipophilic surfactant, whereas a hydrophilic surfactant will have a high HLB value.

In niosomal vesicles, the surfactant HLB value plays an important role in controlling drug entrapment efficiency (Kumar and Rajeshwarrao 2011). Many non-ionic surfactants with a variety of HLB values have been employed for preparation of niosomes. Examples include glucosyl dialkyl ethers, polyglycerol alkyl ethers, crown ethers, polyoxyethylene ethers, and esters such as series of Brijs, Tweens, and Spans (Biswal et al. 2008; Shilpa et al. 2011).

Surfactants with an HLB value ranging from 3 to 8 are favorable for preparing bilayer structures and are considered water-in-oil (W/O) emulsifiers. In addition, oil-in-water (O/W) emulsifiers possess HLB numbers between 8 and 18 (Abdallah et al. 2013).

Critical Packing Parameter (CPP)

Besides the HLB value, chemical structure and a range of other factors play a significant role in the prediction of surfactant vesicle forming ability. CPP is considered another dimensionless scale of surfactants and is described as follows (Uchegbu and Vyas 1998):

$$\mathrm{CPP} = V/l_c a_0$$

where V, hydrophobic group volume; l_c, critical hydrophobic group length; and a_0, area of the hydrophilic head group (Fig. 5.3).

Understanding the CPP value of the selected surfactant may predict the vesicle type. CPP points to the surfactant's capability to form spherical micelles (CPP < 1/3), non-spherical micelles (0.33 < CPP < 0.5), bilayer vesicles (0.5 < CPP < 1), or

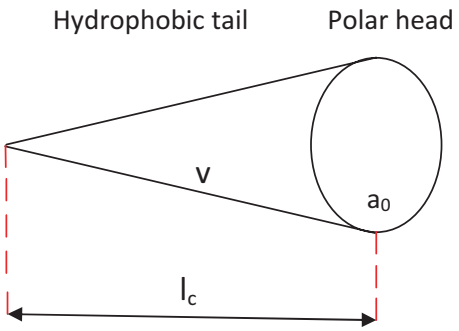

Fig. 5.3 Schematic structure for detection parameters of CPP formulation

inverted micelles (CPP ≥ 1) (Bouwstra and Hofland 1994; Pardakhty et al. 2007; Kumar and Rajeshwarrao 2011).

Additive Compounds

Besides the surfactant type, encapsulated drug characteristics, and preparation method, the incorporation of additives may play an effective role on the self-assembly of surfactants to form niosomes (Uchegbu and Vyas 1998). A range of additives has been utilized for niosome preparation along with cholesterol being the most important and commonly used of these compounds (Moghassemi and Hadjizadeh 2014).

Cholesterol concentration potentially affects vesicle characteristics, e.g., storage time, entrapment efficiency, stability, and release (Biswal et al. 2008; Shilpa et al. 2011). Considering surfactants exhibiting HLB > 6, cholesterol is an essential additive for bilayer vesicle formation. It has been observed that the incorporation of cholesterol allows more hydrophobic surfactants to form vesicles, reducing the propensity of the surfactant to form aggregations (Kumar and Rajeshwarrao 2011). Cholesterol acts to improve vesicle stability in case of surfactants with lower HLB values. The increased stability of the surfactant bilayer when cholesterol is added is attributed to abolishing the gel to liquid transition temperature of the niosomal vesicle (Kumar and Rajeshwarrao 2011).

Also, cholesterol content could affect drug loading capacity (Shilpa et al. 2011). Consequently, cholesterol concentration ought to be optimized for improved characteristics. It has been revealed (Fang et al. 2001a) that increasing cholesterol concentration enhances the stability of enoxacin, which results in higher entrapment efficiency (Agarwal et al. 2004; Verma et al. 2010). Guinedi et al. observed that varying Span to cholesterol molar ratio affects the release rate of acetazolamide from niosomal vesicles (Guinedi et al. 2005).

Dicetyl phosphate is another widespread additive used for niosomes. It stabilizes their bilayers by imparting a negative charge on the niosomal vesicle surface or achieves an electrophoretic motion resembling that of erythrocytes as in the case of encapsulated hemoglobins. Nevertheless, an unnecessary increase in the amount of dicetyl phosphate will prevent the niosomal formation (Waddad et al. 2013).

Niosome Advantages

Compared to liposomes, niosomes provide various advantages (Moghassemi and Hadjizadeh 2014). Niosomes are osmotically active, are more chemically stable, and have longer storage time than liposomes. For example, Manconi et al. showed that higher tretinoin stability was obtained when it was incorporated in niosomes rather than in liposomes (Manconi et al. 2006). Functional groups on their hydrophilic heads permit easy surface formation and modification. Being non-ionic in nature gives them higher compatibility with biological systems and low toxicity; they are also biodegradable and non-immunogenic. They are able to promote the therapeutic efficiency of drugs by protecting them from biological environment, leading to better availability. Access to raw materials used in niosomal vesicles is convenient, with no special precautions and conditions for handling. Characteristics of the niosomes can be easily controlled by modifying type of surfactant, preparation method, cholesterol concentration, surface charge, and size.

Both a permeation enhancer effect (due to the presence of the non-ionic surfactant) and direct vesicle fusion with the stratum corneum may contribute to the enhanced skin permeation of drugs loaded in niosomes (Marianecci et al. 2014). For example, Fang et al. showed that enhancing effect for skin permeation of enoxacin for niosomes was greater than that of liposomes composed of dimyristoyl phosphatidylcholine (Fang et al. 2001a). It could be concluded that niosomes are favored over liposomes in terms of higher stability and accessibility of raw materials used in their preparation as well as in improved drug permeation through skin layers.

Similar to liposomes, a number of trials have been made to modify the structure of niosomal vesicles to achieve better skin penetration. Recently, pH-sensitive non-ionic surfactant vesicles obtained with Tween 20 or Span 60 mixed with cholesterol and cholesteryl hemisuccinate, a derivative of cholesterol as a pH-sensitive molecule, were proposed for topical delivery of ibuprofen. Only niosomes with Span 60 and cholesteryl hemisuccinate showed a significant increase of in vitro skin permeation of the drug (Carafa et al. 2009).

Another promising approach being investigated is the formulation of novel surfactant-based elastic nanovesicles. These vesicles are composed of a non-ionic surfactant along with an edge activator in nano-sized ranges (Kakkar and Kaur 2011); they have been used potentially in topical drug delivery due to enhanced drug penetration, targeting both hydrophilic and lipophilic drugs in a controlled manner over a prolonged period of time, thus enhancing the therapeutic activity, achieving better patient compliance and reducing side effects (Mahale et al. 2012).

These elastic nanovesicles are more advantageous than conventional niosomes as they have a greater ability not only to overcome the stratum corneum barrier but also efficiently penetrate into deep subcutaneous target tissues by squeezing through pores much smaller than their own size and retain their structure (Cevc et al. 2008). Their elasticity allows them to pass through channels that are less than one-tenth of their own diameter (Kumar and Rajeshwarrao 2011). The elasticity of these novel vesicles may be attributed to the edge activators which destabilizes the vesicles and consequently fluidizes the vesicular bilayer by lowering the interfacial tension, thus

promoting better drug penetration (Kakkar and Kaur 2011). Several research works reported elastic niosomes for enhancing the skin permeability (Manosroi et al. 2008, 2011, 2013; Al-Mahallawi et al. 2015; Arslan Azizoglu et al. 2017; Farghaly et al. 2017; Ammar et al. 2018; Aziz et al. 2018; Fahmy et al. 2018).

In summary, niosomes have the ability to provide a better alternative to liposomes as potential transdermal delivery carriers owing to their better stability and the penetration enhancement provided by the surfactants in their structure. Possible modifications in their structure, in a way similar to liposomes, have been investigated in recent years so as to increase vesicle elasticity. Such advancements would pave the way to newer generations of surfactant-based carriers with promoted skin permeation.

Provesicular Technology

One approach to reduce water content in liposomal and niosomal preparations is the formation of a vesicle "preconcentrate" (which is called proliposomes or proniosomes) (Abdelkader et al. 2014). These provesicular concentrates consist of the usual vesicular ingredients in addition to ethanol and merely a trace amount of water. These gel-like systems are assumed to generate vesicles when applied to the skin (i.e., in situ) (El Maghraby and Williams 2009; Ammar et al. 2011). In addition, dry granular provesicular concentrates are prepared using a water-soluble porous powder (e.g., maltodextrin or sorbitol) as coating material (Azeem et al. 2009). Owing to their ease of preparation and scale up, besides the ability to adjust skin delivery of drugs, such provesicular systems have the potential of being a suitable dosage form for TDD (El Maghraby and Williams 2009; Ammar et al. 2011).

Proniosomes

The utilization of provesicular technology for preparation of niosomes was introduced about 20 years ago (Moghassemi and Hadjizadeh 2014). Proniosome is a novel drug carrier preparation method, and it has been used as stable precursors for preparation of niosomal carrier systems (Mokhtar et al. 2008). Several studies have employed the proniosome technology for many therapeutic agents such as 17β-estradiol (Fang et al. 2001b), benzocaine (Abd El-Alim et al. 2014), tenoxicam (Ammar et al. 2011), valsartan (Gurrapu et al. 2012), and vinpocetine (El-Laithy et al. 2011).

Upon storage, niosomes exhibit good chemical stability. However, problems could arise due to physical instability in niosomal dispersions (Azeem et al. 2009). Similar to liposomes, aqueous suspensions of niosomes have a limited shelf life as they could exhibit aggregation, fusion, or leaching. Also hydrolysis of the encapsulated drug could take place (Hu and Rhodes 2000; Mokhtar et al. 2008). Methods of formulation of niosomes such as ether injection and reverse-phase evaporation

methods have the drawbacks of requiring harsh conditions such as use of organic solvents, sonication, and prolonged exposure to elevated temperatures (Weiner 1994).

To overcome such limitations, provesicular approach, which involves the formulation of a dry, non-hydrated product, was developed. These "provesicles" could be hydrated instantly before administration. The idea behind this technology is that vesicle formation is stopped in such a manner that allows the system to "vesiculate" in the location and at the desired manner. Proniosomes can be classified into dry granular proniosomes and liquid crystalline proniosomes (Azeem et al. 2009) (Fig. 5.4).

Preparation of dry granular proniosomes takes place through coating of a water-soluble carrier (e.g., maltodextrin or sorbitol) with surfactant. Thus, a dry formulation is obtained in which water-soluble particles are covered with a thin surfactant film. Proniosomes are reconstituted, at a temperature greater than the transition temperature of the surfactant used in preparation, through addition of aqueous phase (Azeem et al. 2009).

A method for preparing dry proniosomes by "spray coating" was introduced by Hu and Rhodes where sorbitol was used as the inert carrier (Hu and Rhodes 2000). The produced dry product could be hydrated prior to use in order to yield aqueous niosomal dispersion. This gives these "dry niosomes" a great potential for industrial application. Proniosome-derived niosomes were found to be as good as

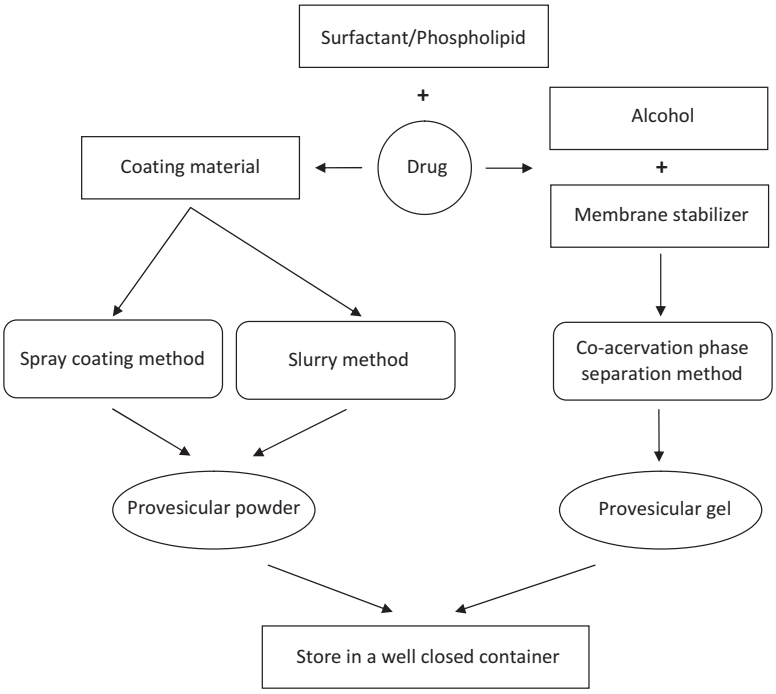

Fig. 5.4 Materials and methods used for the preparation of provesicular systems

conventional niosomes concerning their structure, vesicle size, and drug release behavior (Azeem et al. 2009).

The "slurry method" is another technique employed to prepare maltodextrin-based proniosomes (Blazek-Welsh and Rhodes 2001). Briefly, maltodextrin powder is added to a round-bottom flask, and whole surfactant solution is added directly. The mixture is rotated under vacuum till a free flowing dry powder is obtained (Azeem et al. 2009).

Preparation of liquid crystalline proniosomes involves the technique of "coacervation phase separation." This method involves mixing the drug, the non-ionic surfactant, cholesterol, and lecithin (as a membrane stabilizer) with alcohol in a wide mouth glass tube. The glass tube is tightly covered and warmed on a water bath at around 60–65 °C for a period of 5 min. The aqueous phase is then added, and the mixture is further warmed for a few minutes before being allowed to cool down at room temperature, thus converting the dispersion to a proniosomal gel (Vora et al. 1998; Azeem et al. 2008).

The produced proniosomal structure could be described as "liquid crystalline-compact niosomes hybrid" that, upon hydration, can be convUfasomesUfasomeserted into niosomes straight away (Fang et al. 2001b; Varshosaz et al. 2005; Gupta et al. 2007). Proniosomes, thus, provided the advantages of easy and immediate preparation of niosomes (Azeem et al. 2009).

Proliposomes

Proliposomes are free-flowing particles that have the ability of instant formation of a liposomal system upon hydration (Payne et al. 1986a, b). Proliposomes are composed of phospholipids, the drug, and a water-soluble porous powder. They have the advantage of easy storage and sterilization in dry state. A narrow size range of the reconstituted liposomes could be obtained through controlling the size of the porous powder. Owing to these attributes, proliposomes seem to be a promising substitute to conventional liposomes in formulation of liposome-based dosage forms. Proliposomes offer a versatile model for liposomal systems that can be used with a wide range of therapeutic entities (Choi and Maibach 2005).

Upon application to mucosal membranes and hydration by mucosal fluids, proliposomes are expected to form liposomes. Microscopic observation revealed that proliposomes are converted to liposomes almost entirely after contact with water within few minutes. Upon administration under occlusive conditions in vivo, proliposomes have the ability to form liposomes as they are hydrated by sweat (Ahn et al. 1995). Sustained drug absorption is expected from proliposomes in case of TDD without using complicated measurements since proliposomes themselves may control the release rate of the drug.

In vitro skin permeation of nicotine from proliposomes was reported by Hwang et al. (1997). The flux of nicotine from proliposomes was firstly retarded in contrast to that of nicotine powder. The initial flux of nicotine from the powder was more than twice than that of proliposome preparations. These results denote the

possibility of sustained transdermal delivery of nicotine upon applying proliposome formulations under occlusive condition (Choi and Maibach 2005).

In conclusion, the provesicular technology aims mainly to improve physical and chemical stability of the vesicles and improve their storage condition as the vesicles are actually reconstituted before use. Yet, this novel technology has provided no additional benefit towards improving the shortcomings of the vesicles regarding poor ability to penetrate deep skin layers.

Other Novel Vesicular Carriers

In recent years, several new types of vesicular carriers were introduced which vary in their properties and potential for different applications according to the ingredients used in their preparation. The following section discusses some of these novel vesicular carriers that are considered as promising TDD systems. It is noteworthy to mention that, compared to the vesicular carriers discussed earlier in this chapter, such novel vesicles still require much more research in order to fully study their characteristics and compare them to the older vesicular systems. Examples of recent studies utilizing these novel carriers for drug delivery through the skin could be viewed in Table 5.5.

Ufasomes

Ufasomes or "unsaturated fatty acid vesicles" could be described as suspensions of closed lipid bilayers from employing fatty acids and their ionized species (soaps). Accordingly, these vesicles contain two types of amphiphiles, i.e., the neutral, non-ionized form and the negatively charged, ionized form (Patel et al. 2011). Gebicki and Hicks were the first to report the formation of fatty acid vesicles in the 1970s. The new vesicles were originally named "ufasomes" denoting unsaturated fatty acid liposomes (Gebicki and Hicks 1973; Morigaki and Walde 2007).

Based on information obtained from pressure area measurements on fatty acid surface films and analysis of natural membrane phospholipids, it is suggested that

Table 5.5 Recent studies considering novel vesicular carriers as skin delivery systems

	Drug	Indication	Carrier	References
1	Capsaicin	Topical analgesic	Cubosomes	Peng et al. (2015)
2	Dexamethasone	Antiinflammatory	Ufasomes	Mittal et al. (2013)
3	Etodolac	Antirheumatic	Cubosomes	Salah et al. (2017)
4	Fluconazole	Antifungal	Cubosomes	Prajapati et al. (2014)
5	Methotrexate	Antirheumatic	Ufasomes	Sharma and Arora (2012)
6	Paeonol	Antiallergic/ antiinflammatory	Cubosomes	Li et al. (2015b)
7	Silver sulfadiazine	Treatment of burns	Cubosomes	Morsi et al. (2014)
8	Tetanus toxoid	Immunization	Vesosomes	Mishra et al. (2006)

C-12 to C-22 fatty acids would be appropriate for preparation of stable ufasomes. As a matter of fact, the majority of the studies were confined to the C-18 acids which proved to be of the highest potential in early studies. Only oleic acid and linoleic acid formed membranes that enabled the ufasomes to accomplish these criteria (Patel et al. 2011).

The ratio of non-ionized neutral form and the ionized form is vital for stability of ufasomes. Consequently, the formation of ufasomes is limited to a somewhat narrow pH range (7–9) where roughly half of the carboxylic groups are non-ionized. At higher pH values, fatty acids become too soluble while at lower range unstructured fatty acid precipitates are formed (Patel et al. 2011).

It is well-recognized information that free fatty acids act as penetration enhancers for drugs through the stratum corneum (Naik et al. 1995). However, their use is limited because they can cause skin irritation. Nevertheless, such drawback could be overcome through the use of fatty acid vesicles as drug carriers. It has been revealed that bilayer membrane possesses a fusogenic tendency due to its ability to lower the phase transition temperature of lipids in biological membrane (Mittal et al. 2013). The membrane of the vesicles fuses with skin lipids, leading to release of vesicle contents. Thus, it is assumed that fatty acid vesicles will act as a suitable carrier to enhance drug penetration through the stratum corneum leading to reduced toxicity. Furthermore, fatty acid vesicles have the advantage of ease of formulation and low cost of ingredients (Kanikkannan et al. 2000). Ufasomes have superior entrapment efficiency for both hydrophilic and hydrophobic drugs compared to liposomes. They are also cheaper and more stable than liposomes (Jain et al. 2014).

Polymersomes

Polymersomes are a category of synthetic vesicles made from synthetic amphiphilic block copolymers (Antonietti and Förster 2003; Du and O'Reilly 2009; Massignani et al. 2010; Brinkhuis et al. 2011; Meng and Zhong 2011). Usually, polymersomes consist of void spheres where the core contains an aqueous solution enclosed by a bilayered membrane composed of hydrated hydrophilic coronas at the inside as well as the outside of the lipophilic center of the membrane protecting the fluid core from external environment (Lee and Feijen 2012).

Utilizing polymer chemistry permits the formation of vesicles with a broad range of properties depending on the type of block copolymer utilized. These polymeric chains can be made from two or more covalently bound homopolymer units. They can also be synthesized into diblock, triblock, or multiblock, random, star, and graft units. Upon exposure to water, polymer chains self-assemble into vesicles due to their amphiphilic nature. Such arrangement takes place in order to reduce the interaction between hydrophilic and hydrophobic domains. Polymersomes generally differ from liposomes in that polymer chains have much higher molecular weight. Accordingly, their membrane structures are highly intertwined. This enables the vesicles to bear very high stresses without breaking due to better mechanical properties which make them ideal for transdermal applications (Pegoraro et al. 2012).

Based on chemical and physical properties of the polymers used, polymersomes can be tailored to be more or less deformable and reach the deeper skin layers with different diffusion rates. The high entanglement level of polymer chain means that exceptionally high energy would be needed to rupture the vesicles (Pegoraro et al. 2012). Permeation studies carried out on polymersomes having a diameter ranging from 200 to 400 nm across 50 nm pores revealed that they can transport large macromolecules (molecular weight > 10 kDa) and cross the pores without fragmenting (Pegoraro et al. 2011). When applied ex vivo to the human skin, this polymersome formulation led to a tenfold increase in permeation of fluorescently labeled dextran compared to passive diffusion (Pegoraro et al. 2012).

Vesosomes

Alteration of the composition of the unilamellar liposome while preserving the lipid membrane as the fundamental structural unit is a suitable choice for optimizing the bilayer chemistry and physics (Kisak et al. 2004). Vesosomes are liposome-type carriers that have the ability to encapsulate one or more smaller liposomes in their aqueous core (Singla and Sachdeva 2015). This leads to the creation of a multicompartment system composed of the external lipid bilayer of the vesosome and an inner layer entrapping small liposomes. Vesosomes have the advantage of easy and independent optimization of the interior compartments and increase in drug retention (Paleos et al. 2013). A marked increase in encapsulation of both hydrophobic and hydrophilic small molecular weight molecules could be obtained by these "nested" bilayers. Each additional bilayer provides an extra barrier to degradation by lipolytic enzymes as well as to drug permeation (Kisak et al. 2004).

An advantage of vesosomes is that various drugs can be compartmentalized in different liposomes giving these vesicles the ability to deliver several drugs at the same time (Singla and Sachdeva 2015). The vesosome structure could be used to a fixed ratio of a combination of antibiotics or antimicrobials to desired sites; such drug combinations have been shown to exert synergistic effect when delivered in a single liposome (Schiffelers et al. 2002). Avoiding development of drug-resistant pathogens could be achieved through using such multidrug formulations instead of a single drug (Kisak et al. 2004). Vesosomes have been investigated and proved great promise for transcutaneous immunization (Mishra et al. 2006). Although the prospect of using vesosomes in TDD has not been realized as much as for other drug delivery systems, they hold a great potential owing to their unique structure (Singla and Sachdeva 2015).

Sphingosomes

Sphingosomes could be defined as concentric vesicles with lipid bilayer mainly consisting of natural or synthetic sphingolipid enclosing an aqueous content (Singh et al. 2015). In other words, they are liposomes composed of sphingolipids. The

main constituents of sphingosomes are sphingolipid (sphingomyelin) and cholesterol varying in the range of 75/25 mol% (Jain et al. 2014). The most important sphingolipids that have been employed in sphingosomes formulation include sphinganines, hexadecasphinganine, lysosphingomyelins, lysoglycosphingolipids, N-acylsphingosines, and gangliosides (Jain et al. 2014).

Sphingosomes are characterized by being much more stable to acid hydrolysis than liposomes. They also possess superior drug retention characteristics. Sphingosomes are administered through many routes including the parenteral route (Saraf et al. 2011) and also could be administered orally or transdermally (Webb et al. 1996).

Sphingosomes are built up by only amide and ether linkage which are more resistant to hydrolysis than ester linkage of lecithin; hence they are more stable than liposomes. They also contain less double bonds than lecithin and thus are less subjected to rancidity (Saraf et al. 2011). On the other hand, sphingosomes usually have poor entrapment efficiency and are not economic due to the high cost of sphingolipids (Jain et al. 2014).

Sphingosomes are also employed in the cosmetic industry and TDD. Topically administered sphingolipids have high compatibility with the skin because they belong to the same class of chemical compound as epidermal lipid, giving sphingosomes penetration-enhancing characteristics (Saraf et al. 2011).

Cubosomes

Cubosomes are discrete, submicron, nanostructured particles of bicontinuous cubic liquid crystalline phase (Jain et al. 2017). The term "cubosomes" was given as a reflection of their similarity to liposomes and cubic molecular crystallography.

In presence of polar solvents, the hydrophobic region of amphiphilic molecules self-assembles into an array of thermodynamically stable liquid crystalline phases possessing an adequate level of structural symmetry and molecular orientation in the nanometer range (Jain et al. 2014). Cubosomes contain three dimensional curved bicontinuous lipid bilayer organization resembling honeycombs. These structures are divided into two internal aqueous channels that can be employed to carry various bioactives, such as chemical drugs, peptides, and proteins (Karami and Hamidi 2016).

Glyceryl monooleate (usually called monoolein) is the most commonly used amphiphilic lipid in cubosome preparation (Montis et al. 2015; Murgia et al. 2015). Glyceryl monooleate is a synthetic compound which consists of mixture of glycerides of oleic acid and other fatty acids, consisting mainly of monooleate. This class of amphiphilic lipids has the capacity to form a variety of lyotropic liquid crystals (Lutton 1965; Kulkarni et al. 2011). One of the major and exceptional characteristics of cubosomes is their bioadhesive nature through which they can suitably be applied in topical and mucosal drug delivery (Karami and Hamidi 2016).

5.5 Conclusion

Skin delivery (dermal and transdermal) offers a potential substitute to oral route, particularly for skin diseases or conditions which need site-specific delivery. The chief restriction for skin delivery is the impermeable stratum corneum, which hinders the passage of drugs across the skin. Nevertheless, in the last 40 years, technological and scientific advances have resulted in the development of a variety of vesicular carrier systems for skin delivery. Liposomes, ultradeformable liposomes, ethosomes, and niosomes have revealed successful delivery for various drug molecules. These vesicular carriers are able to overcome the limitations of the penetration of drugs and bioactives especially large molecules like peptides, hormones and antibiotics, other bioactives with poor permeation because of unfavorable physicochemical properties, drugs for immediate and targeted action, etc. Improved delivery of drug molecules across the skin via vesicular carriers opens new opportunities and challenges for the development of new enhanced therapies. In spite of the achievement of vesicular delivery systems, stability issues, scaling-up of manufacturing process, regulatory challenges, and cost are some of the problems which need special consideration in order for these novel carriers to reach their full potential.

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Chapter 6
Nanotechnology in Delivery and Targeting of Phytochemicals

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Abstract Recently dietary and plant-derived phytochemicals are rising into limelight as many people developed a propensity towards opting nature-dependent healthy lifestyle. Scientific advancements highlight the effectiveness of phytochemicals in the treatment of many diseases and for various lifestyle benefits. Traditionally used in medicines, food supplements, and cosmeceutical products, phytochemical compounds are now conjoined with modern science to produce significant health benefits to humans as they possess fiddling risks compared to synthetic chemical entities. Phytochemicals are implemented in different forms and for different purposes such as phyto-, aroma-, and gemmotherapy for their potential health benefits. But the formulation of these phytochemicals for various applications is a major concern primarily owing to their low bioavailability, solubility, and the need to be taken in combination or as whole food. Hence, efficient delivery systems such as nano-engineered formulations are imperative to potentially yield the complete benefits from these phytochemicals. Besides enhancing the solubility and stability of phytochemicals, the nano-delivery systems can also prolong their average blood circulation time. Consequently, the high differential uptake efficiency, enhanced permeation, and retention characteristics in target tissues could prevent phytochemicals from premature interaction with the biological environment, thus resulting in decreased toxicity and favorable dose optimization possibilities. These advanced delivery systems also aid in the targeted delivery approaches. This chapter depicts the major natural products employed for the human benefits, their limitations, and nanotechnological solutions to triumph these limitations.

Keywords Nanomaterials · Phytochemicals · Supplements · Formulations · Bioavailability · Delivery · Inflammation · Cancer · Additive agents · Cosmeceuticals

6.1 Introduction

Phytochemicals are compounds which are derived from the plants and are generally non-nutritive to them. But these phytochemicals provide typical flavor and color to the fruits, vegetables, nuts, spices, grains, beverages, and other dietary plant-derived products (Chuan et al. 2017; Upadhyay and Dixit 2015). Despite providing color, odor, and flavor, they mainly protect the plants from diseases and environmental hazards such as pollution, stress, drought, UV exposure, and pathogenic attacks

(Saxena et al. n.d.). Traditional knowledge and current screening studies confirm similar protective effects in humans under diverse conditions and thus created a huge possibility for significant pharmaceutical applications that could benefit mankind in a more efficacious way than other synthetic medicines.

Phyto-applications have its own advantages like the existence of vast biodiversity of phyto-remediation system, easy availability, and cost and preference among population. Although many phytochemicals show significant promises in nutrient supplementation and in therapy, their hydrophobic nature, poor stability, poor absorption and bioavailability, rapid metabolism, elimination, and low target specificity make them difficult while administering at the therapeutic doses, an issue which can be possibly overcome using nanotechnology (Aqil et al. 2013).

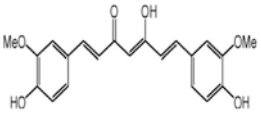
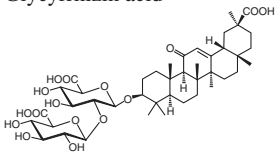
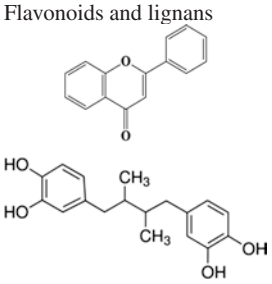
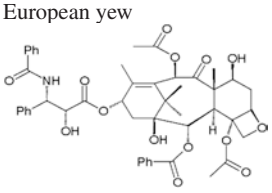
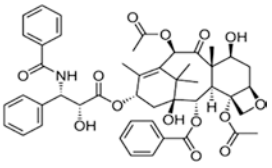
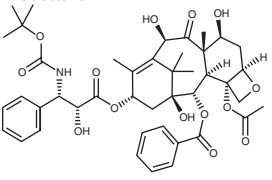
Several phytochemicals have been well recognized for their role as (a) antioxidants (e.g., allyl sulfides from onions, leeks, and garlic, carotenoids from fruits and carrots, flavonoids of fruits and vegetables, polyphenols from tea and grapes), (b) hormones (e.g., isoflavones of soy), (c) enzyme regulatory agents (protease inhibitors from soy and bean, indoles found in cabbages), (d) DNA replication modulators (saponins found in beans, capsaicin from hot peppers), and (e) antibacterial agents (proanthocyanidins from cranberries). These properties provide diverse opportunities in therapeutic, nutraceutical, industrial, and cosmetic applications.

In case of therapeutic application against cancer, phytochemicals can be potentially used to stimulate the weaker immune system; prevent carcinogenic activation; behave as anti-inflammatory, antioxidative principles; induce mutated cells to commit apoptosis; and regulate the hormonal and cellular mitogenic controls. Thus phytochemicals like carotenoids (such as beta-carotene, lycopene, lutein, zeaxanthin), flavonoids (such as anthocyanins and quercetin), indoles and glucosinolates (sulforaphane), inositol (phytic acid), isoflavones (daidzein and genistein), isothiocyanates, polyphenols (such as ellagic acid and resveratrol), and terpenes (such as perillyl alcohol, limonene, carnosol) are attracting serious scientific attention for use in cancer therapy nowadays (Hosseini 2015).

However with the shortage of available lead pharmacological compounds, along with the onset of side effects and resistance to the existing drug molecules, more extensive research is carried out for the implication of phytochemicals in the health industry. Globally, the development of potent plant-based drugs is given more importance hoping to find cures for disorders such as liver damage or pancreatitis for which there are hardly any reliable drugs for treatment (Leema and Tamizhselvi 2018).Thousands of phytochemicals have already been screened, and few potent ones were studied in detail for their favorable pharmacokinetic and pharmacodynamic properties (Table 6.1).

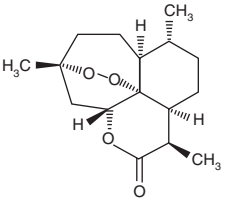
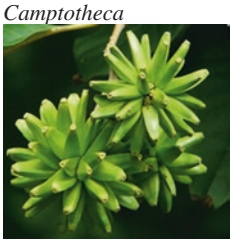
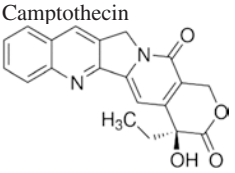
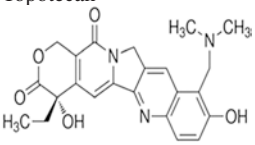
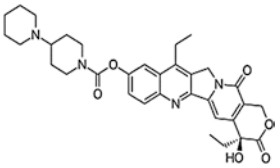
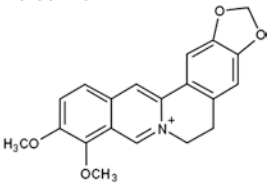
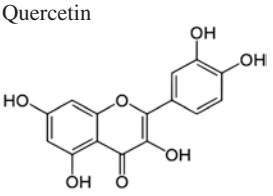
Interestingly several phytochemicals were also reported to have deleterious side effects which prevents them from regular applications. For instance, soybean-based trypsin inhibitors compromise the trypsin function which in turn leads to the release of cholecystokinin and excessive trypsin synthesis by pancreases, amylase inhibitors producing unwanted hypoglycemic effects, saponins interacting with cholesterol in the erythrocyte membrane and lysing the erythrocytes, association of dietary phytoestrogens with infertility and liver disease, and lignans having estrogenic and

Table 6.1 Partial list of active phytochemical molecules used in therapy and biological applications and their source of origin (Conte et al. 2017)

Sources	Active ingredients	Biological activity
Turmeric 	Curcuminoids 	Anticancer and antioxidant
Glycyrrhiza 	Glycyrrhizin acid 	Anti-inflammatory and antihypertensive
Psoralea corylifolia 	Flavonoids and lignans 	Hepatoprotective and antioxidant effects
Pacific yew tree bark 	Taxel 	Anticancer
European yew 	Paclitaxel 	
	Docetaxel 	

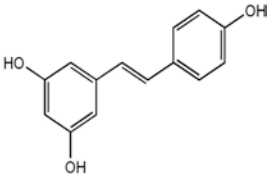
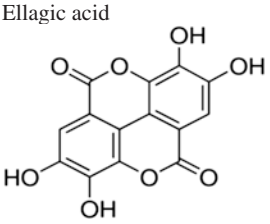
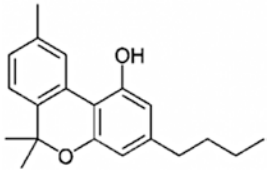
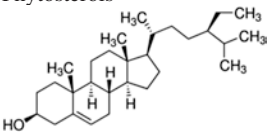
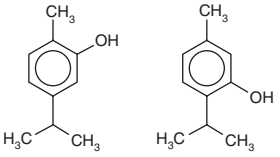
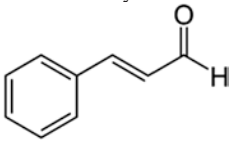
(continued)

Table 6.1 (continued)

Sources	Active ingredients	Biological activity
Artemisia annua 	Artemisinin 	Anticancer
Camptotheca 	Camptothecin  Topotecan  Irinotecan 	Anticancer
Berberis 	Berberine 	Anticancer
Broccoli 	Quercetin 	Antioxidant and anti-inflammatory properties

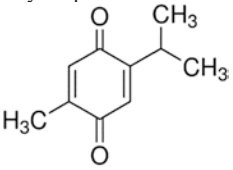
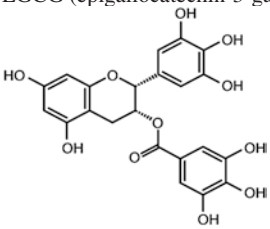
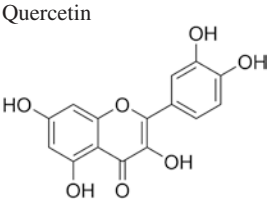
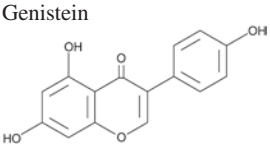
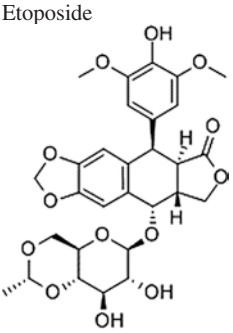
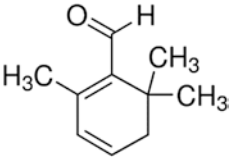
(continued)

Table 6.1 (continued)

Sources	Active ingredients	Biological activity
Red grapes 	Resveratrol 	Anti-inflammatory properties
Pomegranate 	Ellagic acid 	Anti-inflammatory properties
Cannabis 	Phyto**cannabinoids: cannabin** 	Anti-inflammatory properties
Avocado 	Phytosterols 	Anti-inflammatory, anticancerous, and anti-atherogenic activities
Oregano  Cinnamon 	Carvacrol and thymol  Cinnamaldehyde 	Anti-inflammatory, antioxidant, and antimicrobial activity Anti-inflammatory properties

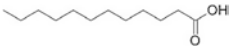
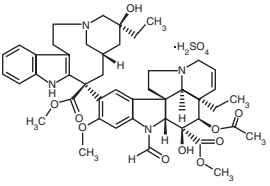
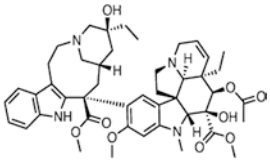
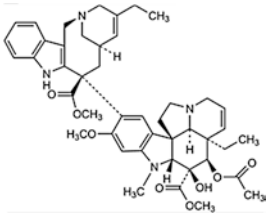
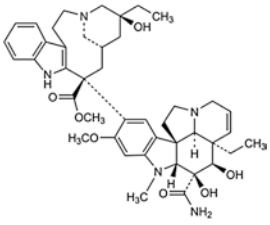
(continued)

Table 6.1 (continued)

Sources	Active ingredients	Biological activity
Nigella sativa (black seed) 	Thymoquinone 	Antimicrobial, anti-inflammatory, antioxidant, anti-diabetic, anticancer, hepatoprotective, and renal protective activities
Green tea 	EGCG (epigallocatechin-3-gallate) 	Anticancer activity
Apple 	Quercetin 	Anticancer activity
Soybean 	Genistein 	Anticancer activity
Himalayan mayapple 	Etoposide 	Anticancer activity
Saffron 	Safranal 	Increased moisturizing effect and anti-UV activity

(continued)

Table 6.1 (continued)

Sources	Active ingredients	Biological activity
Coconut oil 	Lauric acid 	Antimicrobial activity
Catharanthus roseus 	Vincristine  Vinblastine  Vinorelbine  Vindesine 	Hypoglycemic and cytotoxic anticancer effects

antifertility effects. Likewise sugar-binding lectins and hemagglutinin may bind and agglutinate red blood cells (Hagerman et al. 1997).

Though they possess some threats, manipulating and modifying phytochemicals can be done to elicit more benefits and undermine harmful effects. Also, the reluctance among individuals to use phytocompounds is primarily because of treatment time, effectiveness, and nature of application. Thus, there is need for this type of therapy to be updated for the modern trends using technological advancements like nanotechnology. Nanotechnology has the potential in resolving the difficulties associated with phytochemicals with respect to access and delivery, overcoming the complexities of natural product chemistry, and quickening the inherent slow phase of action associated with working of natural products.

6.2 Nanotechnological Applications in Phytochemical Delivery

Although phytotherapy is followed for thousands of years, the mechanism-based phyto-applications are very limited today except with few serendipity-based drugs. In this regard, study on nanoparticles for targeted delivery or enhancing the efficacy of phytochemicals has gained much more importance. Nanotechnology is the emerging science that deals with particles in the range of 10 to 200 nm which serves as the effective vehicle for the drug delivery systems (Xie et al. 2016).

Existing phytochemical formulations face major issues mainly due to their unfavorable pharmacokinetic and pharmacodynamic properties. However when they are doped with nanoparticles, not only the solubility of the drug increases but also the efficiency of phytochemical is enhanced (Siddiqui et al. 2014). There are changes in biodistribution patterns of phytochemicals which, by interfering the therapeutic index of the phytochemicals, greatly enhance the drug efficacy and reduce the toxicity to the normal tissues (Fig. 6.1). The therapeutic index is the ratio of amount causing effective therapeutic activity to the amount causing toxic effect (Granja et al. 2016; Thangapazham et al. 2008).

The phytochemical agents could be loaded into nanomaterials or nanocarriers through encapsulation, conjugation, or adsorption to improve their therapeutic index and pharmacokinetic profiles (Brigger et al. 2002). These formulations enhance their absorption, stability, bioavailability, and prolonged systemic circulation and protect them from enzymatic degradation. Therefore, sustained and controlled release with increased uptake efficiency can be achieved. As a matter of fact, by conjugating through target-specific ligands, potential targeting of cancer cells could be achieved (Fig. 6.2). Already few nanotechnological formulations are approved as cancer therapeutic products in recent times (Siddiqui et al. 2014; Granja et al. 2016; Singh et al. 2014; Creixell and Peppas 2012).

Also it has to be noted that nanotechnologically modified phytochemicals themselves may exhibit some adverse pharmacokinetic profiles and exert negative

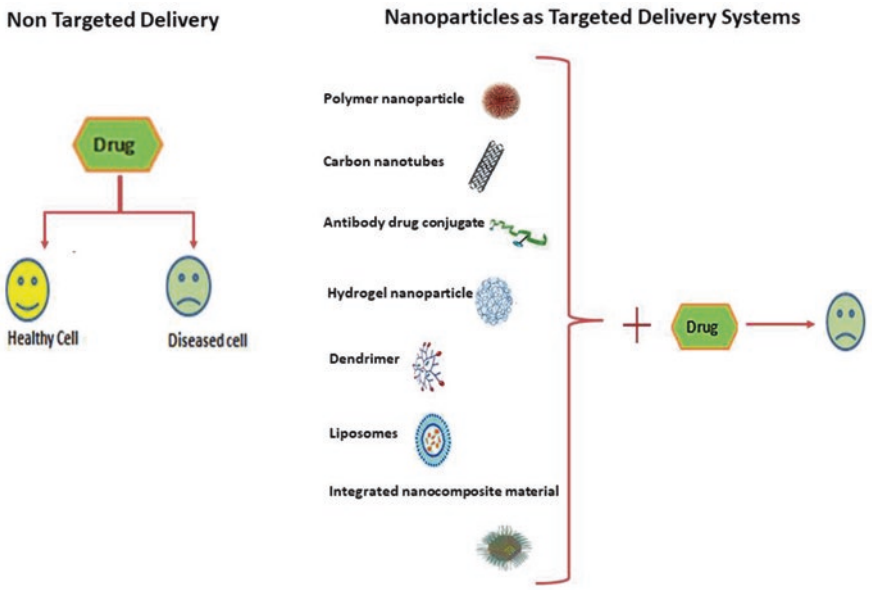


Fig. 6.1 Enhancement of phytochemical delivery using nanocarriers. Compared to the free, conventional form of delivery, nanocarrier-mediated delivery systems enrich the phytochemicals at the target site enhancing the kinetics and dynamics of the drug

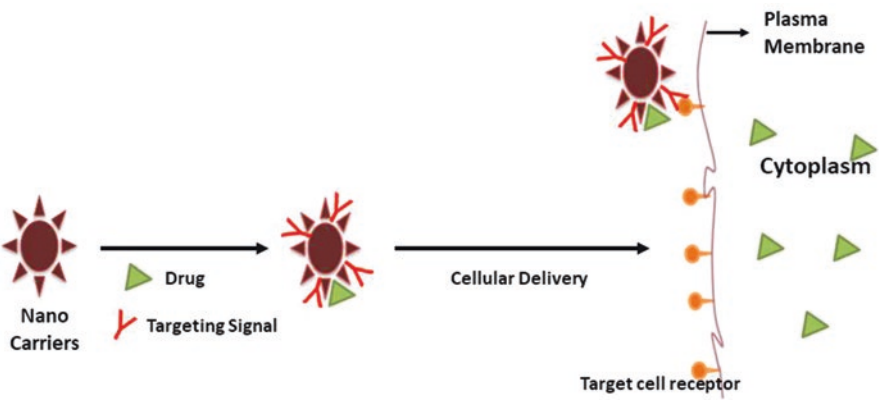


Fig. 6.2 Nanoparticles as targeted drug delivery systems. Drug-loaded, ligand-guided nanocarrier interacts precisely with the target cell receptors/surface molecules, providing specificity for therapy

influence on the therapeutic index or toxicity. However in these cases also, surface modifications of nanocarriers can avoid such toxicity issues and enhance cellular phytochemical delivery through changing biophysical interactions between nanocarriers and cell membrane (Alkilany and Murphy 2010).

Table 6.2 Nano-based applications and the food additive compounds commonly applied in the food industry

Purpose	Example
For improving the food qualities to (1) enhance dispersibility in food products; (2) improve food tastes; (3) enable hygienic food storage, (4) reduce the use of fat, salt, sugar, and preservatives; and (5) improveme the uptake and bioavailability of nutrients and supplements	**Food additives:** synthetic form of the tomato carotenoid lycopene, benzoic acid, citric acid, ascorbic acid, and supplements such as vitamins A and E, isoflavones, ß-carotene, lutein, omega-3 fatty acids, coenzyme-Q10
	Inorganic nanomaterials: transition metals and metal oxides (e.g., silver, iron, titanium dioxide), alkaline earth metals (e.g., calcium, magnesium), and non-metals (e.g., selenium, silicates)

The nanoparticles are made of variety of materials, and the individual composition depends on the purpose and characteristics such as the ability for eventual degradation. Based on the type of material used, nanoparticles can be classified into synthetic biocompatible polymeric nanoparticles and natural degradable biopolymers. Examples for synthetic polymers include poly(lactic-co-glycolic acid) (PLGA) and polylactic acid (PLA) nanoparticles. On the other hand, gelatin-, albumin-, cellulose-, or chitosan-based nanoparticles come under natural, degradable biopolymers (Brigger et al. 2002). So far a variety of nano-applications have been studied, and some proposed nanotechnologically modified phyto-additives and supplements are listed in Table 6.2.

Likewise the few modified phytochemicals available in market as reviewed recently (Bradley et al. 2011; Mamillapalli et al. 2016; Safhi et al. 2016) are:

1. Nanoparticles of *Cuscuta chinensis*: flavonoids and lignans are applied for their hepatoprotective and antioxidative role in the form of oral nano-suspension.
2. Artemisinin nanocapsule: artemisinin molecules are used for anticancer applications using self-assembled nanocapsulation procedure.
3. *Radix salvia miltiorrhiza* nanocapsule: used against heart disorders which applies spray-drying technique for synthesizing the phyto-nanocapsules.
4. Taxel-loaded nanoparticles: anticancer drug paclitaxel is loaded in nanoparticles for enhanced and sustained availability.
5. Berberine-loaded nanoparticles: berberine-loaded nanoparticles are prepared through ionic gelation method for sustained anticancer activities.
6. Nano-herbal cosmetic formulations: St. herb Nano Breast Cream and red blood cell Life Science's "Nanoceuticals Citrus Mint Shampoo" and conditioner.

Various nanostructured herbal formulations have been made as the combinational therapy. As reviewed earlier (Gopi and Amalraj 2016), curcumin, for instance, can be applied in different nano-combinational formulations. Curcumin–diclofenac diethylamine nanocarrier and curcumin–celecoxib-loaded nanoparticles for anti-inflammatory and antioxidative activities, curcumin–hydroxypropyl methylcellulose (HPMC) and polyvinylpyrrolidone (PVP)-loaded nanoparticles for antimalarial

activity, and curcumin–temozolomide-loaded magnetic nanoparticles for antitumor activity are some selected curcumin-based combinational nano-approaches.

In food industries various nutraceuticals are formulated as nanoforms for making the functional food components. Potential phyto-applications in this regard include nanoformulations of hydrophobins and vitamin D_3, folic acid–whey protein and starch, DL-α-tocopheryl acetate and β-carotene, vitamin D_3 entrapped whey protein and calcium, folic acid and calcium, carotenoids and lipids, long chain fatty acids and CoQ10, omega-3-fatty acids and oil soluble vitamins, clove oil and Eugenol, dextran and isoflavone genistein (Gopi and Amalraj 2016).

6.2.1 Advantages of Nanotechnology-Based Interventions

The major benefits of nanotechnological applications during phytotherapy include reducing the toxicity, enhancing the drug release, improving the solubility cum bioavailability, and eventually providing better formulation opportunities and efficacy for drugs (Alkilany and Murphy 2010; Fukumori and Ichikawa n.d.).

Interestingly the enhanced permeability and retention (EPR) effect is the key mechanism for targeting tumors specifically by nanovehicles. The prolonged circulatory presence of drug provides the adequate time required for delivery via the EPR effect (Brigger et al. 2002; Acharya and Sahoo 2011). In order to achieve the target-specific drug delivery in the solid tumors, the structural and architecture abnormalities in the circulatory and lymphatic vasculature of the tumor are exploited in this EPR approach (Maeda et al. 2000). Nevertheless, here the size of the nanoparticle needs to be customized for efficient targeted drug delivery, as anticancer drugs nanoformulated for intravenous administration could possibly escape the renal clearance. Also fine-tuning the physiological characteristics and functionalization through surface modifications and drug conjugations make them resistant to macrophage-based removal. These measures thus reduce the dose requirement through improved stability and circulation time. Moreover, the enhanced permeability and retention (EPR) effect allows the macromolecular compound and its nanocarriers to escape and leak favorably into the neighboring tumor tissue (Torchilin 2011). Further the defective lymphatic drainage in the tumors leads to drug enrichment in the cancer cell surroundings, whereas in normal tissues, this effect cannot be seen (Fig. 6.3) (Yin et al. 2014). The size of the nanoparticles and its biocompatibility are the most crucial parameters for the EPR effect. The minimum molecular size of 40 kDa for macromolecules and particle size of 5 nm for nanocarriers usually have the active EPR effect (Gopi and Amalraj 2016; Hu and Huang 2013). Thus nanotechnology-derived EPR advantage provides the crucial support while designing the phytochemical-based clinical applications.

Though phytochemicals are easily extracted using methanol, chloroform, and acetone, there are no suitable delivery systems available for effective delivery. Also for effective phytochemical activity, in most of the cases, they have to be given in very high dosages. However dose minimization is preferred for patient compliance

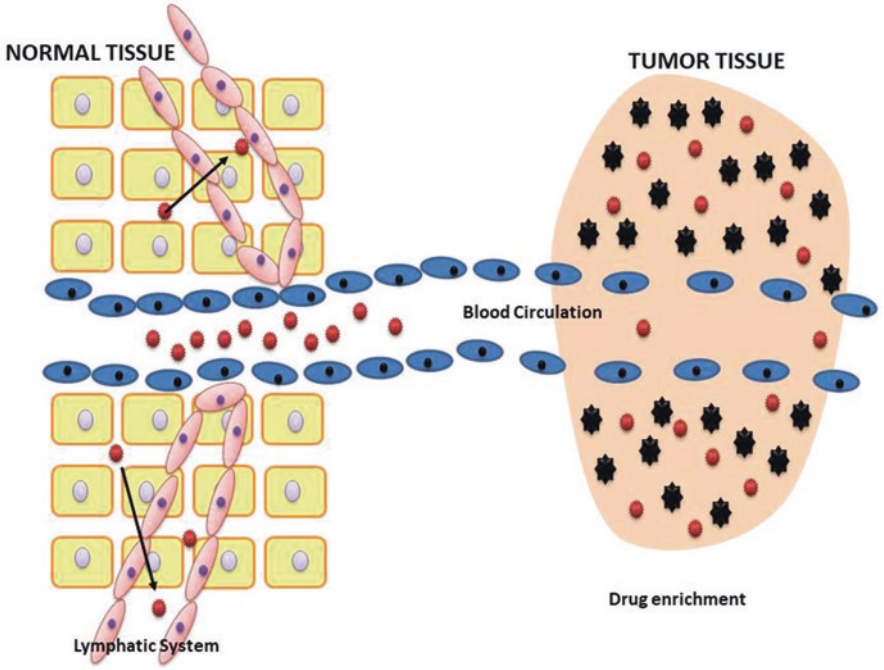


Fig. 6.3 Enhanced permeability and retention (EPR) effect in nanocarrier-mediated drug formulations. Improved stability, circulation time, defective lymphatic drainage, and leaky vasculature of the tumor provide the targeted drug delivery at the tumor sites (Yin et al. 2014)

which can be easily favored through nanoformulations (Ansari et al. 2012). Thus herbal compounds and phytochemicals are the right candidates to be delivered through nano-delivery system (Amol and Pratibha 2014; Ajazuddin and Saraf 2010). Mainly available current phyto-based therapeutic formulations do not have target specificity. However when nano-delivery systems are considered in such cases, they significantly enhance the delivery of phytocompounds at targeted site.

6.3 Nano-delivery Platforms for Phytochemicals

The phytochemicals are vast and diverse in nature, and their customization with respect to various physical and chemical properties is crucial to ensure efficient delivery into the desired system. The major limitations associated with the phytochemicals and herbal medicinal combinations are poor solubility in aqueous media, poor bioavailability, poor stability, and toxicity (Ansari et al. 2012). Using nanoencapsulation and nanoformulation techniques, particles can be designed to have different shapes, sizes, and compositions. These nanoformulations can be functionalized

and modified to have the unique physicochemical properties, thereby improving the delivery characteristics of bioactive molecule.

Both organic biocompatible and biodegradable nanoparticles such as nanoliposomes, nanoemulsions, lipid nanocarriers, phytosomes, micelles, and poly(lactic-co-glycolic acid) (PLGA) nanoparticles and inorganic nanoparticles, e.g., gold, silver, zinc, copper oxide, aluminum oxide, iron oxide, ceramics, and carbon nanoparticles are used in phytochemical studies (Sarker and Nahar 2017). Some prominent forms of nano-delivery approaches attempted recently for phytochemical delivery are discussed below (Fig. 6.4).

6.3.1 *Liposomes*

Liposomes are nanocarriers of size 20 to 1200 nm diameter and have an aqueous core internally and phospholipid bilayer on the external surface. Liposomes are highly advantageous because of their optimization capabilities by altering the surface charge and functionality in addition to the targeted delivery option of anticancer drugs to tumor tissues (Hofheinz et al. 2005).

Already targeted delivery of curcumin is attempted through cyclodextrin-encapsulated curcumin-loaded liposomes (Dhule et al. 2012). Similarly Marqibo®,

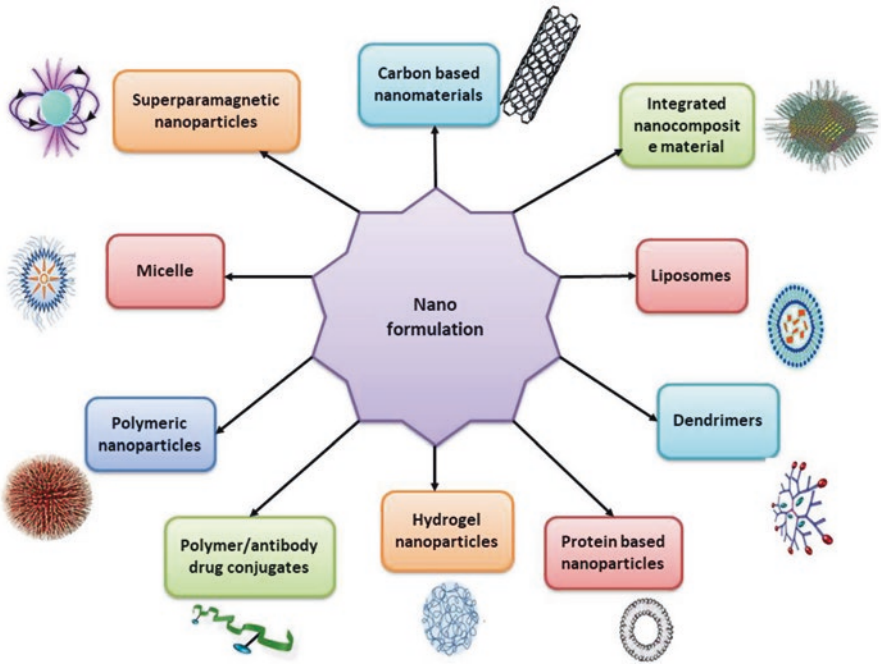


Fig. 6.4 Diagrammatic representation of different drug delivery platforms

a liposomal formulation of vincristine made of sphingomyelin and cholesterol (Silverman and Deitcher 2013; Chang and Yeh 2012), has already been approved by the Food and Drug Administration (FDA), whereas Lipusu®, a liposomal formulation of paclitaxel, has been approved by the Food and Drug Administration in China (Xu et al. 2013; Ye et al. 2013). It is proven that a novel liposomal formulation of doxorubicin has reduced the undesirable delivery of doxorubicin at off-target heart or renal system, increasing the concentration only in the tumor tissues due to EPR effect (Creixell and Peppas 2012; Muggia et al. 1997; Gabizon and Martin 1997).

Likewise, berberine-loaded liposomes are developed as the controlled delivery platform for prolonging the drug release (Sailor et al. 2015; Ai et al. 2014). Also to synergize the therapeutic potential, 5-fluorouracil and resveratrol were incorporated and positively tested using PEGylated liposome (Mohan et al. 2014).

6.3.2 Micelle

Micelles range from 10 to 400 nm in size and are basically smaller in size in comparison with other nanocarriers. Micelles are one of the popular drug delivery carriers for phytochemicals.

Interestingly thymoquinone-based nanoparticles were tested against breast cancer cell growth for their antioxidant and anticancer activities (Ganea et al. 2010). In a targeted approach, the folate-conjugated doxorubicin-loaded micelles were internalized by the cancer cells using receptor-mediated endocytosis (Yang et al. 2010). Similarly copolymeric, biodegradable, and biocompatible encapsulated paclitaxel were specifically targeted using this approach (Liu et al. 2011a; Bamrungsap et al. 2012).

6.3.3 Nanocrystals or Nanoparticles

Nanocrystallite particles ranging from 10 to 100 nm in size with drugs embedded on to the surface are used to deliver less water-soluble drugs with poor dissolution rate. After formulation the surface area of the drug increases and results in enhanced solubility and improved dissolution rate. As a consequence, plasma concentration of the drug is maximized, thus leading to dose minimization of loaded chemicals (Domínguez-Villegas et al. 2014). On the other hand, drug itself can be reduced in size and formulated as nanosized drug which has the ability to act as self-carrier. After nanosizing, these drugs can be administered in different routes for use as oral, nasal, and injectable formulations (Ajazuddin and Saraf 2010; Ramalingam et al. 2016). This formulation could specifically aid in delivering the polyphenols and other phytochemicals, as they suffer from low stability and unfavorable pharmacokinetics. Using this approach, green tea polyphenols are encapsulated in

chitosan-based delivery system, improving the stability of tea polyphenols and preventing their oxidative loss or degradation in the gastrointestinal tract (Liang et al. 2017).

6.3.4 *Polymeric Nanoparticles*

Polymeric nanoparticles are colloidal in nature with the size ranging from 10 to 100 nm. They are formulated as spheres, branched structures, or core–shell structures using natural collagen, albumin, alginate, gelatin, and chitosan molecules. Also it can be fabricated using synthetic yet biodegradable polymers like poly lactide-poly glycolides, poly caprolactones, and poly acrylates (Bhatia 2016). It was reported that the biodegradable polymeric formulation containing *Syzygium cumini* was found to retain the antioxidant activity of plant extract in rat model study (Bitencourt et al. 2016). Similarly green-synthesized AgNPs using extracts of *Vitex negundo* L. retained cell viability inhibition in human colon cancer cell lines (Prabhu et al. 2013). Moreover it is possible to make "smart polymers" which are sensitive to stimuli and can alter its physiochemical properties in response to the surrounding environment. The triggering responses include physical stimuli (temperature, ultrasound, light, electricity, and mechanical stress), chemical niche (pH and ionic strength), or biological signals (enzymes and biomolecules) (Fig. 6.5). Also, it is

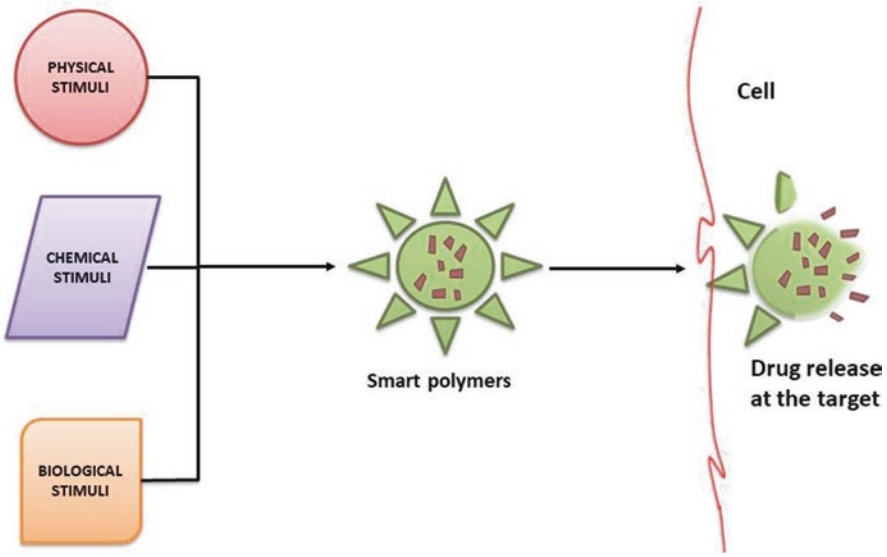


Fig. 6.5 Stimulus-sensitive delivery: various environmental stimuli at the diseased or induced site can alter the chemical properties of nanocarriers, thus releasing the cargo drug in response to the environmental stimuli

possible to tune up drug release in response to the stimulus within a narrow range, thus resulting in more accurate and programmable drug delivery. Currently linear polymers made using covalent chemistry, polymeric micelles from amphiphilic block copolymers, and hydrogels of water-filled depot for hydrophilic drug encapsulation are more common (Kale and Torchilin 2007; Fleige et al. 2012).

6.3.5 Prodrugs

The prodrugs are the polymeric nanocarriers having covalent conjugation of the drug with the linear arm of the polymers. Conjugation of drug with macromolecular polymeric compounds gives more blood circulation time. In addition to peptides or protein drugs, many anticancer drugs of small molecular size are also PEGylated for improved pharmacokinetics. For example, PEG–camptothecin also known as PROTHECAN® entered into clinical trials for the cancer therapy (Joralemon et al. 2010). Table 6.3 lists out the free and various nanoformulations of camptothecin and its derivatives from *Camptotheca* species which are in different process of clinical development (Lerchen 2002). Similarly in CRLX101, camptothecin molecules are conjugated to cyclodextrin–poly(ethylene glycol) copolymers (Fig. 6.6) (Ganesan and Choi 2016; Moody et al. 2015). With this approach there are some limitations like increase in production cost, requirement of additional purification steps, and regulatory issues with approval agencies (Lerchen et al. 2001). Thus there are only a limited numbers of drugs and polymers which have been used to develop polymer–drug conjugates (Table 6.4).

Table 6.3 List of free and various formulations of camptothecin which are in different processes of clinical development (Lerchen 2002)

Drug Name	Delivery System	Source	Status
Irinotecan HCI (CPT-11)	Water soluble	Pharmacia/Aventis	Launched
Topotecan HCI	Water soluble	GlaxoSmithKline	Launched
Rubitecan (9-NC)	Lipophilic	Supergen	Phase III
Exatecan mesylate (DX·8951-f)	Water soluble	Daiichi	Phase III
Lurtotecan (OSI-211)	Liposomal formulation	OSI Pharm.	Phase II
CKD-602	Water soluble	Chong Kun Dang	Phase II
Diflomotecan (BN80915)	Homocamptothecin	Beaufour lpsen	Phase II
Afeletecan HCI (Bay38-3441)	Water-soluble prodrug	Bayer	Phase II
PROTHECAN®	PEG conjugate	Enzon	Phase II
BNP-1350 (karenitecin)	Lipophilic	BioNumeric	Phase I
Gimatecan (ST-1481)	Lipophilic	Sigma Tau	Phase I
DE-310	Polymeric conjugate	Daiichi	Phase I
Camptothecin polyglutamate	Polymeric conjugate	Cell therapeutics	Phase I

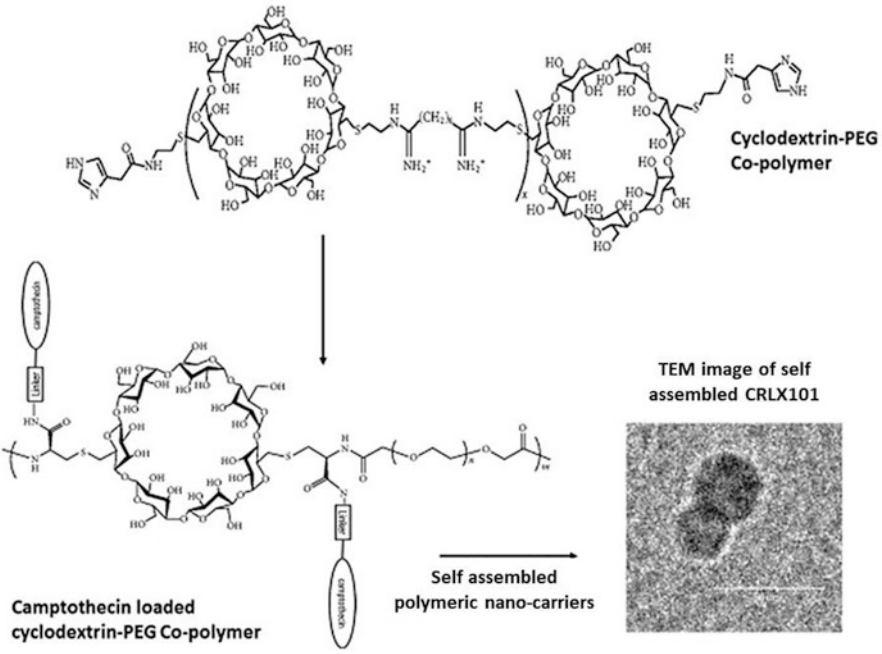


Fig. 6.6 Schematic diagram of CRLX101, a copolymeric nanoparticle formulation, where the phyto-derivative cancer drug camptothecin is conjugated to the linear, cyclodextrin–poly(ethylene glycol) (CD-PEG) (Ganesan and Choi 2016; Moody et al. 2015)

Table 6.4 Commonly used polymers and the corresponding drugs for nano-conjugate preparations

Polymer drug conjugates	**Drug**
	Doxorubicin
	Camptothecin
	PTX
	Platinate
	Polymer
	N-[2-hydroxylpropyl]methacrylamide [HPMA] copolymer
	Poly-L-glutamic acid [PGA]
	PEG
	Dextran

6.3.6 Hydrogel Nanoparticles

Hydrogels are cross-linked networks of hydrophilic polymers that can absorb and retain more water and at the same time maintain the distinct three-dimensional structural network (Bhatia 2016).

Hydrogels are mainly useful for the slow release of drug molecules into the biological system. It was demonstrated that the implantation of hydrogel nanoparticle caused high drug concentration and retention of the drug at the target tissue.

Furthermore the hydrogel could be combined with the magnetic nanoparticles to develop a hybrid hydrogel, which can be transported to the target site by applying magnetic field externally (Bamrungsap et al. 2012; Bhatia 2016; Hamidi et al. 2008). Although nanoparticulate, hydrogel-based drug delivery systems are not commercially applied, owing to their highly biocompatible and efficient drug-loading properties, they have high possibility to be further developed for drug delivery systems in the future.

6.3.7 Dendrimers

Dendrimers are the uniformly branched, macromolecular structures synthesized in a stepwise manner so that they are developed into the size of 1–10 nm particles. This treelike structure is distinct from other linear polymers that the molecular weight and the chemical composition can be precisely controlled. The dendrimers possess internal cavity-like structures where the drugs are encapsulated and which helps for slower, controlled release from the inner core. The dendrimers also allow the embedding of the drugs onto the outer surface using covalent or ionic interactions (Bhatia 2016; Avti and Kakkar 2013).

Dendrimers are synthesized using divergent or convergent techniques. The divergent technique allows synthesis of the inner core, and it is further built into other layers. In the convergent approach, the dendrimer synthesis starts from the outer periphery and ends in the inner core. Dendrimers are good anticancer drug delivery system due to their increased drug solubility, permeability, and intracellular targeted drug delivery (Fig. 6.7) (Fleige et al. 2012; Joralemon et al. 2010; Hamidi et al.

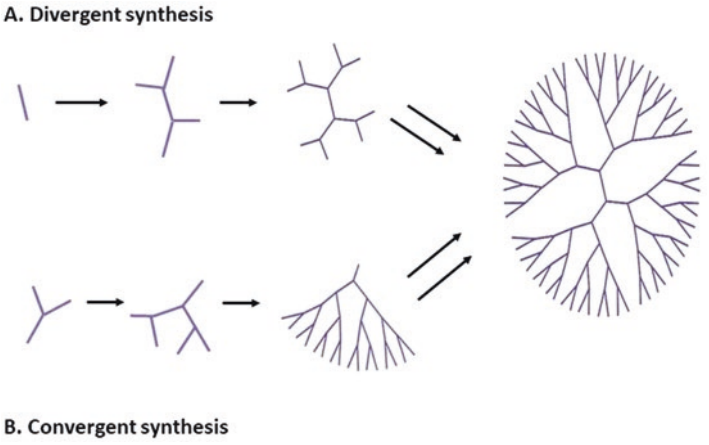


Fig. 6.7 Divergent synthesis (A), where the nanomaterial develops from inner core to other layers, though in convergent approaches (B), the development starts from periphery to inner core for the synthesis of dendrimer as explained thematically in the review (Fleige et al. 2012; Joralemon et al. 2010; Hamidi et al. 2008)

2008) . It was reported that the bioavailability of quercetin could be improved by incorporating it in polyamidoamine (PAMAM) dendrimers (Madaan et al. 2016).

6.3.8 Inorganic Platforms

The gold (Au) nanoparticles are emerging as a more promising drug delivery system due to their advantages like low toxicity from their inertness, ease to synthesize, increased surface area, and tuneable stability. Gold (Au) nanoparticles can be synthesized using biological method via bioreduction of *Piper guineense* aqueous leaf extract, and the drug formulation has highest release efficiency when compared to the drug-alone application (Shittu et al. 2017). It is possible to target the gold nanoparticles into the tumor and destroy the tumor by hyperthermic reactions. Also the gold nanoparticles can be monitored by contrast-based imaging during theragnostic applications. Still the key issue that needs to be addressed with gold nanoparticles is the engineering of the particle surface for optimized properties, such as bioavailability, biocompatibility, and non-immunogenicity (Kuo et al. 2010).

6.3.9 Superparamagnetic Nanoparticles

Recently magnetic nanoparticles are designed with the motive of drug carriers for targeted delivery. Magnetic nanoparticles are embedded in polyelectrolyte capsules and delivered for sustained release of drugs by applying external magnetic field. For example, iron (II) oxide particles are used to deliver microcapsulated drugs by applying magnetic field (Lu et al. 2002). Also after internalization, the magnetic nanoparticles can be induced to produce heat and cause hyperthermic effect. For example, a grafted thermosensitive polymeric system was developed using poly(*N*-isopropylacrylamide)-based hydrogels in which FePt nanoparticles were embedded. Using these nanoparticles, sustained release of loaded drug was attained by increasing the temperature based on the magnetic thermal heating event (Bamrungsap et al. 2012; Pankhurst et al. 2009). Magnetic nanoparticles also could influence the microcapsule permeability by oscillation using external magnetic fields. The major benefits of the magnetic nanoparticles in comparison to conventional cancer treatments are its less invasive nature, accessibility even of hidden tumor, and the reduced side effects.

6.3.10 Carbon-Based Nanomaterials

Recently, carbon-based nanomaterials are gaining popularity in drug delivery since it has the advantage of surface functionalization for grafting of nucleic acids, peptides, and proteins. However the major limitation of the carbon-based

nanocarriers is their cytotoxicity. There are varieties of carbon-based nanomaterials available, such as carbon nanotubes (CNTs), fullerenes, and nanodiamonds. Among these carbon-based nanocarriers, carbon nanotubes are shown to inhibit cell proliferation and cause apoptosis in cells. Also, the toxicity is high in carbon nanotubes because of the presence of the functional groups such as carbonyl, carboxyl, or hydroxyl groups on the surface of the carbon nanotubes (Kang et al. 2007; Liu et al. 2011b).

Betulinic acid (3β-hydroxyl-lup-20(29)-en-28-oic acid) of birch tree effectively induces caspase activation, mitochondrial membrane alterations, activation of reactive oxygen species (ROS), and DNA fragmentation and hence triggers the death of cancer cells. Carbon nanotubes (CNTs) are widely explored to deliver betulinic acid, and a poorly water-soluble drug was formulated using oxidized carbon nanotubes with diameter of 20–30 nm and length of 0.5–2.0 μm by chemical vapor deposition process. Studies confirmed that the CNT-formulated betulinic acid has increased efficiency than the free drug when studied using human lung cancer cells (A549) and human liver cancer cells (HepG2) (Tan 2014).

6.3.11 Integrated Nanocomposite Materials

Combining different nanocarriers helps in improvement of already existing nano-drug delivery platforms. Liposomes when combined with polymeric nanoparticles tend to have the benefits of both the systems. Also, liposomes can be frequently coated with PEG in order to prolong the in vivo plasma circulation time (Gabizon and Martin 1997; Yu et al. 2008; Allen and Cullis 2013). Similarly, liposomes formulated with dendrimers have slow and sustained drug-releasing abilities with improved drug loading capacity. "LipoMag" is the formulation where the inner core is made of magnetic nanocrystal coated with oleic acid, and the outer shell is made of cationic lipid molecules (Fig. 6.8) (Pankhurst et al. 2009; Xie et al. 2010; Namiki et al. 2009).

The bio-efficacy of phytochemicals, especially polyphenols, is improved by edible nanoencapsulation vehicles (ENVs). Efficacy enhancement is through

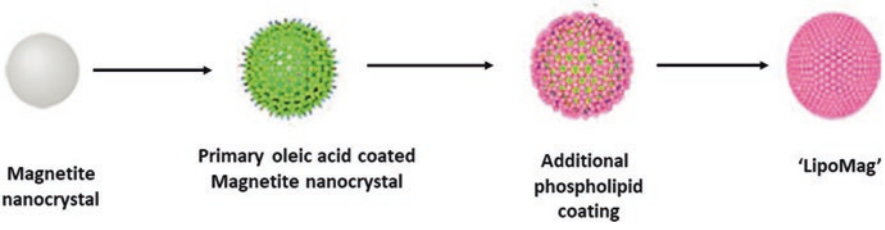


Fig. 6.8 "LipoMag" formulation of oleic acid-coated magnetic nanocrystal core and a cationic lipid shell. These nanoparticles could be magnetically guided to deliver at the specific targeted sites (Pankhurst et al. 2009; Xie et al. 2010; Namiki et al. 2009).

influencing phytochemical dispersion and gastrointestinal stability, rate of release, transportation efficiency across the endothelial layer, systemic circulation and biodistribution, and regulation by gut microflora. Furthermore, the rational design of the size, surface property, matrix materials, and compartment structure of ENVs also influence the bio-efficacy of the ENVs (Xiao et al. 2017).

6.3.12 Traditional and Green Synthesis of Nanoparticles

The production of nanoparticles basically involves two major approaches, namely, the top-down technique and the bottom-up approach of the components. The top-down techniques comprise milling, grinding, and applying laser to shred and break the larger particles into smaller-sized nanoparticles. The bottom-up technique allows the creation and engineering of nanoparticles based on an atom scale arrangement controlled by thermodynamic regulations (Manickam et al. 2017). With the aim of sustainable, pollution-free, nano-chemical synthetic approaches, the development of efficient green chemistry methods has drawn the interest of many researchers in recent years. Basically the aim of green biosynthesis of nanoparticles is to have cost-effective and environmentally friendly alternative approaches compared to the chemical and physical methods. Among the green alternatives, plants and phytoextracts are considered to be the best candidates of choice for the biosynthesis of nanoparticles. The advantages of using plant and plant extracts for the synthesis of nanoparticles include cost-efficiency, prolonged stability, and faster and large-scale synthesis (Sharma et al. 2009).

6.4 Nano-phytochemical Applications Against Inflammation

Inflammation is the process which characterizes the physiological reaction of the body to tissue damage (e.g., stress, irritants, and radiations), infections (microbial and viral), or genetic changes. It is a defensive response which involves immune cells, blood vessel, and different types of mediators (Surh 2003). Many biological processes associated during the inflammatory events include local vasodilatation, increased capillary permeability, accumulation of fluid and blood proteins into the interstitial spaces, recruitment of neutrophils out of the capillaries, and release of inflammatory mediators (Tabas and Glass 2013; Baum and Arpey 2005; Gurtner et al. 2008; Karin and Clevers 2016).

But if the tissue injury is not fixed during the acute inflammatory phase and prolonged, it leads to chronic inflammation which causes various immunopathological changes in the biological system (Baum and Arpey 2005; Hench 2005; Ryan and Majno 1977; Golia et al. 2014). Many diseases and their pathological progression are associated with inflammation which includes diabetes, cancer, cardiovascular disease, neurodegenerative diseases, obesity, asthma, and inflammatory disease like

acute pancreatitis and arthritis (Ryan and Majno 1977; Golia et al. 2014; Montecucco et al. 2017; Amor et al. 2014; Velusamy and Tamizhselvi 2018; Chen et al. 2016; Perretti et al. 2017; Lambrecht and Hammad 2015; Zhong et al. 2017; Crusz and Balkwill 2015; Bhatia et al. 2005).

Many synthetic compounds currently used against these disorders are associated with side effects like liver failure, skin problems, asthma, headache, nausea, ulcer, and gastric problems. To overcome these side effects, the focus is turned on towards a range of natural phytoconstituents including phenolics, alkaloids, and terpenoids for the regulation of inflammatory processes (García-Lafuente et al. 2009). Partial list of some phytochemicals used for anti-inflammatory approaches and the formulations used for effective delivery as reviewed in recently are listed below (Table 6.5) (Conte et al. 2017).

The ability of phytochemicals in inhibiting iNOS activity, reducing iNOS expression, or regulating cyclooxygenase-2 (COX-2) function has been proven in various studies. Also, phytochemicals suppress Akt, protein kinase-C, and mitogen-activated protein kinase (MAPK) signaling pathways by modifying the DNA-binding abilities of transcription factors such as nuclear factor kappa-B (NF-κB) (Fig. 6.9) (Montecucco et al. 2017). During the allergic reactions, by inhibiting the release of histamine, phytochemicals can be used as anti-inflammatory drugs.

In spite of the high potential of raw plant extracts for controlling inflammations, poor solubility, poor stability, short biological half-life, and rapid elimination hamper their clinical use. Likewise, absorption is adversely affected by the massive molecular size and altered pharmacokinetics due to high acidic gastric pH (Milbury et al. 2010).

To overcome these ill effects, nanosized carriers are applied while delivering the anti-inflammatory phytochemicals to the respective delivery site. Various phytochemical-conjugated nanotechnological formulations are applied in enhancing the anti-inflammatory properties and some of which are discussed here.

6.4.1 Polyphenolic Compounds

Polyphenols are most active towards chronic disease by boosting the response of the immune system. Polyphenolic compounds are prominently involved in anti-inflammatory activity. These anti-inflammatory principles are structurally arising due to their primary aromatic ring, oxidation status, and associated functional groups and functionally because of their potent-free radical scavenging properties and interactive abilities with proteins, enzymes, and membrane receptors activity (González et al. 2011). Hence the polyphenolic quercetin, resveratrol, and tannins are acknowledged as painkillers (Etheridge et al. 2013). However, clinical applications of polyphenolic compounds are limited due to both intrinsic (chemical structure, molecular weight, and low hydrosolubility) and extrinsic issues (poor stability in the gastrointestinal environment). Polyphenolic compounds were able to maintain the structural and functional integrity when delivered through the nano-delivery systems (Li et al. 2009).

Table 6.5 Some of the common phytochemicals used for anti-inflammatory, anticancerous, and other therapeutic approaches as reviewed in (Conte et al. 2017)

Phytocompounds	Reported mode of delivery system
Quercetin (polyphenol)	Solid lipid nanoparticles made up of soya lecithin, Tween 80, and PEG
	Poly(lactic-co-glycolic acid) (PLGA) nanoparticles loaded with quercetin
	Quercetin-loaded Eudragit-polyvinyl alcohol nanoparticles
	Lipid-coated nanocapsules
	Quercetin-loaded poly(lactic-co-glycolic acid) (PLGA) NC
Resveratrol (polyphenol)	Encapsulated in PLGA nanoparticles
	Resveratrol in Eudragit RL 100 nanoparticles
	Carboxymethyl chitosan nanoparticles
	Loaded in solid lipid nanoparticles with controlled releasing profile
	Resveratrol loaded in solid lipid nanoparticles
	Cyclodextrin-based nano-sponges
Ellagic acid (phenolic class of tannins)	Ellagic acid loaded in PLGA nanoparticles
	Poly(lactic-co-glycolic acid) (PLGA)–polycaprolactone (PCL) nanoparticles
Curcumin (polyphenol)	Encapsulated in hydrogel-/glass-based nanoparticles
	Oil in water nanoemulsion containing curcumin in oil phase
	Encapsulation of curcumin in liposomes (lipid nanoparticles)
D-9-Tetrahydrocannabinol (phyto-cannabinoid)	Encapsulated in nanostructured lipid carriers
	Loaded in lipid nanoparticles containing lecithin
	Poly(lactic-co-glycolic acid) (PLGA) nanoparticles containing surface-modifying agents such as chitosan, Eudragit RS, lecithin, and vitamin E
	Cannabidiol-loaded PCL particles
	D9-THC-loaded poly(lactic-co-glycolic acid) (PLGA) nanoparticles
Phytosterol	Nanodispersion produced by emulsification–evaporation using hexane
	Phytosterols colloidal particles using anti-solvent precipitation
	Nanodispersion obtained by suspensions of submicron particles of phytosterol
Oregano and cassia essential oil	Encapsulated in corn zein nanocapsules via phase separation techniques
Thymol and carvacrol essential oil	Nanoencapsulation in corn zein nanoparticles via liquid–liquid dispersion method
Thymol and cinnamaldehyde essential oil	Inclusion in cyclodextrin
Lippia sidoides essential oil (50%–70% thymol)	Encapsulated aliginate/cashew gum (biopolymer blend) nanoparticles via spray-drying
Cumin and basil essential oil	Polyamide capsules, release cargo oil under UV-light radiation

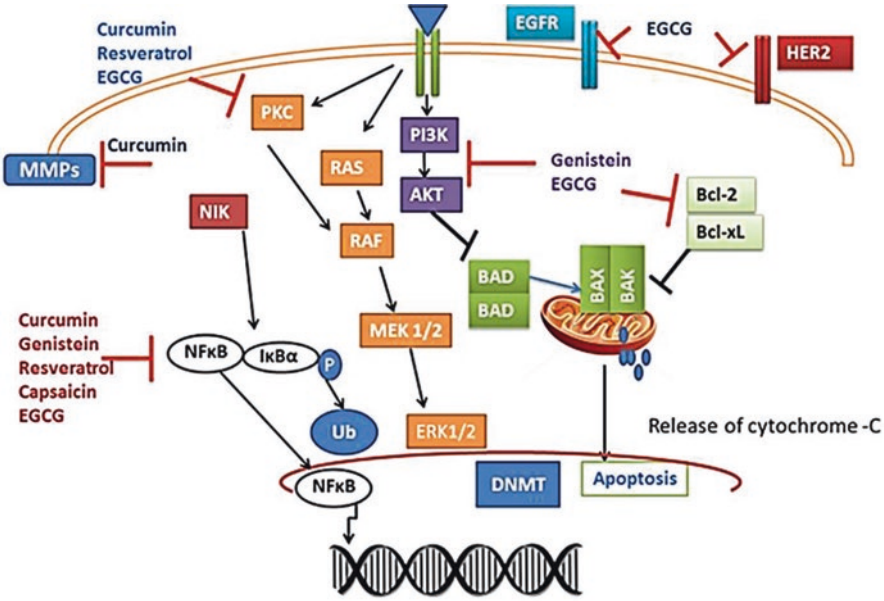


Fig. 6.9 Many phytochemicals target various mitogenic signaling pathways like Akt, protein kinase C (PKC), mitogen-activated protein kinases (MAPKs), MMPs, transcription factors like nuclear factor kappa B (NF-κB) (Montecucco et al. 2017)

6.4.2 Quercetin

Quercetin is semi-lipophilic flavonol present in the plant of tomatoes, leafy green vegetables, and berries. For encapsulation-based delivery of quercetin, solid lipid nanoparticles made of soya lecithin, Tween-80, and polyethylene glycol (PEG) were used, and here 91% encapsulation effectiveness was achieved. Using this approach, there was a 5.7-fold enhancement in the absorption of poorly water-soluble quercetin during the oral delivery (Barras et al. 2009).

Quercetin formulated using lipid-coated nanocapsular approach enhanced the solubility by hundred times compared with the free form of quercetin. Also here the stability was improved by more than 10 weeks without any drastic degradation (Wu et al. 2008). Activity-wise, enhanced antioxidant properties like DPPH scavenging, superoxide anion scavenging, and anti-lipid peroxidation were strengthened, and more efficiency was reached with quercetin-loaded nanoparticles than pure quercetin. Release of quercetin from carriers was increased by 74-fold than the free form, when nanoprecipitation was used with Eudragit E® and polyvinyl alcohol (PVA) during synthesis of quercetin-loaded nanoparticles.

Poly(lactic-co-glycolic acid) (PLGA)-based nanofabrication has been developed for encapsulation and controlled release of phytocompounds. Pool et al. synthesized quercetin-loaded poly(lactic-co-glycolic acid) (PLGA) nanocapsules aimed at preventing oxidative stress in human body against peroxyl radical-induced lipid peroxidation,

thus ensuring more potent applications in anti-inflammatory therapy (Chakraborty et al. 2012; Pool et al. 2012). Even in a rat model, the effectiveness of orally administered quercetin–PLGA nanoparticles was confirmed (Singh and Pai 2014a).

6.4.3 Tannins

Ellagitannins (ETs) and ellagic acid (EA) belong to the family of bioactive polyphenolic class of tannins (Heber 2011) and are abundantly present in pomegranates (Sonaje et al. 2007). To enhance the bioavailability, ellagic acid was loaded with poly(lactide-co-glycolide) (PLGA) and polycaprolactone (PCL) using PEG 400 with DMAB or PVA as the stabilizers. Significantly enhanced intestinal uptake of DMAB-stabilized nanoparticles was observed than carboxymethyl cellulose suspension or when compared with the PVA-stabilized ellagitannins in rats. Evidentially from biochemical and histopathological studies of kidney, it was shown that ellagic acid nanoparticles were capable to check the induced nephrotoxicity in rat models (Chainani-Wu 2003).

6.4.4 Curcuminoids

Curcuminoids are chemicals present in turmeric and show therapeutic potential against different pathological conditions. This group includes mainly curcumin (diferuloyl methane), demethoxycurcumin, and bisdemethoxycurcumin. Curcumin was shown to have anti-inflammatory actions by inhibiting the key molecules mediating inflammation (Zhang et al. 2016). Different attempts were made to increase the efficacy of these molecules. For example, topical application of curcumin captured in hydrogel-/glass-based nanoparticles protects the chondroprotective activity of curcumin and increases its bioavailability in osteoarthritic mouse model (Li et al. 2005). Also liposomes are used as nano-vectors for encapsulating and release of curcumins, and Takahashi et al. developed encapsulation of curcumin in liposomes by using commercially accessible lecithin (Takahashi et al. 2009). These nano-delivery procedures demonstrated that encapsulation enhances the bioavailability and increases the pharmacokinetics of drug.

6.4.5 Phytocannabinoids

Cannabis, commonly known as marijuana, is a product of the *Cannabis sativa* plant, and the active compounds are collectively referred to as *phytocannabinoids*, and to date around 70 phytocannabinoids are reported (Hill et al. 2012). Cannabinoids are anti-inflammatory in nature and mediate their effects through different mechanisms

which include induction of apoptosis, inhibition of cell proliferation, suppression of *cytokine* production, and induction of T-regulatory cells (Nagarkatti et al. 2009).

Cannabinoids in different experimental models such as multiple sclerosis, rheumatoid arthritis, colitis, and hepatitis have reported to guard the host from the pathogenesis through stimulation of multiple anti-inflammatory pathways (Henson 2003; George et al. 2008; Esposito et al. 2016).

To avoid any potential psychotropic drug exploitation and precisely to target the active principle, encapsulated phytocannabinoids have been prepared using nanolipid carriers by ultrasonication. Similarly, a lipid nanoparticle-based cannabinoid formulation was developed against chronic pain (Durán-Lobato et al. 2016).

Also the surface-modified poly(lactic-co-glycolic acid) nanoparticle (PLGANP) was developed using modifying agents like chitosan, Eudragit RS, lecithin, and vitamin E to increase the release rates of the particles (Martín-Banderas et al. 2015). Hernán Pérez de la Ossa et al. used oil in water emulsion–solvent method to prepare suitable dosage form of cannabidiol-loaded PCL particles (Hernan Perez De La Ossa et al. 2012).

From multiple studies it is clear that these nanocarriers maintained the original physicochemical properties and long-term stability and improved the pharmacokinetics of these phytochemicals.

6.4.6 Phytosterols

Phytosterols, the natural components of human diets which involve plant sterols and stanols, and phytosteroids are found mostly in vegetable oils, cereals, fruits, and vegetables.

Experimental (Medeiros et al. 2007; Vitor et al. 2009; Holanda Pinto et al. 2008; De Jong et al. 2008) and clinical (Hallikainen et al. 2008; Aldini et al. 2014) studies have confirmed the anti-inflammatory properties of plant sterols in addition to anticancerous and anti-atherogenic activities (Hu et al. 2017). Recently phytosterol supplementation has shown no significant effect on growth but could extraordinarily decrease diarrhea rate and develop resistance and anti-inflammatory action in animal models like weaned piglets (Leong et al. 2011).

Conversely the absorption rate of phytosterols is less than 2%, and to increase the phytosterol pharmacokinetics, the formulation and characterization of phytosterol nanodispersions are done by using emulsification–evaporation process. These formulations with food application are highly water soluble and characterized by significantly enhanced absorption (Rossi et al. 2010).

Similarly, stable colloidal dispersions having hydroxyl groups on the particle surface and non-ionic stabilizer-based sterically stable formulations were prepared. Turk et al. used the technique which involves a rapid expansion of a supercritical solution using four different surfactants to produce stable suspensions of submicron particles of phytosterol. Most cases bimodal particle size of about 500 nm were obtained and long-term stability was observed (Mancini et al. 2014; Türk and Lietzow 2004).

6.4.7 *Essential Oils*

Essential oils (EOs) are hydrophobic liquid rich with volatile aroma compounds, extracted from aromatic plants as secondary metabolite, and have a significant role in the traditional pharmacopeia. Due to its biological activity and medicinal properties, essential oils are used for antimicrobial, anti-inflammatory, and other pharmaceutical applications (Ajazuddin and Saraf 2010; Elshafie et al. 2015). Additionally essential oils have been applied in various industries while preparing perfumery, cosmetics, feed, food, and beverage-based products. With essential oils, encapsulation techniques are applied for upgrading the bioavailability, pharmacological activities, solubility, targeted delivery, and reduction by biodegradation (Parris et al. 2008). Recently 100% pure essential oil of oregano, red thyme, and cassia have been encapsulated through phase separation into zein nanospheres (Parris et al. 2008; Wu et al. 2012). Similarly, liquid–liquid dispersion method used to encapsulate thymol and carvacrol in the nanoparticles of zein was applied which has improved antioxidant as well as antimicrobial activity (Pinho et al. 2014; Zhou et al. 2018).

Like zein, cyclodextrins have also been used to improve the solubilization and stabilization of natural active product. Cyclodextrin formulations are used to load molecules which are less polar than water (Pinho et al. 2014; Loftsson and Brewster 1996). Alternatively alginate/cashew gum nanoparticles were developed into a biopolymer blend for encapsulation of essential oil using spray-drying method (Fleige et al. 2012; de Oliveira and Paula 2014).

Recent innovative approaches include controlled and triggered release of phyto-compounds using stimuli-responsive materials during the encapsulation methods. In this regard, Bizzarro et al. prepared cumin and basil oil-loaded polyamide capsules, which have the capability of delivering their cargo oil under UV-light irradiation (Bizzarro et al. 2016).

Though many phyto-modifications were proposed using nano-approaches for anti-inflammatory functions, currently they are only in laboratory level for developmental purpose and not been entered into any clinical applications.

6.5 Anticancerous Approaches Using Phyto-nanotechnology

Evidences from the in vitro, in vivo, clinical trial data reveal that the plant-based diet can help to fight many chronic disorders including cancer. Phytochemical compounds control cancer by regulating the crucial pathogenic transformation process like mediating apoptotic cell death, inhibiting angiogenesis, blocking metastasis, and others (Fig. 6.10). Recently, a lot has been studied about the potential role of medicinal plants in anticancer therapy, and it is proven that phytochemical agents are associated with better efficacy and lesser side effects. Evidentially, around 47% of FDA-approved anticancer drugs are derived from plants (Carter et al. 2003). Phytochemicals could be used as a single chemotherapeutic agent or in

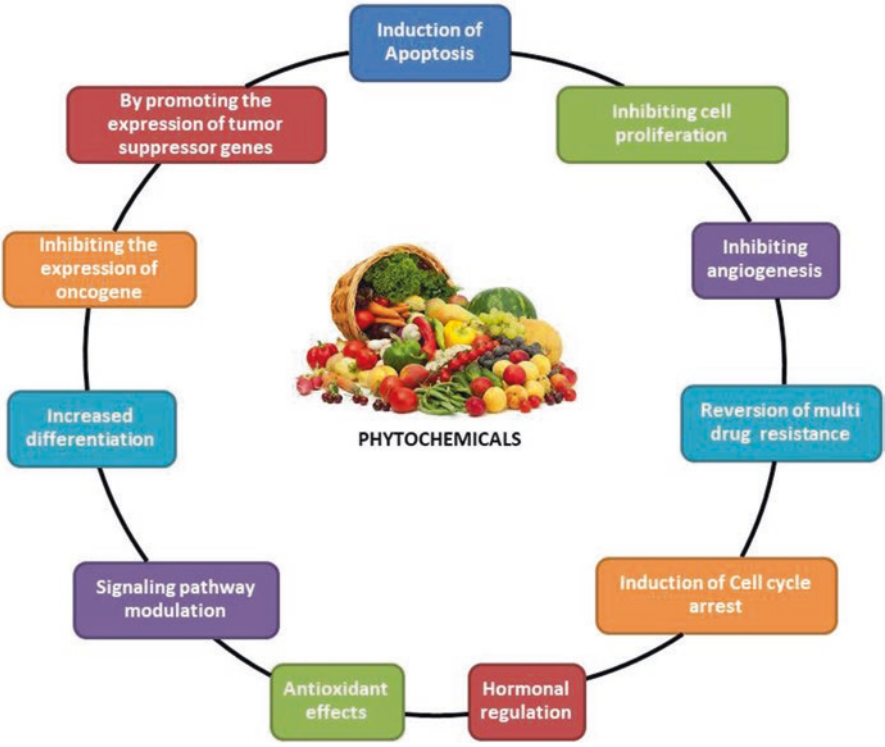


Fig. 6.10 Phytochemical compounds control cancer by regulating the crucial pathogenic events. Phytochemicals combat cancer by the inducing apoptosis, by inhibiting cell proliferation, by cell cycle arrest, by modulating many signaling pathways, by hormonal regulation, by diverting the neoplastic cells for differentiation, by interfering with the expression of oncogenes, by overexpressing tumor suppressor genes, by reverting the drug resistance, by inhibiting angiogenesis and metastasis, and by exerting its antioxidant effects

combination as adjuvants with standard chemotherapeutic drugs to increase their effectiveness while decreasing their side effects.

Some of the promising phytochemicals or its derivatives already marketed for cancer treatment include paclitaxel, vinblastine, and topotecan, and the nanotechnological approaches in formulation and enhancing their efficiency are discussed below.

6.5.1 Nanotechnological Approaches in FDA-Approved Phyto-derivatives for Cancer Therapy

Paclitaxel

Paclitaxel (PX) is a mitotic inhibitor isolated from the bark of Pacific yew (*Taxus brevifolia*). It is considered to be one of the important and most effective chemotherapeutic drugs ever developed, and it exerts its cytotoxic effects against a

broad range of cancers such as lung, ovarian, and breast cancers. It belongs to the class of plant alkaloids. The underlying mechanism of action of paclitaxel for attaining its cytotoxicity is by promoting and stabilizing microtubules and inhibiting late G2 or M phases of cell cycle. It is highly hydrophobic, and due to this reason it is formulated in a mixture of Cremophor EL and dehydrated ethanol (50:50, v/v), a combination known as Taxol. Due to the presence of surfactants like Cremophor EL® (BASF Corp.) for paclitaxel, and Tween-80® (ICI Americas, Inc.) for docetaxel, Taxol has severe side effects. Therefore, there was an ultimate need for the development of alternative Taxol formulations (Lines and Studies 2015). Researchers developed nano-particular albumin-bound paclitaxel (Abraxane®) which has been approved by the FDA and marketed by Celgene for the treatment of metastatic breast cancer and non-small-cell lung cancer (NSCLC).

Abraxane® nanoparticles are 130 nm in diameter and are made of human serum albumin. The albumin-based nanoparticle is formulated to bind to the drug paclitaxel non-covalently and is reversible. This formulation also increases the drug-carrying capacity of the nanocarrier as it can carry about extra 10% of the drug paclitaxel (Green et al. 2006; Miele et al. 2009).

In addition, there are plenty of novel paclitaxel nanoparticle formulations which are in different stages of clinical trials. For instance, paclitaxel-loaded PLA–PEG nanoparticles were synthesized and characterized for their cytotoxic activity on breast (MCF7, MDA-MB-231, and BT-474) and ovarian cancer cell (SK-OV-3) lines, which showed sustainable nontoxic drug-releasing properties. Further in tumor xenograft models, distribution of these nanoparticles was visualized for efficient delivery (Hou et al. 2015). Paclitaxel-loaded poly(lactic-co-glycolic acid) (PLGA) particles were tested for the viability of human hepatocellular carcinoma (HepG2) cells, and this nanoparticle formulation effectively inhibited the proliferation and induces the apoptosis in HepG2 cells. Using this cost-effective nanoformulation approach, sustained release of paclitaxel was achieved (Moudi et al. 2017).

Vinca Alkaloids

Vinca alkaloids are a subset of drugs obtained from the Madagascar periwinkle plant (*Catharanthus roseus*) which are antimitotic and anti-microtubule targeting alkaloids. They possess hypoglycemic as well as cytotoxic effects. Four major vinca alkaloids are in clinical use for cancer: vinblastine, vinorelbine, vincristine, and vindesine. These vinca alkaloids halt the division of cells and cause cell death. During cell division, they bind to the tubulin molecules and disrupt its microtubule function and directly cause metaphase arrest (Lee et al. 2015). Tubulin protein normally works in cells to create “spindle fibers” (also called as microtubules). These microtubules provide cells with both the structure and flexibility they need to divide and replicate. Without microtubules, cells cannot divide. Vinca alkaloids are highly neurotoxic. Moreover vinca alkaloids are susceptible to multidrug resistance in the earlier phase of treatment which limits severely the clinical usage of vinca

alkaloids. To minimize these problems and enhance the therapeutic efficiency of vinca alkaloids, many researchers have developed nanotechnological strategies such as using liposome-entrapped drugs, chemical or peptide-modified drugs, polymeric packaging drugs, and chemotherapy drug combinations. Because of the resistance developed from decreased uptake and increased drug efflux, Wang et.al encapsulated vincristine into folic acid-conjugated PEGylated liposomes to improve the anti-tumor efficacy on multidrug resistant cancers (Wang et al. 2012). These nanoparticles inhibited tumor growth effectively both in vitro on KBv200 cells and on in vivo KBv200 xenograft models.

Etoposide

Etoposide (ETP) belongs to a class of plant alkaloids. It is a semisynthetic derivative of podophyllotoxin, an inhibitor of topoisomerase II, which interfere with structural arrangement of DNA which is necessary during replication.

Etoposide has a significant activity against malignant lymphoma, small-cell lung cancer, stomach cancer, and ovarian cancer. However, because of its low solubility, the short biological half-life (1.5 h), poor bioavailability, and severe side effects (cardiotoxicity and myelosuppression), etoposide (ETP) has limited clinical applications. To overcome these problems, there is a need for finding the new systems of efficient and targeted drug delivery which could deliver anticancer agents precisely to the target cancerous cells. Henceforth etoposide-loaded nanostructured lipid carriers (ETP-NLCs) were synthesized and evaluated for their antitumor activity in vitro and in vivo. ETP-NLCs significantly enhanced the cytotoxic effects in vitro and in vivo antitumor effect against SGC7901 cells and gastric cancer animal model compared to the free drug (Zhang et al. 2017).

Folate (FA)-decorated and etoposide-loaded NLCs (FA-ETP-NLCs) were prepared and analyzed for anticancerous activities both in vitro and in vivo. In vitro cytotoxic effects were on three cell lines CT26, SGC7901, and NCI-H209, and nanostructured carriers were found to increase cytotoxicity compared to the EPT solution form. The in vivo studies on BALB/c nude mice for gastric cancer models illustrated that FA-ETP-NLCs had the best biodistribution in tumor tissue and the highest antitumor activity than free form (Pimple and Manjappa 2012).

For testing the synergistic effect of etoposide and quercetin on lung cancer cell lines, PLGA nanoparticles separately loaded with etoposide (ETP) and quercetin dihydrate (QDN) were designed by using solvent diffusion (nanoprecipitation) technique. In the encapsulated form, the drug-loaded PLGA nanoparticles showed sustained release of drugs as compared to faster clearance of free form. The in vitro cytotoxicity assays on A549 (human lung adenocarcinoma epithelial cell line) revealed significant increase in cytotoxicity with nanoparticle formulations than the free drug form. The comparison was also made with respect to cytotoxic activity of individual drug against combination drugs in the form of free drugs as well as nanoparticles. The combination treatment in the form of nanoparticles is found to produce significant cytotoxic effects (Ma and Mumper 2013).

6.5.2 Other Phytochemicals in Cancer Therapy

In addition to the approved and marketed phyto-derivatives, other promising phytochemicals and their nanotechnical formulations were reported for cancer therapies, and some of which are discussed below.

Resveratrol

Resveratrol (3,5,4′-trihydroxy-*trans*-stilbene) is a phytoalexin produced in plants in response to injury or upon pathogen attack like fungus and bacteria. It is abundantly found in many plant species like the skin of grapes, berries, etc. It is considered to be a major active polyphenol of red wine and exerts numerous health benefits including improved metabolism, cardiac protection, and cancer chemoprevention (Lee et al. 2012; Karthikeyan et al. 2013).

Many in vitro and in vivo studies explained the promising anticancer properties of resveratrol, even though it has many drawbacks when considered for clinical applications. For instance, its bioavailability is highly limited because of various reasons including poor stability, lesser absorption, poor water solubility, and shorter biological half-life. Thus it is very difficult to maintain the therapeutically relevant doses in the bloodstream (Karthikeyan et al. 2013; Singh et al. 2016). To address these limitations, researchers found a way to increase its anticancer properties by developing many biocompatible nanoparticles.

Resveratrol-loaded gelatin nanoparticles were studied for their anticancer properties on lung cancer cell line, NCI-H460, and showed promising anticancer effects than free resveratrol. This increase in anticancer activity is due to enhanced reactive oxygen species and by increase in DNA damage. Also the bioavailability of resveratrol was increased when it is loaded into the gelatin nanoparticles than when applied in its free form (Karthikeyan et al. 2013).

Bu et al. (2013) developed trans-resveratrol-loaded chitosan nanoparticles which are conjugated with two ligands biotin and avidin on the surface. This approach is to target the resveratrol selectively to the hepatic carcinoma, instead of directing towards whole liver. The biotin-bound polymers tend to accumulate in malignant tissue than normal tissues, and avidin-bound polymers are rapidly eliminated from blood circulation and accumulate in the liver. In this study they have concluded that the resveratrol bioavailability dramatically increased when it is loaded into the biotin-coated chitosan nanoparticles (B-CS-NP) and avidin–biotin-coated chitosan nanoparticles (A-B-CS-NP). They have also succeeded in increasing accumulation of trans-resveratrol-loaded chitosan nanoparticles in liver by conjugating either biotin or avidin. In another study resveratrol-loaded poly(ethylene glycol)–poly(lactic-co-glycolic acid) polymeric nanoparticles are studied for their cytotoxic and metabolic effects on CT26 cancer cells by comparing to that of the free compound. Increasing the stability and circulation time of resveratrol (RSV) by loading into nanoparticles allows significant metabolic and antitumor effects in

tumors of live mice models. Convincingly the results provide an encouraging outlook on the potential of PEG–PLA polymeric nanoparticles as an effective method to deliver resveratrol in vivo for cancer therapy (Bu et al. 2013).

With respect to nanocapsules, Carletto et al. (Carletto et al. 2016) has developed resveratrol-loaded nanocapsules with high drug-loading efficiencies and high in vitro cytotoxicity in B16F10 skin cancer cells. In in vivo studies, these nanocapsules loaded with resveratrol significantly reduced the tumor size of B16F10-bearing tumor mice models (Carletto et al. 2016).

In addition to its application in cancer therapy, resveratrol is having anti-inflammatory activities during human applications, and efforts are on to enhance its efficacy through nano-modification procedures during nutrient supplementation for various other disorders. Thus resveratrol turned more efficient when encapsulated as nanoparticulate form than the free one. Orally administered PLGA nanoparticles of resveratrol with particle size of about 170 nm have been reported to be more efficient by 78% than the free form. Furthermore, it was demonstrated that there is significant increase in rate and extent of oral bioavailability of Eudragit-RL-100 formulated trans-resveratrol. It was proven that biodistribution of it in liver and spleen has been significantly affected by Eudragit RL 100 composition (Singh and Pai 2014b). Also carboxymethyl chitosan-encapsulated resveratrol enhanced the solubility and thus enhanced the antioxidative property of resveratrol (Zu et al. 2014).

In an in vitro approach, nanosomes like spherical cyclodextrin have been reported to enhance the solubility and stability of resveratrol with better encapsulation efficiency, however without compromising the biological activity of pure form (Ansari et al. 2011). Similarly Pandita et al. prepared resveratrol-loaded solid lipid nanoparticles with drug integration efficiency of 89%, having improved plasma availability compared to the freely delivered drug (Pandita et al. 2014).

Thymoquinone

Thymoquinone (TQ) (2-methyl-5-isopropyl-1,4-benzoquinone) is a phytochemical compound and is a major active component present in the plant *Nigella sativa* (black seed) with long history of traditional medical applications. The thymoquinone is said to have many therapeutic properties including antimicrobial, anti-inflammatory, antioxidant, antidiabetic, anticancer, hepatoprotective, and renal protective activities. Although it possesses many health benefits, it has major limitations in its clinical properties due to its poor solubility. Its hydrophobic nature causes decreased bioavailability and reduces its formulation characteristics and poor membrane penetration capabilities. The lack of bioavailability and unfavorable pharmacokinetic parameters prevented the use of thymoquinone in clinical settings. To improve the bioavailability and cytotoxicity, thymoquinone-loaded nanostructured lipid carrier (TQ-NLC) was developed and tested using breast cancer cells (MDA-MB-231 and MCF-7) and cervical cancer cell lines (HeLa and SiHa). The TQ-NLC has shown high cytotoxic effects in MDA-MB-231 cells compared to the other cell lines (Fakhoury et al. 2016).

In another study, thymoquinone nanoparticles (TQ-NP) were formulated in poly(styrene-b-ethylene oxide) (PS-PEO) for theranostic approach, i.e., simultaneous imaging and cytotoxicity induction in breast cancer cell lines (MDA-MB-231, MCF-7). By comparing with non-tumorigenic cell line MCF-10A, the authors confirmed the stability, increased cellular uptake, and improved entry into nucleus, and as a consequence cytotoxic potential of thymoquinone was greatly enhanced in cancer cells (Soni et al. 2015).

In a synergistic approach, polymeric biodegradable poly(lactic-co-glycolic acid) (PLGA) nanoparticles encapsulated with thymoquinone and paclitaxel have shown to exert enhanced anticancer potential than the free drugs on MCF-7 breast cancer cells (Ng et al. 2015). In an in vivo setup, thymoquinone encapsulated in biodegradable polymeric nanoparticles was studied for any improvement in bioavailability. When tested on colorectal tumors of murine model, this nanoformulation of thymoquinone showed significant increase in the therapeutic activity by decreasing the tumor volumes and increasing the survival rate of the cancerous animals (Odeh et al. 2017).

Delivery of drug molecules using liposomes is considered to be a promising strategy to increase the therapeutic efficiency of targeted compound and to reduce the drastic side effects exerted by them. Liposomes loaded with thymoquinone were developed to increase the solubility of thymoquinone while reducing its side effects and to check their anticancer potential on breast cancer cells (Odeh et al. 2017).

Curcumin

Curcumin is a potential dietary component of turmeric (*Curcuma longa*), which belongs to the family Zingiberaceae. It is a natural phenolic compound which is responsible for the yellow color of the turmeric. Chemically it is referred to as diarylheptanoid belonging to the group of curcuminoids. Studies have indicated that curcumin shows potential anticancer effects by killing cancer cells and preventing the cells from growing. It has the best anticancer effects on breast cancer, bowel cancer, stomach cancer, cervical cancer, liver cancer, colon cancer, skin cancer cells, etc.

Curcumin is a hydrophobic polyphenol with very low toxicity even at very high doses. Though curcumin is considered to have potential anticancer activities, its low bioavailability makes its less useful for its therapeutic applications. Subsequently, different nanotechnological measures were attempted to enhance the bioavailability and some of which are discussed here. For instance, the curcumin-loaded poly(lactic-co-glycolic) nanoparticles (PLGA-CUR-NPs) were shown to have effective therapeutic potential on prostate cancer cells by inhibiting the cell proliferation in androgen-dependent and androgen-independent prostate cancer cell lines. The inhibitory role is achieved in both in vitro and in vivo models by inhibiting cell proliferation, inducing apoptosis, by interfering β-catenin, AKT, and STAT3 signaling pathways. Moreover the PLGA-CUR-NPs show to downregulate the oncogenic miRNA *miR21* and upregulate the beneficial *miR-205* (Saeed et al. 2017).

In a recent study, PLGA (poly(lactic-co-glycolic acid)) nanospheres encapsulated with curcumin (NCur) were tested on PC3 prostate cancer cell lines for their cytotoxic effects. In these cells, NCur has shown increased cell death, which is mediated mechanistically by both apoptosis and autophagy compared to the free curcumin (Wang 2017).

Nanostructured lipid carriers (NLCs) are the second-generation solid lipid nanoparticles.

These nanocarriers possess many important properties which make them as a preferred drug carrier. It is considered to increase the stability and improve the releasing properties of the loaded drug. NLC can be administrated through oral, pulmonary, intravenous, and percutaneous routes. NLC loaded with curcumin was studied in vitro for their anticancer properties on A549 lung cancer cell lines, and its pharmacokinetic effects were studied in rat models injected with curcumin-loaded NLCs (Cur-NLCs). This study has revealed that the intraperitoneally injected Cur-NLC showed very good in vivo tissue distribution characteristics of curcumin. Evidently the in vitro results proved the promising antiproliferative effect of Cur-NLC on A549 cell line by inhibiting the cell proliferation and directing the cells to apoptosis. Like in vivo results, with in vitro model also, the cellular uptake of curcumin has increased significantly when delivered through NLC than free curcumin (Yin et al. 2013).

In a study using different types of curcumin-loaded nanoparticles, which when tested against the lung cancer cells, it was proven to have potent anticancer activities. Three types of curcumin-loaded nanoparticles (mPEG4k PCL20k, mPEG2k PCL4k, mPEG10k PCL30k) were prepared as amphilic methoxypoly(ethylene glycol) (mPEG)–polycaprolactone (PCL) copolymers and were tested on A549 lung cancer cell lines for their anticancer potentials. Among them, mPEG10k PCL30k has shown highest drug-loading efficiency and sustained release pattern. The curcumin-loaded nanoparticles have exerted their anticancer activity through apoptosis, which is better when compared to the free form of curcumin (Li et al. 2005).

Epigallocatechin-3-gallate

EGCG (epigallocatechin-3-gallate) is a biologically active and most abundant catechin found in green tea (*Camellia sinensis*). Its chemotherapeutic role was studied extensively using in vitro and in vivo studies. It exerts its anticancer activity by inhibiting the proliferation of cancer cells, inducing apoptosis through Bcl-2 and BCl-xL proteins, by inhibiting epidermal growth factor receptor (EGFR) (Masuda et al. 2003) and human epidermal growth factor receptor-2 (HER2) (Fang et al. 2003), by blocking the DNA methyltransferase to interfere at DNA hyper methylation (Li et al. 2013), by regulating cell cycle, and by suppressing the angiogenesis by downregulating VEGF through HIF-1α (Hsieh et al. 2011) (Fig. 6.11). Intake of high amounts of green tea polyphenols can exert toxic effects against cancer cells. Despite its potential anticancerous properties, its use is still limited due to its excessive toxicity, less bioavailability, and ineffective systemic

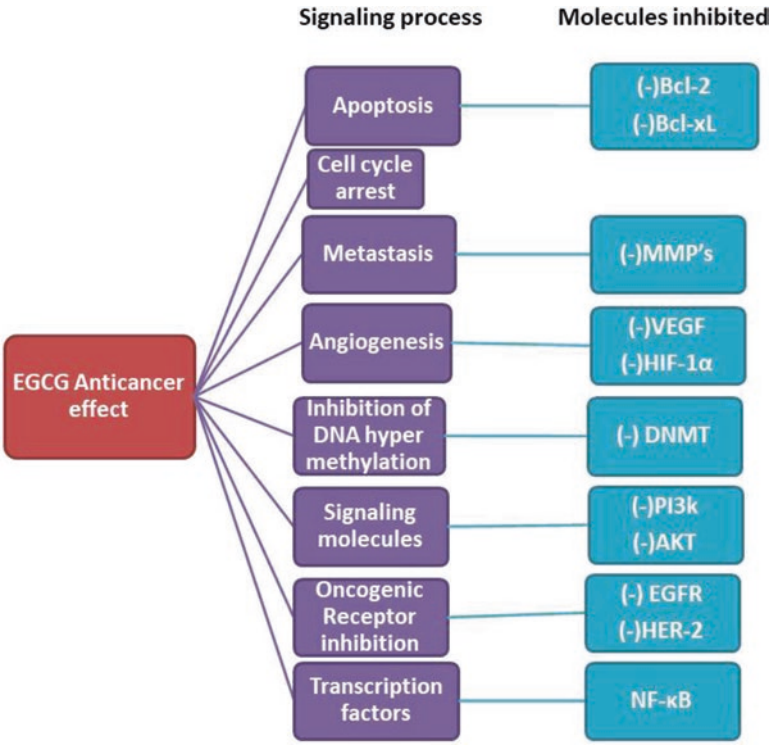


Fig. 6.11 Chemotherapeutic principle of epigallocatechin-3-gallate (EGCG) is mediated through negative interference of various molecules in different cell signaling processes. EGCG exerts its anticancer activity by inducing apoptosis by inhibiting survival-related genes like *BCL2* and *BCl XL*, by inhibiting the activation of receptors like epidermal growth factor receptor (EGFR) and human epidermal growth factor receptor-2 (HER2), by blocking the DNA methyltransferase (DNMT) to inhibit the DNA hypermethylation, by regulating cell cycle, and by suppressing the angiogenesis by downregulating VEGF through HIF-1α, and it prevents metastasis by inhibiting matrix metalloproteinases (MMPS), by inhibiting signaling molecules like PI3K and AKT, and by preventing nuclear translocation of transcription factor NF-κB

delivery. To improve its pharmacokinetic, pharmacodynamics properties and deliver as the optimized therapeutic agent, nanotechnological aspects can be implemented.

Gold nanoparticles (AuNPs) are gaining more interest as they offer many advantages. They are very much biocompatible and nontoxic, and these nanoparticles possess unique optical and biochemical properties. Hence gold nanoparticles are not only used for drug delivery, but it exerts its applications in diagnosis also. In a study using gold nanoparticles, EGCG was conjugated to the surface of the gold nanoparticles (EGCG-pNG) and tested for its anticancer potential on bladder cancer. It was found to kill effectively the bladder cancer cells (MBT-2) by intrinsic pathway of apoptosis, however without showing any toxic effects to the normal cells. Compared to free EGCG, it also effectively reduced the tumor size in C3H/HeN mice cancer model induced by MBT-2 cells (Hsieh et al. 2011).

Also EGCG nanocarriers were developed using chitosan specifically for the oral administration. *This nano-EGCG when checked using melanoma cell-based xenograft model was found to have many advantages than the native EGCG.* In vitro *studies of EGCG on Mel 928 cells showed the cytotoxic effects even at eight fold lower doses than the native EGCG by inducing apoptosis and cell cycle inhibition. In* in vivo *setup also, it inhibited the growth of Mel 928 tumor xenograft implanted in nude mice* even at tenfold lower dose than the native agent. It was reported that nano-EGCG inhibited the expression of proliferative marker proteins like PCNA and ki-67 (Siddiqui et al. 2014).

Quercetin

Quercetin (3,3′,4′,5,7-pentahydroxyflavone) is a polyphenol which is present excessively in fruits, seeds, vegetables, tea, coffee, bracken fern, and natural dyes. It is considered to have very strong antioxidative and anti-inflammatory properties. However quercetin is highly hydrophobic, and delivery is limited by its poor percutaneous permeation and skin deposition. Quercetin significantly inhibits the growth of cancerous cells like leukemia and breast, hepatic, ovarian, colorectal, gastric, and endometrial cancers. Several studies have shown that quercetin controls the growth of cancer cells by inducing apoptosis, regulating specific signaling pathways, decreasing oncogene expression, and inhibiting angiogenesis (Kumar et al. 2014). Though discussed in the previous section with respect to enhancing the anti-inflammatory roles, various attempts were also made using nanoformulation procedures for enhancing the pharmacological applications of quercetin against cancer.

Magnetic Fe_3O_4 nanoparticles were conjugated with quercetin was tested for their in vitro anticancer properties on MCF-7 breast cancer cell line. A simple precipitation method was used to conjugate quercetin on the surface of dextran-coated Fe_3O_4 via carboxylic/amine group using nanoprecipitation method. The cytotoxicity of quercetin-conjugated Fe_3O_4 nanoparticles increased significantly in comparison with pure quercetin, and this was supposedly due to the increased bioavailability of the compound (Guan et al. 2016).

In a study, quercetin-loaded poly(lactic-*co*-glycolic acid)-d-α-tocopheryl polyethylene glycol succinate nanoparticles (QPTN) were prepared for targeted treatment of liver cancer. These nanoparticles were prepared by ultrasonic emulsification–solvent evaporation technique, and for these study three different nanoformulations, QT-loaded PLGA-TPGS NPs (QPTN), QT-loaded PLGA NPs (QPN), and QT/coumarin-6-loaded PLGA-TPGS NPs (QCPTN) were prepared. In vitro studies on HepG2 and HCa-F/T cells showed efficient uptake and internalization of the fluorescently labeled QPTN nanoparticles. In case of cell viability studies, QPTN showed higher cytotoxicity than QPN. They both induced apoptosis in liver cancer cells in a dose-dependent manner. However in the in vivo studies, QCPTN nanoparticles were highly targeted towards liver than QPN (Sarkar et al. 2016).

Hence these nanoformulations can be effectively used both for diagnosis and treatment.

Folic acid (FA) armed mesoporous silica nanoparticles (MSN-FA-Q) loaded with quercetin nanoparticles were tested on breast cancer cells (MDA-MB-231 and MCF-7) for their anticancer properties. In this nanoformulation, the bioavailability of quercetin was increased by reducing its hydrophobicity. These nanoparticles caused apoptosis by increasing BAX proteins and downregulating phospho-AKT. Additionally these nanoparticles restricted the migration of breast cancer cells (Tian et al. 2013). Thus nanoformulative measures provide us the opportunity in unraveling and utilizing the complete antioxidative and anti-inflammatory and anticancerous properties of quercetin.

Genistein

Genistein is a major isoflavone constitute present in soybean and considered to be a most important phytochemical in cancer therapy. It is a natural angiogenesis inhibitor and also a phytoestrogen as its structure is similar to the 17β-estradiol. The anticancer properties of genistein were demonstrated in many in vitro and in vivo studies. It shows its anticancer effect in different cancer cell types including breast, prostate, colon, gastric, non-small-cell lung cancers, as well as in leukemia. Many recent reports demonstrated that genistein could act as potent agent which can be used as chemopreventive agent either individually or in combination with conventional cancer drugs. Interestingly in Asian populations, the lower incidence of breast and prostate cancer is due to the regular dietary intake of soy products which is rich in genistein. Genistein is poorly soluble in water which limits its clinical applications, and so the nano-technical modifications were proposed. In this regard, genistein-loaded biodegradable TPGS-b-PCL (d-α-tocopheryl polyethylene glycol 1000 succinate-b-poly(ε-caprolactone-ran-glycolide)) nanoparticles were tested on cervical cancer cells. Genistein-loaded TPGS-b-PCL NPs were more effective in suppressing cancer in both in vitro and in vivo than the native form of genistein. These nanoparticles increased the bioavailability of genistein and helped in increasing its therapeutic potential (Yang et al. 2015).

6.6 Nano-cosmeceuticals

In addition to their pharmaceutical and nutritive applications, the bioactive components derived from the plant and plant extracts have increased beautifying cosmetic effect in human beings. The cosmeceutical industry is a rapidly blooming industry which has been using several phytochemicals as active component since ancient times. Phytochemicals are applied to treat variety of skin and other diseases including aging, hair loss, inflammation, psoriasis, and protection from ultraviolet (UV) radiations (Kapoor 2005).

Variety of plant-derived compounds used in synthesis of beauty and healthcare products includes catechins, epicatechins, gallic acids, quercetin, ascorbic acids, curcumin, luteolin, alpha- and beta-carotene, complex polysaccharides, and hydroxyl benzoic, cinnamic, and other fatty acids (Ganesan and Choi 2016).

Still there are significant challenges with respect to particle size and solubility resulting in low-quality cosmeceuticals, reduced skin penetration, and prolonged non-beneficial effects. These challenges lead to the quest for finding more novel and efficient technologies to synthesize more enhanced cosmetics and associated products. Nanotechnological applications attempt to solve the abovementioned challenges like enhancing the efficiency of the phyto-derived cosmeceutical products. In order to increase the activity of the nano-cosmeceutical products, different nano-delivery systems and methods are attempted (Hu and Huang 2013). These include nanoemulsions, dendrimers, hydrogels, and lipid nanoparticles (Bhatia 2016). Some of the phyto-derived cosmeceutical products and the application of nanotechnology as reviewed in (Ganesan and Choi 2016) are listed in Table 6.6.

Evidentially the use of nano-based phytochemicals is eventually increasing in sunscreens and other skin protectants. At present, a variety of natural and synthetic cosmetics having nanoformulations are marketed with multiple effects like skin

Table 6.6 Nanotechnology in phyto-derived cosmeceutical products (Ganesan and Choi 2016)

Phytocompounds	Nano-delivery methods	Size (nm)	Applications
Rice bran oil	Nanoemulsion	69	Moisturizer Antiaging Skin care
Rice bran and raspberry seed oil	Lipid nanocarriers		Sunscreens
Lavender extracts	Polymeric poly(lactic-co-glycolic)acid [PLGA] nanoparticle	301–303	Antiaging, antioxidant
Rosemary extracts	Solid lipid nanocarriers Nanostructured lipid carriers	57 68	Antioxidant(skin) Antioxidant
Aloe vera extract	Nano-liposome	200	Skin care
Safflower extracts	Nanostructured lipid carriers	100	Hair care
Lutein	Nanostructured lipid carriers and nanoemulsion	150–350	Skin care
Quercetin	Nanostructured lipid carriers	215	Skin care
Ganoderma triterpenoids	Nanostructured lipid carriers gel	179	Skin enhancement
Hinokitiol	Poly(epsilon-caprolacton) nanocapsules	223	Hair care
Hinokital	Bilayer vesicles		Hair growth
Curcumin	Nanoencapsulation	190 and 276	Skin care
Tocopherol	Nanostructured lipid carriers and nanoemulsion	67 and 576	Skin care
Resveratrol	Solid lipid nanocarriers versus nanostructured lipid carriers	287.2 and 110.5	Skin care

whitening, UV protectant, and moisturizing capabilities. Recently, polymeric nanoparticles of sizes 70 nm, 156 nm, and 202 nm were tested to build a nanoemulsion system of flavanones obtained from *Eysenhardtia platycarpa* leaves to increase the efficiency of antiaging activity (Domínguez-Villegas et al. 2014). Besides, co-encapsulating the phytochemicals such as curcumin and resveratrol showed high antioxidant and antiaging activity due to the enhanced delivery measures (Ramalingam and Ko 2016; Siddiqui et al. 2015).

6.6.1 Moisturizers and Skin Enhancers

The skin at adverse conditions loses its moisture content leading to dryness and skin damage. The moisturizer on the other hand forms a thin layer or film on the outer surface of skin in order to retain the skin moisture content and acts as a protectant. Plant-derived natural compounds are gaining more significance in cosmetic products and formulations since they have the ability to protect the skin from both endogenous and exogenous damaging agents. The nanocarriers like emulsions, liposomes, and solid lipid carriers are used in moisturizing formulations with phyto-bioactive compounds to restore skin hydration and increase the efficiency of the beauty products (Mota et al. 2017). Moreover the solid lipid nanoparticle carriers with reduced viscosity and greasiness provide more efficient moisturizing and skin hydrating effects (Wissing and Müller 2003) (Fig. 6.12).

Plant-based moisturizing nano-delivery systems are now in the early stages of its development. One such product is safranal, a terpenic phytochemical obtained from saffron, constructed using solid lipid nanoparticles of 100 nm diameter. It provides an increased moisturizing effect and anti-UV activity (Golmohammadzadeh et al. 2011). Similar to safranal, the nanoemulsions from rice bran oil is also developed to subsidize the effect of many skin diseases like psoriasis and dermatitis (Bernardi et al. 2011; Rigo et al. 2014; Wu et al. 2013). Besides these products, the nanoemulsions from the extract of *Opuntia ficus-indica* (*L.*) and variety of vegetable oils are developed with varying particle size to study their potential moisturizing effect (Ostrosky et al. 2015; Klang et al. 2010).

6.6.2 Skin Cleansing Agents

Skin cleanser is an important skin care product in maintaining the skin health. Skin cleansing plays an active role in reduction of skin odor by directly eliminating the bacteria inhabiting the outer surface of the skin. Phytochemical ingredient found in the cleansing formula helps in opening of pores and oil content of the skin promoting the cleansing activity.

Further, the usage of phytochemicals in treatment of skin problems like acne is very well studied. In case of lauric acid, when constructed as nanosized liposomes

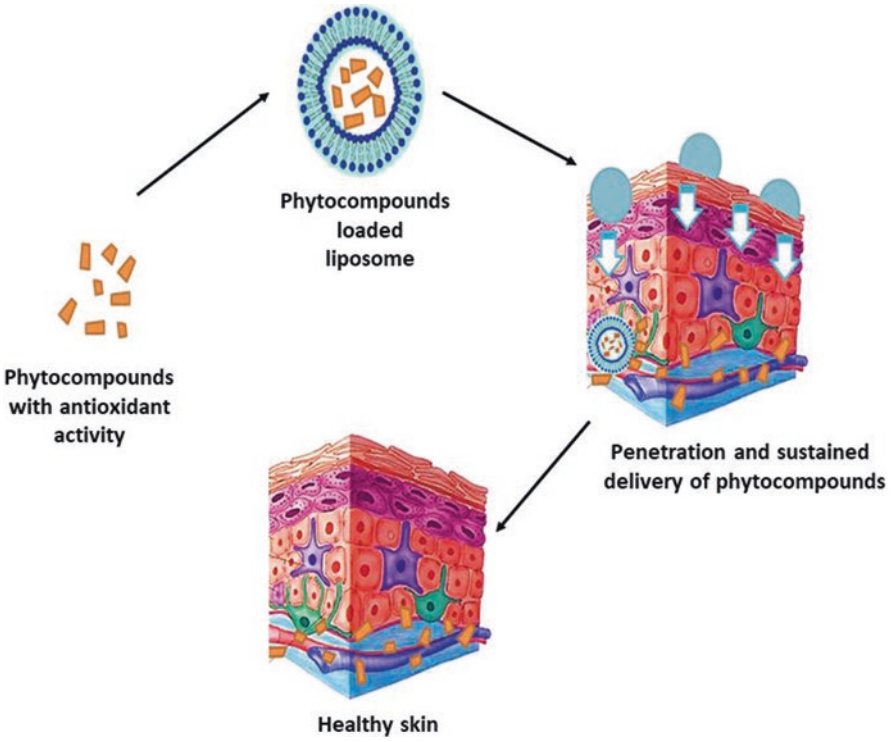


Fig. 6.12 Liposomes and other lipid carriers loaded with the phyto-active compounds are used to restore skin hydration, enhance the moisturizing effect, and increase the efficiency of the beauty products (Wang 2017; Yin et al. 2013)

of 113 nm showed increase in the antimicrobial activity against the acne (Ganesan and Choi 2016; Garg 2016). Recently niosomes are discussed for their applications as drug carriers in cosmeceuticals. Niosomes are nanocarriers formed by association of non-ionic surfactants and cholesterol as bilayer in an aqueous phase (Fig. 6.13). Non-ionic surfactants possess no charged groups in the hydrophilic heads which renders them non-immunogenic and readily biodegradable (Hamishehkar et al. 2013). They also have a prolonged shelf life and high stability and show good target specificity. Also lauric acid and curcumin, when combined and rendered using niosomes, enhance the antimicrobial activity to prevent the skin infections from acne (Amol and Pratibha 2014).

6.6.3 Sun Protective Agents

Sunscreens and protective agents are available commercially as lotions and vanishing creams that contain compounds protecting against harmful radiation from the sun. Sunscreens block the penetration of UV and other harmful irradiations, thus

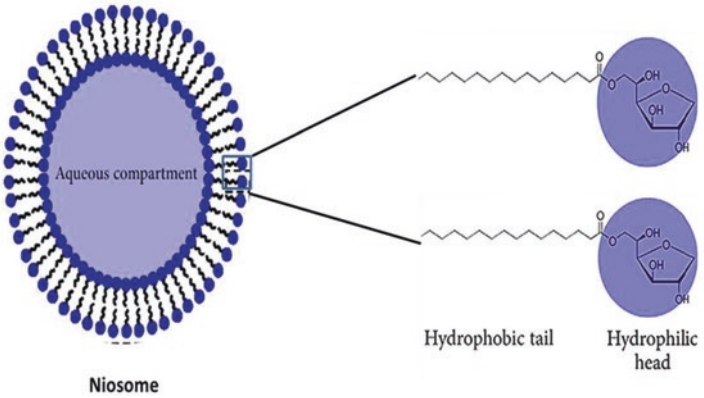


Fig. 6.13 Nano-phyto-carriers like niosomes are made of non-ionic surfactants and cholesterol as bilayer in an aqueous phase. They are non-immunogenic, are readily biodegradable, prolong the shelf life, have high stability, and show very good specificity (Amol and Pratibha 2014; Hamishehkar et al. 2013)

preventing from irritation and other skin problems. However, the plant bioactive compound-based sunscreens are more advantageous over the conventional synthetic ones. Synthetic sunscreens form a chalky layer and create greasiness over the skin and are also toxic. Some of these disadvantages are avoided with natural sun protectants containing phyto-based active ingredients. Bulla et al. have recently studied the use of plant-based bioactive compounds in sunscreens using the extracts of *Schinus terebinthifolius Raddi* and Brazilian *Lippia* species and showed that they have enhanced antioxidant and photo-protective activities (Bulla et al. 2015; Vivina et al. 2007).

Although phyto-based natural active ingredients in sunscreens show enhanced photo-protective effects, the use of nano-delivery systems in creams and lotions play an active role in providing stability and skin protective effects. Recently, safranal in the nano-range of 103 to 233 nm built with solid lipid nanoparticles increased the sun protective activity (Antunes et al. 2017; Khameneh et al. n.d.). Similarly, rice bran oil, pomegranate seed oil, and raspberry oil with lipid nanocarrier enhance the sunscreen activities with higher antioxidant and UV protection (Badea et al. 2015).

6.7 Conclusion

Today we are innovating ourselves into personalized diets and therapy using informatics and artificial intelligence (AI)-based approaches to solve food and pharma problems. Traditionally plant and plant-derived compounds are used for food supplementation and cosmeceutical and medical applications. Taking the cue from conventional knowledge of nontoxic nature and cost-effectiveness of phyto-materials,

and with the deliberations of issues such as high cost, time taken for new drug development, and high drug attrition rate associated with synthetic drug discovery process, herbal or natural sources for the development of lead compound are evolving to be the global trend in the pharmaceutical industry (Pan et al. 2013).

However in most of the cases, phytochemicals work effectively only when delivered in a mixed combination. Therefore by applying the formulative nanotechnological knowledge in surface science, organic chemistry, molecular biology, delivery, and molecular engineering, the scope of phytochemical applications can be inflated. Using nanotechnology, it is possible to increase the solubility and stability of phytochemicals, enhance their absorption, enhance permeation and retention in target tissues, increase bioavailability, protect them from premature degradation in the body, exhibit high differential uptake efficiency in the target cells, and prolong their circulation time (Sarker and Nahar 2017). Nanotechnology provides us with the fertile ground for future research, development, and application with respect to sustainable use of plant materials, biomass waste, and by-products (Griffin et al. 2017).

Nanotechnology has already made a huge impact in the field of synthetic drug delivery and is now influencing the phytochemical research and drug delivery process. Enhanced bioavailability and net effectiveness of phytomolecules including quercetin, genistein, naringin, sinomenine, piperine, glycyrrhizin, and nitrile glycoside have been demonstrated using various nanotechnological formulations. Currently approved nanomaterials in pharma industry are based on relatively simple and established nanoparticles like PEGlyated liposomes. However the future prospects for nanotechnology are claimed to be with actively targeting the delivery chemicals, multifunctional materials, and more complicated materials.

It has to be noted that observed properties of nanomaterials differ entirely from those of their constituent atoms and molecules and from those of the bulk material. Nano-engineered substances can have substantially altered bioavailability and thus come with new safety issues from their non-engineered counterparts. Human use of existing nano-enabled phytocompounds has not yet been thoroughly investigated, and further research is needed with regard to safety and effectiveness. Specifically knowledge on conjugated phytochemicals, the unique nanoformulation-specific signaling pathways, toxicological profile, and the complete disposal machineries are deficient. There exist a hiatus in information about safety, effectiveness, environmental impact, and regulatory status of the developed nano-products mainly because of weak, insufficient regulatory mechanisms.

Regarding the toxicological and regulatory side, there exists a huge void in the dosing, reproducible toxicological evaluation, and standardized reporting of newly engineered nano-products. As there is nanomaterial originated interference in colorimetric cytotoxicity assays, standardization has to be performed using nanoparticle noninterfering techniques like flow cytometry (Kumar et al. 2015).

In case of safety-related screening studies, it is perfunctory to obtain knowledge mainly from in vitro⁄ex vivo systems which usually is incomplete for nanoformulations, and therefore obtaining knowledge on systemic toxicity using in vivo models should be given priority. Other than the beneficial effects, the

clearance system from body after the indented action and environmental impact have to be analyzed in detail. These testing and approval have to be coordinated among different institutions, regulatory agencies, and approval committees, so that fast decision can be reached during discovery and developmental cycle.

Moreover a repository of toxicological data can be established where toxicity response of available novel delivery systems can be listed. This database can be predictively used during new product development using the clues of past success and failures. Also big data or informatics-based predictive platforms have to be established exclusively for the nano-conjugated lead phytocompounds. This could save the time, and also the prediction-derived alternative strategies could be implemented at the designing stage itself. Naturally existing nano-mechanisms could be closely evaluated for designing safe nanoformulation-based products.

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Chapter 7
Nanopharmaceuticals: Healthcare Applications and Safety Evaluations

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Contents

Abstract The prospects of nanotechnology in enhancing the quality of healthcare delivery cannot be overemphasized. Indeed, the advancement in nanotechnology is now a motivation for the increasing and wider acceptance of nanotechnology for applications in healthcare improvement particularly for diagnostic and therapeutic purposes. The use of nanotechnology to enhance the quality of pharmaceutical

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delivery forms the bulk of the emerging field referred to as the nanopharmaceuticals. This has created an interdisciplinary approach which has the potential of improving pharmaceutical delivery which is among the most promising and exciting innovations in healthcare strategy. As revealed in this chapter, nanopharmaceuticals offers remarkable prospects for improved healthcare delivery by reason of their additional potentials including increased surface area, enhanced solubility, increased oral bioavailability, dosage reduction and ease of attachment to functional groups amongst others. These unique features of nanopharmaceuticals are part of the merits which are conspicuously nonexistent with the conventional/traditional pharmaceuticals. Thus, this chapter discusses the nanopharmaceuticals vis-a-vis the applications and safety evaluations.

Keywords Drug delivery and targeting · Nanomedicine · Nanomaterials · Nanotoxicology · Safety assessment

7.1 Introduction

The pharmaceutical industry (PI) discovers, develops, produces, and markets drugs or pharmaceutical drugs for use as medications. Pharmaceutical companies may deal in generic or brand medications and medical devices. Pharmaceuticals are a vital part of human and/or animal survival, and its value has been associated with the balance between its effectiveness and side effects (Chan et al. 2013). Pharmaceuticals contribute significantly to the health and well-being of both humans and animals. Consequently, the PI has a responsibility in ensuring balance in their efficacy, side effects, and cost value (Ding et al. 2013a). As a result, the PI is encumbered with research and development (R&D), productivity, and investments (Ding et al. 2013b). It is noteworthy that the PI is more highly regulated than any other industry so as to afford the maximal service and support to healthcare while also providing investor value.

Over the years, the PI has had a continuous growth of 4–7% per year, and it currently approaches a market scope of 1 trillion USD (Ding et al. 2013a). The PI sustains itself by spending heavily on producing new prescription drugs through patents that make firms sponsor its expenses and also prevent competitions (Kappe 2013). As at 2000, 802 million USD was the average requirement to develop a drug, and the financial requirement for R&D in 2008 was 50 billion USD, rising to 160 billion USD in 2016 (DiMasi et al. 2003; Statista 2017). The increase in the cost of pharmaceutical R&D has been attributed to the need to cover the loss due to patent expiry, because this gives opportunities to generic drugs to compete (Paul et al. 2010; Kappe 2013). In the meantime, statistical studies suggest that the number of generic drugs in the market have increased from 18.6% of unit sales in 1984 to 78% in 2010 (Kappe 2013). Another reason for the increased cost in R&D is the continuous increase in the amount needed to acquire a regulatory approval for new drugs

(DiMasi and Grabowski 2007). Further, the demand by healthcare practitioners and sponsors for new, improved, and cheaper drugs with extensive clinical reports on its properties has placed pressure and strain on the PI (Betz et al. 2013). In summary, comparative analysis of reports on the current status of the PI reveals that the industry is plagued by concerns on its reliability and transparency in reference to drugs' efficacy and safety, issues pertaining to patent expirations, increased regulatory demands, lower inflow of funds for R&D, lack of innovation, and technological and societal/environmental problems (Khanna 2012; Ding et al. 2013a, b; Paul et al. 2010; Kappe 2013). Taken together, the PI needs to be more innovative in order to outgrow most of the limitations that threaten its survival. Perhaps the limitations bedeviling the PI have forced it to identify and pursue new ways of sustenance while being able to also add value to healthcare delivery at reasonable costs. Among the new strategies is the deployment of nanotechnological advancements for pharmaceutical purposes and this has birthed the emerging field of research currently termed nanopharmaceuticals (Pepic et al. 2014). A large number of innovations in health sector have been exploiting nanotechnology (Berkner et al. 2016). Applications of nanotechnology for pharmaceutical purposes include the development of efficient and intelligent drug delivery systems which possess the enhanced ability to bypass biological barriers and interact directly with target tissues. The use of nanotechnology for pharmaceutical development also enhances drug bioavailability, stability, and action, thereby reducing the dosing frequency (Thakur and Agrawal 2015). Other areas of benefits include applications in vitro rapid and portable diagnostics (Pautler and Brenner 2010), in vivo imaging, and as active implants.

7.2 Nanopharmaceuticals

The development of various pharmaceutical dosage forms in the range of 10–1000 nm using nanotechnological tools is referred to as naonopharmaceuticals or nanopharmaceutical dosage forms. Nanopharmaceuticals also comprise colloidal drug delivery carriers not exceeding 1000 nm in size (Bawa 2008; Gaur and Bhatia 2008). A recent definition describes nanopharmaceuticals as pharmaceuticals in which the nanomaterial plays the pivotal therapeutic role or adds additional functionality to the previous compound (Rivera et al. 2010). In a recent review, Weissig et al. (2014) proposed that prospective nanopharmaceuticals must satisfy the dual conditions of being manufactured via a nanoengineering process and an inherent therapeutic activity of the nanomaterial.

There are two types of nanopharmaceuticals: [i] those where the therapeutic molecules are themselves the drug (i.e., the therapeutic compound itself also functions as its own carrier) and [ii] those where the therapeutic molecules are directly coupled (functionalized, entrapped, or coated) to a nanoparticle carrier. As there is no universal nomenclature system for the classification of nanopharmaceuticals, different nanoscale structures of different shapes are often classified as

nanopharmaceuticals. In fact, some of the common shapes of nanopharmaceuticals include spheres (hollow or solid), tubules, particles (solid or porous), and tree-like branched macromolecules (Bawa 2008, 2009).

7.2.1 Applications of Nanopharmaceuticals

Materials in sizes ranging from about one nanometer up to several hundred nanometers exhibit interesting physical properties that are different from bulkier scales and, hence, present prospects for novel applications in medicine. Indeed, nanotechnology has played key role in the new lift and approach used in Science globally. It has brought scientific innovations at the intersection of engineering, medicine, and biotechnology (Bawa 2011). In recent times, the nanopharmaceuticals have been receiving attention due to their potential to reform drug delivery systems (Park 2007; 2017). Researchers have implicated nanopharmaceuticals for drug delivery and therapeutics (Adeyemi and Sulaiman 2015; Bawarski et al. 2008; Peer et al. 2007; Wagner et al. 2006), as well as for the enhancement of drug solubility and increase in drug half-life, among others (Pepic et al. 2014). Further, nanostructures like solid nanoparticles, polymeric micelles, quantum dots, and dendrimers have been explored for therapeutic and/or diagnostic purposes in conditions like infectious diseases, cancer, and pain (Bawarski et al. 2008; Pepic et al. 2014).

A recurring dilemma of pharmaceutical delivery is the accurate targeting of the pharmaceutical to the cells or tissues of choice. Since this is generally unachievable, active agents have to be administered in excessively high doses, thereby increasing the likelihood of toxicity. Nanopharmaceuticals have enormous potential in addressing this failure of traditional therapeutics. This precision targeting reduces toxic systemic side effects, resulting in better patient compliance. Also, a great number of drugs are unable to transverse the tight epithelial junctions of skin and taut endothelial interface of blood-brain barrier. However, with the advent of nanopharmaceutical delivery systems, drugs can be targeted to every part of the body (Ali et al. 2013). Compared to traditional pharmaceuticals, nanopharmaceuticals possess the following advantages (Bawa 2008):

(a) Increased surface area
(b) Enhanced solubility
(c) Increased oral bioavailability
(d) Dosage reduction
(e) Ease of attachment to functional groups

Moreover, nanotechnology has been implicated for prospects in the integration of diagnostics with therapeutics and the facilitation of the development of specific therapeutics best suited for an individual (Jain 2008).

7.2.2 *Drug Delivery*

Recently, use of nanopharmaceuticals has been reported as potentially circumventing the inability of conventional drugs to deliver effective drug dose at target sites during diseases treatment or control (Namdari et al. 2017). Rapoport (2007) reported the use of polymeric micelles for anticancer drug delivery. The author described polymeric micelles as spherically shaped core-shell structure with a hydrophobic core and hydrophilic shell as shown in Fig. 7.1. They are unique for their core-shell structure and can be conjugated with other functional groups. In drug delivery concept, the most commonly used hydrophilic group in polymeric micelle is the poly(ethylene oxide) which has the ability of reducing residence time by way of preventing micelle opsonization. This has great advantage in targeting tumor cells as it promotes permeability and retention effect (Rapoport 2007). Since most known anticancer agents have low aqueous solubility, polymeric micelles deployed as nanopharmaceuticals are used as solubilizing agents to increase the solubility of these anticancer agents. This is one of the most important applications of nanopharmaceuticals. Presently, this has given room to the encapsulation of hydrophobic drugs in micelle cores for proper targeting of disease sites.

Foldvari and Bagonluri (2008) also reported the use of carbon nanotubes (CNTs) in drug delivery. CNTs have the ability to perform controlled and targeted drug delivery which can be achieved via interaction with pharmaceutical agents in three ways. Firstly, the interaction can be viewed as a porous absorbent to entrap active pharmaceutical agents within CNTs mesh or CNTs bundle (Fig. 7.2a); secondly, it could be via functional attachment of active pharmaceutical agents to the exterior

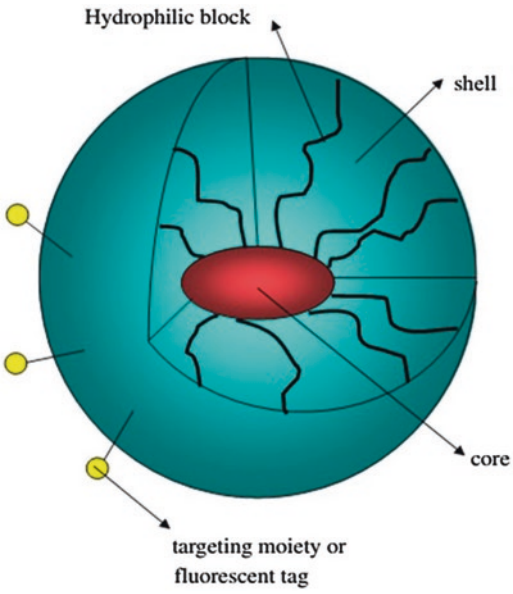

Fig. 7.1 Schematic representation of block copolymer micelle; lipophilic drug (red color) is encapsulated in the micelle core. The core-shell structure can be conjugated with other functional groups (Rapoport 2007)

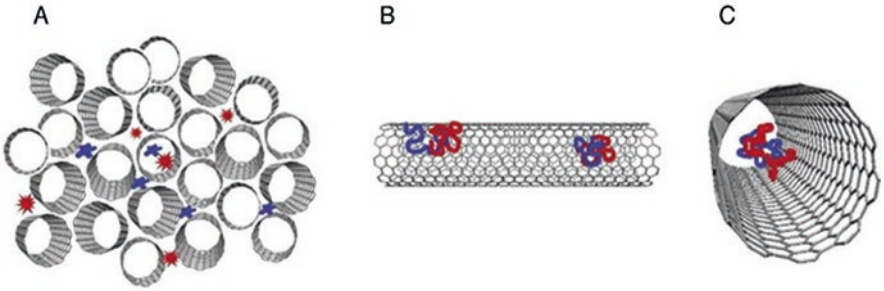

Fig. 7.2 Schematic representation of (**a**) a bundle of CNTs as a porous matrix encapsulating drug molecules between the grooves of individual CNTs, (**b**) moieties attached to the exterior of a CNT either by covalent bonding to the CNT wall or by hydrophobic interaction of moieties with the CNT walls, and (**c**) the encapsulation of moieties within the internal nanochannel of a CNT (Foldvari and Bagonluri 2008)

walls of CNTs (Fig. 7.2b); and, thirdly, this can be achieved using CNTs channels as nanocatheters (Fig. 7.2c). As nanopharmaceuticals, CNTs can be applied more specifically by controlling the conjugation of active pharmaceutical agents on it rather than making use of the bulk property of CNTs. Such conjugation can be carried out either exohedrally or endohedrally. In exohedral conjugation, the active pharmaceutical agents are bonded to the exterior of the CNTs for delivery into the cells but when endohedral, the active agents are encapsulated and transported through the inner cavities of the CNTs to the target site of delivery.

7.2.3 Cell Imaging Agents

Cellular imaging, defined as the use of a system/technology capable of visualizing a cell population, single cell or subcellular structures, applied in combination with image-analysis tools, is emerging as a crucial tool enabling the integration of biological complexity into drug discovery. Detection systems include microscopes, fluorescence macro-confocal detectors and fluorometric imaging plate readers (FLIPR) used with charge-coupled device (CCD) cameras. These systems generate a two-dimensional pixel array of information (a digital image) extracted from a particular biological event or tissue type. Various image-analysis tools have been developed to process the information in the digital image into meaningful parameters (Lang et al. 2006). Presence of nanopharmaceuticals in living organisms has revealed the involvement of nanopharmaceuticals in the differentiation of cells, cell–cell and host–pathogen interactions, immune response, etc. (Corfield and Berry 2015; Jones 2015; Pinho and Reis 2015). Studies have also revealed that the occurrence of specific structure of nanopharmaceutical agent correlates with disease invasion and the capacity to metastasize target organs at specific site which is an indication that nanopharmaceuticals can be used as disease biomarkers to screen, predict, monitor, and/or diagnose diseases most especially at early stage (Christiansen

et al. 2014; Dosekova et al. 2017). The principle of operation during cell imaging is based on selective delivery which makes use of binding receptors. Theranostic concept has been used overtime. For practical and clinical applications, Webster et al. (2015) suggested that theranostic nanomaterials should effectively combine therapeutic agents, targeting moieties, and imaging agents. However, there are situations where the nanopharmaceutical agent is self-imaging. In this case, the nanopharmaceutical agent does not require the presence of a binding receptor due to its ability to fluorescence (An et al. 2015; Dosekova et al. 2017). This has brought a major enhancement to the use of NMRi (nuclear magnetic resonance imaging) and computed tomography imaging of tissue or single cell. Theranostic medicines can provide insights into the availability of a molecular target in the tissue, the vascular permeability and retention of the molecule, the drug release from the particle, and the response of the target tissue (Kiessling et al. 2014). A recent study provided insight on the application of magnetic targeting and pH-responsive lipophilic anticancer drug delivery. A theranostic nanocage system, formed from biogenically synthesized Fe_3O_4 nanoparticles and decorated with an anticancer drug and a saponin-based biosurfactant, was developed for the targeted delivery of two anticancer agents: camptothecin and luotonin A. These theranostic nanocomposites showed better chemotherapeutic efficacy when examined in MCF-7 and HeLa cancer cell lines with a specific targeting capacity (Kesavan et al. 2018). Additionally**,** a prostate-specific membrane antigen targeted gold nanoparticle for theranostics of prostate cancer has been synthesized. The theranostic agent, AuNP-5kPEG-PSMA-1-Pc4, loaded with a fluorescent photodynamic therapy drug, Pc4, is envisioned to provide surgical guidance for prostate tumor resection and therapeutic intervention when surgery is insufficient (Mangadlao et al. 2018).

Targeted magnetic nanoparticles (MNPs) have also found use in noninvasive molecular imaging and therapy. They can be used as target-specific agents, to selectively enhance the contrast in molecular level, if functionalized, for instance, by incorporating them with antibodies. Targeted compounds improve the lesion detectability of certain pathologies and more importantly provide the localized therapy as drug delivery systems (Amiri et al. 2013). Targeted MNPs have been used to detect Aβ plaques of Alzheimer's disease (AD). For instance, Poduslo et al. (2002) targeted amyloid-β plaques of AD using a putrescine-gadolinium-amyloid-beta peptide probe detectable by magnetic resonance imaging. According to their study, the plaque-to-background tissue contrast-to-noise ratio, which was precisely correlated with histologically stained plaques, was enhanced more than ninefold in regions of cortex and hippocampus following intravenous administration of this probe in AD transgenic mice.

7.2.4 Cancer Treatment

Cancer is a leading cause of death worldwide in countries of all income levels. To add to the existing burden, the number of cancer cases and deaths is expected to grow rapidly as populations grow, age, and adopt lifestyle behaviors that increase

cancer risk (Torre et al. 2016). Cancer, which is characterized by uncontrolled proliferation of cells and dysregulation of the apoptotic mechanism, requires very complex process of treatment. Because of complexity in genetic and phenotypic levels, it shows clinical diversity and therapeutic resistance. A variety of approaches, including surgical removal, chemotherapy, radiation, and hormone therapy, are currently deployed in cancer treatment. Unfortunately, each of them has some significant limitations and side effects. Chemotherapy, for example, which involves the delivering of anticancer drugs systemically to patients, suffers from nonspecific targeting and poor delivery of these agents (Jabir et al. 2012; Zhao and Rodriguez 2013). Nanopharmaceuticals, through passive and active targeting, have been designed to overcome lack of selectivity and aqueous solubility deficiencies of conventional cancer chemotherapy. Selected delivery systems used to achieve passive targeting are liposomes, polymeric nanoparticles, nanocrystals, inorganic nanoparticles, micelles, dendrimers, etc. Active targeting involves conjugation of targeting molecules (like antibodies, ligands, peptides, nucleic acids, etc.) on the surface of nanoparticles with receptors overexpressed on a tumor cell surface (Van Vlerken et al. 2007; Lammers et al. 2008; Gullotti and Yeo 2009). When conventional nanoparticles are used as carriers in chemotherapy, the cytostatic drug is usually delivered to the mononuclear phagocytes system by endocytosis/phagocytosis of their tissue localized macrophages (Moghimi et al. 2005). Moreover, enhanced chemotherapy with nanopharmaceutical formulations has been shown when treating cancers, such as breast (Goldman et al. 2017; Park 2017), ovarian, (McQuarrie et al. 2004) and lung (Das et al. 2016). The underlying mechanism is that nanopharmaceuticals trapped by organs of the mononuclear phagocyte system are able to work as a pool and release anticancer agents to cancerous cells. Apart from nontargeted drug delivery, tumor drug resistance is another key concern in conventional chemotherapy. In many cancer types, nearly 40–50% of the patients diagnosed with cancer has P-glycoprotein overexpression in the malignant tissues. A defining strategy used to overcome P-glycoprotein-mediated multidrug resistance is to encapsulate antitumor drugs with various drug delivery systems, including *N*-(2-hydroxypropyl) methacrylamide drug conjugates, micelles, hybrid lipid nanoparticles, lipid-based nanocapsules and nanoparticles, liposomes, and cyanoacrylate-type nanoparticles. Reported mechanisms included enhancement of cellular uptake of drug via endocytosis and ion-pair formation, ATP depletion, influence of function and expression of P-glycoprotein, and change of P-glycoprotein downstream signaling pathways (Dong and Mumper 2010).

Among a wide variety of proposed nanopharmaceuticals, only a handful has been approved for use in the treatment of cancer. Doxil, with the brand name Caelyx®, was obtained by encapsulating doxorubicin within liposomes. This nanoformulation boosted pharmacokinetic indices such as longer circulation half-life and maximal drug accumulation in target tissues. Clinical validation of the use of doxil in the treatment of metastatic breast cancer, ovarian cancer, and multiple myeloma has been reported. The non-PEGylated liposomal doxorubicin formulations, Myocet and DaunoXome, have also been used for the treatment of metastatic breast cancer and Kaposi sarcoma, respectively. Abraxane, a co-condensate of

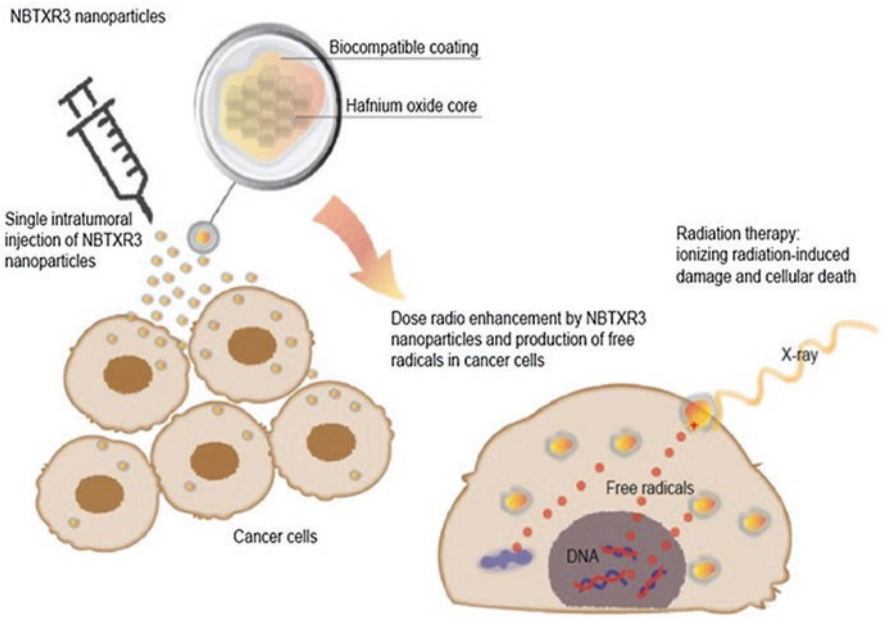

Fig. 7.3 Schematic representation of the radio enhancement mechanism of NBTXR3 nanoparticles in cancer cells after an intratumoral injection. When activated by a radiation source, these potential therapeutic agents generate high amounts of reactive oxygen species when they find their way into tumor resulting in cellular damage. (Weissig and Guzman-Villanueva 2015)

albumin and paclitaxel, is another nanopharmaceutical which has demonstrated significantly higher tumor response rates and longer times to tumor progression in patients with metastatic breast cancer (Huynh et al. 2009; Gradishar et al. 2005; Montana et al. 2011). Several strategies have also been adopted to enhance the effects of anticancer agents. Previous works reported potential therapeutic agents with high electron density which allowed the deposit of large amounts of energy within living cells due to ionization (Weissig and Guzman-Villanueva 2015; Marill et al. 2014). When activated by a radiation source, these potential therapeutic agents generate high amounts of reactive oxygen species when they find their way into tumor resulting in cellular damage (Weissig and Guzman-Villanueva 2015; Marill et al. 2014), this is illustrated in Fig. 7.3. Surface modified promising therapeutic agents are currently being screened as radio-enhancer for treatment of tumors, this ongoing research has shown high prospect with the hope of a better healthy living. Presently, there are ongoing trial studies on metal-based therapeutic agents for biopsy. Antigen-specific ligands have been employed in surface modification of nanomaterials as nanopharmaceutical agents in active targeting approach. Several studies have revealed the prospect of nanopharmaceuticals as agents for biopsy, most of the studies have shown promising results (Adolphi et al. 2010; Jaetao et al. 2009).

7.2.5 HIV/AIDS Treatment

Despite concerted efforts at mitigating the acquired immune deficiency syndrome (AIDS) menace, millions of individuals worldwide are still HIV-1-infected and rate of new infections remain unabated. Antiretroviral therapy (ART) effectively controls viremia in virtually all HIV patients and partially restores the primary host cell (CD4+ T cells) but fails to eliminate HIV-1 from latently infected T cells (Gandhi et al. 2010). In latently infected $CD4^+$ T cells, integrated proviral DNA copies persist in a dormant state but can be reactivated to produce replication-competent virus when T cells are activated, resulting in rapid viral rebound upon interruption of antiretroviral treatment. Therefore, most HIV-infected individuals, even those who respond very well to ART, must maintain lifelong ART due to the ability of virus to establish anatomical or cellular reservoirs which escape the action of antiviral drugs (Kaminski et al. 2016).

These reservoirs include the following (Clarke et al. 2000):

(a) Extracellular virions trapped on the surface of follicular dendritic cells within the lymphoid tissue
(b) Latently infected and resting $CD4^+$ T cells
(c) Microglial cells of the brain, pulmonary alveolar macrophages of the lung, and macrophages within the spleen and lymph nodes
(d) Brain tissues, such as the brain

Many antiviral drugs present problems that reduce their efficacy, such as limited solubility, a short half-life or slow, incomplete or highly variable absorption. Consequently, high doses and frequent administration are required that, in turn, can negatively affect patient compliance, causing severe side effects. Besides solubility and permeability, other factors that affect the oral bioavailability of an antiviral include the action of intestinal metabolizing enzymes, efflux transporters, and food. The oral administration of an antiviral with a low or variable bioavailability thus requires the use of higher doses and prolonged treatment durations in order to eradicate the virus. Another problem of antiviral agents is that the chronic treatment with such drugs can produce moderate levels of drug toxicity, which might lead to serious complications in the patient. Moreover, prolonged antiviral therapy increases the likelihood of drug-resistant virus strains emerging (Emery 2001; Sharma and Garg 2010; Williams and Sinko 1999).

To improve the therapeutic activity of currently available antivirals, it is possible to change the conventional dosage forms, either by modification of their formulations or by the design of novel nanopharmaceuticals. As with conventional drugs, a major concern of deploying nanopharmaceuticals in treatment and prevention of disease is the ability to reach target site(s) in their active form. This challenge may be overcome by optimizing the physicochemical properties of the nanopharmaceutical or by modifying its surface by attachment of ligands or agents that prevent opsonization, in order to facilitate transport across membranes or enable targeting (Alexis et al. 2008).

Dou and coworkers (2007) showed that effective delivery to various tissues could be achieved with a nanosuspension of the drug indinavir, stabilized by a Lipoid 80 surfactant system. The indinavir nanosuspensions were loaded into macrophages, and their uptake was investigated in mice. Results showed high distribution of the drug in the lungs, liver, and spleen. More significantly, the intravenous administration of a single dose of the nanoparticle-loaded macrophages in a rodent mouse model of HIV brain infection resulted in significant antiviral activity in the brain and produced measureable drug levels in the blood up to 14 days posttreatment (Dou et al. 2009). Furthermore, macrophages, which are the major HIV reservoir cells, have various receptors on their surface such as formyl peptide, mannose, galactose, and Fc receptors, which could be utilized for receptor-mediated internalization. The drug stavudine was encapsulated using various liposomes (120–200 nm) conjugated with mannose and galactose, resulting in increased cellular uptake compared with free drug or plain liposomes and generating significant level of the drug in the liver, spleen, and lungs. The drug zidovudine, with half-life of 1 h and low solubility, was also encapsulated in a mannose-targeted liposome made from stearylamine, showing increased localization in lymph node and spleen. In another study, the drug efavirenz was delivered to monocytes and macrophages in vitro using a mannose-targeted poly(propyleneimine) dendrimer nanocarrier. The targeted nanocarrier resulted in 12-fold increase in cellular uptake compared with free drug. A similar system was used to deliver the drug lamivudine in vitro, resulting in significantly higher anti-HIV activity for the targeted and nontargeted dendrimer systems compared with free drugs (Dutta and Jain 2007; Dutta et al. 2007; Kaur et al. 2008). Hopefully, these nanopharmaceuticals would present the needed platforms for improving targeted delivery of antiretroviral drugs to the cellular and anatomical reservoirs of HIV.

7.2.6 Intravaginal Microbicides

Intravaginal application of drugs has long been of interest to researchers, who had explored it for the local delivery of therapeutic agents such antimicrobials (Vanić and Škalko-Basnet 2013). Intravaginal route of drug administration bypasses the gastrointestinal tract and delivers directly into the vagina. This avoids loss of active compound through incomplete absorption or degradation in the acidic environment of the stomach and duodenum or bacterial flora in the gut. More importantly, the first-pass effect in the liver, which could have resulted in structural modifications of the nanopharmaceutical, is circumvented. The vagina could also provide a platform for systemic treatment if drug is resorbed through the vaginal venous plexus into the body. The vaginal venous plexus empties into the iliac or hemorrhoidal veins that do not pass the liver circulation, thus avoiding the first-pass effect. But vaginal route of administration is not a straightforward approach. Drug formulations for vaginal application must be retained at the site for the sufficient period, in spite of the normal vaginal clearance and discharge. To achieve an optimal retention,

mucoadhesive and muco-penetrative delivery systems have been explored. However, the presence of cross-linked mucin fibers limits drug penetration across the vaginal tract. In order to penetrate the mucus, delivery vehicles must be small enough to overcome significant physical hindrance by the dense mucin fiber mesh (Ensign et al. 2014; Katz et al. 2011). Nanopharmaceuticals offer an opportunity to achieve uniform epithelial delivery to the vagina. The choice of optimal nanocarrier will be dependent on the characteristics of the particular drug and expected dosage regimen of the therapy. Since majority of the drugs of interest for vaginal administration have limited solubility, nanocarriers that can solubilize these drugs enhance their bioavailability. In addition, nanopharmaceutical formulations could increase the retention time of the drugs at the vaginal site if composed of substances capable of promoting mucoadhesion (Caramella et al. 2015; Wong et al. 2014). Additionally, the small size of nano-agents facilitates their cellular internalization and release of the drug directly to the cytosol.

Furthermore, the small size of nanoparticles means that some of these particles can interact with viral agents and thus may offer protection against STDs such as HIV (Notario et al. 2017). For example, studies have demonstrated in vitro viral adhesion with silver nanoparticles, while silver-coated PVP nanoparticles have demonstrated antiviral activity ex vivo at nontoxic concentrations (Lara et al. 2010). Their large surface area improves the dissolution and absorption of slightly soluble drugs and also allows optimization of these nanoparticles according to their functionalization; they can bind to specific targets by multivalent conjugations and attach at the drug release site. These nanosystems can either exhibit HIV inhibitory activity by themselves or serve as a vehicle for drug delivery. Recent research has focused on the possibility of developing microbicides based on nanoparticles for HIV prevention (Notario et al. 2017). These nanoparticles consist of cross-linked polymer chains formed thanks to crosslinking agents, creating a structure within which to load the drug. Nanoparticles have been loaded with antiretroviral microbicides such as dapivirine (DPV) and tenofovir (TFV) in order to improve cellular internalization of the microbicides (Yang et al. 2013). PLGA nanoparticles loaded with the antiretroviral drug saquinavir have been conjugated to the anti-CD4 antibody. The nanoparticles thus bind to the $CD4^+$ immune cells, and the drug is specifically released inside them. Nanoparticles of PLGA and methacrylic acid copolymer (Eudragit® S-100) have been loaded with TFV and are capable of releasing the drug in a pH-dependent manner in the presence of seminal fluid (Zhang et al. 2011). Another example of release in response to stimuli is the nanoparticles of hyaluronic acid loaded with TFV, which release the drug in the presence of semen due to the degradation of hyaluronic acid in the presence of the enzyme hyaluronidase (Agrahari et al. 2014). An alternative option is to include the nanoparticles in a stimuli-sensitive dosage form, such as temperature-sensitive gels that are liquid at room temperature, convenient to apply and gain consistency at body temperature, thus avoiding vaginal seepage after application and maintaining the nanoparticles in contact with the mucosa for longer. Formulations with PLGA nanoparticles loaded with TFV or with rilpivirine (Destache et al. 2016) have been developed using these gels. For all these reasons, despite the current scarcity of microbicides based on

nanosystems for the prevention of HIV, coming years will see a boom in research in this field, since nanoparticles provide a delivery strategy for targeted and controlled delivery of drugs to the vagina (Notario et al. 2017).

7.2.7 Enhancement of Anticancer Agents

Previous works reported potential therapeutic agents with high electron density which allowed the deposit of large amounts of energy within living cells due to ionization (Weissig and Guzman-Villanueva 2015; Marill et al. 2014). When activated by a radiation source, these potential therapeutic agents generate high amounts of reactive oxygen species when they find their way into tumor resulting in cellular damage (Weissig and Guzman-Villanueva 2015; Marill et al. 2014), this is illustrated in Fig. 7.3. Surface modified promising therapeutic agents are currently being screened as radio-enhancer for treatment of tumors, this ongoing research has shown high prospect with the hope of a better healthy living. Presently, there are ongoing trial studies on metal-based therapeutic agents for biopsy. Antigen-specific ligands have been employed in surface modification of nanomaterials as nanopharmaceutical agents in active targeting approach. Several studies have revealed the prospect of nanopharmaceuticals as agents for biopsy; most of the studies have shown promising results (Adolphi et al. 2010; Jaetao et al. 2009).

7.3 Safety Evaluations

It is undeniable that nanotechnology has the potential to appreciably lower drug production costs, while providing prospects for success in areas where the traditional pharmaceuticals have failed (Moghimi et al. 2011). Thus the emerging field of nanopharmaceuticals is increasingly being accepted globally. However, there are still safety concerns as well as potential of the unknown complications that may arise as a result of long-term exposure to nanoformulations (Cao and Sim 2007; Bawarski et al. 2008; Pepic et al. 2014). Though the safety concerns associated with the use of nanopharmaceuticals may be overshadowed by the benefits and prospects that the nanopharmaceuticals offer, nevertheless, these concerns are real and, therefore, necessitate consideration.

The physical and chemical properties of materials tend to become very different from those of their parent compounds when they become highly reduced in size (Ray et al. 2009). Exposure to nanomaterials may proceed through inhalation (respiratory tract), topical application (skin contact), intravitreal, transscleral, suprachoroidal, subretinal, oral administration (ingestion), and injection (blood circulation) routes (Oberdorster et al. 2005; Kompella et al. 2013). Due to their extremely small dimensions and large surface-to-volume ratio, nanomaterials may gain assess into both the circulatory and lymphatic systems and induce irreversible injuries through

promotion of oxidative stress (Fu et al. 2014). Therefore, in a bid to maximize the benefits and minimize the potential harms that could be caused by exposure to nanomaterials, it becomes expedient to consider the safety impacts of nanopharmaceuticals through the lens of public health (Pautler and Brenner 2010). For the safe development of nanotechnology and the safe use of commercial nanomaterials, investigations regarding the toxicity and safety profiling of nanomaterials are needed (Fu et al. 2014).

7.3.1 Potential Health Risk

At the cellular level, nanomaterials could interact with vital cell components such as the nucleus, mitochondria, and membrane and thus exert adverse effects such as damage to organelles or DNA, apoptosis, oxidative stress, mutagenesis, and protein up-/downregulation (Pan et al. 2009). The toxicity profiling of nanomaterials has been investigated in vitro by studying the effects of the nanomaterials on cell culture over a given period of time, e.g., 24–48 h. Effects such as cell proliferation and survival, membrane permeability, inflammatory cytokine levels, and ATP production have been reported (Mo et al. 2007). However, considering the complexity of a whole organism as compared to that of a single cell, the safety evaluation of nanopharmaceuticals in vivo is more dependable and should be sought for. The evaluation in the whole animal should include investigation of general health indicators such as weight loss, mortality percentage, average life span, and behavioral abnormality (Alkilany and Murphy 2010). As with the conventional pharmaceuticals, the absorption, biodistribution, metabolism, and excretion of nanopharmaceuticals must be explored and put into consideration prior to acceptance by regulatory bodies (Alkilany and Murphy 2010). Important pharmacokinetic parameters such as the volume of distribution (V_d), maximum plasma concentration (C_{max}), half time in the blood ($t_{½}$), and total body clearance (Cl) are also necessary, and these can also be obtained by the application of classical pharmacokinetic models (Cho et al. 2009).

Currently, the most sought-after emerging therapeutics in biomedical applications include the biocompatible and biodegradable nanomaterials. It is wisdom that carrier materials for pharmaceutics should ideally be easily metabolized to safe products in order to afford ease of clearance from the system, or if the material is intended to remain in the body, it should be inert, biocompatible, with no adverse effects even after a long-time exposure. Organic materials such as carbohydrates, lipids, and proteins are being used in the preparation of nanomedicines, as well as inorganic compounds such as gold and porous silica. However, toxicity concern arises with the use of inorganic materials as they persist without breaking down unlike the organic materials. The key physicochemical properties influencing the biocompatibility of nanomaterials include the composition, shape, size, surface charge, surface modifications, and lipophilicity (McNeil 2005). Also, the route of administration, dose, dose frequency, and patient idiosyncrasies should be put into consideration in order to minimize potential toxicity.

For example, the mechanism of the clearance of a nanomaterial, following periocular administration, was recently investigated in live and dead animals (Amrite and Kompella 2005; Amrite et al. 2008). According to their reports, at 6-h post-administration of nanomaterials in both living and dead animals, only 45% of the nanomaterial was retained at the periocular site in living animals, while 77% was retained in the dead animals. The percent retained in dead animals was however close to the observed retention immediately after dosing. Their results further revealed a possible breakdown of some of the transport barriers in the dead animals as the tissue level of the nanomaterials in the sclera-choroid were 19-fold higher in the dead animals than the live animals. Again, the particles were found in the retina and vitreous of the dead animals but absent in the live animals (Amrite et al. 2008). Perhaps, the role played by the reticuloendothelial system (RES) in the clearance of intravenous injection of particulate systems cannot be over emphasized (Gèze et al. 2007; Schipper et al. 2009). In separate studies with fluorescent latex particles (size 200 nm) where particles were instilled into the conjunctival cul-de-sac, the conjunctival follicle-associated epithelium in rabbits was able to bind to and translocate particles. The visualization of the translocated particles in the cervical lymph nodes after being translocated from the conjunctival epithelium was an indication of the role of the lymphatic circulation in the clearance of these particles (Liu et al. 2005). The presence of inflammatory cells as observed in the histological sections of periocular tissue 60 days following the administration of 200 and 2000 nm particles suggests the possibility of the inflammatory cells playing a role in the clearance of the particle after administration (Amrite and Kompella 2005). Taken together, the potential risks posed by nanomaterials seem to be dependent on the route of delivery, the nanoparticle composition, and target tissue. These risks include immune stimulation, immunosuppression, inflammation (Zolnik et al. 2010), aggregation, membrane disruption, accumulation in nontarget tissues (Panessa-Warren et al. 2009), hemolysis, generation of oxidative stress (Medina et al. 2007), and adsorption of plasma proteins onto the surface. A summary of the health risk of nanopharmaceuticals is presented in Table 7.1.

7.3.2 Environmental Risks

Understanding the implications of the process of syntheses, products, and byproducts of nanotechnology on the environment and human health is crucial to its acceptable utilization. With over 1000 nano-enabled consumer products in the market, knowledge gaps still exist regarding their fate and transportation within humans, the environment, and ecosystems (The Project on Emerging Nanotechnologies Consumer Products 2010). Some nanoparticles appear to persist more in the environment than others, hence, the need to explore and obtain more information as touching risk assessment and management (Kahru and Dubourguier 2010). Further, many topical creams which are intended for direct application, for example, sunscreens, contain nanoparticles (The Project on Emerging Nanotechnologies Health

Table 7.1 Summary of in vitro *and* in vivo nanoparticle toxicity

Nanoparticle	Side effect	Experimental model/organ/tissue	References
AgNP; 1, 10, and 100 μg/ml; incubated at 1, 4, and 24 h	No hemolysis observed for 1 and 10 μg/ml washed red blood cell, hemolysis observed for 100 μg/ml washed red blood cell incubated at 24 h	In vitro red blood cell	Laloy et al. (2014)
AgNP	Chromosome instability	In vitro normal human lung fibroblast	AshaRani et al. 2009
AgNP	Mitotic arrest (although normal human fibroblast eventually recovered while cancer cells did not recover)	In vitro normal human lung fibroblast	AshaRani et al. 2009
AgNP	Alterations in cell morphology	In vitro normal human lung fibroblast	AshaRani et al. 2009
AgNP	Oxidative stress and apoptosis	Rat liver cell	Hussain et al. (2005)
AuNP; 4, 12 & 18 nm; spherical; 0.001–0.25 μM; 72 h incubation period	Nontoxic	K562 human leukemia cell line	Connor et al. (2005)
AuNP	Nontoxic	Dendritic cells	Villiers et al. (2009)
AuNP; 2 nm, spherical, quaternary ammonium, carboxylic acid. 0.38–3 μM dose; incubation time 1–24 h	Cationic nanoparticles were toxic while anionic were not	COS-1 mammalian cells, red blood cells, *E. coli*	Goodman et al. (2004)
AuNP; 10, 50, 100, 250 nm; spherical; intravenously administered; 77–108 μg/rat	No side effect. Most nanoparticles were found in spleen and the liver; the 10 nm particles were also found in the brain, heart, kidney, testis, and thymus	Rat liver, spleen, brain, and heart	De Jong et al. (2008)
AuNP; spherical; 0–4 mM dose, 24–144 h incubation time	Decreased cell proliferation rate, adhesion and motility	Human dermal fibroblast	Pernodet et al. (2006)
AuNP; 15–20 nm; spherical; intravenous 0.8–1.88 mg/gold/kg	AuNP accumulated. No hematological or renal side effects	Pig liver, lung, kidney, and blood	Kattumuri et al. (2007)

and fitness 2010). Some of these materials could be absorbed through the epidermis, while, some, while being washed off, enter into the public waste water systems. Also, nanomaterial utilized for medical interventions have multiple entry points into the environment; while some nano-enabled drug products are excreted into waste

water, some novel imaging agents and medical devices are disposed at the end of their life cycle into a landfill. The Food and Drug Administration (FDA) and Environmental Protection Agency (EPA) currently provides guidelines on how to dispose of unused medications to the public as this and other byproducts could cause harmful effect to the environment (U.S. Environmental Protection Agency 2010; U.S. Food and Drug Administration 2010). The ability of bionanomaterials to accumulate in the environment, food chain, and work force represents a direct proportional rise in the risk of environmental damage (Nel et al. 2006). More so, certain toxins have been found to enter the food chain by bioaccumulating in organisms. Hence, the ecological fate of nanomaterials needs to be considered and the environment monitored for potential threats (Patil et al. 2015).

For example, researchers studied the fate of CTAB-coated gold nanorods (65 nm length 9 15 nm width) in replicate estuarine mesocosms which was modeled to mimic a high tidal marsh creek (Ferry et al. 2009). The mesocosms consisted of seawater, sediment, fish, snails, microbial biofilms, clams, and shrimps. The authors of the study found out that the nanomaterials were differently partitioned into most of the organisms to varying extents, with a low concentration remaining in water. The largest accumulations of nanomaterials were the microbial biofilms and clam (filter feeders). Nevertheless, no death was reported at the dosage used (Ferry et al. 2009). In a separate report by Bar-Ilan et al. (2009), the toxicity of different sizes (3, 10, 50 and 100 nm) of gold and silver nanoparticles were assessed using zebrafish embryo. The study demonstrated that the gold nanoparticles unlike the silver nanoparticles of comparable sizes were not toxic to zebrafish. The silver nanoparticles were highly toxic, inducing 100% death after 120-h postfertilization. In addition, other environmental fates of nanoparticles particularly in the marine ecosystem include its ability to sink very slowly to the ocean floor, where it may pose a risk to pelagic species, deposition in sediments where it may pose a risk to benthic species and accumulation in the surface microlayers of the oceans (Wurl and Obbard 2004). Furthermore, crops may uptake nanomaterials if exposed to nanopesticides and the uptake varies, depending on the plant species, the source of growth media, nanomaterials, and mode of application. For example, exposing lettuces and cilantro to nanopesticides via soil resulted in the uptake of nCu, nCuO, and the two $nCu(OH)_2$ nanopesticides leading to the accumulation of Cu mostly in the roots, with little translocation to the stems (Hong et al. 2014; Zuverza-Mena et al. 2015). Also, when the mode of application of the nanopesticides was foliar, a much larger fraction of the Cu taken up by the plant remains in the leaves or fruits.

7.4 Conclusion

At present, use of nanopharmaceuticals is cogent in sustaining the future growth of the pharmaceutical industry (PI) in both developed and developing nations of the world. Research efforts are being made to advance the applications of nanopharmaceuticals that will better benefit the healthcare industries. However, as

nanotechnology emerges as promising tool in the field of medicine and particularly for the healthcare industry, the environmental exposure will continue to rise. Therefore, investigations aimed at profiling the safety and environmental fate of these particles become highly essential. Further, the general public needs to be educated on the safe disposal of byproducts of nanomaterials in order to ensure safe community health while the government needs to put in place policies to monitor and adequately regulate the synthesis and utilization of nanomaterials for biomedical and for pharmaceutical applications.

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Chapter 8
Potential Ecotoxicological Risk of Nanopharmaceuticals in the Aquatic Environment

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Contents

Abstract Nanopharmaceuticals are an emerging innovative domain of research that integrates nanotechnology and biotechnology applications. This technological development will permit producing unique nanopharmaceutical compounds used in the medical field, particularly in drug delivery. This book chapter focuses on organic (polymeric and lipid nanoparticles, dendrimers) and inorganic (magnetic nanoparticles and quantum dots) materials used to produce nanopharmaceuticals with different characteristics such as size, structure, chemical composition, and behavior

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enabling their use in different fields, one of which the drug delivery systems. Within drug delivery systems, special emphasis is given to vesicular (liposomes) and nanoparticulate carriers as they are the most explored at the market level. The biotechnological development, main features, and examples of applications of some types of nanostructures are discussed. Moreover, data available on sources, pathways, and effects of nanopharmaceuticals in the aquatic environment are discussed, with special emphasis on the environmental impact of these nanopharmaceuticals to the aquatic environment. Results indicate that there is no standard protocol for ecotoxicological testing and limited information exists on environmental impact assessment of nanopharmaceuticals. Thus, human and environmental safety guidelines are urgently needed to protect both the human health and the environment.

Keywords Aquatic organisms · Biomarkers · Drug delivery · Ecotoxicity · Environmental risk assessment · Nanopharmaceuticals · Nanotoxicology · Public health

8.1 Introduction

Nanotechnology applications have revolutionized different activities, among them, industry and medicine. Within the medical field, this technology, known as nanomedicine, includes a broad range of nanomaterials that can detect at molecular and cellular level and, at much earlier stages, diseases that affect organs and tissues and help to preserve and restore human health and well-being (Bawarski et al. 2008.). Therefore, nanopharmaceuticals are an emerging domain that integrates nanotechnology, biotechnology, and pharmaceuticals (Jain 2008) and were defined by Rivera Gil et al. (2010) as "pharmaceuticals where nanomaterials play the pivotal therapeutic role or add additional functionality to the previous compound" (Rivera Gil et al. 2010).

Nanomaterials by definition have a size range in the sub-100 nm scale (ISO 2015), different shapes, large surface area, and great reactivity. At this size scale, quantum effects may alter the specific physicochemical properties of the bulk material, which allow them to cross biological barriers and be used in drug delivery, therapy, *in vivo* imaging, *in vitro* diagnostics, biomaterials, active implants, and regenerative medicine (Wagner et al. 2006). Due to this wide range of applications, the size of the individual particles tested for drug delivery and imaging may range from 2 nm to 1000 nm. This led the European Medicines Agency to adopt, in 2010, a broader definition, considering the application of nanomaterials in nanomedicine, in the size range of 1 nm to 1000 nm – even including compounds surpassing these limits – if they are manufactured on purpose of drug delivery (Berkner et al. 2016; Souza and Amaral 2017). A wide range of nanomaterials were created with several therapeutic applications and include particulate and vesicular systems, dendrimers and drug-polymer conjugates, colloidal gold, iron oxide crystals, quantum dots, and

solid nanostructures (fullerenes and carbon nanotubes) (Bawarski et al. 2008). They can be classified in two categories: "hard" and "soft" nanomaterials. "Hard" nanomaterials are formed by ionic or covalent bonds, such as metal and metal oxide nanoparticles, while the "soft" ones are formed via weak interactions, such as liposomes, dendrimers, and micelles (Mahapatra et al. 2013). The main features and examples of application of some types of nanostructures, such as polymeric nanoparticles, magnetic nanoparticles, and liposomes, will be presented in this chapter.

The materials used to produce nanopharmaceuticals have different chemical composition and behavior when present in the aquatic environment. A successful example of a nanopharmaceutical is Abraxane® (Abraxis, Los Angeles, California), an albumin-bound nanoparticle formulation of paclitaxel with application in metastatic breast cancer, which allowed to overcome the limitations associated with drug hydrophobicity while avoiding the need to use toxic organic solvents (Bawarski et al. 2008). Therefore, not only the produced nanopharmaceuticals will be unique, but also their interactions with drug molecules will be distinctive. The impact of their discharge to the aquatic environment will produce interactions with abiotic and biotic components of the aquatic ecosystems that in some cases can be toxic. For example, colloidal gold, iron oxide nanoparticles, and quantum dots generally used in nanomedicine are known to be toxic (Nogueira 2014; Rocha et al. 2015a, 2017; Lefevre et al. 2015; Valdiglesias et al. 2016). Therefore, human and environmental safety guidelines are urgently needed.

Nanopharmaceutical research has focused on drug formulation to improve biodistribution, bioavailability, and pharmacokinetics and on the specific delivery of existing drugs, especially in mammal species (Chen and Guan 2011). After being administered they are excreted from the human body, introduced in hospital waste water sewage or present in industrial effluents, ending up in waste water treatment plants where their elimination is reduced. Clearly, the nanopharmaceutical formulations or their metabolites will be in contact with several other organisms during the elimination process until they reach the aquatic environment. Available data on the behavior and effects of nanoparticles in the aquatic environment, such as aggregation, evidence the need for models that allow predictions, inclusive of their concentrations and potential ecotoxicity. Ecotoxicity effects of nanoparticles in aquatic organisms include oxidative stress, genotoxicity, neurotoxicity, behavior changes and immunotoxicity (Rocha et al. 2017). However, ecotoxicological studies about the behavior, fate, and impact of nanopharmaceuticals in nontarget species remain scarce (Yegin et al. 2017). Given the wide range of applications, nanopharmaceuticals evolved and grew in recent years, but safety issues were not taken into account and possible undesirable effects on humans were not studied properly. In addition, little attention was paid to the potential nefarious effects caused by the starting materials that result in the nanomaterials. As such, the environmental impact assessment of the fabrication process and the problematic effects that may arise from the environmental release of these compounds were also disregarded (Linkov et al. 2008; Berkner et al. 2016). Therefore, the main objective of this book chapter is to highlight the potential effects of nanopharmaceuticals in the aquatic environment as

a result of their applications in drug delivery systems, with special emphasis on liposomes and nanoparticles, as these are the most explored, even at the market level.

8.2 Types and Uses of Nanopharmaceuticals

The pharmaceutical area is, at present, the focus of great innovation. Along with new molecules being discovered and others undergoing chemical modifications to meet specific requirements, several molecules already in clinical practice are being studied in different formulation strategies that include the use of the so-called drug delivery systems (Wu et al. 2017; Tarhini et al. 2017). These systems are gaining increased interest and are developed to fit specific needs that cover a wide range of possibilities. Usually, the delivery systems act as carriers for the molecule of interest and its formulation provides improved stability and protection towards degradation (Zhang et al. 2013; Petros and DeSimone 2010). Additionally, in many cases, the delivery of drugs to a site of interest or the modification of the kinetic profile is envisaged (Almeida and Grenha 2014; Sarwar et al. 2017). Overall, the use of drug delivery systems is also expected to allow the reduction of side effects and potentiate the efficacy of the drug (Zhang et al. 2013; Petros and DeSimone 2010). There are many drug delivery systems, differing in characteristics such as the structure, composition, and size. Nanoscaled carriers are currently gathering much attention, because of several advantages comparing with micron-sized counterparts. These advantages include higher control over drug release (Lopes et al. 2016), increased drug absorption (Csaba et al. 2006), and great ability for surface functionalization (Singh Jr. and Lillard 2009), among others, that make nanocarriers viable therapeutic alternatives.

As of this day, there are several nanopharmaceuticals already approved by the Food and Drug Administration (Weissig et al. 2014) that will be briefly discussed later. Figure 8.1 shows a chronology in which the discovery of different carriers is highlighted, showing some marketed formulations of relevance.

8.2.1 Vesicular Drug Delivery Systems

Vesicular drug delivery systems correspond to liposomes, which were first described by Bangham in 1965 (Bangham et al. 1965) and used in clinic since 1997, when the first products were approved by the Food and Drug Administration (Weissig et al. 2014). Liposomes are vesicles comprised of a lipid bilayer, usually obtained using phospholipids (Lasic 1988). Their amphiphilic structure allows the encapsulation of hydrophilic drugs inside the formed cavity or hydrophobic molecules within the membrane (Gulati et al. 1998). This flexibility is one of the key features of these systems, along with the reported biodegradability and biocompatibility, chemical flexibility, and stability provided to drug molecules, namely, by preventing or

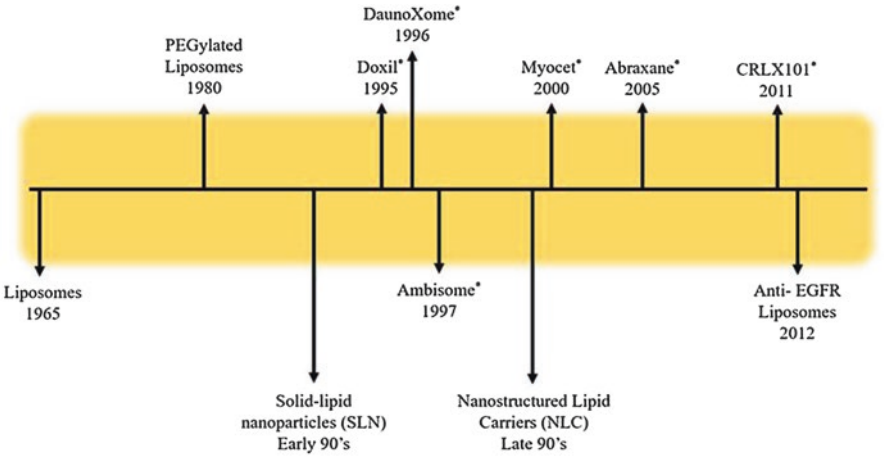

Fig. 8.1 Chronological order of development of several drug nanocarriers and approval of nanopharmaceuticals

delaying their degradation. All these characteristics have fostered their use as drug carriers. Furthermore, liposomes also provide protection to organisms receiving the drugs, as described for amphotericin B. This antifungal drug causes severe toxicity and its liposomal formulation (AmBisome®) soon revealed to be the solution for problems of therapeutic incompliance (Akbarzadeh et al. 2013). Regrettably, liposomes present low solubility and short shelf-life. Furthermore, due to their composition, the possibility of oxidation and hydrolysis of phospholipids needs to be considered, as these compromise liposome usefulness, leading to vesicle disintegration with consequent drug leakage (Akbarzadeh et al. 2013). Fortunately, these problems can be addressed by a process of freeze-drying, which ensures the removal of almost all the water of the formulation, improving liposome's shelf-life and greatly inhibiting oxidation and drug leakage (Miyajima 1997).

Liposomes are mainly composed of phospholipids, being phosphatidylcholine and phosphatidylethanolamine the most commonly used materials for their production (Laouini et al. 2012). Nevertheless, it is very frequent to include other molecules in liposome formulations to confer specific characteristics. Depending on the length and saturation of the lipid chain of the phospholipids, rigid or fluid liposomes may be formed (Akbarzadeh et al. 2013). Cholesterol is included very often, as it makes the liposomal membrane more rigid and less flexible, allowing a better control over the release of the drugs (Tardi et al. 2016). The amount of cholesterol plays a relevant role in this regard (Briuglia et al. 2015). Additionally, it is suggested that cholesterol helps increasing the vesicle's circulation time (Kirby et al. 1980). Polyethylene glycol is also used frequently in liposomal formulations, as it hampers the process of opsonization and, consequently, the detection of the vesicles by the immune system. This delays the elimination of the liposome (Milla et al. 2012; Immordino and Cattel 2006) and, thus, potentiates the drug effect.

Lipid film hydration and the solvent injection methods are two of the most used techniques to produce liposomes. These techniques share two common steps: (1) the dispersion of lipids in organic solvent and (2) the addition of an aqueous solution to form the vesicles (Akbarzadeh et al. 2013; Meure et al. 2008). More recent approaches include the methods of microfluidic channel and of supercritical fluid injection and decompression, but this requires expensive equipment to effectively produce the vesicles (Meure et al. 2008). The lipid film hydration involves solubilization of phospholipids using organic solvents, solvent evaporation to form the lipid film, and subsequent addition of an aqueous solution, either with or without the drug to be encapsulated (Brandelli 2012). Liposomes are formed instantaneously in this case. After their production, further processing is usual to tailor sizes to the desired outcome. A technique of extrusion is frequently applied for this end, as well as sonication (Schroeder et al. 2009). The literature displays a comprehensive review on the methodologies to produce liposomes (Meure et al. 2008) and on techniques to optimize the produced vesicles (Mozafari 2010). In fact, size and zeta potential are two of the most relevant characteristics of nanocarriers. Zeta potential indicates the surface charge of the vesicle and is naturally dependent on its composition. Along with size, it plays an important role on the interaction with involving environment, including epithelial surfaces and proteins in the blood, among others (Manaia et al. 2017).

After production and further refinement, liposomes are classified according to three categories, as shown in Fig. 8.2.

Different types of liposomes can be obtained: small unilamellar vesicles (SUVs), large unilamellar vesicles (LUVs), and multilamellar vesicles (MLVs). Small unilamellar vesicles (SUVs) are formed by a single phospholipid bilayer and can present 100 nm or smaller size; large unilamellar vesicles (LUVs) also have a single bilayer, but range between 200 and 800 nm; finally, multilamellar vesicles (MLVs) are comprised of many concentric bilayers, reaching sizes up to 5000 nm (Torchilin 2008). Fortunately, the myriad of production processes and refinement methods allow the production of vesicles of different sizes and structures that are studied and used depending on the given purpose.

Liposomes are thus efficient drug delivery systems that are strongly used in clinics and are still subject of intense study, as will be addressed in Sect. 8.2.3.

8.2.2 Nanoparticulate Drug Delivery Systems

Nanoparticulate drug delivery systems, along with the vesicular systems revolutionized therapeutics and the field of drug delivery. A great part of research is conducted with several delivery routes being tested, as well as encapsulation strategies that enable and improve the efficiency of certain drugs (Mallipeddi and Rohan 2010; Pachuau 2015).

As for liposomes, size and zeta potential are two of the most relevant characteristics. The International Organization for Standardization (ISO) defines

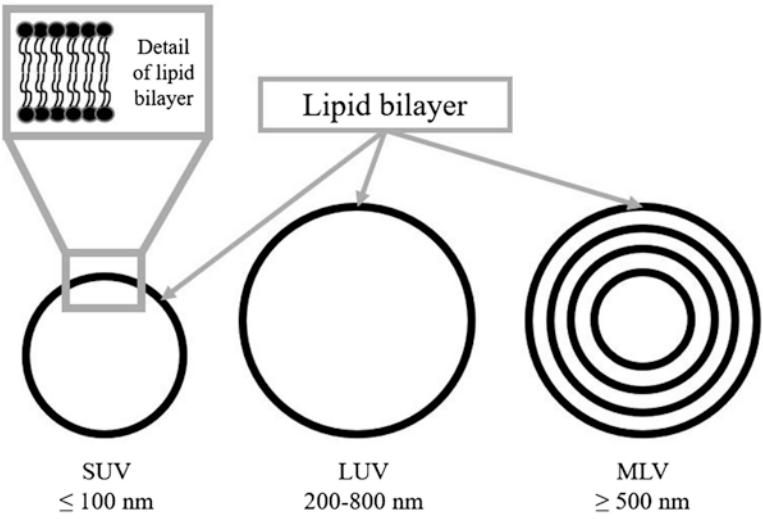

Fig. 8.2 Depiction of the different types of liposomes that can be obtained: small unilamellar vesicles (SUVs), large unilamellar vesicles (LUVs), and multilamellar vesicles (MLVs). Liposomes are vesicular drug delivery systems, mainly composed of phospholipids organized in bilayers. These are represented by the black bold line. Depending on the size, and on the refinement techniques, liposomes can have only one bilayer (the case of SUV and LUV) or more than one concentric bilayer (the case of MLV)

nanoparticles as particles having at least one dimension less than 100 nm (ISO 2015). However, carriers with sizes up to 1000 nm are also considered nanoparticles by most of the scientific community (Wilczewska et al. 2012), including the European Medicines Agency (Berkner et al. 2016). The broader definition is the one considered in this chapter.

Nanoparticulate carriers can have different composition, either organic (polymers or lipids) or inorganic (metals or silica). This chapter will focus on organic nanoparticulate drug delivery systems (polymeric and lipid nanoparticles, and dendrimers), and on magnetic nanoparticles and quantum, which will be detailed below.

Organic Systems

Organic nanoparticulate drug delivery systems enclose two sub-categories: polymeric particles, produced with either synthetic or natural polymers, or lipid particles. These carriers are typically divided in the categories of nanocapsules and nanospheres. Nanocapsules are nano-reservoirs comprised by a shell structure and a core that can be either aqueous or oily and liquid or semi-solid. The core or cavity is the place where the drug of interest is mostly encapsulated/associated. On the other hand, nanospheres are matrix nanoparticles, meaning particles that are solid, having the drug of interest distributed virtually anywhere (Vauthier and Bouchemal 2009).

In the category of polymeric systems, polyesters and, more particularly poly(lactide-co-glycolide) acid (PLGA), are the most used synthetic materials, while polysaccharides and proteins are used as natural ones. PLGA evidences ability to control drug release, along with low toxicity and high biocompatibility, which enable its inclusion in several formulations already in the market for several biomedical applications (Sharma et al. 2016). Chitosan, alginate, and hyaluronic acid are some examples of polysaccharides that are being studied for application in formulations of therapeutic relevance (Huh et al. 2017). Chitosan has been widely studied over the recent decades and is the most explored polysaccharide. This is justified by its unique cationic character, which brings very relevant characteristics such as strong mucoadhesivity. Additionally, it is also reported as biocompatible in many routes of administration and useful in various studies of clinical relevance, as in colon cancer (You et al. 2016), detection of tumors via image diagnosis (Hong et al. 2017), and for the delivery of antibiotics (Madureira et al. 2015), among others. Polymeric nanoparticles have a slight immunomodulatory activity. Thus, when used to carry immunomodulatory vaccines or peptides, they may potentiate the activity of the carried molecules (Amaral et al. 2010; Ribeiro et al. 2015). Despite the exhaustive study of the referred polysaccharides and the demonstration of favorable characteristics such as biocompatibility, low toxicity, and biodegradability (Martínez et al. 2012), there is no nanopharmaceutical formulation approved with these materials. In turn, albumin is one of the most studied proteins as matrix component of drug delivery systems and has one formulation approved (Abraxane®) for the treatment of breast cancer and non-small-cell lung cancer (Bernabeu et al. 2017).

Dendrimers are also an emerging class of polymer-based carriers. These are spherical structures with high surface area derived from the highly branched polymers used in their preparation. Because of this, they present huge internal space to incorporate bioactive molecules (Rimondino et al. 2017). Features such as the spherical shape and symmetrical architecture, coupled with specific physicochemical properties, make these structures fascinating for drug delivery applications. Dendrimers are typically composed of a central component (called core), by several internal cavities created according to the branched polymer used and by a surface that can be formed by different functional groups (Srinageshwar et al. 2017). These groups on the surface promote delivery of dendrimers to different cell types and even simplify their penetration into tissues or cells. Dendrimers are classified according to the number of layers, where each layer is called generation (G): a four-layer dendrimer is called "dendrimer generation 4 (G4)." Most likely because of their small size, usually around 10 nm, and surface charge, dendrimers are able to penetrate diverse tissues of the body (Albertazzi et al. 2010). Upon injection into the carotids of mice, dendrimers with a slight cationic surface were able to cross the blood-brain barrier (Srinageshwar et al. 2017). Dendrimers surface charge strongly correlates with the cell penetration ability, as neutral or negatively charged dendrimers show reduced internalization (Perumal et al. 2008). This attribute can be improved by coupling molecules such as peptides to their surface (Jiang et al. 2016).

Lipid-based nanoparticulate carriers are another category of organic-based systems. Within this category, solid lipid nanoparticles and nanostructured lipid

carriers are those gathering the highest attention. Solid lipid nanoparticles are comprised of a matrix of solid lipids only and were first reported in the early 1990s to avoid some limitations shown by liposomes and polymeric nanoparticles (Nunes et al. 2017). As for the nanostructured lipid carriers, they appeared in the late 1990s as an improvement of solid lipid nanoparticles. A mixture of both solid and liquid lipids is required to prepare nanostructured lipid carriers, which display increased stability provided by the liquid lipids present in the matrix (Li et al. 2017), when compared with solid lipid nanoparticles. The methods of production of solid lipid nanoparticles and nanostructured lipid carriers are described in Lin et al. (2017) and Tamjidi et al. (2013).

Polymeric nanocarriers can be obtained by the polymerization of a monomer or from preformed polymers, the latter being the most used of the approaches. In this context, methodologies involving emulsification such as emulsification/solvent evaporation, emulsification/solvent diffusion, solvent displacement and interfacial deposition, among others, are very frequent and allow the production of both nanocapsules and matrix nanoparticles. Matrix nanoparticles also frequently produced in processes mediated by electrostatic interactions or involving desolvation. The latter consists in adding a desolvating agent, a salt or a non-solvent of the polymer that is miscible with water, to the polymeric solution. Macromolecular aggregation or particle formation is brought about by the partial desolvation of fully solvated polymer molecules (Vila and Lastres 2001). Methods such as polyelectrolyte complexation and ionic gelation are those involving electrostatic interactions, in which nanoparticles form upon interaction between oppositely charged molecules. Several comprehensive reviews exist on the methodologies to produce polymeric nanocarriers, featuring their advantages and disadvantages (Pinto Reis et al. 2006; Vauthier and Bouchemal 2009). To produce solid lipid nanoparticles and nanostructured lipid carriers, the method most commonly applied is of high pressure homogenization. In this method, the solid lipid components are melted and mixed afterwards with the liquid lipids (when applicable) and drugs (if the production of drug-loaded carriers is envisaged). This mixture is then added to a hot aqueous solution containing surfactants, being stirred by a high-shear mixing device, to form an emulsion. Homogenization is repeated until nanodroplets are obtained.

Inorganic Systems

Inorganic systems comprise those nanoparticles that are composed by inorganic materials. Magnetic nanoparticles are one of the most used, with structures with about 7 nm. When close to a magnetic field, they can suffer alterations that influence their behavior (Loebinger et al. 2009; Issa et al. 2013). Metals used to prepare magnetic nanoparticles should be carefully chosen to avoid toxicity. Cobalt, nickel, and neodymium-iron-boron are used. However, they may suffer oxidation during *in vivo* applications (Dias et al. 2011). Iron oxide materials such as maghemite and magnetite are safer and, thus, often used to produce these nanoparticles (Dias et al. 2011). Their interest relies on multifunctional characteristics, such as small size,

supermagnetism, and low toxicity to mammals, easiness of synthesis and functionalization (Wu et al. 2009; Dias et al. 2011). However, from the two materials, maghemite presents iron in the oxidized state, further reducing its toxicity (McBain et al. 2008).

To improve the stability of these structures and to prevent them from clumping, they are functionalized by surface-binding to organic substances, thus forming the magnetic fluids. In this way, the components connected to or incorporated in them will also be influenced by the magnetic field (Shi et al. 2012). Another property of these functional groups is to reduce the toxicity of certain metals and increase their biocompatibility (Lin et al. 2010; Kolhatkar et al. 2013). Gallo et al. (2013) presented an extensive review in which several types of materials used for the coating of magnetic nanoparticles are mentioned, including dextran and bovine serum albumin. According to the nature of the functional groups incorporated in magnetic nanoparticles, they can carry drugs and nucleic acids, as well as substances for contrast in magnetic resonance examinations (Loebinger et al. 2009). They may also be associated with other nanostructures such as liposomes, and, thus, by responding to an external magnetic field, they can be manipulated to be target-directed. Currently, several applications are being focused on these magnetic nanoparticles, but the main applications are in treatment and detection of tumors.

Quantum dots are another group of inorganic systems being strongly explored. They are a class of engineered nanoparticles formed by fluorescent semiconductor nanocrystals with nanometer diameters ranging from 1–10 nm. These nanoparticles are classified in two categories: cadmium-based quantum dots and cadmium-free quantum dots. The quantum dots core can be made of a variety of metal complexes, such as group II–IV series (CdSe, CdTe, CdSeS, ZnS, ZnSe, and PbSe) or group III–V series (InP, InAs, GaAs, and GaN). This core determines their color, while the inorganic shell or ligand(s) can enhance stability, brightness, water solubility, and conjugation capacity (Michalet et al. 2005; Nguyen et al. 2013). The most common quantum dots core used for biological and medical applications are CdSe and CdTe, which can be coated with a shell and additional capping layer or ligands (Michalet et al. 2005; Smith et al. 2008; Rocha et al. 2017).

The quantum dots' shell consists mainly of a second semiconductor material (e.g., ZnS) and protects the core from oxidation and degradation. Surface ligands can be hydrophilic, hydrophobic, or amphiphilic polymers, such as mercaptoacetic acid (MAA), mercaptosuccinic acid (MAS), thioglycolic acid (TGA), dihydrolipoic acid (DHLA), and amphiphyllic polymers like modified polyacrylic acid (PAA). These ligands increase the quantum dots' water solubility and compatibility for applications in biological systems (Maysinger et al. 2007). Furthermore, quantum dots can also be conjugated with biomolecules (e.g., peptides and oligonucleotides), antibodies, and/or drugs for identification and action in specific biological targets (Smith et al. 2008; Rizvi et al. 2010).

Due to their physicochemical properties and biological interactions, quantum dots are applied in many fields. These include electronics (i.e., light-emitting diode (LED), organic light-emitting diode (OLED), photovoltaic, and lasers), solar panels, photo-chemistry (i.e., photoelectrodes), analytical chemistry, pharmacy,

molecular and cell biology (i.e., live cell imaging, co-localization of genes/proteins, multicolor staining, and flow cytometry), and nanomedicine (i.e., molecular profiling of cancer, antimicrobial agents, in vivo tumour imaging, photodynamic therapy, diagnosis, and development of disease- and patient-specific therapies) (Michalet et al. 2005; Deerinck 2008; Rizvi et al. 2010).

Nanoparticulate drug delivery systems comprise a wide topic in the pharmaceutical field. Although their market presence is limited so far, they are considered the future alternative in therapeutics, thus looking for new options on how drugs will be delivered to the human body. The most remarkable achievements will be addressed in the following section.

8.2.3 Nanopharmaceuticals as a Viable Therapy

Many different materials can be used to prepare various carriers that are intended to encapsulate a variety of drug molecules. Vesicular and nanoparticulate drug delivery systems are two of the most studied approaches. As shown in Fig. 8.1, the discovery of liposomes occurred before 1980s. Afterwards, when it was possible to alter the outer membrane of the liposomes by adding specific molecules, the PEGylated liposomes appeared, bringing uncountable advantages regarding drug half-life. From this point on, important liposome-based formulations appeared in the market: Doxil®, DaunoXome®, Ambisome® and Myocet®. Apart from Ambisome®, all the other products encapsulate anticancer drugs, and cancer is, indeed, one of the greatest fields of application of liposomal formulations. Naturally, pharmaceutical formulations comprising advanced technologies result in more expensive products, which find application more easily in diseases permitting higher investment. Ambisome® encapsulates a potent antifungal drug (amphotericin B), enabling the decrease of the strong side effects caused by the administration of the drug per se (Stone et al. 2016). In an interesting approach, thermosensitive liposomes were recently proposed to provide external targeting of drugs to solid tumors, in combination with local hyperthermia or high-intensity focused ultrasounds (Al Sabbagh et al. 2015; Novell et al. 2015).

Another important event in the timeline shown in Fig. 8.1 is the approval of Abraxane® in 2005, the only marketed formulation comprised of nanoparticles. This formulation is composed by albumin conjugated with paclitaxel, currently having an application in metastatic breast cancer and non-small-cell lung cancer. Weissig and Guzman-Villanueva provide a comprehensive review on these products, along with many others that are not subject of this chapter (Weissig et al. 2014).

Nanopharmaceuticals are an alternative approach to conventional therapeutic strategies, also having a role in the field of diagnostics. They can have important roles on smoothing severe adverse effects or on mediating active targeting of the encapsulated molecules to specific cells/tissues. There are several formulations that are currently on clinical trials for a possible approval soon. One example is CRLX101, a cyclodextrin-PEG nanoparticle encapsulating camptothecin, another

anticancer drug. It is an intravenous formulation, and it has finished phase II clinical trials, being proposed for the treatment of rectal, ovarian tubal, and peritoneal cancer (Svenson et al. 2011; Ragelle et al. 2017). Another example included in Fig. 8.1 is the anti-EGFR immune-liposomes, which recently completed phase I clinical trials. This strategy is based on the encapsulation of doxorubicin, to be potentially used in solid tumors (Mamot et al. 2012). Furthermore, several metal-based delivery systems are currently in phase I clinical trials for potential use in radiotherapy and the treatment of prostate cancer by application of a magnetic field (Ragelle et al. 2017). A recent review addresses the market prospects of these nanopharmaceuticals, further referring to many other formulations undergoing clinical trials (Ragelle et al. 2017). Interestingly, it also describes three products currently in phase III: one for hepatocellular carcinoma, another for respiratory syncytial virus infection, and the last one for metastatic breast cancer. The results of these clinical trials will dictate the possibility to obtain marketing authorization. Other strategies, more specific for the cancer therapy area, are described in a novel review by Li et al. (2017), proving that the versatility of this strategy is being taken into consideration for the next generation of therapeutics.

Dendrimers and magnetic nanoparticles are not so advanced in their positioning to market. Nevertheless, there are very interesting applications being reported. Dendrimers formed by polyamidoamine polymer (PAMAM) have a high density of amine groups with empty inner cavities and functional groups that promote high solubility and reactivity (Jiang et al. 2016). Because of this, they are widely used to deliver anticancer drugs, such as doxorubicin. Although toxic, in animal model experiments, doxorubicin was better tolerated by animals when dosed twice the tolerable limit to cause toxicity (Kaminskas et al. 2012). Gene therapy applications have also been reported (Nam et al. 2015; Hemmati et al. 2016). Dendrimers were successfully used to carry siRNA, evidencing ability for incorporation into their internal cavities and to promote cell internalization (Liu and Peng 2016). Highly branched dendrimers comprised of glutamic acid-modified hyperbranched polyamidoamine (HPAMAM) also evidenced efficient gene transfection, with decreased toxicity (Hemmati et al. 2016).

Regarding magnetic nanoparticles, superparamagnetic iron oxide nanoparticles (SPIONs) have been used in magnetic resonance imaging (Pour and Shaterian 2017; Xiong et al. 2017), benefiting from the attachment of specific receptors to SPIONs surface, which allow greater affinity and precision in detecting tumors. Magnetohyperthermia is also an innovative approach gathering attention, consisting in the generation of heat at different intensities by the application of a magnetic field over magnetic nanoparticles. This technique is applied to destroy tumor cells, which are more sensitive to heat changes (Miranda-Vilela et al. 2014), but it can also be used to promote release of the bioactive compound from magnetic nanoparticles (Tang et al. 2017).

The potential of the systems has been demonstrated in many cases, but regulations have generally tightened up regarding the approval of new drug formulations, with restrictions gaining emphasis for nanopharmaceuticals. For these formulations, it has become even more important to correctly and exhaustively assess the

biological implications of their delivery to and permanence within the human body. In this regard, Kipen and Laskin have stated that *nanotechnology yields nanotoxicology* (Kipen and Lasking 2005). While this is not necessarily true, it reveals the lack of knowledge and information on toxicological effects of engineered nanomedicines. These effects are not restricted to those felt by the organism receiving the formulations but also encompass the effects imposed to the environment. It must be reminded that, after delivery to humans, the formulations or their metabolites end up reaching the environment via debris. The next sections of this chapter address the impact of nanopharmaceuticals on the aquatic environment. In fact, the toxicological effects of engineered nanomedicines are one incredibly important feature in the approval process of a nanopharmaceutical.

8.3 Biotechnology and Production of Nanopharmaceuticals

Nowadays, there are various nanostructured formulations for drug delivery in clinical use. Applications of these nanostructured formulations involve treating various diseases, such as cancer and fungal infections. In Table 8.1 a few examples of drugs at nanoscale currently in clinical use are listed.

The delivery of bioactive molecules, such as drugs, peptides, and nucleic acids, can be performed through different materials, in accordance with their nanoproperties (Amaral and Felipe 2013). When these bioactive molecules are incorporated within nanostructures, they present better stability, improving its therapeutic efficacy (Kaminskas et al. 2012). Drug delivery systems at nanoscale can be prepared by different methods and types of materials, but it is essential to consider the nature of the molecule to be encapsulated and its destination when used for biological purposes.

Table 8.1 Examples of approved clinical nanomedicines and their clinical indications

Medicine	Drug	Composition	Clinical indication	References
Abraxane®	Paclitaxel	Albumin-bound	Breast, lung and pancreatic cancer	Vallo et al. (2017)
Ambisome®	Amphotericin B	Liposome	Fungal and protozoan infections	Stone et al. (2016)
Doxil®	Doxorubicin	Liposome	Ovarian cancer and Kaposi's sarcoma	Kakar et al. (2016)
Epaxal®	Inactivated Hepatitis A virus	Virosome	Hepatitis A infection	Bovier (2008)
Gemzar®	Gemcitabine	Liposome	Several types of solid tumors	Federico et al. (2012)
Inflexal®	Influenza particles	Virosome	Influenza vaccine	Herzog et al. (2009)
Opaxio®	Paclitaxel	Polymer conjugates	Several types of tumors	Galic et al. (2011)

The modern biotechnological techniques to manipulate nucleic acids have allowed the development of drugs with improved pharmacological properties, such as peptides and DNA vaccines (Amaral et al. 2012; Amaral and Felipe 2013). However, because of the physicochemical nature of these molecules, which need to keep their original conformation to preserve the activity, they are easily degraded when in contact with the physiological environment by the action of enzymes. When incorporated into these nanostructures, many drugs may have their concentration increased where the therapeutic activity is needed and thus reducing toxicity (Kaminskas et al. 2012).

In vivo experiments proved to improve peptides and DNA vaccines' bioavailability when incorporated within nanoparticles (Amaral et al. 2010; Ribeiro et al. 2015). Using the murine model of fungal infection *Paracoccidioidomycosis*, it was possible to increase the immunomodulatory activity of a peptide of 10 amino acid residues, called P10, when incorporated into polymeric nanoparticles (Amaral et al. 2010). Similar results were remarked for the same experimental model when a DNA therapeutic vaccine is delivered within polymeric nanoparticles or liposomes (Ribeiro et al. 2015). Both formulations were able to enhance in four times the antifungal activity of the vaccine compared with the DNA vaccine administered in the free form.

8.4 Sources of Nanopharmaceuticals Release into the Aquatic Environment

The sewage effluent is the major source of nanopharmaceuticals in the aquatic environment. Human nanopharmaceuticals are released into the sewage system as a mixture of the unchanged, metabolized or conjugated compounds. The elimination of nanopharmaceuticals by patients occurs via excretory or hepatobiliary system followed by fecal or biliary excretion. In addition, nanopharmaceuticals applied to veterinary medicine are also a potential source of pollution, while the sludge from waste water treatment plants is an additional source of soil and aquatic pollution. The direct or indirect release of nanopharmaceuticals in effluents of wastewater treatment plants from hospital, communities, and industrial facilities will result in the exposure of aquatic organisms to nanopharmaceuticals. Although the concentration of nanopharmaceuticals in the aquatic environment is unknown, the environmental levels of pharmaceuticals is increasing due to an ageing, increase on life expectancy and growing of human population, as well as the increase production and use of new products, indicating that nanopharmaceuticals may follow the same environmental fate of pharmaceutical compounds. Mahapatra et al. (2013) indicated that the release form and environmental fate and exposure of nano-enabled medical products have not been investigated and little or no data exists in the literature, confirming the urgent need to investigate the potential hazards and risks associated to nano-enabled medical products, such as the nanopharmaceuticals.

After its release in the aquatic systems, different processes may influence the environmental behavior and fate of nanopharmaceuticals and their metabolites, as reported for other engineered nanoparticles: physicochemical transformation, aggregation/agglomeration, macromolecular interactions, and biologically mediated reactions (Dwivedi et al. 2015; Rocha et al. 2017). However, these processes have not yet been investigated for freshwater, estuarine, and marine environment.

8.5 Effects of Nanopharmaceuticals in the Aquatic Environment

The potential pathways for ecotoxicological research of nanopharmaceuticals in the aquatic environment are summarized in Fig. 8.3. Nanopharmaceuticals exhibiting novel and multifunctional properties, such as high surface area and saturation solubility, resistant to settling, fast dissolution, and improved adhesion to biological surfaces, may give rise to potentially new ecotoxicological effects and environmental risks.

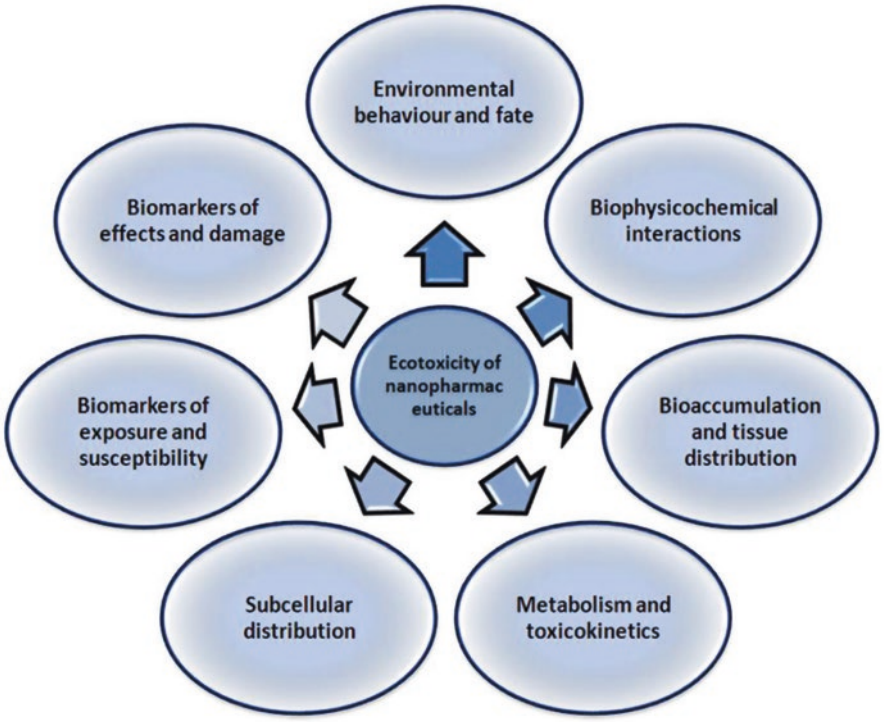

Fig. 8.3 Potential pathways for ecotoxicological research of nanopharmaceuticals

In studies about the mode of action and toxicity of nanopharmaceuticals, a comprehensive knowledge of how these nanomaterials interact with biological systems is fundamental (Fig. 8.3). The interaction between nanomaterials applied to personalized medicine and biological systems is mediated by protein-binding, ligand-mediated interactions and interactions during intracellular processing (Zhang et al. 2012). Furthermore, upon contact with biological fluids (e.g., hemolymph, blood, interstitial fluid, or mucosal secretions), nanomaterials are coated with proteins and/or other molecules, forming the protein corona (bio), which may change their nano-specific properties, such as surface charge (zeta potential) and hydrodynamic diameter. According to Canesi and Corsi (2016) and Canesi et al. (2017), the interaction of nanomaterials with plasma proteins in non-mammal species also induces the formation of the protein corona, changing its uptake and toxicity in target cells. On the other hand, the interaction of nanomaterials with the external environment (i.e., natural organic matter and clays) forms the eco-corona, which changes its environmental behavior and fate in distinct compartments of the ecosystems (aqueous phase, sediments, biota) (Rocha et al. 2015a, 2017; Canesi and Corsi 2016).

The interaction and bioaccumulation of nanomaterials in aquatic organisms are directly related to their mode of action and toxicity (Fig. 8.3). Recently, Yegin et al. (2017) showed that paclitaxel-loaded nanoparticles (84 ± 4 nm; 0.2–16.2 μg ml^{-1}) were adsorbed on cell surfaces of the freshwater algae *Raphidocelis subcapitata* and *Chlamydomonas reinhardtii* and decreased the algal growth rate (72 h IC_{50} of 1.6 ± 0.1 μg paclitaxel ml^{-1} for *R. subcapitata* and 120 h IC_{50} of 1.1 ± 0.1 μg paclitaxel ml^{-1} for *C. reinhardtii*), as well as inhibited the photosynthesis efficiency more than molecular (free) paclitaxel after 5 days of exposure. Furthermore, the polymer poly(ethylene oxide)-block-poly(ε-caprolactone) (PEO-*b*-PCL) used in the formation of nanocarriers was nontoxic to both algae species. In this sense, Yegin et al. (2017) indicated that the nanotechnology can increase the ecotoxicity effects of insoluble (lipophilic) drug molecules and that paclitaxel-loaded nanoparticles have algaecide properties. In this sense, the algal community that form the basis of aquatic food web represents an important target for biological interaction and ecotoxicity effects of nanopharmaceuticals. On the other hand, the tissue and subcellular distribution, metabolism and toxicokinetics of nanopharmaceuticals on aquatic organisms deserve further studies.

Fish are considered a suitable model for ecotoxicity assessment of magnetic nanoparticles in the aquatic environment (Table 8.2). The zebrafish *Danio rerio* and *Oryzias latipes* are the main fish species used to assess the environmental impact of magnetic nanoparticles. However, there is limited data for other economically important fish species, such as *Oreochromis niloticus* (Ates et al. 2016) and *Poecilia reticulata* (Qualhato et al. 2017) (Table 8.2).

Li et al. (2009) described oxidative stress and hypoxia in *Oryzias latipes* after exposure to nZVI nanoparticles and nFe-oxide nanoparticles (49 nm; 1–100 mg L^{-1}) for 14 days and revealed that the mortality observed was dependent on the nanoparticle composition [Fe(II) > CMC-nZVI > nFe-oxides]. Recently, Qualhato et al. (2017) showed that ecotoxicity of iron oxide nanoparticles in fish species is exposure time and concentration dependent. In general, data indicate that oxidative stress

Table 8.2 Magnetic NPs effects in fish species

Nanoparticles			Species	Exposure conditions		Accumulation	Cell/ Tissue[b]	Effects[c]	References
Type	Capping layer[a]	Size (nm)		Concentration (mg L^{-1})	Time(h)				
Fe_3O_4	SiO_2 and DTC	100	*Anguilla anguilla*	2.5	72	–	G*	Co-exposure: Hg + NPs; ↓TGSH; ↑GR; GPx↑; ↑GST; ↑LPO. Synergistic response to co-exposure.	Srikanth et al. (2014)
α-Fe_2O_3	–	30	*Danio rerio*	0.1–100	168	–	E	168 h NOEC <50 mg L^{-1}; 168 h LC_{50} = 53.35 mg L^{-1}; ↓embryo-hatching (≥10 mg L^{-1})	Zhu et al. (2012)
Fe_2O_3, Fe_3O_4	–	80–90; 140–160		4, 10	E: 28 days; D: 24 days	x	W	Similar accumulation in both concentrations; elimination (~24 days); uptake by gastrointestinal tract.	Zhang et al. (2015)
γ-Fe_2O_3	DMSA	5.7		4.7–74.4	96	x	Bl, L	↑DNA damage; ↑NAs; low LPO; differential expression gene.	Villacis et al. (2017)
Fe_2O_3	–	100 x 200	*Labeo rohita*	500	25 days	–	G, Bl	↑hemoglobin; ↑red blood cell; ↑hematocrit; ↓cellular volume; ↓white blood cell; ↓Na^+; ↓cl^-; ↓K^+; ↑gill Na+/K + -ATPase	Ribeiro et al. (2015)
α-Fe_2O_3, γ-Fe_2O_3	–	20–90, 40–120	*Oreochromis niloticus*	0.1, 0.5, 1.0	E: 60 days; D: 30 days	x	S, I, K, L, G, Bl, mu	Accumulation (S > I > K > L > G > B > mu); elimination (except in L, S); no changes in haematological parameters and respiratory burst; ↓GLU; ↑GOT; ↑GPT; ↑LDH; ↓LA; ↑MPO.	Ates et al. (2016)

(continued)

Table 8.2 (continued)

Nanoparticles			Species	Exposure conditions		Accumulation	Cell/ Tissue[b]	Effects[c]	References
Type	Capping layer[a]	Size (nm)		Concentration (mg L^{-1})	Time(h)				
nFe	SPA	30	*Oryzias latipes*	0.5–50 μg mL^{-1}	8–14 days	–	E, G, I, L, B	E: ↓SOD, ↑LPO. Adult: ↑histopathological alterations (cell swelling, hyperplasia, granulomas), ↓SOD; no LPO; ↓GSH.	Li et al. (2009)
nZVI; nFe-oxide	CMC	49.3		1–100	12–14 days	x	W, I	Mortality [Fe(II) > CMC-nZVI > nFe-oxides]; ↓dissolved oxygen level (induced hypoxia); ↑ROS; ↑CAT mRNA expression; no histopathological alteration (I)	Chen et al. (2013)
nZVI; nFe_3O_4	CMC	27.1, 30.1		25–200	7 days	x	E	Accumulation (nFe_3O_4 > nZVI); ↑ROS; ↑developmental abnormalities (nZVI > nFe_3O_4); ↓hatchability; ↑SOD; ↓CAT; ↑GR.	Chen et al. (2013)
Fe_2O_3	Citrate	3.97	*Poecilia reticulata*	0.3	3–21 days	–	Bl	↑DNA damage (3–21 days); ↑ NAs (14–21 days). Genotoxicity and mutagenicity: Long-term exposure > acute exposure.	Qualhato et al. (2017)

[a]*CMC* carboxymethyl cellulose, *DTC* dithiocarbamate, *DMSA* meso-2, 3-di-mercaptosuccinic acid, *SPA* sodium polyaspartate
[b]*Bl* blood, *B* brain, *G* gills, *I* intestine, *K* kidney, *L* liver, *M* muscle, *S* spleen, ∗in vitro test
[c]*GLU* glucose, *GOT* glutamic oxaloacetic transaminase, *GPT* glutamic pyruvic transaminase, *GPx* glutathione peroxidase, *GR* glutathione reductase, *GST* glutathione sulfotransferase, *LDH* lactate dehydrogenase, *LA* lysozyme activity, *MPO* myeloperoxidase, *NAs* nuclear abnormalities, *TGSH* total glutathione

associated to changes in activities of antioxidant enzymes and oxidative damage (i.e., lipid peroxidation and DNA damage) is one of the main modes of action of toxicity of magnetic nanoparticles in fish species (Table 8.2). *Anguilla anguilla* exposed to SiO_2 and dithiocarbamate (DTC)-coated Fe_3O_4 nanoparticles (100 nm; 2.5 mg L^{-1}; 72 h) showed changes in the activity of glutathione reductase, glutathione peroxidases and lipid peroxidation, as well as a synergistic response after co-exposure to Hg (Srikanth et al. 2014). Genotoxic (DNA damage) and mutagenic effects (nuclear abnormalities) were observed in peripheral erythrocytes of the guppy *Poecilia reticulata* exposed to citrate-coated Fe_2O_3 nanoparticles (3.97 nm; 0.3 mg L^{-1}; 3–21 days) (Qualhato et al. 2017) and in the zebrafish *D. rerio* exposed to meso-2, 3-di-mercaptosuccinic acid (DMSA)-coated γ-Fe_2O_3 nanoparticles (5.7 nm; 4.7–74.4 mg L^{-1}; 96 h) (Villacis et al. 2017), indicating that the comet assay associated to micronucleus test and erythrocyte nuclear abnormalities assessment are a suitable approach to detect the clastogenic and aneugenic effects in fish species after exposure to magnetic nanoparticles (Table 8.2).

Similar to magnetic nanoparticles, the most used fish species in ecotoxicological research to assess the effects of quantum dots is *D. rerio*. The biological effects were assessed in different life stages, such as embryos, adults, and *in vitro* (Rocha et al. 2017). Reactive oxygen species production, lipid peroxidation, and changes in gene expression and in antioxidant enzymes activities in fish species exposed to different types of quantum dots were identified. In addition, mollusc bivalve species, namely *Mytilus galloprovincialis* and *Mytilus edulis*, were indicated as a target group for quantum dots ecotoxicity (Canesi and Corsi 2016; Rocha et al. 2015a, 2017). As filter feeders, the mussels take up quantum dots aggregates/agglomerates from seawater, specially by endocytosis and/or phagocytosis in the digestive system, following tissue distribution and metabolism, wherein the digestive gland is the main organ for storage, metabolism, and elimination of quantum dots (Rocha et al. 2015a, b, 2017). Similar mode of action and toxicity of quantum dots in fish were identified in bivalve species, while the mechanism of genotoxicity for both species remains unknown. In addition, it was demonstrated that hard nanomaterials such as quantum dots and iron oxides nanoparticles are toxic at different trophic levels (Rocha et al. 2015a, 2017; Nogueira 2014; Lefevre et al. 2015; Valdiglesias et al. 2016).

Among the important aspects in NanoEcoSafety, the development of nanomaterial safety standards is a priority. In this context, chitosan (polymer derived from chitin by deacetylation) effectively protected the freshwater crustaceans *Ceriodaphnia cornuta* and *Moina micrura* by enhancing the survival rate and repair of lost parts (Vijayakumar et al. 2016). ZnO nanoparticles (40.9 nm; 160 μg L^{-1}) induce 100 and 76% mortality in *C. cornuta* and *M. micrura* neonates, while the co-exposure to chitosan at 100 μg ml^{-1} significantly reduced the mortality of *C. cornuta* (36%) and *M. micrura* (14%) after 24 h of exposure (Vijayakumar et al. 2016), indicating that chitosan decreases the toxicity of metal-based nanoparticles.

The knowledge of the biological effects of nanopharmaceuticals and of the mode of action in aquatic organisms is limited and no standard protocol for ecotoxicological tests exists. In this sense, bioassays or biomarker assessment should focus on

specific mechanisms of nanopharmaceuticals action on nontarget species. The revised data indicate that there is an urgent need to develop guidelines for ecotoxicological test using aquatic species at different trophic levels, as well as the development of new biomarkers by OMICs technologies (e.g., proteomics, transcriptomics, and metabolomics) to assess the impact of nanopharmaceuticals in aquatic organisms.

8.6 Environmental Risk Assessment of Nanopharmaceuticals

Environmental Risk Assessment (ERA) includes four components: hazard identification, toxicity assessment, exposure assessment, and risk characterization. In the European Union, ERA for medicinal products follows the Guideline of the ERA of Medicinal Products for Human Use (EMA 2011) but is only foreseen for medicinal products within the marketing authorization procedure, not including the conformity assessment for medical devices (EU 2001). The assessment is conducted only taking into account the active ingredient (API) but not excipients. Metabolites and transformation products are covered by the "total residue approach" that assumes the same effects for parent compounds, metabolites, and transformation products (EMA 2011). This still leaves excipients, medical devices, and disposal of the medicinal products out of the current ERA.

ERA consists of a 2-tiered approach. In phase I, the environmental concentration of the API present in the water is measured (MEC) or predicted (PEC). To define PEC it is assumed that APIs are taken up by patients, excreted, and end up in urban sewage, which is then un-treated, partially treated or treated in waste water treatment plants and then introduced in the aquatic environment. For the ERA, it is crucial to determine the amount the patient excretes and in which form, because these nanosized compounds form aggregates/agglomerates in water, particularly in seawater (Berkner et al. 2016). Persistent Bioaccumulation and Toxic (PBT) data must be collected to identify the potential toxicity of these compounds. For this purpose, the octanol/water partitioning coefficient (log K_{OW}) has been used as an indicator of possible toxicity. If log K_{OW} is equal to or above 4.5, within an environmental relevant pH range, information on their fate in aquatic and sediment systems and on bioaccumulation and long-term ecotoxicity are required. Nanopharmaceuticals are formed by a core and a coating. Therefore, information based on partitioning coefficient cannot be used to predict the bioaccumulation potential because it may induce an over estimation (OECD 2014). The assessment of the persistence of the compound can be carried out using the criteria as defined under European Regulation on Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) (ECA 2008).

Within ERA, it is assumed that a certain percentage of the population consumes the maximum daily dose of the API. The fraction of inhabitants taking

nanopharmaceutical compounds is either estimated by default (value of 0.01) or based on epidemiological data. The amount of API taken daily is divided by the amount of waste generated (water/inhabitant/day) taking also into account a dilution factor. The obtained PEC is compared with action limit below 10 ng L^{-1}, according to EMA, or to 1 μg L^{-1} according to FDA. If the PEC is below the action limit, there is no risk. However, if PEC is equal or above the established limits, the assessment has to proceed to phase II. Phase II comprises the knowledge of the physicochemical properties of API based on pharmacokinetics (EMA 2011), its fate in the environment, as well as its ecotoxicity in water and sewage sludge. As a result, a non-observed effect concentration (NOEC) is established along with a predicted no-effect concentration (PNEC). To assess if the nanopharmaceutical compound poses a risk to the environment, a risk quotient is calculated based on the ratio between PEC and PNEC. If this ratio is higher than one the compound poses a risk to the aquatic environment and other measures need to be taken to minimize the risk.

Although to date there is no information available on how to conduct an ERA for nanopharmaceuticals, the EMA stated that before marketing a new product, toxicology, and ecotoxicology for a specific nanopharmaceutical need to be assessed (EMA 2006, 2011). For that purpose, the more appropriate methods to assess the fate and toxicology of nanopharmaceuticals need to be established (Berkner et al. 2016). As a prerequisite for a nanopharmaceutical ERA, besides its physicochemical composition, information on size, shape, distribution, morphology, and surface properties (e.g., chemistry, reactivity, surface area) but also aggregation/agglomeration and dissolution behavior need to be taken into account, because normal size ranges are not adapted for nanosized molecules (Gondikas et al. 2012; Tejamaya et al. 2012; Ottofuelling et al. 2011; Misra et al. 2012, Sant'Anna et al. 2013; Rocha et al. 2017). When dissolution of ions occurs, the nano-character of the particles is lost. However, there is a lack of data and some scientific uncertainty (Sant'Anna et al. 2013) regarding all these aspects, namely, what are the particle characteristics that affect toxicity and transport in the different compartments of the environment, their routes of exposure, and the best metric to measure their exposure.

Linkov et al. (2008) proposed that environmental information should be incorporated into engineering nanomaterials and nanomedicine development. In order to avoid the increase on the complexity of the decision, he proposed that this could be achieved combining toxicology, potential health risks, risk assessment modelling, and tools developed in the field of a multi-criteria decision analysis (MCDA). This tool should be used for regulatory decision on nanomaterials and could be used to support the weight-of-evidence approach for evaluating possible health or environmental risks of nanomaterials.

There is a wide variety of nanopharmaceutical compounds, and there is a need to establish guidelines to assess their impact on the marine environment. Therefore, there might be a need to diversify solutions for the correct establishment of an ERA of these compounds.

8.7 Conclusions

There is no doubt that nanostructured systems, more specifically those aimed at health applications for both diagnosis and treatment, represent an important technological advance, bringing many benefits and positive impacts to improve the population quality of life and well-being. However, because these so tiny "entities" are not naturally found in the environment, it is crucial to assess their environmental safety and impact in order to ensure that these nanopharmaceutical compounds do not pose undesirable effects on humans and the environment in the future. For this reason, there is an urgent need to establish appropriate ecotoxicological assays essential for regulatory purposes and environmental and human safety guidelines to protect human health and the environment for the safe use of nanopharmaceutical compounds.

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Chapter 9
Recent Advances on Nanostructured Materials for Drug Delivery and Release

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Abstract Nanomedicine has become a hot field of research, as it has the potential for developing several innovations in healthcare and, in particular, new pharmaceutical formulations. The need of innovative ways for drug transportation and delivery has accelerated the advances in the field of nanomaterials for pharmaceutical applications. The ultimate purpose of designing nanomaterials for drug delivery must be to ensure that the drug to be released exerts its pharmacological effect at the lowest possible dose, with the least number of side effects and equal benefits to a high dose. These so-called "nanopharmaceuticals" may possess distinctive features useful to improve the stability of the drugs, extend their systemic half-lives, enhance efficiency, increase bioavailability, and delay clearance. There is no doubt that nanopharmaceuticals are a promising strategy to overcome traditional pharmacokinetic limitations. Researchers around the world have been making important efforts to design and test novel nanoformulations, especially in *in vitro* and *in vivo* model studies. Virtually, all routes of drug administration have been investigated at this level. Compared to the high number of nanoformulations that are currently in the discovery and preclinical stages of the development pipeline, there are still very few nanopharmaceuticals in clinical trials and even less already in the market. This current scenario points to the need to accelerate nanomedicine endeavors in order to spur these formulations through the drug discovery pipeline.

In this chapter, we will present some of the several opportunities for the design and use of nanomaterials (nanoliposomes, micelles, carbon nanostructures, dendrimers, polymeric, and inorganic nanoparticles) for pharmaceutical formulations. The experimental challenges, associated with moving from bench to bedside, will be addressed, as well as concerns about the precise control of drug release, their biodistribution or fate, and their toxicity, especially when they do not biodegrade. The need to validate and standardize protocols for early detection of toxicity, as well as an in depth understanding of the interaction among nanoparticles and tissues, organs, cells, and biomolecules, will be stated. Finally, the importance of developing a close interaction between scientists, regulators, institutions, and industry in order to help accelerate the efforts in the field will be indicated. The application of several innovative approaches to the design of new nanopharmaceuticals may allow achieving innovation and disruptive advances, providing safe, convenient, and cost-effective drug formulations to patients.

Keywords Nanomaterials · Dendrimers · Polymeric nanoparticles · Inorganic nanoparticles · Micelles · Nanoliposomes · Graphene · Graphene oxide · Carbon nanotubes · Drug delivery · Controlled release · Theranostics · Pharmaceutical formulation · Nanotoxicity

9.1 Introduction

Nanomaterials have dimensional features in the range from 1 to 100 nm (1 nm = 1 × 10^{-9} m). Their sizes are smaller or comparable to those of common biologically relevant cells, bacteria, or virus or even of their molecular components such as proteins, nucleic acids, antibodies, and other important biomolecules. That characteristic may allow them to cross most physical or biological barriers (Fig. 9.1) or to generate different kinds of specific interactions. For example, once a nanomaterial comes in contact with a biological fluid, a nanoparticle-protein complex is formed (the process is known as "protein corona" formation). This complex will determine the fate of the nanomaterial, its systemic circulation, biodistribution, bioavailability, and even its toxicity. Nanomaterials, on the other hand, can be designed to be able to selectively recognize certain cell types or tissues by chemical modification of their surfaces. By selecting the type of nanomaterial and the specific chemical modification of their surfaces, different applications such as new tools for biomedical diagnosis or treatments, fortified foods, water pollution treatment, or advanced materials for textiles or construction can be achieved. In that way, the chemical and physical interactions of a nanomaterial can be finely tuned, selectively controlling how this affects their unique new properties and uses.

Nanotechnology exploits the physical properties of materials in the nanoscale. At that scale, optical, mechanical, magnetic, electronic, and chemical properties suffer drastic changes, making these properties highly attractive to be exploited in innovative technological applications (Halappanavar et al. 2018; Mostafalou et al. 2013). The great interest risen by use of nanomaterials in biomedical applications has originated a new multidisciplinary field called *nanomedicine*, where pharmaceutical nanotechnology is a very recent and attractive branch (Juillerat et al. 2015; Khan

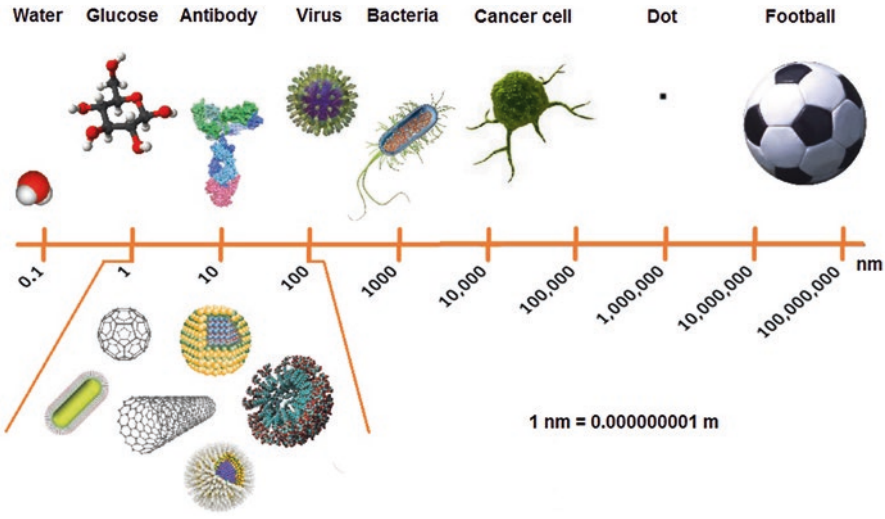

Fig. 9.1 Relative size comparison among common nanomaterials and biologically relevant structures

2012; Chekman 2010). In terms of economic impact, it is expected that nanomedicine will grow in value by about 9% annually; it is a market with a value of nearly 112 billion dollars, as estimated in 2016, and it is predicted to reach nearly 261 billion dollars by 2023 (Grand View Research 2017). In particular, advanced drug delivery systems account for nearly 12 billion dollars (11% of the total), with half of that market consisting of systems for controlled release, liposomal drug delivery, gene therapy, and polymer-based systems, among others. The development of nanostructured materials for pharmaceutical applications ("nanopharmaceuticals") such as controlled drug delivery and release carriers, agents for imaging and diagnosis, as well as innovative therapeutic vehicles, both as active pharmaceutical ingredients (API) and components of the pharmaceutical formulation, is currently a very active field of research (Beyer et al. 2016). Nanopharmaceuticals are a relatively recent class of therapeutic agents containing nanomaterials with unique physical and chemical properties because of their small size. They present new opportunities for the transport and stabilization of poorly soluble or unstable APIs (synthetic drugs, phytochemicals, proteins, nucleic acids, genes, etc.), as well as targeting them towards a specific site of the organism. However, the idea of nanopharmaceuticals must be handled carefully, as there is no consensus in the field. For the purposes of this chapter, a nanopharmaceutical will be considered as a complex system where an API is associated with an excipient (usually, the nanomaterial), as previously indicated. Due to their characteristics, these nanopharmaceuticals may be multifunctional, having better biological tissue distribution and becoming useful to mobilize drugs more easily through different biological barriers. They are interesting, versatile, and potent vehicles for the therapeutic treatment of different diseases (Mendez-Rojas et al. 2014; Sasaki and Akiyoshi 2010). Several of the actual challenges that current pharmaceutical formulations present may be overcome by the use of nanomaterials. So far, several advances and studies have been made with respect to the use of nanotechnology for the treatment and improvement of pharmaceutical formulations that may be useful against several diseases, rising very high expectations concerning positive results and benefits for society. Nanomaterials of different composition (organic, inorganic and composites) such as polymer-based, liposomes, quantum dots, iron oxide nanoparticles, or gold nanoparticles have been developed to provide fast and sensitive detection of disease-related molecular indicators.

We can safely consider that, in the near future, nanomaterials will be found more frequently in novel pharmaceutical formulations as they can bring solutions to the current challenges and limitations found in drug delivery and release systems, offering advantages and opportunities that will finally benefit both patients and the pharmaceutical industry. In order to reach a point where nanotechnology and all the applications mentioned above can be effective and applicable, joint efforts are needed between scientists, clinicians, the pharmaceutical industry, and legislative bodies to successfully implement the design and application of nanosystems in the treatment of several diseases that are currently challenging our health systems worldwide (Luque-Michel et al. 2017).

9.2 Current Approaches to Optimize Drug Deliverability

There are several important challenges in drug delivery and release in today's pharmaceutical industry (Onoue et al. 2014; Chekman 2010). Nanotechnology offers a novel approach for solving these problems, advancing the industry from the model of high sales volume of popular, financially profitable drugs to a more "personalized medicine" model, where attention is given to the individual and not to a general, faceless, complex market. The pharmaceutical industry faces several challenges in order to develop new pharmaceutical formulations. Drug solubility in physiological conditions needs to be improved, while drug release has to be finely controlled. Biocompatibility and safety of the formulation has to be increased by avoiding drug clearance and increasing the plasmatic life of the drug in order to avoid the use of higher therapeutic doses. Drug transport to specific organs, tissues, or systems needs to become specific to avoid secondary effects, optimizing the drug pharmacokinetics and developing the co-delivery of multiple therapeutic drugs against different targets. Finally, real-time monitoring of drug delivery and distribution, as well as effective post-therapy assessment outcomes, to facilitate a faster development of improved APIs with minimal safety concerns has to be considered (Onoue et al. 2014; Chekman 2010).

Nanomaterials may help to solve most of these challenges since efficacy, safety, patient convenience, and compliance are demanding tasks that encourage to seek continuous optimization of drug delivery systems. Today there are very promising and highly complex high-tech APIs (e.g., biotech drugs), for which it would be very disappointing if deficiencies in their formulation and release systems would limit their bioavailability, their arrival at the target site, and their *in vivo* performance in general. Efficient dosage forms depend on a deep understanding of the pathways of the physiologic disposition of a drug since many physicochemical, biopharmaceutic, and pharmacokinetic factors can result in incomplete bioavailability and in the need of using a high amount of the drug thus incrementing costs and side effects.

A drug delivery system can be defined as a formulation or a device that enables the introduction of a pharmaceutical compound in the body and improves its efficacy and safety by controlling the rate, time, and place of its release (Bruschi 2015). Therefore, the route of administration is intimately related to the concept of drug delivery. Based on drug solubility and toxicity, drug delivery systems aim to prolong residence of a drug in biological fluids, to enhance solubility for improving its bioavailability and to ensure targeted action (Demina and Skatkov 2013).

The solubility of an API is relevant to select the right formulation approach and manufacturability and, as mentioned before, is one of the factors governing bioavailability. Thus, in 1995 the US Food and Drug Administration agency initiated the Biopharmaceutics Classification System (BCS) to support the waiving of bioequivalence studies of certain orally administered generic dosage products. The BCS classifies APIs in four classes according to their solubility in aqueous medium and their intestinal permeability properties (WHO 2016). BCS class I drugs (highly soluble, highly permeable) are readily eligible for biowaivers and class IV (poorly

Table 9.1 Examples of drugs of each BCS class

Class I	**Class II**
Enalapril	Carbamazepine
Fluvastatin	Ketoprofen
Metoprolol	Levodopa
Valacyclovir	Verapamil
Class III	**Class IV**
Atenolol	Acyclovir
Cimetidine	Several cephalosporins
Losartan	Furosemide
Ranitidine	Hydrochlorothiazide

Source: Drug Delivery Foundation. Biopharmaceutics Classification System (BCS) database. Copyright 2015-2019. The Drug Delivery Foundation. http://www.ddfint.org/bcs-about. Accessed April 18, 2020

soluble, poorly permeable) are not. Class II drugs (poorly soluble, highly permeable) and class III drugs (highly soluble, poorly permeable) are eligible for biowaivers if they dissolve very rapidly either at pH values typical of the small intestine or under all physiological pH conditions, respectively (Chavda et al. 2010) (See Table 9.1).

The BCS can be usefully applied as a prognostic tool for designing or selecting drug delivery technologies (See Table 9.2). Drug release can be modulated using controlled release technology for class I drugs or increasing dissolution rate for class II drugs. Class III drugs technologies include manipulating the site or rate of exposure or incorporating functional agents into the dosage form to modify the metabolic activity of the enzyme systems. Class IV compounds are generally not suitable for oral drug delivery and are rarely developed to reach the market because of their erratic and poor absorption as well as their inter- and intra-subject variability (Chavda et al. 2010). Certainly, most of the class IV drugs are substrates for P-glycoprotein (resulting in low permeability) and substrates for cytochrome P450 3A4 (prompting to extensive pre-systemic metabolism) which further potentiates the problem of poor therapeutic potential of these drugs (Ghadi and Dand 2017).

The BCS is still evolving since there may be a risk of misclassification of some drugs because it is based on highest dose and on rigid definitions of solubility and permeability (Chavda et al. 2010). Moreover, Daousani and Macheras (2016) correlated the heterogeneous aspects of oral drug absorption with the biopharmaceutic classification of drugs. They found that for class I drugs no time dependency is expected for both absorption and non-absorption processes, while due to the biopharmaceutical properties of class II, III, and IV drugs, these drugs travel throughout the GI tract, and therefore, both absorption and non-absorption processes will exhibit time dependency. Therefore, the BCS is a very useful guiding tool primarily for the development of oral drug delivery formulations and technologies.

Table 9.2 Methods for enhancing deliverability according to BCS class[a]

BCS Class I systems	**BCS Class II systems**
Multiporous oral drug absorption system	Prodrug approach
Single composition osmotic tablet system	pH adjustment
Constant surface area drug delivery shuttle	Use of salts, solvates, and hydrates
Diffusion controlled matrix system	Use of selected polymorphic forms
Delayed pulsatile hydrogel system	Micronization
Dual release drug absorption system	Lyophilized fast-melt systems
Granulated modulating hydrogel system	Surfactants
Intestinal protective drug absorption system	Emulsion or microemulsion systems,
Microparticle drug delivery technology	Solid dispersion
Pelletized pulsatile delivery system	Complexing agent such as cyclodextrins
Bioerodible enhanced oral drug absorption system	Softgel (soft gelatin capsule formulation
Programmable oral drug absorption system	Zer-Os tablet technology (osmotic system)
Spheroidal oral drug absorption system	Triglas and nanosized carriers Such as nanoemulsion, nanosuspension, and nanocrystals
Solubility modulating hydrogel system	**BCS Class IV systems**
Stabilized pellet delivery system	Lipid-based delivery systems
BCS Class III systems	Self microemulsifying drug delivery systems (e.g., Cremophor, Labrafil)
Use of permeation enhancers (e.g., synthetic surfactants, bile salts, fatty acids, and derivatives, chelators, cyclodextrins, and derivatives, mucoadhesive polymers)	Polymer-based nanocarriers
Oral vaccine system	Crystal engineering (nanocrystals and co-crystals)
Gastric retention system	Liquisolid technology
High-frequency capsule	Self-emulsifying solid dispersions
Telemetric capsule	Miscellaneous techniques addressing the P-gp efflux
	Self-nanoemulsifying drug delivery systems

[a]With information from Chavda et al. (2010), Sachan et al. (2009) and Ghadi and Dand (2017)

Recently, the Biopharmaceutics Drug Disposition Classification System (BDDCS) was proposed (Benet 2013). After classifying the drugs of 500 bioequivalence studies according to BCS and BDDCS, Cristofoletti et al. (2013) found that the final outcome of a bioequivalence study is strongly influenced by the solubility of the drug, but not by its intestinal permeability or extent of metabolism. Thus, solubility outweighs any effect of the extent of drug absorption and determines the need for particular drug delivery and release approaches.

As for therapeutic peptides and proteins, their biophysical stability, low bioavailability, and metabolic liability comprise the main challenges to overcome and succeed in oral formulation development and final bioperformance. Particularly, their oral bioavailability is limited by chemical and enzymatic degradation in the gastrointestinal tract, efflux pumps, first-pass gut, and hepatic metabolism, as well as their inability to cross the epithelial barrier of the gastrointestinal tract (Bak et al. 2015). Therapeutic peptides and proteins are usually classified as class III or class IV drugs by the BCS system (e.g., cyclosporine A, an immunosuppressant peptide, as a class IV compound), which means that low permeability is their main biopharmaceutical challenge together with the aforementioned limitations concerning oral drug product development. Considering that, excipients play a pivotal role in formulation development. Peptide stabilizers, pH modifiers, antioxidants, or metal chelators to minimize degradation, peptidase inhibitors, surfactants to better solubilize peptides, and biocompatible mucoadhesive polymers to promote peptide absorption are carefully chosen together with enteric coating approaches and appropriate packaging to preserve the integrity of the molecules and to overstep the oral delivery barriers (Bak et al. 2015).

In some cases, direct structural modifications of therapeutic peptides and proteins are needed. The example of success is represented by cyclosporine A, for which cyclization and therefore its decreased flexibility may confer to this drug a superior absorption after oral administration (Bruno et al. 2013). In addition, the covalent conjugation of polyethylene glycol to therapeutic peptides and proteins, called "pegylation," improves drug delivery by increasing water solubility, enhancing stability, and half-life, reducing immunogenicity and limiting antigenic reactions (Milla et al. 2012). Moreover, the introduction on nonnatural amino acids as in the case of the Hybridtide® technology grants therapeutic peptides and proteins of proteolytic stability and dramatically enhanced half-life, facilitating oral delivery and overall improving pharmacokinetics (Horne et al. 2009). Protein lipidization, vitamin B_{12} conjugation, prodrug synthesis, and locking the conformation of therapeutic peptides and proteins by linking some residues to a synthetic hydrocarbon backbone are other effective strategies to improve stability and oral absorption (Bruno et al. 2013).

Likewise, carrier systems are rapidly evolving in very interesting ways to advance oral deliverability. Some interesting examples are the bilosomes, which are bile salt stabilized delivery nanovesicles that act as very stable penetration enhancers to promote oral bioavailability of large molecular weight proteins and peptides (Ahmad et al. 2017). Orally administered bilosome-based vaccine formulations (e.g., influenza, tetanus, and hepatitis B) represent a major step forward in vaccine technology by preventing antigen degradation and enhancing mucosal penetration (Chilkwar et al. 2015).

IgG antibodies as nanoscale proteins may also act as drug carriers in the so-called antibody-directed enzyme prodrug therapy and antibody-targeted drug conjugates, allowing for targeted drug delivery. Ibritumomab-tiuxetan-90Yttrium, a

B-lymphocyte antigen CD20-directed radiotherapeutic IgG1k (mouse monoclonal aldolase C antibody) indicated for relapsed or refractory, low-grade or follicular B-cell non-Hodgkin lymphoma, is a leading example of this kind of systems (Tridente 2014).

Drug delivery has also been evolving to stimuli-responsive systems that improve bioavailability, reduce the cost of production, and increase patient compliance by allowing pH, temperature, light, ultrasound energy, magnetic or electric fields, swelling processes, or specific chemical agents or enzymes to regulate drug release (Halappanavar et al. 2018; Bajpai et al. 2010). Moreover, according to intended therapy, drug delivery and release is also classified in the following scenarios: rapid therapeutic onset (e.g., for drugs for acute pain or insomnia treatment), multiphasic or fixed-dose combination delivery (e.g., for antihistaminics or antimigraine drugs), delayed or chronotherapeutic onset (e.g., for oral antidiabetics, proton pump inhibitors, or antihypertensive drugs), and maintenance of target exposure (e.g., for some antibiotics or Alzheimer's disease drugs) (Selen et al. 2014). This is known as therapy-driven drug delivery and is also extremely relevant to design and selection of excipients and drug delivery systems. Ultimately, linear, pulsed, or delayed release profiles enabled by the previously mentioned systems and others face always the challenge of being predictable and reproducible as well as allowing for minimum fluctuation in plasma drug levels.

The ultimate purpose of drug delivery strategies must be to ensure that the drug to be released exerts its pharmacological effect and, if possible, that it does so at the lowest possible dose and causing the least amount of adverse effects. Furthermore, patient compliance and treatment cost are also of the utmost importance because if the patient does not adhere to treatment or does not have access to it, all the research behind a medication will be wasted.

9.3 Routes of Administration for Nanopharmaceuticals

Along with the physical-chemical properties of a drug and the dosage form in which that drug is given, the route of administration plays an important role on the rate and extent of systemic drug absorption. Nanoformulations currently available for clinical use are typically administered orally or parenterally by the intravenous, subcutaneous, and intramuscular routes (Table 9.3). Some other administration routes have been meagerly explored by approved nanodrugs, either because of their complexity or because there are few active substances that, based on their pharmacodynamics and indication, require delivery to a very specific site of action. However, among the nanopharmaceuticals that are currently under investigation, many other administration routes are now being examined such as the transdermal, vaginal, pulmonary, and ophthalmic routes.

Table 9.3 Selected commercially available nanopharmaceuticals and their routes of administration (Ventola 2017)

Generic name	Trade name	Nanoformulation features	Route of administration
Morphine sulfate	Avinza	Nanocrystals, provide greater bioavailability	Oral (extended release capsules)
Aprepitant	Emend	Nanocrystals, increased aqueous solubility of the active substance	Oral (capsules)
Megestrol acetate	Megace ES	Nanocrystals, lower dosing needed	Oral (suspension)
Liposomal amphotericin B lipid complex	Abelcet	Liposome nanoparticles, decreased toxicity	Intravenous (infusion)
Liposomal irinotecan	Onivyde	Liposome nanoparticles, protect irinotecan from early conversion to its toxic metabolite	Intravenous
Liposomal verteporfin	Visudyne	Liposome nanoparticles, increased delivery to site of action	Intravenous (plus photodynamic therapy activation)
Glatiramer acetate	Copaxone	Polymer nanoparticles, controlled clearance	Subcutaneous
Leuprolide acetate and polymer	Eligard	Polymer nanoparticles, continuous release, longer circulation time	Subcutaneous
Pegvisomant	Somavert	Polymer nanoparticles, greater stability	Subcutaneous
Iron dextran	Infed	Inorganic nanoparticles, increased dose	Intramuscular
Paliperidone palmitate	Invega Sustenna	Nanocrystals, slow release of low-solubility drug	Intramuscular
Micellar estradiol emulsion	Estrasorb	Micelle nanoparticles, controlled delivery	Topical (on dry skin of both legs)
Triamcinolone acetonide	Zilretta	Polymer nanoparticles, extended release	Intra-articular
Pegaptanib	Macugen	Polymer nanoparticles, greater stability	Intravitreal
Liposomal morphine sulfate	DepoDur	Liposome nanoparticles, extended release	Lumbar epidural
Liposomal cytarabine	DepoCyt	Liposome nanoparticles, increased delivery to tumor site, decreased toxicity	Intrathecal
Poractant alfa	Curosurf	Liposome nanoparticles, increased delivery, decreased toxicity	Intratracheal

9.3.1 Oral Administration

The oral route is widely recognized as the most convenient, noninvasive, safe, conventional, cost-effective, and traditional way to administer drugs. However, it offers many disadvantages that nanocarrier-based formulations may help to solve. Targeted drug delivery is a big challenge when drugs are administered orally; this route

usually requires formulating high amounts of the active substance increasing the production costs. As revised in the previous section, poor water solubility plays a crucial role when trying to improve the oral bioavailability of an API. The oral route is also challenging for APIs labile to gastrointestinal pH, bacteria, and enzymes.

To date, an important amount of knowledge has been gathered regarding the performance of different types of oral nanosystems for drug delivery. It has been reported that positively charged particles are absorbed more efficiently through the gastrointestinal tract as well as small nanoparticles (50–100 nm) which are absorbed in greater proportion than larger ones (500 nm) and distribute better to the kidneys, liver, spleen, lungs, and even brain. It is also known that particulate carrier systems administered orally could also undergo paracellular uptake from the digestive tract into blood circulation as well as the lymphatic system. On the other hand, it has been described that biodegradable (e.g., poly lactic acid) and lipid-based nanoparticles could suffer a significant degradation in gastric and intestinal fluids due to its surface composition (Hamidi et al. 2013). Much of this data has been applied to optimize oral drug delivery.

For example, by reducing the particle size to less than one micron using wet-milling techniques, the NanoCrystal® technology has overcome the solubility problem and allowed for various oral nanomedicines to be prepared and marketed, e.g., fenofibrate tablets (TRICOR®) and megestrol acetate oral suspension (MEGACE® ES) (Agarwal et al. 2018).

As far as lipid-based nanosystems are concerned, they are known to mimic food, improve gut solubilization and mucosal permeation, inhibit intestinal metabolism and/or P-glycoprotein efflux, and improve lymphatic uptake resulting in oral bioavailability augmentation (Borišev et al. 2018). A great challenge is represented by the hydrophilic low-permeability anticancer drug doxorubicin, which exhibits low oral bioavailability due to active efflux from intestinal P-glycoprotein. Thus, its oral administration remains a problem and no oral formulation for doxorubicin is marketed, till date (Ahmad et al. 2018). Attempts to tackle these obstacles were reported by Daeihamed et al. whose doxorubicin-loaded non-PEGylated, 120-nm-sized positively charged rigid liposomes attained a fourfold increase in oral bioavailability in a preclinical study (Daeihamed et al. 2017). Previously, another study in rats demonstrated a 384% enhancement in oral bioavailability compared to solution of a doxorubicin hydrochloride loaded lipid-based nanocarrier (LIPOMER) (Benival and Devarajan 2012).

9.3.2 Parenteral Administration (Intravenous, Intramuscular Subcutaneous)

Low bioavailability can be completely overcome by administering drugs intravenously and to some extent by using the intramuscular or the subcutaneous routes. Invasiveness, safety, and toxicity issues of injections together with pain and patient

compliance concerns reduce as a whole the therapeutic value of parenterally administered drugs for long-term management of certain diseases. Yet, most nanopharmaceuticals already on the market are designed for parenteral administration.

Intravenous administration of nanoparticles allows for intracapillary passage followed by an efficient cellular uptake, also for macrophage endocytosis and for passive drug delivery to inflammatory sites with leaky vasculature (Gelperina et al. 2005). Thus, depending on the desired site of action and the nature of the nanoformulation, this route offers clear advantages.

The antimalarial drug artemisinin, for example, has no intravenous formulation available due to its poor aqueous solubility. Ibrahim et al. reported the preparation of a promising nanoformulation based on biodegradable albumin-bound artemisinin suitable for intravenous injection which enabled direct contact of artemisinin with infected erythrocytes. In *in vitro* experiments as well as in *Plasmodium falciparum*-infected "humanized" mice, the nanoparticles proved to be highly effective (Ibrahim et al. 2015).

In the case of solubility problems of drugs such as paclitaxel, an interesting approach to surpass this limiting factor was its binding with albumin, a natural carrier of endogenous and exogenous molecules. The high-solubility 130 nm albumin-bound particle form of paclitaxel contained in Abraxane® increased drug penetration into the tumor cells after intravenous administration. Unfortunately, P-glycoprotein mediated resistance affecting the antitumoral activity of paclitaxel could not be overcome by the nanoparticle formulation (Zhao et al. 2015).

Intramuscular injection offers the advantages of sustained release and long action; and compared to the intravenous route, it allows relative avoidance of the reticuloendothelial system (RES), the natural particle-removal system of the body (Hamidi et al. 2013). Increased bioavailability, bypassing the intestinal metabolism, and reduced toxic effects are some of the benefits of the intramuscular route that make it very popular. Moreover, intramuscular long-acting formulations provide great opportunities for chronic patients who benefit from once monthly administration or even less frequently by improving adherence, variability in drug exposure and treatment costs.

Recently, Zhou et al. improved the delivery, biodistribution, and viral clearance profiles of the antiretroviral drug cabotegravir by creating its myristoylated prodrug and formulating it into nanoparticles of stable size and shape. The particles exhibited enhanced monocyte-macrophage entry, retention, and RES depot behavior demonstrated *in vitro* as well as in animal models by means of viral restriction evaluations. The nanoformulated prodrug also showed the possibility of extended dosing intervals towards maximizing patient convenience (Zhou et al. 2018).

The subcutaneous route allows good absorption especially for drugs with a low oral bioavailability. Long-acting and targeted drug delivery are favorable outcomes from subcutaneous administration. Depending on size and composition, particles reach the circulation via the lymphatic system. Size plays also an important role when sustained release is the major objective since large nanoparticles persist longer at the injection site (Hamidi et al. 2013). The keratinous subcutaneous layer is the major barrier the nanoparticles encounter upon administration. It is a

hydrophobic and rigid structure, which is difficult for particles to cross unless penetration enhancers (e.g., monoolein) are used to promote drug diffusion and solubility within this layer. Besides, the presence of an immunological barrier made of Langerhans and dendritic cells below the subcutaneous layer requires the use of nanocarrier coating (e.g., with polyethylene glycol) in order to prevent macrophage elimination (Bose et al. 2014). When the drug finally reaches the well-irrigated dermis layer of skin, it can diffuse into the systemic circulation.

An illustrative example of this administration route comes from the application of nanocarriers in traditional herbal medicine. Indeed, the nanonization of phytoceuticals seeks to advance phytotherapeutics by improving their pharmacokinetic and pharmacodynamic profile. Curcumin, a very promising natural anticancer agent, has very poor aqueous solubility and a very limited systemic bioavailability. Thus, Ranjan et al. formulated a prolonged subcutaneous delivery nanosystem that showed improved effectiveness on a non-small cell lung cancer xenograft model (Ranjan et al. 2016).

9.3.3 Transdermal Administration

Unlike topical formulations, transdermal medications are intended to exert clinical effects at distant or deeper tissue sites. Transdermal is a route of administration wherein active ingredients are delivered across the skin in order to reach the dermal layer by means of transcellular, intercellular, or hair follicles pathways for becoming available for systemic absorption via the dermal microcirculation.

The transdermal route requires sufficient lipophilicity of the active substance to be administered even when penetration enhancers or fasteners such as limonene may be added to the formulation to ease the permeation of drugs through the skin barrier. Transdermal delivery systems are useful to achieve controlled release of the drug over long periods while avoiding gastrointestinal effects or first-pass metabolism in the liver for certain drugs if they were administered orally. Moreover, they are noninvasive systems that have better patient compliance and can be easily removed by the patient when necessary (Gönüllü and Şaki 2017).

Skin penetration of large and hydrophilic drugs, or even macromolecules, is limited but nanocarriers have been successful crossing this barrier and even more so when the skin is disrupted, e.g., in diseases like psoriasis and atopic dermatitis. Regarding these two skin diseases in particular, in recent years several murine models have been used to develop and optimize transdermal nanocarrier formulations loaded with drugs like tretinoin, methotrexate, tacrolimus, cyclosporin A, and ketoprofen showing very promising results (Palmer and DeLouise 2016). Also, the treatment of psychiatric disorders can use nanosystems for transdermal administration; Iqbal et al. produced a solid lipid nanoparticle-based formulation for delivery of olanzapine whose favorable performance will allow its inclusion in and production of transdermal patches (Iqbal et al. 2017).

9.3.4 Pulmonary Administration

Pulmonary delivery implies a noninvasive route capable of granting a rapid onset of action of which most important advantages are its large absorptive surface area, its large absorptive mucosal membrane, and its high vascularity. To achieve a sustained therapeutic effect from the pulmonary route of administration, a nanodrug needs to avoid the pulmonary clearance processes mediated by the mucociliary apparatus and the alveolar macrophages. Enzyme degradation is also an obstacle, but metabolizing enzyme activities are limited compared to the gastrointestinal tract and liver. In cases of infections, the delivery systems should resist entrapment and inactivation of drugs by bacterial biofilms. Moreover, if the pharmacological action is meant to take place locally, sufficient lung-tissue retention needs to be guaranteed and systemic absorption minimized in order to avoid rapid elimination of the drug and undesirable systemic effects (El-Sherbiny et al. 2015; Lee et al. 2015).

Particle size, shape, and orientation allow for deposition at the targeted site in the respiratory system and influence avoidance of clearance mechanisms. By attaching or coating the drug with a stealth material, e.g., hyaluronic acid or polyethylene glycol, it is also possible to evade the pulmonary clearance features. Regarding the most promising type of nanosystems, the liposomal-based aerosol formulations have shown to prolong the retention half-life as well as solid lipid nanoparticles, polymeric micelles, and cyclodextrins (El-Sherbiny et al. 2015).

Due to their particle size, inhalable pharmaceutical forms using nanocarriers pose the risk of being exhaled during breathing. Thus, several strategies can help to solve this limitation: nebulization of nanocarriers as a colloidal suspension; mixing nanocarriers along with inert carriers, e.g., lactose and mannitol; or embedding the nanosized system into microparticles (Moreno-Sastre et al. 2015). One example is the suspension consisting of amikacin sulfate encapsulated in liposomes for inhalation (Arikayce™) which maximizes delivery to the lungs due to particle size as well as the antimicrobial efficacy, due to the ability to penetrate and diffuse through sputum into the bacterial biofilm. This formulation also decreases the potential for systemic toxicity. Presently it is undergoing clinical trials and FDA scrutiny (ClinicalTrials.gov 2018a). Also, in the final stretch for approval remains the cisplatin-based formulation named SLIT cisplatin. This formulation was planned for inhalation by patients with relapsed/progressive osteosarcoma metastatic to the lung. The acronym SLIT comes from "sustained release lipid inhalation targeting" and consists of aerosolized cisplatin loaded into lipid vesicles. The goal of this delivery system is to achieve drug accumulation in lungs while reducing exposure to other organs and thus the risk of hemotoxicity, nephrotoxicity, ototoxicity, and neurotoxicity (ClinicalTrials.gov 2018b; Lee et al. 2015).

Certainly, one major disadvantage of nanosystems is their potential toxicity. Most of the nanoparticles for drug delivery are usually made with well-tolerated materials, "generally recognized as safe" (GRAS), aimed to avoid toxic effects (Moreno-Sastre et al. 2015). Nevertheless, it has been reported that nanoparticles get absorbed from the olfactory mucosa into the central nervous system through the

olfactory nerve, which can be considered a good approach to crossing the blood–brain barrier and delivering nanoparticles to the brain but at the same time this could act as a toxic outcome pathway (Hamidi et al. 2013).

9.3.5 Vaginal Administration

The vaginal route offers a high contact surface and a rich blood supply for drug absorption to obtain local (i.e., intravaginally delivery), uterine, or systemic effects (i.e., transvaginally delivery). The vagina is an effective drug administration route for local delivery of microbicide, contraceptive, and anticancer agents. When it comes to systemic active compounds, it represents a noninvasive route that allows for controlled transmucosal delivery. The vaginal route avoids the gastrointestinal environment and the hepatic first-pass metabolism, but the cervicovaginal mucus, the menstrual cycle, the vaginal pH, and its fluids (Leyva-Gómez et al. 2018) challenge the biodistribution and retention of a formulation. Thus, in order to extend their cervicovaginal residence time, nanosystems aimed to deliver drugs via vaginal mucosa must possess surface properties capable of interacting with O-glycosylated macromolecules, the main component of the mucus responsible for mucoadhesion. Polymer-based nanoparticles have the greatest potential for bioadhesion propensity and increased penetration capacity. For example, solid lipid nanoparticles based on polyoxyethylene (40) stearate containing the antifungal drugs ketoconazole and clotrimazole showed, under pH conditions simulating the pathologic environment, potential utility against vaginal infections caused by *Candida albicans* (Cassano et al. 2016). More recently, clotrimazole loaded into poly (d,l-lactide-co-glycolide) nanoparticles with chitosan-modified surface showed enhanced antifungal activity and mucoadhesive properties also for treating vaginal candidiasis (Martínez-Pérez et al. 2018).

9.3.6 Ophthalmic Administration

Nanoparticles have also shown great potential for ophthalmic formulations. The eyes have very poor retention of dosage forms and many anatomical and physiological barriers that cannot be penetrated easily. Low bioavailability, limited dose and volume capacity, and the presence of ocular tissue enzymes and efflux proteins are also concerns when designing ophthalmic products. Improving corneal residence time is one of the main objectives of pharmaceutical nanoformulations since tears, blinking and solution drainage result in loss of therapeutic drug levels on the precorneal surface. For treating some ocular diseases, intravitreal, subretinal, or subconjunctival injections could deliver adequate amounts of drug to the posterior segments of the eye, but this method causes pain and carries bleeding, toxicity, or

infection risks. Thus, topical instillation remains a more convenient alternative (Tahara et al. 2017; Xu et al. 2013).

Liposomes are considered the ideal drug delivery systems because of their biocompatibility and their capacity of enclosing both hydrophilic and hydrophobic drugs. In addition, several studies have proved the efficacy of nanosuspensions for improving the bioavailability of corticosteroids such as dexamethasone and prednisone (Wang et al. 2016). Tahara et al. (2017) developed submicron-sized PLGA nanoparticles loaded with coumarin-6 as a model drug and marker and which surface was modified by chitosan, glycol chitosan, or polysorbate 80. The nanosystem improved the drug delivery efficiency to the retina after administration as topical eye drops to mice. Drug eluting contact lenses are also a good alternative for sustained delivery. A preclinical study with dogs showed that silicone hydrogel vitamin E-loaded contact lenses prolonged the release of the drug timolol and increased its bioavailability with only one-third of the loaded drug compared to eye drops (Xu et al. 2013). Lastly, a research group from the University Eye Hospital Tübingen (Germany) launched a nanocarrier system based on lipid-modified DNA strands that self-assemble into micelles with a hydrophobic core and a hydrophilic corona. The so-called "nano-I-drops" technology can be equipped with different drugs by hybridization with an aptamer. This DNA nanoparticles show excellent adherence to the corneal surface for extended periods reducing the need for frequent application and thereby minimizing side effects (Willem de Vries et al. 2018).

There is no doubt that nanopharmaceuticals are a promising strategy to overcome traditional pharmacokinetic limitations. Researchers around the world have been making countless efforts to design and test novel nanoformulations, especially in *in vitro* and in animal model studies. Virtually, all routes of drug administration have been investigated at this level. However, compared to the high number of nanoformulations that are currently in the discovery and preclinical stages of the development pipeline, there are still very few nanopharmaceuticals in clinical trials and even less already marketed.

9.4 Nanocarriers: Composition, Structure, and Properties of Selected Recent Systems

Pharmaceutical technology allows us to select among several materials for designing the most appropriate pharmaceutical form to prepare a medicine, according with their physical and chemical characteristics and use. Nanocarriers, nanosuspensions, and nanogels are considered as some of the most common systems for the formulation of potentially useful nanopharmaceuticals. Nanosuspensions and nanogels chemical and physical characteristics have been reviewed and discussed previously in the scientific literature (Dhanapal and Ratna 2012; Asadian-Birjand et al. 2012) and won't be further discussed here. Nanocarriers, in the other hand, are colloidal systems with sizes in the range in between 10 and 100 nm; they have been widely

used for diagnostics, treatment, and tracking of biomarkers. There are several types of nanocarriers, most of them designed to contain APIs encapsulated into their structures. Nanocarriers for controlled delivery are usually designed to avoid unintended exposure of the individual to the API, protecting simultaneously from being detected by the host's immune and clearance system. Additionally, surface functionalization of nanocarriers is used to achieve delivery of the drug contents with great specificity (Halappanavar et al. 2018). Direct delivery of drugs into the site of action helps to reduce side effects. This approach is limited to skin, ocular, or mucosal pathologies using drops, creams, lotions, or emulsions. However, tissue or organ selectivity can be achieved with surface modified nanopharmaceuticals (Fig. 9.2).

During the last 5 years, more than 2000 papers were published containing the keywords "nanocarrier," "drug delivery," and "release." The majority (90%) are devoted to micelles, nanoliposomes, niosomes, polymeric nanoparticles, and dendrimers, while the remaining 10% explore the use of single-walled carbon nanotubes (SWCNTs), multi-walled carbon nanotubes (MWCNTs), lipid-core nanoparticles, and inorganic nanoparticles. Tekade et al. (2016) presented the state-of-the-art on the interface of nanotechnology and combination chemotherapy, which has shown a remarkable promise in the therapy of resistant tumors. Anticancer drugs in combination with small interfering RNAs (siRNAs), such as VEGF, XLAP, PGP, MRP-1, BCL-2, and cMyc, are some examples that are mentioned in their article. The siRNAs have shown an immense promise of eliminating drug resistance genes, as well as recovering the sensitivity of tumors resistant to cancer therapy. Following this same line, Jeetah et al. (2014) mentioned different classes of

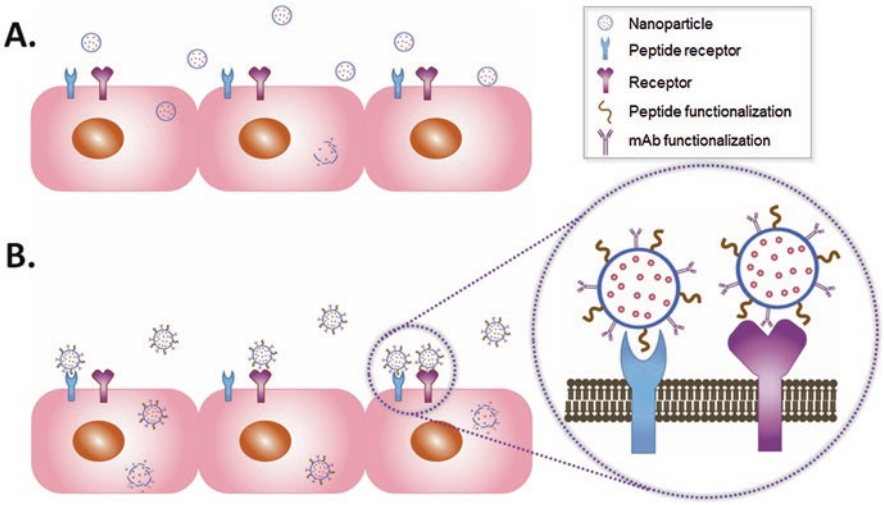

Fig. 9.2 Surface modification of nanocarriers for targeted drug delivery. (**a**) Traditional pharmaceuticals without surface modification. (**b**) Nanopharmaceuticals with surface modification. Diana cells can be targeted with the drug nanocarrier using ligand and receptor interactions like antibody-antigen interaction, allowing a specific drug release

phytochemicals and some of their members that have been encapsulated in nanovehicle systems for chemotherapeutic or chemopreventive properties. They focused mainly on block copolymer nanomicelles, nanoparticles, polymer-drug conjugates, liposomes, and solid lipid nanocarriers. Nearly 20 different phytochemicals were reviewed and the advantages of trapping in nanocarriers were evaluated. Petrenko et al. (2014), also mention that the nanoencapsulation of anticancer drugs improves their therapeutic indexes by virtue of the improved retention and permeation effect, which achieves passive targeting of nanoparticles in tumors. Derived from the aforementioned, we realize that, indeed, the research and use of nanotechnology for the treatment of cancer is taking a primordial focus at present. The controlled release of drugs is another crucial point in the use of nanotechnology in medicine. A drug carrier should ideally be able to deliver drug molecules to the site of action and to interact specifically with target cells. In 2016, Pastorino's research group reviewed different studies where different organic and inorganic nanosystems have been proposed and tested. An interesting technique is the layer-by-layer self-assembly of the nanoengineering shells onto sacrificial templates. Attention has been focused on the possibility of synthesizing calcium carbonate nanoparticles in a very controlled manner, which has opened new perspectives for this type of carrier systems. One issue related with drug delivery is the transfollicular drug delivery. Hansen et al. (2014), reported improved needle-free transcutaneous immunization by means of a more efficient drug supply making use of nanotechnology. Nanotechnology can facilitate transfollicular delivery because the nanoparticles penetrate deeper and to a higher extent into hair follicles than solutions. In addition, nanoencapsulation can stabilize antigens and increase their antigenicity. Therefore, the development of more efficient adjuvant-coupled nanocarriers with high antigen payload is a solution to improve the supply of drugs. Naumenko et al. (2014) described the recent advances in the manufacture and utilization of nanoparticle-labeled cells, showing that one of the most promising techniques is the layer-by-layer polyelectrolyte assembly on cells and intracellular and extracellular labelling with magnetic nanoparticles. Among the applications that stand out include the tissue engineering and tumor therapy, showing that nanotechnology not only has application in transport of drugs but also in different medical therapies.

Pescina et al. (2015) reviewed the literature on the most recent advances on blindness and visual impairment treatment using nanopharmaceuticals. They mention that the nanoencapsulation of peptides and proteins presents a series of advantages for their ocular delivery, since it can protect the drug from metabolic activity, control, and maintain the release and increase the bioavailability of the drug after topical or intravitreal administration. The nanoparticulate formulations contribute to improvements in ocular treatments, it is possible to overcome the ocular barriers, the residence time in the eye is improved, and the local level of the drug is increased. In this case, proteins are also used for the preparation of nanovehicles for ophthalmic administration, so that they have a function as therapeutic agents and in turn as carriers.

It is worth highlighting the current interest regarding DNA research. DNA is also emerging as intelligent material to construct nanovehicles for targeted drug

delivery. Okholm et al. (2016) mention that although the applications of DNA nanostructures are still in the early stages of research, there are great expectations to offer solutions for targeted therapy. With the use of these DNA nanostructures, the selection and crossing of biological barriers will be facilitated. These nanostructures functionalized with molecules such as polymers, proteins, peptides, small molecules, nucleic acids, or lipids have found applications in the study of subdiffraction resolution fluorescence imaging, membrane channeling, enzyme cascades, molecular walkers, plasmonic chirality, and molecular electronics. In addition, the DNA nano vehicles can be designed to function autonomously in the body. With more specific knowledge of the molecular characteristics of diseases and the behavior of nanoparticles *in vivo*, it would be expected that nanoparticles could be customized in the future to provide safe and efficient individualized treatment for patients.

Some recent advances on some of the most common nanostructured materials used as nanocarriers for drug transport and release, such as micelles, nanoliposomes, carbon-based nanostructures (nanotubes, graphene, and graphene oxide), dendrimers, polymeric nanoparticles, and inorganic nanoparticles are discussed in the following paragraphs in order to better understand their potential impact on the development of nanopharmaceuticals.

9.4.1 Polymeric Nanoparticles

Polymeric nanoparticles are spherically shaped particles with a large surface to volume ratio; due to their size, they can biodistribute easily in the organism, making them of interest for biomedical applications as imaging, therapeutic, or diagnostics agents. Drug transport and release can be improved when nanoparticles are used as carriers, as they can cross through biological barriers without problems, decreasing the needed dose for pharmacological action and potential toxicity. Oncological applications are among the most promising fields for lipid nanoparticles (Mostafalou et al. 2013). Usually, polymeric nanoparticles are biocompatible, biodegradable, and nontoxic. Synthetic or natural polymers, shaped as nanocapsules (empty core) or nanospheres (porous structures), have been explored. In nanocapsules, the polymeric membrane surrounds a central cavity where the API is confined, while in nanospheres the drug is dispersed in the polymeric matrix (Fig. 9.3). Some common materials used to prepare them are albumin, chitosan, alginate, poly(lactide-co-glycolide), polylactide, and polyethylene glycol, among several others. Their surfaces can be easily modified and functionalized.

Cancer is one of the health problems that have attracted a larger number of research groups to find potential solutions. Luque-Michel et al. (2017) have recently reviewed the use of polymer-based nanocarriers for cancer therapy. Polymer-based nanocarriers have been used to maximize the effectiveness of cancer treatment and minimize the adverse effects of standard therapy. As chemotherapy may induce undesirable side effects, the development of novel therapeutic formulations that are able to reduce or avoid them is desirable. For example, BH3-mimetic ABT-737 is a

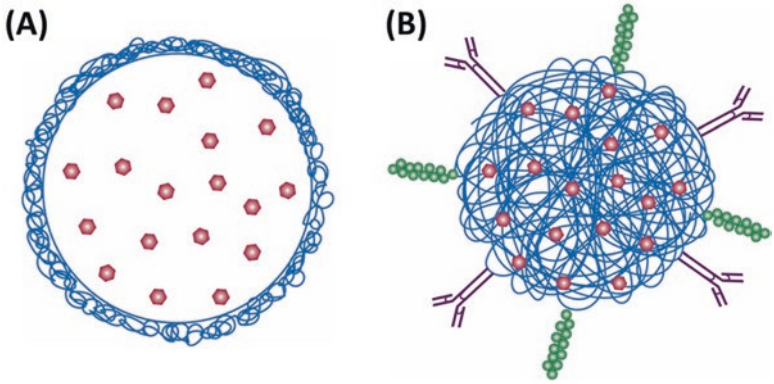

Fig. 9.3 Schematic representation of a drug loaded (left) and surface modified (right) polymeric nanoparticle

chemotherapeutic agent for cancer treatment that induces thrombocytopenia. Schmid et al. (2014) found that this side effect could be reduced through the encapsulation of BH3-mimetic ABT-737 in PEGylated poly(lactide-co-glycolide) nanoparticles. Side effects of camptothecin, another anticancer compound that can cause leukopenia and gastrointestinal toxicity, were decreased when encapsulated in the same system, in contrast with the administration of free camptothecin. When both anticancer compounds, BH3-mimetic ABT-737 and camptothecin, were co-encapsulated in a single polymeric nanoparticle, synergistic induction of apoptosis in both *in vitro* and *in vivo* colorectal cancer models was found, decreasing substantially the undesired effects in the animal model. This successful strategy to decrease toxicity and secondary effects, enhancing the clinical efficacy of synergistic drug combinations may be explored in future nanopharmaceutical formulations in order to tackle that specific challenge. Another challenge where PNPs may find useful application is as stabilizing agent of sensitive, easily degradable, biomolecules with therapeutic action, such as proteins, genes, and nucleic acids. For example, tenfibgen, the carboxy-terminal fibrinogen globe domain of tenascin-C, nanocapsules with sizes under 50 nm were used as nanocarriers to protect DNA/RNA chimeric oligomers for tumor-directed delivery targeting casein kinase 2 (CK2) α-α′ xenograft tumors in mice. Systemic delivery of s50-TGB-RNAi-CK2 specifically targets malignant cells, including tumor cells in the bone, while low doses reduce size and CK2-related signals in orthopedic primary and metastatic xenograft prostate cancer tumors (Trembley et al. 2014; Ahmed et al. 2016). This approach may be used one day for the design of effective and affordable gene therapy.

Several biocompatible and biodegradable biopolymers such as polysaccharides (chitosan, carboxymethylcellulose, starch), poly(lactide-co-glycolide), polycaprolactone, and others have also been explored as building blocks for the design of polymeric nanoparticles. Some of those biopolymers can be pH- or thermally sensitive, allowing activation of drug releasing under specific chemical or physical environments, improving their performance as controlled transport systems and

protecting, at the same time, sensitive molecules with pharmaceutical activity. Bahreini et al. (2014) prepared chitosan-tripolyphosphate nanoparticles loaded with the lyophilized enzyme L-asparaginase II by an ionotropic gelation method; the immobilized enzyme showed an increased *in vitro* half-life and good thermal and pH stability, in comparison to the free enzyme. Loading efficiency was tuned by changing the chitosan and tripolyphosphate concentrations. In other approach, poly(lactide-co-glycolide) or polycaprolactone nanoparticles loaded with a novel antiplatelet N-substituted-phenylamino-5-methyl-1H-1,2,3-triazole-4-carbohydrazide derivative were prepared as a potential promising therapy for the treatment of thrombotic disorders. As current commercial antiplatelet treatments produce undesirable side effects, the use of biocompatible nanocarriers was considered for the design of better therapeutic agents. Their controlled release profile and their *in vitro* and *in vivo* evaluation in a thromboembolism pulmonary animal model was analyzed over 21 days, showing promising activity and low toxicity (Sathler et al. 2014).

Other promising area using polymeric nanoparticles is focused in the treatment of obesity and overweight. Obesity affects, along with overweight, a third of the global population. In recent years, Leptin (Lep), an adipocyte-secreted hormone to control appetite and thermogenesis, has been evaluated combinated with a copolymer Pluronic p85 (Lep@NP85). This conjugate, administered intranasally using the nose-to-brain (INB) route, has shown higher affinity upon binding with the leptin receptor. Many cases of obesity are associated to a leptin resistance. The Lep@Np85 improve not only the accumulation of the leptin as a part of the conjugate in the animal brain, also is observed a significant weight loose. This modified form of leptin show the same activity of the alone hormone after intranasal administration. The LepNP85 with optimized conjugation chemistry is a promising candidate for treatment of obesity (Yuan et al. 2017).

9.4.2 Micelles, Nanoliposomes, and Lipid-Core Nanocapsules

Micelles are spherical structures with sizes usually under 20–50 nm (liposomes are regularly range from 100 nm to 3 μm). They are composed by amphiphilic chains self-assembled in solution as a closed-cage because of polar/nonpolar interactions (Fig. 9.4a, b). Usually, the internal cavity (core) is hydrophobic while the exterior (shell) is hydrophilic (direct micelle), but they can also have a hydrophilic core and a hydrophobic shell (inverse micelle). This duality allows micelles to be applied on the selective solubilization of polar and nonpolar drugs, depending on the administration route. At the internal cavity of direct micelles, small molecules, poorly soluble in water, can be stored, protected, and stabilized by the external layer (Fadeel et al. 2012). These versatile systems can be used for transportation and release of water-insoluble drugs and imaging agents. They are highly stable in physiological conditions, being able to circulate during prolonged times and accumulate in specific target sites when functionalized with appropriate ligands in their surfaces. Nanoliposomes, on the other

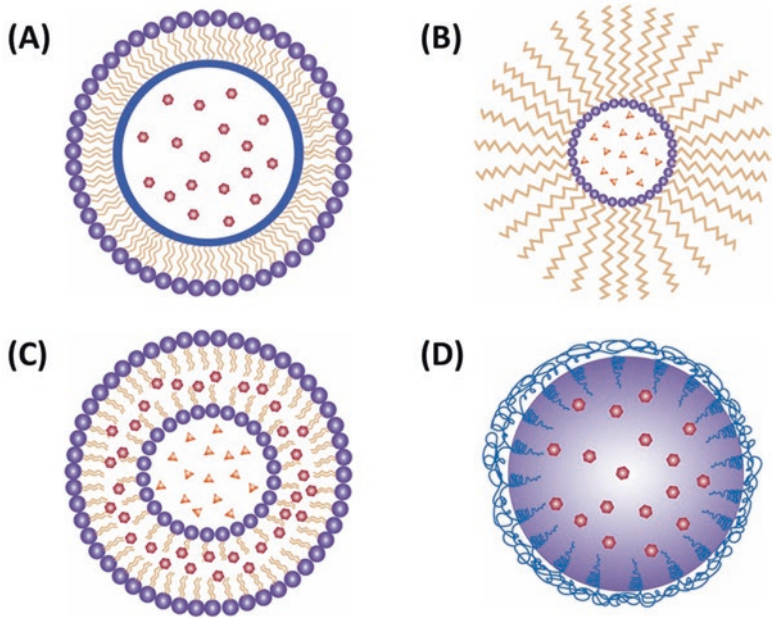

Fig. 9.4 Schematic representation of a: (**a**) direct micelle; (**b**) inverse micelle; (**c**) nanoliposome; (**d**) lipid-core nanocapsule

hand, are nanometric versions of liposomes. Spherical in shape, they can be produced from natural phospholipids, cholesterol, and their derivatives (Fig. 9.4c). Most used systems for encapsulation of APIs and for the design of controlled release systems can be classified, according to the number and size of bilayers as multilamellar and large or small unilamellar vesicles. Size, as well as lipid composition, determines properties such as fluidity, permeability, stability, and structure. There are five types of nanoliposomes, in terms of their composition and intracellular internalization mechanism: conventional, pH-sensitive, cationic, long circulating, and immunoliposomes. When a vesicle is formed from non-ionic surfactants, it is called *noisome*; although their properties are close to those of a liposome, they have larger chemical stability but higher production costs (Chekman 2010). Nanoliposomes have been successfully tested in Food and Drug Administration clinical tests, and some of them have received authorization for developing cosmetics and therapeutic agents as Daunoxome® and Ambisome® for cancer treatment. Several amphiphilic molecules have been used for the formation of stable micelles and nanoliposomes for nanopharmaceutical formulations: polyethylene glycol lipids, pluronic, poly(amino acid)-b-polyethylene glycol (amino acid = glutamic, aspartic), polycaprolactone-b-methoxy-polyethylene glycol, methoxy poly(ethylene glycol)-b-poly(d, l-lactide), chitosan grafted with palmitoyl, and poly(N-isopropylacrylamide)-poly(vinylpyrrolidone)-poly(acrylic acid), among several others (Fig. 9.5).

The fact that micelles and nanoliposomes improve solubility of poorly soluble molecules, as well as protecting encapsulated substances from degradation and

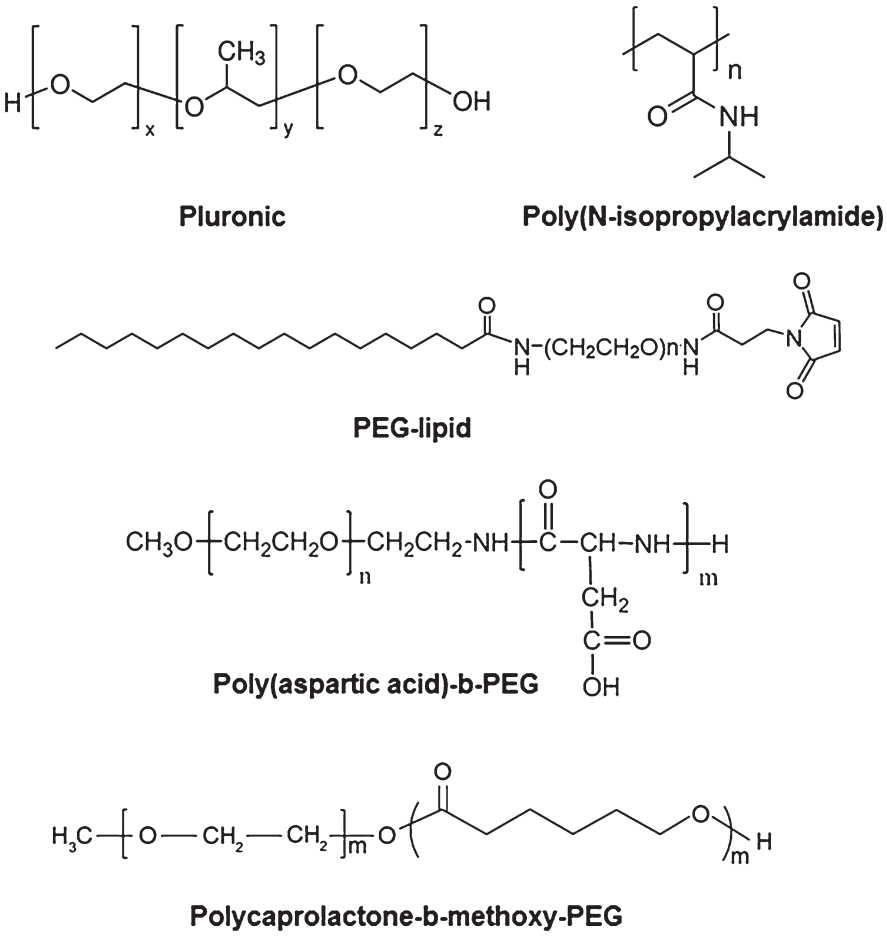

Fig. 9.5 Selected polymers used for micelle formation (*PEG* polyethylene glycol)

clearance, among other properties, makes them attractive for their use as nanocarriers in drug delivery. They have also been explored as efficient vehicles for improving the drug's pharmacokinetics, biodistribution, and cellular uptake, decreasing biodegradation, inadequate tissue distribution, and toxicity. Recently, Haratifar et al. (2014) prepared casein micelles to encapsulate epigallocatechin gallate, the major catechin found in green tea. Epigallocatechin gallate has antiproliferative activity on colon cancer cells, and it was shown that epigallocatechin gallate containing micelles decreased the proliferation of HT-29 cancer cells *in vitro*. These results indicate that protecting epigallocatechin gallate or other sensitive therapeutical molecules in a polymeric matrix may be of utility for the stabilization of sensitive APIs and biomolecules, improving their biodistribution and therapeutic efficiency. For example, the transport and release of antioxidants has an ample market of applications, both for pharmaceutical use as well as for food fortification.

Several potent antioxidants, as well as other useful natural molecules with benefic therapeutic effects, have poor solubility or are unstable at physiological conditions, many of them being prone to enzymatic biodegradation. Nanocarriers may become an alternative to improve both their solubility and stability. Resveratrol and curcumin, two polyphenols well known by their antioxidant properties, were encapsulated in poly(lactide-co-glycolide) lipid-core nanocapsules (Fig. 9.3d). The *in vitro* antioxidant activity against hydroxyl radicals, as well as their antioxidant release profile improved after nanoencapsulation. Co-encapsulation of both antioxidants was also explored, and it is a promising strategy to enhance performance when treating diseases associated with oxidative stress (Coradini et al. 2014, 2015). Docosahexanoic acid, an omega-3 polyunsaturated fatty acid known for its health benefits in the development of infants, has also protective effects against *H. pylori* gastric infection. Docosahexanoic acid was encapsulated in a lipid-core nanocapsule (average size of 302 nm) produced by hot homogenization and ultrasonication using a mixture of commercial surfactants (Precirol ATO5®, Miglyol-812® and Tween 60) and showed inhibition of *H. pylori* growth in vitro (Seabra et al. 2017). Rice bran oil, a natural extract obtained from the hard outer brown layer of rice (rice husk) traditionally used to protect skin from UVB radiation damage as well as for deep-frying cooking. UVB radiation may induce skin damage and cancer, so protection against it may help to prevent these problems. The extract was encapsulated in lipid-core nanocapsules (medium size ~200 nm) and its ability to prevent ear edema induced by UVB irradiation showed a 61% efficiency, reducing at the same time oxidative stress and carcinogenesis response (Rigo et al. 2015). In a similar work, Badea et al. (2015) developed an integrative approach against basal cellular carcinoma. They encapsulated two anticancer drugs (5-fluorouracil, a hydrophilic chemotherapeutic drug and ethylhexyl salicylate, a lipophilic UVB sunscreen agent) in nanostructured lipid carriers made of bioactive squalene (50.8% w/w) obtained from amaranth seed oil, as a chemoprotective agent. The co-loaded nanocapsules (100 nm in diameter) were able to block UVB light efficiently, as well as to scavenge free radicals (70%);*in vitro* drug release showed sustained release of 5-fluorouracil, suggesting this system may become an effective preventive agent against photoaging, skin cancer, and skin damage. Finally, N,O-carboxymethyl chitosan nanoparticles were loaded with 5-fluorouracil and curcumin, and their *in vivo* pharmacokinetics was evaluated. The loaded nanocarriers were blood compatible, releasing the drug over a period of 4 days in a pH range from 4.5 to 7.4 and showing good anticancer effects against colon cancer cells (HT-29) (Anitha et al. 2014).

9.4.3 Carbon Based Nanomaterials

Carbon-based nanomaterials for drug delivery are a rapidly growing field. There are several types of carbon-based nanomaterials. Figure 9.6 shows some of the most representative systems, such as fullerenes, graphene, carbon sponges, nanocones, single-walled carbon nanotubes, and multi-walled carbon nanotubes, among others.

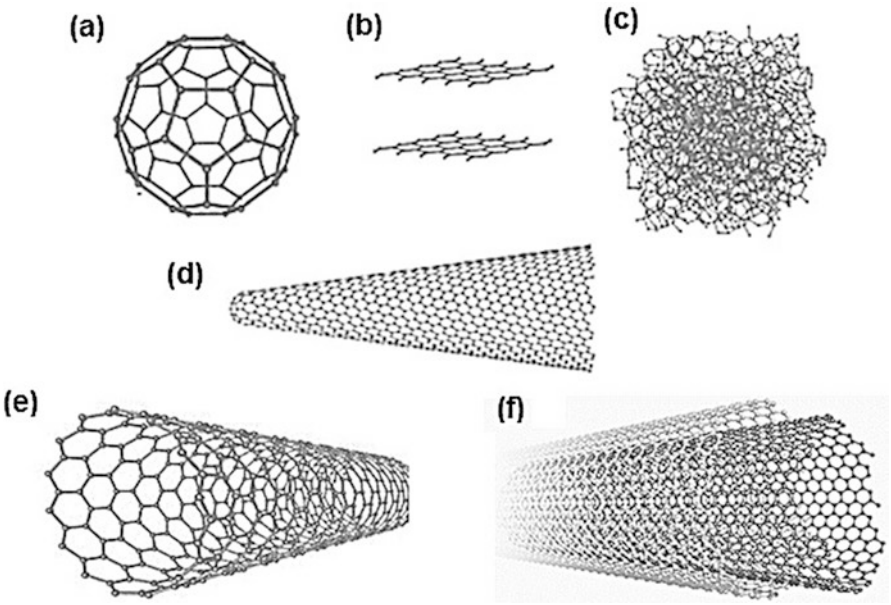

Fig. 9.6 Some selected common nanostructured carbon allotropes: (**a**) fullerene, C_{60}; (**b**) two layers of graphene; (**c**) carbon sponge; (**d**) nanocone; (**e**) single-walled carbon nanotube (SWCNT); (**f**) multi-walled carbon nanotube (MWCNT)

Carbon nanotubes are usually formed by hexagonal open or closed networks of carbon atoms, sometimes presenting different kinds of defects (substitutional or geometrical) and may have diameters from 1 nm (single-walled carbon nanotubes) to several hundreds of nanometers (multi-walled carbon nanotubes) and lengths from 1 nm to several micrometers. Carbon nanotubes have been extensively studied for clinical use, as they are able to penetrate easily the cell membrane, carrying APIs. Due to their high aspect ratio, they have a large capacity for storage inside several small molecules, and their surfaces can be easily modified to improve their specificity, biodistribution, and biocompatibility. Their low solubility in aqueous systems is a problem, being prone to agglomeration. Multi-walled carbon nanotubes are potentially toxic after modifying their solubility, as they become more bioavailable (Hilder and Hill 2007).

The use of graphene-based nanocarriers for drug delivery applications has been recently reviewed and discussed (Liu et al. 2013). The easiness for chemical modification of their surfaces to tune their biocompatibility and toxicity or controlling releasing mechanisms (pH-sensitive, thermal, photo- and magnetic induction) opens numerous possibilities for the development of efficient therapeutic and diagnostic systems. For example, dopamine conjugated graphene oxide nanoparticles were recently prepared and used as nanocarriers for cellular delivery of the anticancer drug methotrexate. The loaded nanocarriers were tested in a human breast adenocarcinoma cell line showing significant antitumor activity and improving drug

delivery. The design of novel nanocarrier conjugates is then a promising field of research for the development of useful therapeutical agents (Masoudipour et al. 2017). In other work, a conjugated graphene oxide-gallic acid drug-delivering system was recently developed by Dorniani et al. (2016) and characterized by several analytical techniques (X-ray diffraction, Fourier transform infrared spectroscopy, high-resolution transmission electron microscopy, Raman and Ultraviolet/Visible spectroscopy). The nano-conjugate was able to release gallic acid in phosphate buffer system at pH 7.4 in a sustainable way; *in vitro* evaluation against normal fibroblast (3 T3) and liver cancer cells (HepG2) showed good inhibitory effect on cancer cells without affecting normal cell growth.

Carbon nanotubes have emerged as an exciting alternative for transporting therapeutical molecules. Functionalization of carbon nanotubes decreases toxicity and immunogenic response, displaying a promising potential to become platforms for drug delivery of peptides, proteins, nucleic acids, and drugs. They can be used as components in multifunctional composites for use as theranostic agents. For example, a composite of multi-walled carbon nanotubes and cobalt ferrite nanoparticles has been designed for use as a MRI contrast agent. Coating the multi-walled carbon nanotube@$CoFe_2O_4$ nanocomposite with mesoporous silica resulted in increased biocompatibility and loading efficiency. When loaded with doxorubicin, the nanocomposite showed good pH-responsive drug release within 48 h (Fan et al. 2017). Molecules with low solubility such as curcumin, a potent antioxidant that protects against oxidative stress-related injuries and anticancer activity, have been also loaded in carbon nanocarriers. Multi-walled carbon nanotubes functionalized with polyvinyl alcohol and loaded with curcumin were evaluated *in vitro* showing good release performance at physiological pH (7.4–5.5); at low pH values, release increased (25–30%) than at higher pH values (Zawawi et al. 2017).

9.4.4 Inorganic Nanoparticles

Numerous nanomaterials with different chemical compositions (magnetite, Fe_3O_4; silica, SiO_2; zinc oxide, ZnO; zerovalent metals such as Ag, Au, Pt; CdS and ZnSe quantum dots), which may present an ample variety of shapes (rods, wires, tubes, particles, sheets) and structures (core-shell, multilayered, organically/inorganically coated, hollow o porous, among others), have been explored as drug delivery systems (Fig. 9.7). Coupling of APIs on the surface of inorganic nanoparticles (surface functionalization) changes the stability of the nanomaterial, as well as its biocompatibility. Inorganic nanomaterials can be easily chemically modified on their surfaces, in order to achieve more stable systems, with increased half-life, to be exploited as drug delivery and controlled release systems (Vargas-Gonzalez et al. 2016). They are easy to modify in their surfaces and have been explored for drug delivery, imaging, diagnosis, etc.

Metallic and metal oxide nanoparticles can act as drug nanocarriers or also as antimicrobial agents themselves. Aside from their microbicide activity, inorganic

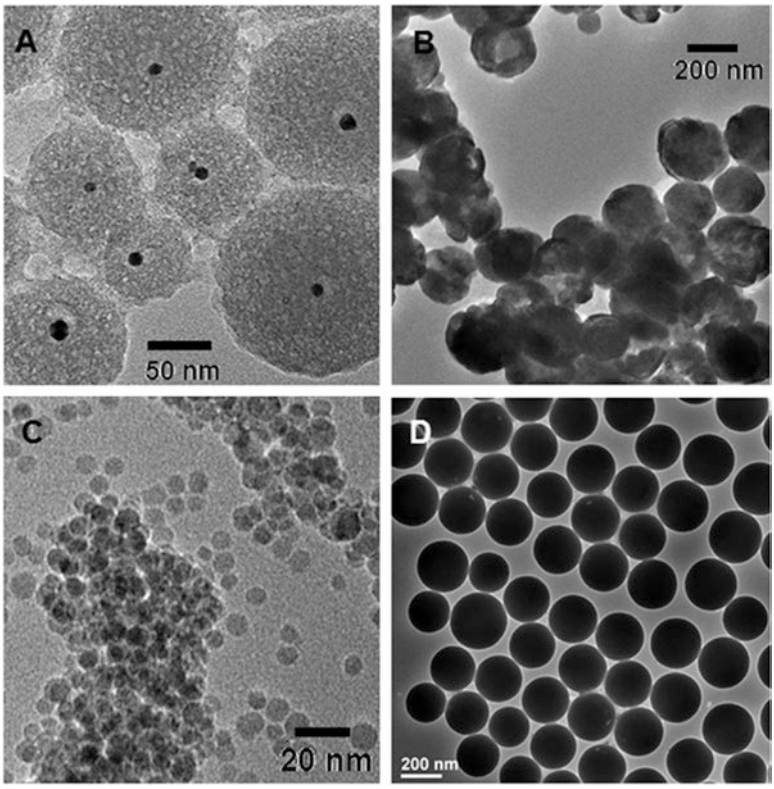

Fig. 9.7 TEM images of (**a**) mesoporous Au@SiO_2 nanoparticles, (**b**) magnetic hollow nanoparticles, (**c**) Fe_3O_4 superparamagnetic nanoparticles, (**d**) SiO_2 spherical nanoparticles (Source: author's laboratory)

nanoparticles may possess interesting physical properties such as magnetism, catalytic activity, redox active behavior, and fluorescence, among others. These properties make them useful for the design of multifunctional nanocarriers, with great potential for theranostic applications. The use of metal-based nanomaterials as antimicrobials, as well as the mechanisms of action, has been discussed by Raghunath and Perumal (2017). In particular, the toxicity of silver nanoparticles in biological systems has been explored by several research groups, and it is a very active field; the antimicrobial activity of silver nanoparticles and its potential use for the design of novel nano-antibiotics has been recently reviewed (Vazquez-Muñoz et al. 2017). For example, Marslin et al. (2015) used extracts of *Withania somnifera* to reduce $AgNO_3$ and prepare a cream formulation containing silver nanoparticles with antimicrobial activity; the cream was reported to be effective against *S. aureus*, *P. aeruginosa*, *P. vulgaris*, *E. coli*, and *C. albicans.* This formulation may be an alternative to the use of conventional antibiotics or for the treatment of antibiotic-resistant pathogens (Marslin et al. 2015). Other metal oxides have been used as support for

silver nanoparticles immobilization. Recently, hollow TiO_2-coated CeO_2 nanocarriers were prepared and loaded with silver nanoparticles and their Ag^+ ion releasing performance evaluated. These systems showed excellent antibacterial activity against *E. coli*, although they were also cytotoxic against a model epithelial barrier cell type (A549 cells) (Gagnon et al. 2016). Further research on the use of silver nanoparticles is necessary in order to avoid toxicological effects that may affect their antibacterial performance.

Mesoporous materials, such as hollow nanoparticles, have been explored as alternatives to carry different kind of biologically active molecules in their inner space, becoming great choices for drug transport and delivery. Further modification of the surface of nanomaterials, both to enhance molecular recognition of specific targets or to attach pro-drugs that can be carried until the right conditions (pH, enzymatic activity) break the bond, releasing the active principle, is an active field of research. Recently, the antitumor performance of ZnO hollow nanocarriers containing the anticancer drug PTX, against breast cancer in an animal model was reported (Puvvada et al. 2015). The surface of the hollow ZnO nanoparticles was modified with folate groups, improving their uptake by breast malignant cells; a drug release efficiency of 75% within 6 h in the characteristic low, acidic, pH of the tumor microenvironment was determined. Fluorescence of the nanocarrier increased because of drug release, becoming thus a useful way to evaluate the nanocarrier's performance. This dual, pH-sensitive, and fluorescent nanocarrier may be useful for improving chemotherapy tolerance and anticancer efficiency and to develop flexible theranostic tools for both diagnostics and anticancer therapy. The development of multifunctional inorganic-organic, hybrid, nanocarriers is a very exciting field. Landarani-Isfahani et al. (2017) reported the development of magnetic nanoparticles conjugated with G2 triazine dendrimers (Fe_3O_4@SiO_2/G2), loaded with methotrexate, that are pH-responsive; the chemotherapeutic hybrid nanocarrier was tested against in vitro using MCF-7, HeLa, and Caov-4 cell lines, showing good cytotoxicity. These nanocomposites were biocompatible and degradable as indicated by blood safety analyses and could be used as effective drug carriers for anticancer applications. In other work where dendrimers and inorganic nanoparticles were mixed, a system consisting of polyamidoamine dendrimers conjugated with magnetic nanoparticles was prepared and characterized; the nanocomposite performance as a stimuli-responsive drug carrier for thermally activated chemotherapy of cancer was evaluated (Nigam and Bahadur 2017). When alternating current magnetic fields were applied to the doxorubicin-loaded formulation, a synergistic effect on the inhibition of cervical cancer cell growth was found. These novel hybrid systems may be of interest for the development of innovative combinatorial therapeutic agents.

Finally, innovative ideas on the design of inorganic nanocarriers conjugated with bioactive molecules, such as enzymes or proteins with antiviral or anticancer activity, have been explored. First, a tyrosine kinase conjugate with gold nanorods was prepared by Liu et al. (2017) and evaluated as potential platforms for targeted drug delivery and photothermal tumor ablation. In the absence of laser irradiation, moderate necrosis of human metastatic renal carcinoma cells in a nude mice model was

observed; however, under irradiation, both with or without gold nanorods, tumor necrosis improved, although irradiation in the presence of gold nanorods showed a synergistic complete tumor necrosis. Finally, momodicas anti-HIV protein, a 30 kDa single-stranded, type-I ribosome inactivating protein with antitumor and anti-HIV activities was encapsulated in zirconium egg- and soy-phosphatidylcholines nanoparticles; the nanocarriers were characterized by transmission electron microscopy and X-ray diffraction. The obtained nanoformulation showed positive results in antimicrobial and anti-HIV assays, with low toxicity and good first order releasing kinetics (Caizhen et al. 2015).

9.4.5 Dendrimers

Dendrimers are hyperbranched, tree-like structured polymers, of large size and complexity, but with a well-defined chemical structure (Fig. 9.8). Dendrimers grow branches from a central core. Their usual size does not exceed 15 nm, having a relatively dense surface with an almost empty core, having also very low polydispersity index, high bio-permeability and biocompatibility. These large molecules present numerous internal voids and channels that can be used to trap host molecules (Onoue et al. 2014). They can be used to improve solubility of APIs and have been explored in the formulation of several controlled release systems. Dendrimers can

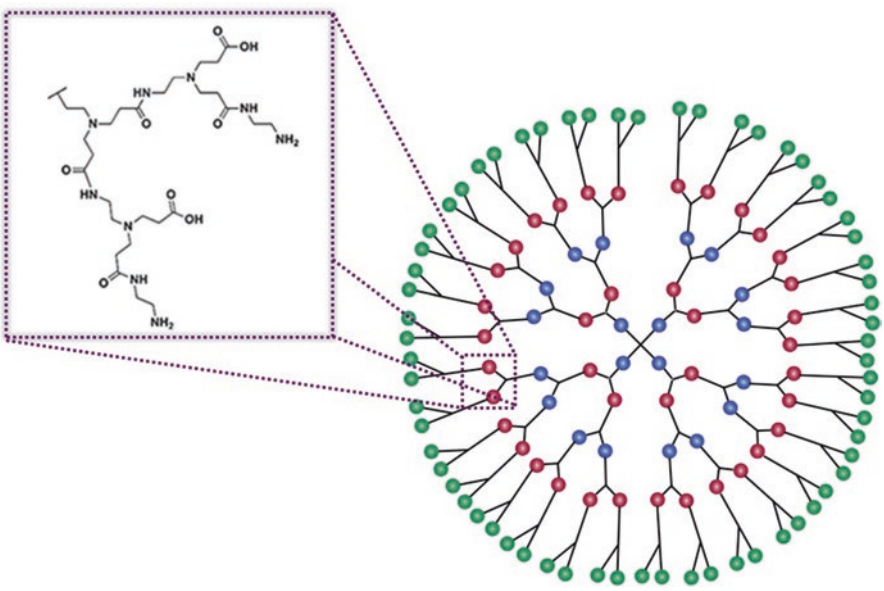

Fig. 9.8 Schematic representation of a dendrimeric structure. Inset shows a representative molecular fragment with typical chemical groups

be designed for multiple drug target-specific controlled release, but there are several concerns related to their toxicity profile. According to the nature of their chemical components, there are several types of dendrimers: polyamidoamine, poly(glycerol), melamine, triazine, polyethylene glycol, carbohydrate, or citric acid derivatives, among several others.

Sundry reviews on the use of dendrimers as drug carriers have been published recently (Elkin et al. 2017; Viswanath and Santhakumar 2017; Sheikhpour et al. 2017). Dendrimers containing terminal amine groups are pH-stimuli-responsive, becoming useful for controlled release of drugs. There are several examples of these nanosized highly branched fractal-like macromolecules, both as pure dendrimers or as in combination with other nanomaterials. As dendrimers can be designed to overcome limitations that most common drugs present such as low solubility, stability, biodistribution, or specificity, they can be tuned to be able to reach specific targets, to avoid immune clearance, and to present reduced toxicity. Dendrimers can enter into the cells through phagocytosis or endocytosis, improving the therapeutic efficiency. The use of dendrimers containing biomolecules, such as amino acids, peptides, or antibodies is an active field of research as these derivatives may be highly effective to recognize specific targets. For example, Kim et al. (2017) prepared a four-branched arginine-glycine-aspartic acid tripeptide (RGD) dendrimer, bound to polyethylenimine-grafted chitosan containing a targeted gene for alpha-beta-integrin. The dendrimer was capable to inhibit the growth of a solid tumor in vivo in a mouse xenograft model. When mixed with other nanomaterials, multifunctional dendrimer-containing nanocarriers can be obtained. A multifunctional dendrimer conjugated to gold nanoparticles and loaded with doxorubicin was designed as a novel nano-platform for pH triggered doxorubicin intracellular delivery. Exploiting the luminescent properties of gold nanoparticles, cell internalization, and doxorubicin release was monitored using confocal laser scanning microscopy, and *in vitro* studies showed increased cytotoxic effect. This development could lead to the design of a promising nanocarrier for imaging the intracellular transport of several anticancer drugs (Khutale and Casey 2017). In other similar work, the utility of multifunctional dendrimers to serve as molecular theranostic agents was explored where an anionic linear globular dendrimer G2 was conjugated with an AS1411 aptamer to target human breast cancer cells (MCF-7) and deliver iohexol. The nanoconjugated toxicity on nucleolin-positive MCF-7 cells and nucleolin-negative HEK-293 cells was assessed by the 2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide cell viability and apoptosis/necrosis assays, and *in vivo* computerized tomography imaging, showing promising effects after reducing the number of cancer cells (Mohammadzadeh et al. 2017).

The good transfection efficiency and low toxicity of dendrimers make them promising for gene therapy and as nuclei acids carriers. Askarian et al. (2017) reported the preparation and characterization of polyamidoamine-pullulan conjugate nanoparticles with sizes in the range from 118 to 194 nm. These systems showed good efficiency as transfection agents in HepG2 (receptor-positive) and N2A (receptor-negative) cell lines, improving delivery of nucleic acids into the liver cells expressing asialoglycoprotein receptor with minimal transfection in

nontargeted cells. In another work, Lee et al. (2017) recently reported a similar system, based on a G4-polyamidoamine dendrimer containing the cathepsin B-enzyme-sensitive sequence (glycine-phenylalanine-leucineglycine, GFLG), which presented excellent transfection efficiency and low cytotoxicity in HeLa cells. These dendrimer nanocarriers, with controllable sizes and architectures, low toxicity, and improved targeting properties, may become one day an efficient nanocarrier for gene therapy, among other applications.

Table 9.4 summarizes some of the selected examples of nanocarriers recently reported.

In summary, there are several opportunities on the design and use of nanostructured materials for drug delivery and release. These nanomaterials may help to overcome several of the already identified challenges associated with traditional pharmaceutical formulations such as specificity, controlled release under specific conditions or external stimuli, stabilization of unstable drugs or biomolecules, and theranostic multifunctionality (e.g., imaging + diagnosis + drug transport), among several others. Polymeric nanoparticles rise as some of the most studied systems, but others such as dendrimers, carbon-based, and inorganic nanoparticles are also becoming prominent and their unique physical properties make them very promising.

9.5 Challenges Associated with the Use of Nanomaterials in Pharmaceutical Formulations

As previously discussed, the unique physical characteristics of nanostructured materials make them very attractive for use as components in the development of new pharmaceutical formulations. However, practical and commercial applications should consider that chemical composition, including purity, crystallinity, and physical properties of the components, as well as their reduced dimensions that affect directly the effective surface area, may affect not only their solubility but also their chemical reactivity. Furthermore, the role of the surfactant agents (organic, inorganic, or composite) as well as that of the chemical functions present on the nanomaterial's surface may play a decisive role not only in their stability in solution but also in the biocompatibility and biodistribution (Fig. 9.9). Chemical or physical interactions among nanostructured carriers and the physiological media components (proteins, sugars, ions) will also affect the stability of the nanopharmaceutical, as well as its drug delivery/release kinetics. In biological systems, these properties have a big impact on pharmacokinetics and toxicity, as they affect directly the nanocarriers' biodistribution and effective internalization in cells and tissues (Halappanavar et al. 2018; Juillerat et al. 2015; Gracssian 2008).

Chemical modification of the nanocarriers' surface may be used as a way to increase stability, solubility, or biocompatibility, decreasing the probability of clearing by the reticuloendothelial system. However, physical and chemical degradation of the surfactants may generate reactive oxygen species or yield other toxic

Table 9.4 Selected examples of recently developed nanocarriers

Nanocarrier	Challenge	Solution	References
Polymeric nanoparticles	To reduce induced thrombocytopenia, leukopenia and gastrointestinal toxicity in the pharmaceutical formulation	Small molecule B-cell lymphoma 2 (Bcl-2) homology 3 (BH3-mimetic ABT-737) and camptothecin, with potential pro-apoptotic and antineoplastic activities. Co-encapsulated in PEGylated poly(lactide-co-glycolide) nanocapsules	Schmid et al. (2014)
Polymeric nanocapsules	Protection of DNA/RNA oligomers from degradation	Tenfibgen nanocapsules with sizes under 50 nm for direct delivering to CK2 a-a'-xenograft tumors in mice	Trembley et al. (2014) and Ahmed et al. (2016)
Polymeric nanoparticles	Controlled release of an API for a long period	Poly(lactide-co-glycolide) or polycaprolactone nanoparticles loaded with a antiplatelet N-substituted-phenylamino-5-methyl-1H-1,2,3-triazole-4-carbohydrazide derivative	Sathler et al. (2014)
Polymeric nanoparticles	Thermal and pH stabilization of enzymes for pharmacological use	Chitosan-tripolyphosphate nanoparticles containing L-asparaginase II	Bahreini et al. (2014)
Micelles	Improve solubility of poorly soluble molecules and protect from degradation and clearance	Casein micelles loaded with epigallocatechin gallate, a compound with antiproliferative activity against colon cancer cells	Haratifar et al. (2014)
Lipid-core Nanocapsules	Stabilization of antioxidants for food fortification, improving drug release	Poly(lactide-co-glycolide) nanocapsules containing resveratrol and curcumin	Coradini et al. (2014, 2015)
Lipid-core Nanocapsules	Protection and stabilization of drugs in acidic environments (gastric infections)	Nanocapsules of Precirol ATO5®, Miglyol-812® and Tween 60 loaded with docosahexaenoic acid for *H. pylori* growth inhibition	Seabra et al. (2017)
Lipid-core Nanocapsules	Encapsulation and stabilization of UVB radiation protective agents	Polycaprolactone and sorbitan monostearate as solid lipid-core nanocapsules loaded with rice bran oil	Rigo et al. (2015)

(continued)

Table 9.4 (continued)

Nanocarrier	Challenge	Solution	References
Lipid-core Nanocapsules	Controlled release of lipophilic chemotherapeutic drugs and UVB protective agents	Squalene nanocapsules loaded with 5-fluoroacyl and ethylhexyl salicylate	Anitha et al. (2014)
Graphene oxide	Drug transport and release of anticancer drugs	Graphene oxide-dopamine conjugate loaded with MTX to treat adenocarcinoma tumors; graphene oxide-gallic acid delivering system for growth inhibition of liver cancer cells (HepG2) cells.	Masoudipour et al. (2017) and Dorniani et al. (2016)
Multi-walled carbon nanotube	pH-responsive drug delivery and imaging agent	Multi-walled carbon nanotube/$CoFe_2O_4$@SiO_2 nanocomposite loaded with DOX	Fan et al. (2017)
Multi-walled carbon nanotube	Transport of poorly soluble free radical protective drugs and pH controlled release	Multi-walled carbon nanotubes functionalized with polyvinyl alcohol loaded with curcumin	Zawawi et al. (2017)
Silver nanoparticles	Alternatives to conventional antibiotics against antibiotic-resistant strains	Cream formulation containing silver nanoparticles; TiO_2-coated CeO_2 nanoparticles loaded with silver nanoparticles	Marslin et al. (2015) and Gagnon et al. (2016)
ZnO	Targeted delivering, imaging and controlled release of anticancer drugs	Hollow, fluorescent, nanoparticles loaded with paclitaxel	Puvvada et al. (2015)
Au nanorods	Targeted drug delivering, and photothermal tumor ablation	Gold nanorods conjugated with tyrosine kinase	Liu et al. (2017)
Zirconium phosphatidylcholine	Protection of anti-HIV protein from degradation and lowering of toxicity	Nanocapsules loaded with momodicas anti-HIV protein with antimicrobial and anti-HIV activity.	Caizhen et al. (2015)
Fe_3O_4@SiO_2/ dendrimer	pH-responsive drug delivery and imaging system	G2 triazine dendrimer modified with biocompatible magnetic nanoparticles loaded with methotrexate	Nigam and Bahadur (2017)

(continued)

Table 9.4 (continued)

Nanocarrier	Challenge	Solution	References
Dendrimer	Targeted drug delivering for cancer treatment	Four-branched arginine-glycine-aspartic acid tripeptide (RGD) dendrimer functionalized with polyethylenimine-grafted chitosan loaded with α−β-integrin	Kim et al. (2017)
Dendrimer	pH-responsive drug delivery and imaging system	Au nanoparticle dendrimer (Au-polyethyelene glycol-polyamidoamine) loaded with doxorubicin	Khutale and Casey (2017)
Dendrimer	Computerized tomography imaging and anticancer targeted drug-delivering system	G2 linear globular dendrimer conjugated with an AS1411 aptamer loaded with iohexol	Mohammadzadeh et al. (2017)
Dendrimer	Gene and nucleic acids carriers for gene therapy with low toxicity	Polyamidoamine-pullulan conjugates loaded with DNA and G4-PAMAM dendrimer-containing cathepsin B-enzyme-sensitive sequence (Gly-Phen-Leu-Gly)	Askarian et al. (2017) and Lee et al. (2017)

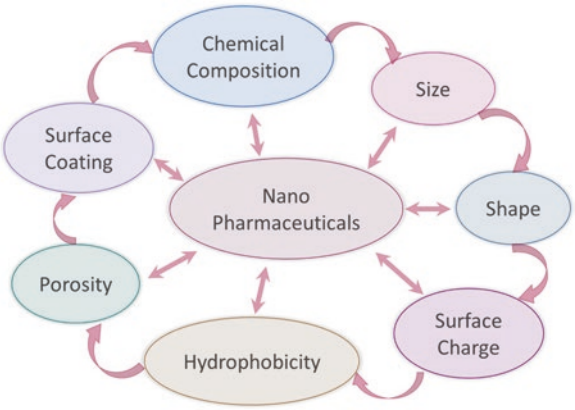

Fig. 9.9 Physical and chemical factors that affect the stability, biocompatibility, and toxicology of nanocarriers used in pharmaceutical formulations

derivatives that may negatively affect the organism. Furthermore, the interaction of the nanomaterials with biomolecules present in the physiological medium may result in the formation of a biological corona on its surfaces that may drastically affect their properties; these properties may be completely different to those of the original nanomaterial, changing their bioavailability, toxicity, or reactivity (Halappanavar et al. 2018; Mahmoudi et al. 2011).

Undesirable adverse reactions to some components of nanopharmaceuticals could become a potential problem, limiting their functionality. Moreover, the large

reactivity derived from the aspect ratio, may be responsible of some observed adverse responses. However, as nanopharmaceuticals can be administered in low doses, these adverse reactions may also decrease. A new discipline called nanopharmacovigilance must be aware of these potential hazards in order to be able to assess the risk of nanotechnology applied to drug development and thus to design proper risk minimization plans for nanomedicines to intervene in a timely manner (Salas-Rojas et al. 2017).

Some recent reports on cytotoxicity, DNA damage, inflammatory responses, and generation of reactive oxygen species, among other adverse reactions have been observed (Holden et al. 2013; Fadeel et al. 2012). The toxicity of nanomaterials (also known recently as nanotoxicity) is an active research field that deals with the possible toxic effects on health of nanomaterials, when they are in contact with human or other living organisms (Halappanavar et al. 2018; Oberdörster et al. 2005). Although currently there is no consensus on the safety or toxicity of several commonly used nanomaterials, it is probable that their unique surface properties derived of their small size may result in adverse effects. However, more research in this field is required in order to better understand the risks and impacts of the use of nanomaterials in pharmaceutical formulations (Saiyed et al. 2011).

To date, very few nanopharmaceuticals are reaching the final phases of development of new medicines and approval for commercialization. Researchers agree that in order to move from the bench to the bedside, several experimental challenges need to be addressed. There is still concern about the precise control of drug release of nanoformulations, about their biodistribution or their fate, especially when they do not biodegrade, and, of course, their toxicity. Thus, there is consensus regarding the need of validated and standardized protocols for early detection of toxicity and for nanoparticle characterization using *in vitro* assays and appropriate animal models of disease (Mendez-Rojas et al. 2016). *In vivo* studies need to go in depth into the understanding of how nanoparticles interact with target organs, tissues, cells, and intracellular molecules, to what extent they remain stable and what is their potential to accumulate (Hua et al. 2018; Ventola 2017; Min et al. 2015).

A close interaction between regulators, academic institutions, research centers, and the industry can help accelerate the translation of nanomedicine efforts. In addition, through the application of the "quality by design" approach, sound science and quality risk management can merge to achieve innovative and even disruptive breakthroughs in new nanopharmaceuticals development and at the same time to provide of safe, convenient, and cost-effective drugs to patients.

9.6 Conclusions

The promises and impacts of nanotechnology are expected to be bigger than the dimensional scale where it is usually defined. Without any doubt, nanomaterials will allow the development of novel products that overcome the different obstacles and challenges currently found in the pharmaceutical industry for targeted drug

delivery such as limited bioavailability, low stability, limited permeability across biological barriers, immune surveillance, and target specificity. The development of intelligent, implantable, and biocompatible devices that can be used for the automatic administration of nanopharmaceuticals directly in the organism is an advance that may be useful for cancer therapy, vaccination, and gene therapy or for the treatment of neurodegenerative diseases. In addition, the exploration of alternative administration routes such as pulmonary drug delivery or transdermal application of drugs will expand the therapeutic choices for the treatment of different diseases. The use of multifunctional materials with unique magnetic, optical, thermal, or mechanical properties widen the possibilities for the design of theranostic systems capable of not only carrying and delivering drugs but also to help with imaging, diagnostics, and even therapeutic uses. However, the large-scale production of safe, biocompatible, and economical nanomaterials suitable for pharmaceutical use is still a limitation that needs to be solved in order to have real applications in human health. A clear understanding of the interactions between nanoformulations and cell components is necessary for the success of such applications. In this sense, combining computational models, bioinformatics tools, and quantitative molecular techniques might allow a deeper understanding of the interaction of nanoformulations with the cell dynamic and the biological systems. This comprehensive understanding is critical, particularly with respect to potential toxicological effects.

There are still several questions and challenges in the field that need to be solved in order to understand not only the toxicological effects of nanoformulations in living organisms but also the environmental effects of them and the opportunities for regulatory approval and commercialization. Nanopharmaceuticals are on the rise, and their impact on the development of a more personalized medicine, with important benefits for patients and physicians, is still beyond our imagination.

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