Chapter 19
Nanomaterials Used for Delivery of Bioactives

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19.1 Introduction

Progression in genetic engineering has led to propagation of gigantic diversity of bioactives which demands effective means of carriers for intracellular delivery in order to achieve specific objectives such as selective tumor targeting, genetic vaccination, regenerative medicine, and treatment of functional loss. Generally, these biologics are prone to enzymatic degradation and deactivation. Hence, immense arrangement of efforts has been made to develop nanometric size vehicles which could not only deliver the medicaments to the desired site of action but also protect for unwanted degradation. In this regard, nanoparticles have been shown great promise as a delivery vehicle for smaller molecules, plus large bioactives, i.e., proteins, peptides, vaccines, or nucleotides by either restricted or tissue-specific delivery. In addition, formulation scientists are fascinated about nanocarriers as a delivery vehicles as proportion of quantity of surface atoms or molecules to the total count of atoms or molecules enhanced drastically hence effective surface area multiplied exponentially (Hadjipanayis et al. 2010). Further, nanoparticles are in great number and could access regions of poor access such as injured tissues, tumor cells, inflamed organs, etc. due to their tiny size (Jong and Borm 2008). Nanotechnology concentrates on encapsulating drugs in bio-friendly nanocomposites, i.e., polymeric nanoparticles, nanoliposomes, solid lipid nanoparticles, micellar systems, and bioconjugates. A schematic diagram of different varieties of nanocarriers used for delivery of bioactives is depicted in Fig. 19.1. These carriers are usually explored to enhance oral bioavailability, to sustain medicament release

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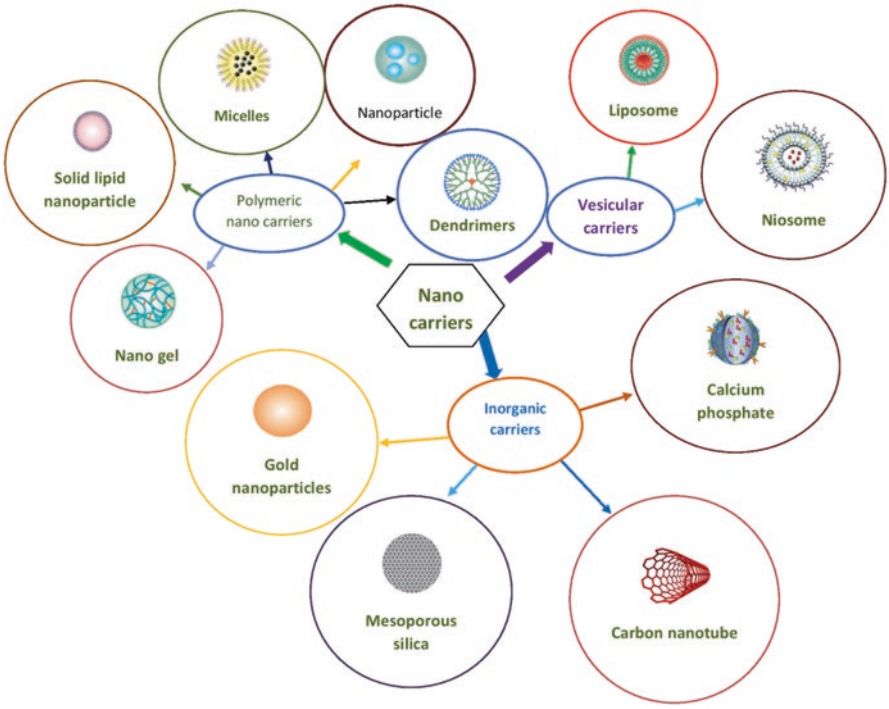

Fig. 19.1 Classification of most commonly used nanocarriers for drug delivery

in desired organ, to dissolve the therapeutics for intravascular administration, to enhance the drug stability against enzyme-mediated degradation, and to achieve targeted (cellular/organ) delivery of drugs. In addition, the release of encapsulated cargo from nanocarriers can be controlled in the organ of interest in order to produce desired therapeutic level for desired period of time to generate maximum therapeutic benefits. Usually, nanoparticles have shown greater cellular uptake compared to microparticles (Desai et al. 1996). Several important human disorder-related diseases have demonstrated significant improvement after treatment of protein-/peptide-loaded nanocarriers (Yu et al. 2016). Macromolecules have large size, high hydrophilicity and susceptibility to physical and chemical degradation, structural fragility, and complexity; hence, these characteristics strongly affect pharmacokinetic and pharmacodynamic behavior in vivo. Moreover, development processes such as higher temperature, exposure to organic solvents, etc. additionally compromise the stability of these macromolecules. To overcome all these challenges, extraordinary efforts are made to incorporate therapeutics in hydrogels, micellar systems, nanocapsules, nanoemulsions, nanoliposomes, and niosomes. Afterward, surface modification of these carriers by specific ligands was explored with an aim to deliver protein therapeutics into the organs of interest in active form. Nanomedicines approved by FDA are enlisted as per variety of carrier/material used in development of the formulation (Table 19.1). The key objective of

Table 19.1 List of nanomedicines approved by FDA classified by type of carrier/material used in the preparation of the formulation

Commercial name (Company)	Ingredient active	Carrier	Application	Advantage	Year approved
Doxil®/Caelyx™ (Janssen)	Doxorubicin	Liposomes	Kaposi's sarcoma; ovarian cancer; multiple myeloma	Increased site-specific delivery (tumor) and decreased systemic toxicity	1995; 2005; 2008
Abelcet® (Sigma Tau)	Amphotericin B lipid complex	Liposomes	Fungal infection	Decreased toxicity	1995
DaunoXome (Galen)	Daunorubicin	Liposomes	Kaposi's sarcoma	Increased site-specific delivery (tumor) and decreased toxicity	1996
DepoCyt© (Sigma Tau)	Cytarabine	Liposomes	Lymphomatous meningitis	Increased site-specific delivery (tumor) and decreased toxicity	1996
AmBisome® (Gilead Sciences)	Amphotericin B	Liposomes	Fungal and/or protozoal infections	Reduced nephrotoxicity	1997
Curosurf /poractant alfa (Chiesi Farmaceutici)	Proteins SP-B and SP-C	Liposomes	Lung activator for stress disorder; pulmonary surfactant for respiratory distress syndrome	Decreased toxicity and increased delivery for smaller volume	1999
Visudyne (Bausch and Lomb)	Verteporfin	Liposomes	Ocular histoplasmosis, myopia, decreased vision	Increased site-specific delivery, (lesion vessels) photosensitive release	2000
DepoDur® (Pacira Pharmaceuticals)	Morphine sulfate	Liposomes	Prolonged release	Loss of pain (postoperative)	2004
Marqibo® (Onco TCS)	Vincristine	Liposomes	Acute lymphoblastic leukemia	Increased site specific delivery (tumor) and decrease toxicity	2012
Onivyde® (Merrimack)	Irinotecan	Liposomes	Pancreatic cancer	Increased site specific delivery (tumor) and decrease toxicity	2015

(continued)

Table 19.1 (continued)

Commercial name (Company)	Ingredient active	Carrier	Application	Advantage	Year approved
Adagen (Sigma Tau Pharmaceuticals®)	Pegademase bovine	PEGylated adenosine deaminase enzyme	Immunodeficiency disease	Improved circulation time in body and decreased immunogenicity	1990
Oncaspar (Enzon Pharmaceuticals)	Asparaginase	PEGylated asparaginase	Acute lymphoblastic leukemia	Improved protein stability due to PEGylation	1994
Copaxone® (Teva)	Glatopa	Glutamate, alanine, lysine, and tyrosine random polymer	Multiple sclerosis	Regulation of clearance and polymer with controlled molecular weight	1996
Renagel® (Sanofi)	Sevelamer hydrochloride or sevelamer carbonate	Poly(allylamine hydrochloride)	Chronic renal diseases	Increased site-specific delivery and increase in circulation time in body	2000
PegIntron® (Merck)	Interferon-alpha (IFN-α2b)	PEGylated IFN-α2b protein	Hepatitis C	Improved protein stability due PEGylation	2001
Pegasys® (Genentech)	Interferon-alpha (IFN-α2a)	PEGylated IFN-α2a protein	Hepatitis B and C	Improved protein stability due PEGylation	2002
Eligard® (Tolmar)	Leuprolide acetate	Polymer (PLGH (poly(dl-lactide-co-glycolide)	Prostate cancer	Prolonged drug delivery and circulation time in body	2002
Neulasta® (Amgen)	PEG-filgrastim	PEGylated granulocyte colony- stimulating factor (GCSF) protein	Neutropenia induced by chemotherapy	Improved protein stability due to PEGylation	2002
Somavert® (Pfizer)	PEG-visomant	PEGylated HGH receptor antagonist	Acromegaly	Improved protein stability due to PEGylation	2003
Macugen® (Bausch & Lomb)	PEG-aptanib	PEGylated anti vascular endothe- lial growth factor aptamer	Macular degeneration; neovas cular age-related (decreased vision)	Improved stability due to PEGylation	2004

Mircera® (Hoffman-La Roche)	Methoxy polyethylene glycol-epoetin beta	Chemically synthesized erythropoiesis-stimulating agent	Anemia associated with renal failure due to diseases	Improved stability due to PEGylation	2007
Cimzia® (UCB)	Certolizumab pegol	PEGylated antibody fragment(Certolizumab)	Crohn's disease; rheumatoid arthritis; psoriatic arthritis and ankylosing spondylitis	Increased stability and circulation time in body	2008; 2009; 2013; 2013
Krystexxa® (Horizon)	Pegloticase	PEGylated porcine-like uricase	Chronic gout	Improved protein stability due to PEGylation	2010
Plegridy® (Biogen)	Interferon beta (IFNβ 1a)	PEGylated IFN-β1a protein	Multiple sclerosis	Improved protein stability due to PEGylation	2015
ADYNOVATE (Baxalta)	Factor VIII	PEGylated factor VIII	Hemophilia	Improved protein stability due to PEGylation	2015
Rapamune® (Wyeth Pharmaceuticals)	Sirolimus	Nanocrystals	Immunosuppressant	Increased bioavailability	2000
Megace ES (Par Pharmaceuticals)	Megestrol acetate	Nanocrystals	Anti-anorexic	Reduced posology	2001
Aviriza (Pfizer)	Morphine sulfate	Nanocrystals	Mental stimulant	Prolonged release and increased bioavailability	2002/2015
Ritalin LA® (Novartis)	Methylphenidate HCl	Nanocrystals	Mental stimulant	Increased drug loading and bioavailability	2002
Zanaflex® (Acorda)	Tizanidine HCl	Nanocrystals	Muscle relaxant	Increased bioavailability and decreased posology	2002
Emend® (Merck)	Aprepitant	Nanocrystals	Antiemetic drug	Increased absorption and bio- availability	2003
Vitoss® (Stryker)	Calcium phosphate	Nanocrystals	Bone substitute	Mimics bone structure by cell adhesion and growth	2003
OsSatura ® (IsoTis Orthobiologics)	Hydroxyapatite	Nanocrystals	Bone substitute	Mimics bone structure by cell adhesion and growth	2003

(continued)

Table 19.1 (continued)

Commercial name (Company)	Ingredient active	Carrier	Application	Advantage	Year approved
Ostim® (Heraseus Kulzer)	Hydroxyapatite	Nanocrystals	Bone substitute	Mimics bone structure by cell adhesion and growth	2004
Tricor® (Lupin Atlantis)	Fenofibrate	Nanocrystals	Hyperlipidemia	Increased bioavailability	2004
Focalin XR® (Novartis)	Dexmethylphenidate HCl	Nanocrystals	Mental stimulant	Increased bioavailability	2005
NanOss (Rti Surgical)	Hydroxyapatite	Nanocrystals	Bone substitute	Mimics bone structure by cell adhesion and growth	2005
EquivaBone® (Zimmer Biomet)	Hydroxyapatite	Nanocrystals	Bone substitute	Mimics bone structure	2009
Invega® Sustenna® (Janssen Pharms)	Paliperidone palmitate	Nanocrystals	Schizophrenia schizoaffective disorder	Decreased release of poor water-soluble drugs	2009/2014
Ryanodex® (Eagle Pharmaceuticals)	Dantrolene sodium	Nanocrystals	Malignant hypothermia	Allows higher administration at higher doses	2014
Estrasorb® (Novav)	Estradiol	Micelles	Menopause hormone therapy	Sustained release	2003
Abraxane® (Celgene)	Paclitaxel (ABI-007)	Albumin-bound paclitaxel nanoparticles	Breast cancer; non-small cell lung cancer and pancreatic cancer	Increase site-specific delivery (tumor) and solubility	2005, 2012, 2013
INFeD (Sanofi Aventis)	Iron	Iron dextran (low MW)	Chronic kidney failure with iron deficiency	Increased dose capacity	1957
DexIron/Dexferrum (Sanofi Aventis)	Iron	Iron dextran (high MW)	Chronic kidney failure with iron deficiency	Increased dose capacity	1957
Feridex/Endorem (AMAG Pharmaceuticals®)	Superparamagnetic iron oxide nanoparticles (SPION	SPION coated with dextran	Imaging material	Superparamagnetic character	1996, 2008
Ferrlecit® (Sanofi Aventis)	Sodium ferric	Sodium ferric gluconate	Chronic kidney failure with iron deficiency	Increased dose capacity	1999

Venofer (Luitpold Pharmaceuticals)	Iron oxide	Iron sucrose	Chronic kidney failure with iron deficiency	Increased dose capacity	2000
GastroMARK; umirem (AMAG Pharmaceuticals™)	Superparamagnetic iron oxide nanoparticles (SPION	SPION coated with silicone	Imaging material	Superparamagnetic character	2001/2009
Feraheme (AMAG pharmaceuticals)	Ferumoxytol ultrasmall superparamagnetic iron oxide nanoparticles (SPION)	Ferumoxytol SPION with polyglucose sorbitol carboxymethyl ether	Chronic kidney failure with iron deficiency	Prolonged steady release and decreased number of doses	2009
Nanotherm (MagForce)	Iron oxide	Aminosilane-coated Iron nanoparticles	Brain tumor	Thermotherapy for destroy tumor cells or sensitized for additional therapies	2010

preparation of nanocarriers is to organize particle size, surface characteristics, as well as efficient delivery in fully active forms. As a result, critical characterizations play a vital role in controlling in vitro as well as in vivo behavior of nanoparticles.

19.2 Classification of Nanocarriers

19.2.1 Liposomes

Liposomes are made of phospholipid bilayer enclosing aqueous cavity which could encapsulate small molecular weight drugs, peptides, proteins, and nucleotides (Yousefi et al. 2009; Patel et al. 2011). Usually, they have a particle size from 25 nm to several micrometers as per the number of bilayers present in the liposomes. Liposomes are proved to be versatile carriers because they offer flexibility in terms of vesicle size, surface charge, bilayer composition, and encapsulation ability which also make these carriers useful for delivery of bioactives. Lipid combination made of distearoyl phosphatidylcholine, dimyristoyl phosphatidylglycerol, and cholesterol (Anderson et al. 1999); a blend of phosphatidylcholine, castor oil, and polyethylene glycol attached to distearoyl phosphatidylethanolamine (Song et al. 1996); and a blend of distearoyl phosphatidylglycerol, distearoyl phosphatidylcholine, and cholesterol (Rezler et al. 2007) are usually used for the preparation of liposomes. Usually aqueous compartment contains hydrophilic agents, whereas lipophilic drugs are inserted into the lipid bilayer (Patel et al. 2009). Bioactives which are prone to degradation are prepared using reverse-phase evaporation process which bypasses unwanted exposure of/to harmful solvents. Liposomes having nano size range can also prolong the release of entrapped bioactives, warranting sustained effect at the site of interest. Pulmonary delivery of insulin is achieved via liposomes which has shown number of advantages as an alternative to insulin injection such as sustained blood glucose levels with a higher pharmacological bioavailability compared to dry powder inhalation (Bi et al. 2008). Further, Bak proteins and voltage-dependent anionic channel (VDAC) were loaded into the liposomes and produce apoptosis into mammalian cells through cytochrome C and caspase activation (Liguori et al. 2008).

Removal of liposomes from blood circulation takes place in a size-dependent manner through membrane fusion actions and interactions with specific serum proteins (opsonins). Despite the fact that cell surface receptors cannot detect liposome directly as a foreign particle, they identify liposomes via cellular and serum proteins that are adhered to liposomal surface (Ishida et al. 2002). Circulation time of liposomes can be enhanced, and also macrophage uptake can be reduced by means of PEGylation which provides anti-opsonizing properties to the liposomes, once it is decorated on the exterior surface by forming a hydrophilic layer of polymer, hence sheltering the charge associated with liposome surface. Usually, RES cells identify and react with liposomes through opsonins, but hydrophilicity of PEG layer helps in escaping the liposomes. Kedar et al. (1994) have significantly enhanced survival

time of mice with metastatic carcinoma (earlier subjected to cyclophosphamide chemotherapy) was enhanced by two to six times followed by treatment with SSL-IL-2 compared to IL-2 even after lower doses and fewer administrations compared to plain interleukin-2 (IL-2). Enhanced cellular translocation of TAT peptide (transactivator of transcription of human immunodeficiency virus) was reported by incorporating it into the liposomes (Carsten et al. 2004). Dioleoyl phosphatidylethanolamine (DOPE) and guanidinium-cholesterol cationic lipid liposomes successfully entrapped the β-galactosidase enzyme and the anti-cytokeratin^{8} (K8) antibody and enhanced cellular delivery of β-galactosidase devoid of any negotiation in its activity (Chatin et al. 2015). In recent time, the applicability of bilosomes as a viable approach for oral administration of larger peptides and proteins along with associated biopharmaceutical challenges has been reviewed by Ahmad et al. (2017).

Niosomes are spontaneously gathered non-ionic surfactants vesicles generally made of alkyl or dialkyl polyglycerol ether class surfactants are (Malhotra and Jain 1994). Lamellar structures fashioned by mingling cholesterol and non-ionic surfactant and followed by hydration which leads to closed bilayer vesicle upon providing some energy such as physical agitation. Such bilayer structures are spontaneous arrangement of surfactant in such a way that allows hydrophilic heads to remain exposed to the aqueous phase while lipophilic tails acquaint themselves with the lipid phase. Niosomes are extensively investigated as a cheaper substitute of liposomes however is of synthetic origin. These vesicles as a carrier systems are closely having similar physical characteristics, in vitro release behavior, as well as in vivo behavior to the liposomes. These carriers not only have maximum advantages of liposomes, but also the economy, enhanced stability, and flexibility in storage conditions make them as ideal alternatives to phospholipids vesicles. Cholesterol is used to facilitate vesicle preparation, which are less leaky. Additionally, stabilizers could also be added to avoid aggregation of niosomes by repulsive, steric, or electrostatic effect. Theoretically niosome formation necessitates the existence of a specific type of amphiphile which possesses an aqueous head and a liphophilic tail and aqueous solvent. The lipophilic unit may consist of one or two alkyl or perfluoroalkyl moieties or, in certain cases, a single steroidal group usually from C12 to C18. Unit chain alkyl as lipophilic tail is more toxic compared to respective dialkylether chain although the ester-based surfactants are chemically somewhat unstable compared to ether-type surfactants. $C_{16}EO_5$ (polyoxyethylene cetyl ether) or $C_{18}EO_5$ (polyoxyethylene stearyl ether) surfactants are mainly utilized for synthesis of polyhedral vesicles. Various aspects manipulating the formation of niosomes are as follows: nature of surfactants, membrane composition, nature of encapsulated drug, and hydration temperature which is responsible for shape and size of the niosomes. The perfect condition for hydration is favored to be above the gel-to-liquid phase transition temperature of the system. The following are applications of niosomes: delivery of anti-cancer drugs, i.e., doxorubicin, paclitaxel, methotrexate; delivery of peptide drugs, i.e., insulin, oligonucleotide; ophthalmic drug delivery; and also transdermal drug delivery. Niosomes demonstrated greater stability to both temperature and oxidation in contrast to the major components of liposomes, i.e., phospholipids, hence are easy to handle without any serious precautions to be taken

in storage (Fang et al. 2001). The vasoactive intestinal peptide (VIP) is therapeutically utilized for the management of Alzheimer's disease however unable to cross the blood–brain barrier (BBB) similar to other endogenous peptides; hence, its parenteral delivery is restricted. The entrapment of VIP in glucose decorated niosomes significantly enhances brain transport of peptide compared to control niosomes (up to 86%, in 5 min) (Biswal et al. 2008). Niosomes containing ethanol showed elastic behavior, hence increasing the intracellular translocation and greater stability of the Tat-GFP fusion protein against chemical degradation (Manosroi et al. 2011). Niosomes were used as a transporter of insulin and demonstrated noteworthy plasma glucose-lowering effect which continued for prolonged period of time (Khaksa et al. 2000). Further niosomes are also used as adjuvant for oral vaccination which demonstrated significant enhancement of antibody levels after encapsulation of ovalbumin into niosomes and shown better immune response compared to Freund's complete adjuvant (FCA), in the BALB/c mice (Rentel et al. 1999).

19.3 Particulate Carriers

19.3.1 Polymeric Nanoparticles

Nanoparticles are known as particulate suspensions or dried particles with a size in the range of 10–1000 nm. As per the method used for their preparation, nanoparticles, nanospheres, or nanocapsules could be produced. Contrasting to nanospheres in which drugs are dispersed in polymer matrix, nanocapsules are vesicular systems in which the drug exits as a core in an aqueous or oily cavity which is covered by coating of polymer; hence, they are "reservoir" system. Nanoparticles not necessarily exhibit size-dependent characteristics which alter greatly in fine particles or bulk materials. The drug/bioactive may be solubilized, dispersed, encapsulated, or adhered to a nanoparticle matrix. A supplementary trustworthy technique to increase the stability of bioactives is encapsulation into a nanoparticle, which protects it from the non-friendly atmosphere inside the biological system and improves the delivery at the site of action (Niven et al. 1994). Encapsulated bioactives were released from the nanoparticles by different mechanisms, i.e., diffusion, erosion, swelling, or polymer degradation. Nanoparticles provide an appropriate way of transporting low molecular weight therapeutics, plus larger bioactives, i.e., proteins, peptides, or genes through localized or targeted delivery to the desired organ. Nanotechnology concentrates on encapsulating therapeutics in biocompatible nanocomposites, i.e., nanoparticles, nanocapsules, micellar systems, and nanoconjugates. These carriers could be utilized to offer targeted drug delivery (cellular/tissue) in order to enhance oral bioavailability, to prolong drug/gene response at the desired site, to dissolve drugs for IV administration, and to stabilize therapeutic agents against enzymatic degradation. Additionally, the release of an entrapped material from nanoparticles to attain wanted therapeutic response in target site for

necessary extent should be managed. Nanoparticles have comparatively higher intracellular uptake than microparticles. Polymeric nanoparticles hold remarkable assurance for the efficient cure of disorders as they have marvelous physicochemical characteristics, i.e., size, surface charge, hydrophilicity, and lipophilicity, hence considered as prospective carriers for bioactives, i.e., vaccines, peptides, anticancer drugs, genes, etc. Eventually, nanoparticles offer numerous advantages compared to free drug, such as protection from unwanted reactions with biological moieties and breakdown, improving the absorption into a desired organ (tumors), and escalating the pharmacokinetics of the therapeutics. Additionally, rate of medicament release from nanoparticles can be amended effortlessly to match up desired therapeutic levels into target organ for desired period of time. Once designed properly, nanoparticles can serve as a model delivery vehicle by preferentially picked up by cancer cells or tumor mass and also avoids early degradation of medicament during its transport. Further, intracellular delivery of the associated agents could be accomplished by engulfment through endocytosis/phagocytosis of nanoparticles. Moreover, characteristics of a polymer could be easily altered; nanoparticles comprise of a versatile carrier system that can be modified to synthesize the nanoparticles which are capable to cross through the biological obstacles and transport the load into the cells and/or intracellular space. As per the rate and extent of prolonged and controlled release of the incorporated protein, various natural and/or artificial polymers have been explored for the formulation of nanoparticles, i.e., chitosan, dextran, starch, albumin, gelatin, 2-methoxyethyl vinyl ether, copolymer of maleic anhydride and cyanoacrylates, poly(lactic acid) (PLA), poly(lactic acid-co-glycolic acid) (PLGA), PEG-PLA block copolymers, and poly(n-hexadecyl cyanoacrylate) (Soppimath et al. 2001; Solaro and Chiellini 2006; Rytting et al. 2008; Solaro 2008; Duan et al. 2009). A list of polymers which are frequently used for development of nanoparticles as protein carriers is shown in Table 19.2. Out of different techniques reported for the development of polymeric nanoparticles, majority of them reports

Table 19.2 Most widely used polymers for development of nanoparticles as drug carriers (Vauthier and Bouchemal 2009; Vyas and Khar 2002)

Synthetic homopolymers	Copolymers	Natural polymers	Colloid stabilizers
Poly(lactic acid)	Polylactide-poly(ethylene glycol)	Chitosan	Dextran
Poly(lactide-*co*-glycolide)	Poly(lactide-co-glycolide)-poly(ethylene glycol)	Alginate	Pluronic F68
Poly(ε-caprolactone)	Poly(ε-caprolactone)-poly(ethylene glycol)	Gelatin	Poly(vinyl alcohol)
Poly(isobutylcyanoacrylate)	Poly(hexadecylcyanoacrylate-co-poly(ethylene glycol) cyanoacrylate)	Albumin	Tween® 20 or Tween® 80
Poly(isohexylcyanoacrylate)		Agarose	
Poly(n-butylcyanoacrylate)		Pullulan	
Polyacrylates and polymethacrylates			
Polystyrene			

two key steps: the first step is emulsion formation, and the second is solvent evaporation or gelation/precipitation of polymer or polymerization. A schematic demonstration of commonly used techniques for development of polymeric nanoparticles is shown in Fig. 19.2. Great attention has been given to biodegradable polymers which are self-eliminating, hence overcoming the concern of surgical removal of carrier system. Permeation of insulin and tetanus toxoid were increased through oral and nasal mucosa by encapsulating it into PEG and chitosan-coated PLA/PLGA nanoparticles, respectively (Vila et al. 2002). Nanoparticles composed of biodegradable polymers poly(ε-caprolactone) (PCL) and blend of PLA and PLGA not only protected insulin from proteolytic degradation into the GI tract but also fruitfully decrease plasma sugar concentration after oral administration to diabetic rats (Damge et al. 2007). Higher toxoid levels were observed in the blood stream and lymph nodes with PEGylated nanoparticles containing tetanus toxoid after

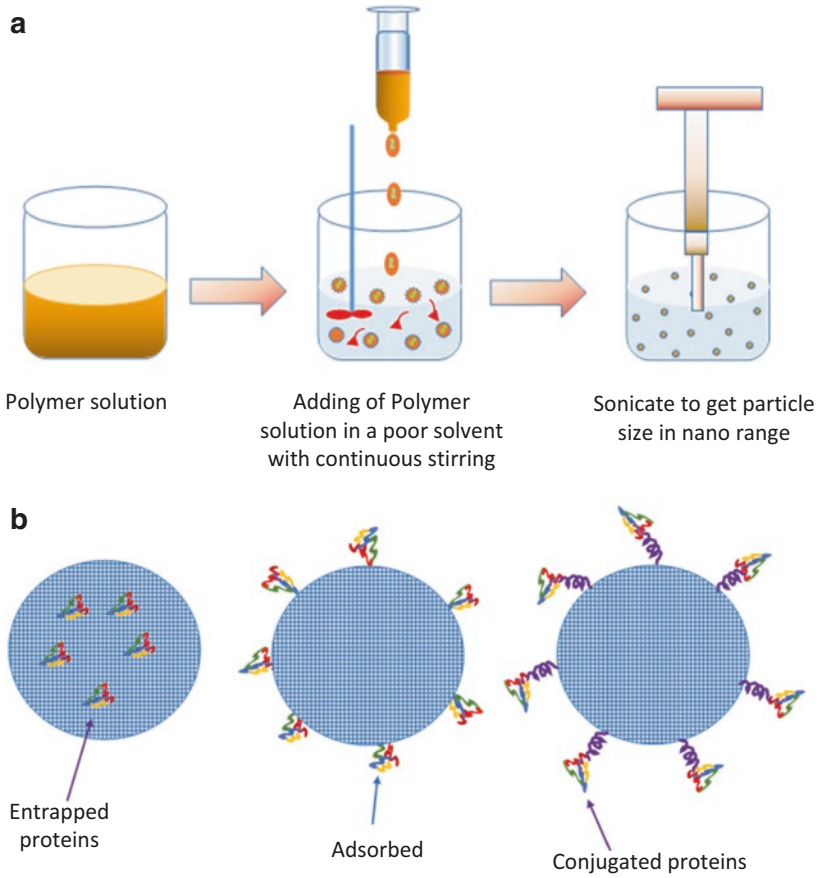

Fig. 19.2 (**a**) Schematic representation of the commonly used method for the preparation of polymeric nanoparticles. (**b**) Schematic of the entrapment of protein in nanocarrier composites

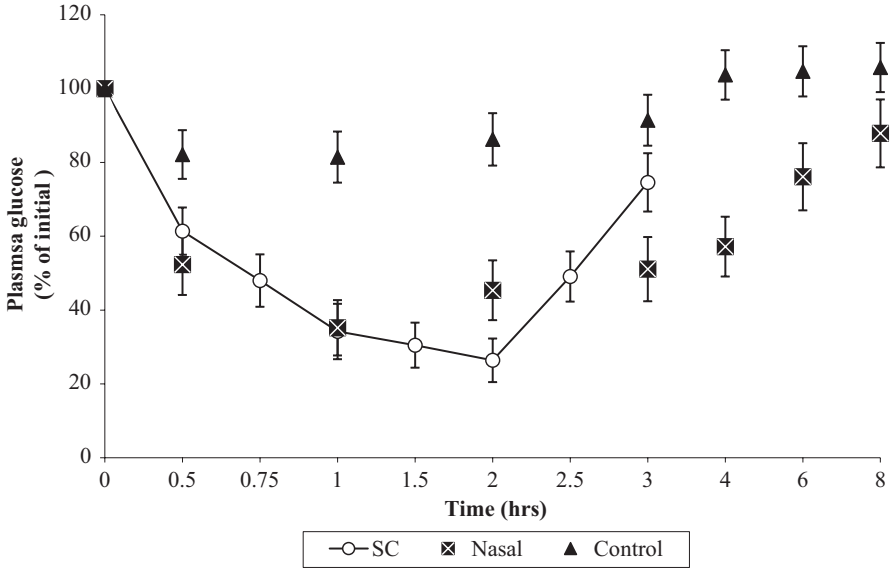

Fig. 19.3 Hypoglycemic effect of EE NPs in the presence of Na glycocholate after nasal administration and plain SC insulin to STZ-induced diabetic rats (mean ± SE, $n = 5$). ∗Hypoglycemic action of SC insulin is higher ($P < 0.05$) at 2 h compared to nasal NPs. ∗∗Hypoglycemic action of nasal insulin is significantly higher compared to SC and control. EE NPs = epichlorohydrin cross-linked starch NPs made via emulsion method. (With kind permission from Elsevier Jain et al. (2008))

nasal and oral administration to rats (Vila et al. 2005). Previously, our research group has established the usefulness of starch nanoparticles for nasal delivery of insulin (Jain et al. 2008) which showed superior hypoglcemic response compared to nasal insulin solution (Fig. 19.3). One of the approaches which is widely used for protein delivery by conjugation with various nanocarriers is receptor-mediated endocytosis (vide infra). In this regard, our research group previously tested the in vivo efficacy of vitamin B12 (VB12) coupled dextran nanoparticles after oral administration of VB12 coupled dextran NPs containing insulin (Fig. 19.4). We have also demonstrated the prevalence of VB12-mediated RME uptake of NPs by co-administering the large excess of VB12 to saturate the receptors 1 h prior to dosing of VB12-conjugated nanocarriers (Chalasani et al. 2007). Carboxymethyl-β-cyclodextrin-decorated chitosan (CMCD-g-CS) nanoparticles showed higher in vitro release profile of BSA in simulated intestinal fluid compared to gastric fluid (Song et al. 2017). Conjugation of cell-penetrating peptide (CPP) to the surface of the 15 nm chitosan nanoparticles (prepared by nanoemulsion method) was carried out and tested in Caco-2 cell line for translocation of insulin across the cell monolayer and found to be effective with 15–19% increase in insulin levels (Barbari et al. 2017). An exhaustive inventory of nanomedicines which are granted FDA approval and are classified as per type of carrier/material utilized in production of the nano formulation is shown in Table 19.1.

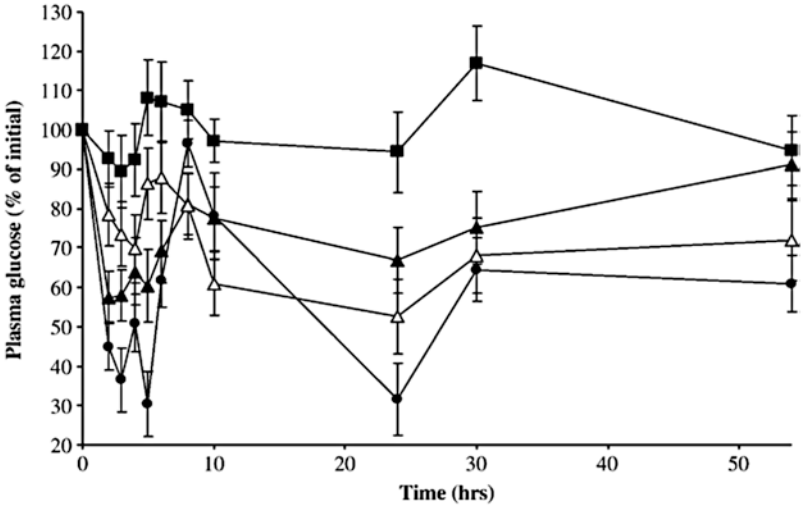

Fig. 19.4 VB12-mediated uptake study of dextran NPs of medium crosslinking density conjugated with O'Hexyl derivative of vitamin B12 (NPs-M35'OH) in terms of plasma glucose reduction after oral administration to STZ diabetic rats ($n = 3$, mean ± S.E.) (Δ) VB12 + 20 IU/kg. (▲) 10 IU/kg. (●) 20 IU/kg.∗ (■) Plain insulin control. ∗ $n = 6$. (With kind permission from Elsevier Chalasani et al. (2007))

19.3.2 Solid Lipid Nanoparticles (SLNs)

SLNs are composed of solid lipids which are stabilized by means of surfactants in an aqueous suspension. They hold close resemblance to nanoemulsion; the only difference is that liquid lipid is substituted with a solid lipid. This replacement of oils with solid lipids controls drug release in an outstanding style, since solid lipid lowers mobility of drug significantly, as compared to oil phase (Martins et al. 2007). SLNs have gained attention in the last few years as carrier system for macromolecules (Marcato and Duran 2008). SLNs are coupled with few advantages and at the same time circumvent some drawbacks of many different carriers such as nanoparticles, liposome, ethosomes, and lipid emulsion.

The advantages offered by SLNs include:

(i) They have drug targeting and controlled drug release ability.
(ii) Protection of labile drugs against photochemical, chemical, and oxidative degradation.
(iii) Limited toxicity compared to polymeric nanoparticles, as SLNs are made of physiological and biocompatible lipids.
(iv) Equally suitable for both hydrophilic and lipophilic drugs.
(v) Bypass exposure of macromolecules to non-friendly hydrophobic solvents.
(vi) SLNs are useful for delivery of macromolecules through various routes such as oral, pulmonary, intravenous, ophthalmic, and dermal (Garcia-Fuentes et al. 2003; Hou et al. 2003; Liu et al. 2008; Joshi and Muller 2009).

Delivery of macromolecules particularly proteins, peptides, and genes might face problems pertaining to their entrapment, as most of them have hydrophobic moieties that show adsorbing behavior onto surfaces (e.g., plastic and glass). This tendency could explain the ways to discrete losses in the amount of bioactives which reaches to the site of action (Duncan et al. 1995).

Mainly researchers described three models for incorporation of drugs or bioactives into the SLNs (Muller et al. 2000; Mehnert and Mader 2001), namely, (i) the drug-enriched shell model, (ii) the homogeneous matrix model, and (iii) the drug-enriched core model. These three models have different formulation composition such as chemical composition of the lipid, bioactive, and surfactants, along with the production method. Contrasting to most of the microsphere and nanoparticle which are made of polymers, SLN preparation techniques avoid potentially toxic organic solvents which may deteriorate bioactives. In addition, under prominent conditions, SLNs can be produced to entrap a huge variety of drugs and emerge to accomplish the desires of optimum nanocarriers (Muller et al. 1995; Wissing et al. 2004). The synthesis of SLN depends on solidification of dispersed phase. As a result, due to hydrophilic nature of bioactives, they have shown poor entrapment into the lipophilic material of SLN that tends to distribute into the aqueous phase during the course, which is promoted by the utilization of emulsifier (surfactants) and stabilizers. Solid lipid nanoparticles are usually prepared by solid lipid nanoparticles by hot homogenization technique (Fig. 19.5). Also, SLN can demonstrate partial

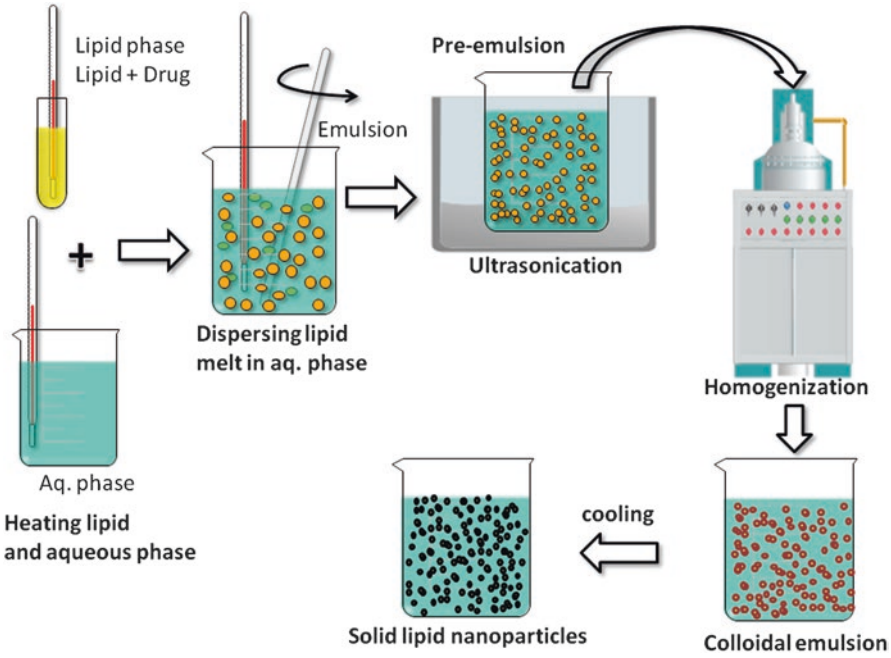

Fig. 19.5 Schematic depiction of various steps involved in the preparation of solid lipid nanoparticles by hot homogenization technique

entrapment owing to drug leakage during storage. In the last few decades, researchers have continuously publicized encouraging results regarding encapsulation of bioactives in SLNs. Numerous peptides such as somatostatin, lysozyme, LHRH, insulin, malaria antigens, calcitonin, and HBsAg have been encapsulated into the SLNs and studied for their stability, in vitro drug release kinetics, and in vivo performance (Table 19.3).

Various strategies have been explored to load macromolecules in ligand-decorated SLNs to enhance oral bioavailability and showed protein activity via both caveolae- and clathrin-mediated endocytosis (Fan et al. 2014). However, mucus was an obstacle to the translocation of SLNs. The absolute bioavailability of peptides was improved by 2.45 to 1.98 times compared to unmodified SLNs, suggesting their effectiveness in oral bioavailability enhancement of proteins. In another report, the gonadorelin (a model peptide) was incorporated into the SLN developed by solvent diffusion technique and evaluated for various parameters. (Hu et al. 2004). Lysozyme (a model peptide) was solubilized into the melted lipid phase, and it remained intact during the course of the process without diminishing its activity and found solubility-dependent entrapment efficiency of the peptide in the hydrophobic phase (Almeida et al. 1997). In another study, physiochemical stability, intracellular uptake by Caco-2 cells, and in vitro cytotoxicity of beta-carotene (BC) SLNs were evaluated and found minimum cytotoxicity of SLNs particularly after dilution (≥10 times) (Yi et al. 2014). Goppert and Muller (2005) studied adsorption pattern of human plasma protein on the outermost layer of SLNs after IV injection and found SLN as a potential targeted drug delivery formulation.

19.4 Inorganic Nanocarriers

Current evolution in nanotechnology has led to the utilization of different inorganic nanoparticles such as mesoporous silica nanoparticle, carbon nanotube, calcium phosphate, and gold nanoparticle for drug delivery (Malmsten and Zauscher 2013). Gradually inorganic nanoparticles are adding weight; among them are carbon nanotubes, gold nanoparticles, and nanospheres, hence widely studied as drug carrier, as their nanometer size enables them to move easily inside the body. The macromolecules could be either placed inside of the nanotube or bound to the particle surface. The major advantages of inorganic nanocarriers are hydrophilic nature, low toxicity profile, biocompatibility, their resistance to microbial growth, and higher stability.

19.4.1 Silica Nanoparticles

Silicates and surfactant co-assembled to produce mesoporous silica, a class of surfactant-templated inorganic compound. Biocompatible nature of mesoporous silica nanoparticles (MSNs) is ideal for biological uses, hence used for delivery of

Table 19.3 Peptide and protein molecules successfully incorporated into the solid lipid nanoparticles

S. no.	Protein/peptide entrapped into solid lipid nanoparticles	Method of development of solid lipid nanoparticles	Entrapment efficiency	References
1.	BSA	Adsorption onto SLN	n.a.	Gualbert et al. (2003)
2.	Calcitonin	Solvent evaporation (w/o/w)	>90%	Garcia-Fuentes et al. (2005a, b)
3.	CyA	HPH hot dispersion HPH hot dispersion HPH hot dispersion HPH hot dispersion Warm microemulsion (o/w)	96.6–97.8% 88.4% n.a. 96.1% 13% protein content	Olbrich et al. (2000) Zhang et al. (2000) Radtke and Muller (2001a, b) Muller et al. (2006) Ugazio et al. (2002)
4.	Gonadorelin	Solvent displacement	50.4–69.4%	Hu et al. (2004)
5.	HAS	Adsorption onto SLN	12.4–32.4% (stealth) 7–13.5%	Cavalli et al. (1999)
6.	Insulin	Solvent displacement Solvent evaporation (w/o/w) Solvent evaporation (w/o/w) Supercritical CO_2 (PGSS)	26.8% n.a. 67.9% 75%	Zhang et al. (2006) Garcia-Fuentes et al. (2003) Zhang et al. (2006) Caliceti et al. (2006)
7.	[D-Trp-6] LHRH	Warm microemulsion (w/o/w)	90%	Morel et al. (1994)
8.	Lysozyme	HPH cold dispersion	43.2–59.2%	Almeida et al. (1997)
9.	Ovalbumin	Adsorption onto SLN Melt dispersion (o/w)	70–97% >80%	Videira et al. (2002) Videira et al. (1998)
10.	Thymopentin	Warm microemulsion (o/w with ionic pair or w/o/w)	5.2% or 1.7%	Morel et al. (1996)
11.	Yak interferon-α	Double emulsion solvent evaporation (w/o/w)	83.7%	Li et al. (2010)

BSA bovine serum albumin, *CyA* cyclosporine A, *HPH* high-pressure homogenization, *LHRH* luteinizing hormone-releasing hormone, *n.a.* not available, *PGSS* particles from gas-saturated solution technique

therapeutics (Radin et al. 2002; Lai et al. 2003); however, they are not bioresorbable. Pore size and pore structure could be altered by ease by selection of surfactant and co-assembly conditions. Higher porous surface along with huge effective surface area of Mesoporus silica nanoparticles (MSN), hence MSN are being exploited as potential nanocarriers for bioactive molecules. Dimension, shape, and surface functionalization of MSN control the release rate of encapsulated bioactives. FITC-cytochrome C was effectively delivered into the HeLa cells after encapsulation into the MSN through a diffusion-mediated process. Also, it was demonstrated by confocal images that FITC-cytochrome C effectively runs away from the endosomal degradation pathways (Tan et al. 2004; Lu et al. 2007). These cellular uptake and endosomal breakout processes of MSNs are energy dependent, whereas surface modification of MSNs by amine and guanidinium makes them able to infiltrate into the cells by a clathrin- and covalent-free pathway (Xing et al. 2005; Slowing et al. 2006). Prasetyanto et al. (2016) published a straightforward approach to encapsulate a highly cytotoxic protein by encapsulating into the organosilica matrices which disintegrate while contact to a chemical response and demonstrated successful cellular delivery into the C6 glioma cells. Alternatively, ligands and antibodies govern the cellular translocation of MSNs by receptor-mediated endocytosis. Moreover, MSNs have been coated with release retarding agents such as chitosan-PEG copolymers, to modulate the release rate of entrapped material (Tan et al. 2004; Lu et al. 2007). Encapsulation efficiency of OVA (ovalbumin) was multiplied by 2.5-fold after surface modification of NPs with positively charged amine because of electrostatic interaction. Amino group modified the zeta potential of the particle from negativity toward positivity (Mahony et al. 2013). Amino-modified silica nanospheres along with photoluminescent CaF2:Tm,Yb nanocrystals have shown higher protein loading and prolonged release of the encapsulated cargo and showed usefulness in cell uptake of bioactives (Li et al. 2017). Besides its use as a delivery vehicle, MSNs were investigated as an immune booster which elicit immune response even at smaller OVA levels. These NPs are synthesized under controlled conditions allowing the modification of pore shape and surface groups (Vallet-Regi et al. 2007), which could be later on conjugated with various mAb structures. Larger pores offer chances for higher loading of mAbs. Surface-functionalized MSN has been encapsulated with a mAb against tumor cell surface protein and administered to mice with melanoma (Lei et al. 2010). The administration of particle-encapsulated mAb demonstrated enhanced suppression of tumor enlargement when compared to pure mAb injection. Findings suggest that encapsulation of mAb into MSN did not change the therapeutics effect or immunological potential, however and by assisting prolonged release of antibody results in enhancement of the half-life of the mAb at the tumor tissue. Mice with malignant mesotheliomas treated with intraperitoneal injection of doxorubicin (DOX) encapsulated into the MSNs which were attached to mesothelin-specific antibody (Macura et al. 2013). The surface-decorated MSN were shown higher effectiveness compared to plain DOX at lower dose level hence able to significantly diminish the undesired effects

of DOX. MSN were also attached to antibody directed against epidermal growth factor in order to treat lung cancer cells, with cytotoxic drug pyrrolidone-2, and demonstrated a 38% reduction in tumor growth with low systemic toxicity (Sundarraj et al. 2014). Also, silica nanospheres composed of vacant core pooled with pH-sensitive chitosan transported encapsulated protein into breast cancer cells through targeting antibody ErbB2 and found higher levels in tumors because of lower local pH at tumors (pH 4.0 Vs physiological pH) upon administration to mouse model (Deng et al. 2011)

19.4.2 Gold Nanoparticles

Gold nanoparticles proved their applicability in biomedical field since they are bio-inert, are biocompatible, have low toxicity, have flexibility of surface modifications, and have cellular imaging ability. Delivery of β-galactosidase into the cells using gold nanoparticles of 2.5 nm in size are explored as useful transporter for and found that β-galactosidase was effectively reached inside the cell membrane of HeLa cells (Ghosh et al. 2010). The mechanism through which gold NPs internalized depend on the surface characteristics, i.e., surface charge and/or size, mainly taken up by endocytosis, whereas antibody-decorated GNPs followed the receptor-mediated endocytic pathway (Tkachenko et al. 2003; Connor et al. 2005). Also, gold NPs were covalently conjugated with protein antigens without any chemical interactions and acted as adjuvant in order to produce a vaccine for cancer immunotherapy. Five nm gold nanoparticles were utilized for protein delivery by treating Balb/c mouse with OVA and AUNPs and were found to be highly effective in producing anti-OVA IgG antibodies (Tang et al. 2013). Gold nanocarriers, including nanorods, multi-functional nanocarriers made of a gold nanoshell, and conventional nanospheres, were conjugated with remedial mAbs and proven to be extremely selective to the target cells without losing any antibody functionality (Shao et al. 2011; Bisker et al. 2012; Cho et al. 2014; Lee et al. 2014; Shen et al. 2014).

19.4.3 Calcium Phosphate Nanoparticles

Calcium phosphate nanoparticles (CaP) having a diameter of 40–50 nm were developed, and their surface was altered by PEG; hence, these modified nanoparticles had zeta potential very close to zero and are used for protein delivery. Coating of pH-responsive material which solubilizes intestinal pH protected encapsulated insulin against the gastric degradation. In vitro release of insulin was negligible in acidic atmosphere whereas insulin was released for a time span of 8 h in intestinal pH (Ramachandran et al. 2008). Zinc is reported to be used for

retarding insulin release (long-acting insulins); thus, calcium phosphates, zinc calcium, and zinc phosphates look attractive contenders for developing ceramic-based insulin carriers. Oral delivery is not only the most favored way of administration of drugs but also provides advantage of patient compliance. Preferably, the absorption of nanoparticles takes place via Peyer's patches region which arrived at the lymphatic system hence bypasses the first-pass metabolism during which insulin degraded significantly. BioSante Pharmaceuticals, a US-based company, successfully synthesized calcium phosphate nanoparticles and entered into the first stage of toxicity studies.

19.5 Concluding Remarks

Delivery of macromolecules is required to be more creative approach than smaller molecular weight therapeutics and demand safer administration, deserve enormous assurance for the treatment of complex diseases. In vivo delivery of macromolecules is emerging importance day by day particularly after latest advancement in recombinant technology which leads to commercial supply of huge variety of therapeutically effective macromolecules. The survival of these agents in the biological environment is of vital importance due to possible denaturation or enzymatic degradation in the absence of an ideal carrier. The selected carrier must provide the complete protection and should be able to translocate the cargo as per desired needs. Hence, it is critically important to design a suitable carrier of appropriate size, composition, and surface behavior as well as biocompatible. Out of the huge range of the carriers studied, nanocarriers emerged as an outstanding choice during the last decades for their successful therapeutic effects through oral, pulmonary, buccal, nasal, as well as parenteral routes.

Because of the biocompatible nature of lipids utilized in synthesis of nanoliposomes and solid lipid nanoparticles, these carriers are proved to be more safe than other types of nanocarriers. Moreover, lipid-based carriers serve as strong immunological adjuvants, capable of eliciting cellular and humoral response against a range of infectious agents related to human disease. However, generally they show considerable instability because of inadequate shelf-life and shorter half time. Indeed, because of high stability of polymeric nanoparticles over other nanocarriers, they emerged as promising carriers of bioactives to meet specific requirements. In addition, surface decoration of these nanoparticles with specific ligands RES uptake can be drastically decreased which is a key constraint with these carriers. On the other hand, synthesis of polymeric nanocarriers generally involves use of organic solvents which could have deleterious effect on macromolecules. Therefore, these drawbacks could be beaten by the picking up protein friendly method for particle synthesis by employment of self-assembling water-soluble polymers.

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