DRUG SYNTHESIS METHODS AND MANUFACTURING TECHNOLOGY

NANOSIZED FORMS OF DRUGS (A REVIEW)

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The main directions of research in the field of nanosized carrier systems for targeted delivery of drugs are reviewed. The fields of research and the results from characterization of the pharmacological properties of nanosized forms of drugs, including nanospheres, liposomes, dendrimers, fullerenes, nanocapsules, and nanocrystalline forms of drugs are considered. Prospects of the search for new forms of drugs based on achievements in nanobiotechnology are considered with a view to the reduction of side effects and improvement of the bioavailability of drugs.

Key words: drugs, nanosized forms, targeted delivery of drugs.

Nanosized colloidal carriers of drugs represent one of the approaches to discovering new methods of pharmacotherapy. Colloidal delivery systems can increase the effectiveness of action by optimizing the biodistribution and toxicodynamics of drugs [1 – 4]. The ability to deliver a drug using a particular particle directly to a biological target, overcoming histohematic barriers (HHB), is exceedingly attractive and is moving more and more from a pure idea to practical implementation [5].

It has been reported that colloidal systems consisting of particles with sizes from several to thousands of nanometers are capable of delivering drugs contained in them (bound to them) to certain biological targets [6 – 8].

A review of the literature [9] identified the main requirements for targeted delivery systems as:

the reliability and use of available raw materials;

the capability to retain activity during delivery in the vasculature for a given and controlled time;

a highly selective interaction with biological targets;

the required amount of delivered drug and its high effectiveness at the target;

the maximum effect on a given number of target cells;

the ability to control both the strength and time of drug action;

flexibility for various chemical classes of drugs and targets.

Figure 1 shows a hypothetical nanoparticle corresponding to all these requirements.

This list of requirements is incomplete and is far from strictly followed for any of the existing systems. However, even partial adherence to these requirements in a certain sys-

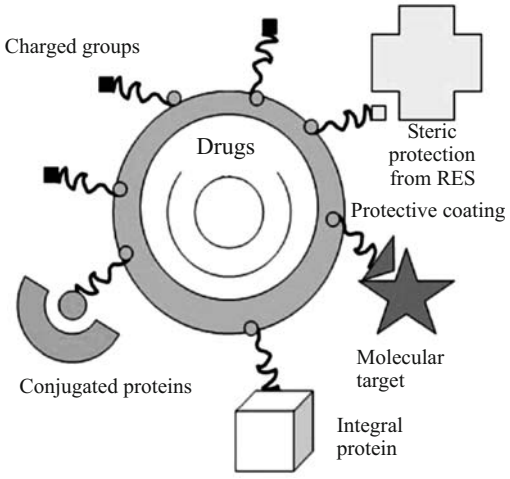

Fig. 1. Hypothetical nanoparticle-carrier of drugs.

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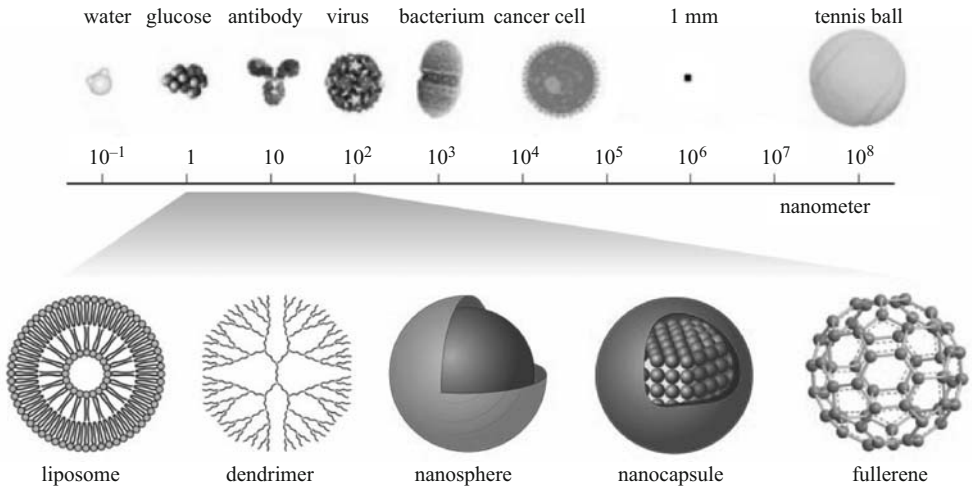


Fig. 2. Nanocarriers of drugs.

tem makes it much more effective than the drug without a carrier.

It should also be noted that the effectiveness of drugs based on nanosized carriers will be highly individualized and will depend on the drug structure, the chemical nature of the carrier, etc. A clearly individualized approach is necessary for creating nanosized forms of a particular drug. In several instances its fabrication will not give the expected result. The activity of enzyme systems can be changed through the action of nanosized particles [10, 11]. This will affect the dosing of nanosized forms of drug. In particular it was found that C_{60} fullerene is complementary to the active center of HIV protease and is bound to it through van-der-Waals forces, thereby inactivating the enzyme [12].

A change of the bioavailability of a drug bound to a nanosized carrier and the targeted delivery of an active principle directly to the target organ will also change the amount of drug required to achieve a given biological response and will decrease possible side effects.

Figure 2 shows a whole series of structures that can act as nanosized carriers according to the literature [13 – 19].

Nanoparticles. The idea that nanotechnology will help to make therapy of many diseases more targeted and selective corresponds with the interests of practical pharmacology. In fact, the distribution of drugs in the organism can be changed so that they reach only their site of action. This problem can be solved by using nanosized carriers, also called nanoparticles (nanosystems), for targeted delivery of drugs that take into account biochemical peculiarities of the organism. Nanosystems for delivering drugs can overcome low solubility and unsatisfactory absorption (assimilation) of the newest generations of drugs because they have the unique feature of an extremely developed (compared with traditional materials) surface. Thus, nanoparticles themselves are used not only for therapy of some pathologies or others but also for diagnosis of diseases [20 – 31]. Therefore, the discovery of new nanosized materials with both therapeutic and diagnostic application has become very important.

Nanosized carriers are modified by amphiphilic surfactants in order to increase the effectiveness of delivery of nanoparticles to the target organ. Such modification prevents capture of the particles by liver and spleen macrophages, i.e., assists their distribution outside the reticulo-endothelial system (RES) [20]. A promising area of this technology is the use of modified nanoparticles for delivery of drugs through the blood-brain barrier (BBB). Use of nanoparticles may turn out to be especially critical for chemotherapy of brain tumors because the BBB prevents the access of many drugs to the brain in therapeutic concentrations whereas local (intracerebral) administration often does not provide the desired results in view of limited diffusion of the drug from the site of administration into brain tissue [22].

Thus, it has been shown that incorporation of doxorubicin (DR) into biodegradable and biocompatible nanopar-

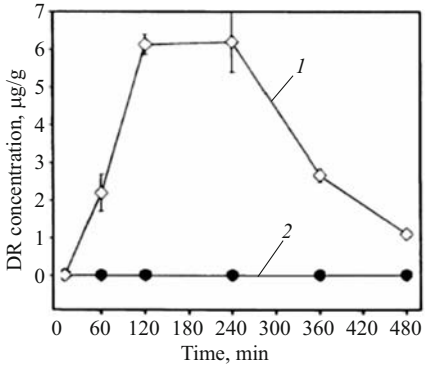


Fig. 3. Kinetics of concentration change of doxorubicin (DR) in rat brain after administration (i.v.) at a dose of 5 mg/kg: DR bound to polypropylcyanacrylate nanoparticles coated with polysorbate-80 (*1*), DR bound to polypropylcyanacrylate nanoparticles, DR together with polysorbate-80, and DR solution (*2*).

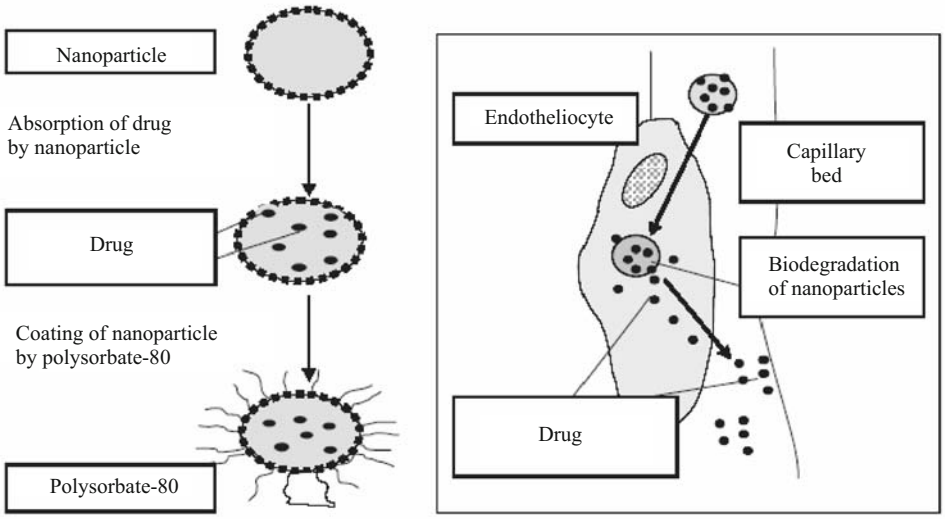


Fig. 4. Construction of drug targeted delivery systems through the blood-brain barrier based on nanoparticles [17].

ticles of polybutylcyanacrylate (PBCA) modified with polysorbate 80 (Tween® 80) can deliver it to the brain because, being a substrate of Pgp (P-glycoprotein-transport protein, which eliminates the antitumor preparation from the cell), DR in the free state does not penetrate the BBB [23, 24] (Fig. 3).

A study of the chemotherapeutic activity of this drug form toward a glioblastoma 101/8 brain tumor model in rats showed that it was highly effective compared with the control and increased the lifespan by 84%. Also, 23% of the animals went into long-term remission (>6 months) [24].

The mechanism of delivery of drugs into the brain using nanoparticles has not been fully established. According to one of the hypotheses, PBCA nanoparticles modified by polysorbate 80 penetrate into endothelial cells of brain capillaries through receptor-mediated endocytosis [25]. This hypothesis was based on several experimental results indicating that modification of the surface of PBCA nanoparticles by polysorbate 80 leads to adsorption on their surface of apolipoprotein E circulating in plasma. Receptors for this protein are expressed in membranes of endothelial cells of brain capillaries. It can be hypothesized that adsorbed apolipoprotein E interacts with the receptors and, thereby, facilitates capture of the particles by endothelial cells. The nanoparticles are degraded in the cell and release the drug, which then penetrates into brain tissue (Fig. 4). Thus, nanoparticles are an effective medium for delivering drugs into the brain. This area presents new interesting possibilities for non-invasive therapy of various CNS pathologies.

It is highly probable that targeted delivery systems for antitumor preparations will combine the main advantages of colloidal carriers and of specific vectors that deliver drugs to these molecular targets.

Fullerenes. Fullerenes must also be discussed as nanosized carriers. Technologies enabling the production of stable colloidal solutions of fullerenes in water have now been developed. Thus, successive addition of a benzene solution of fullerene to THF and acetone followed by addition to water with distillation of the organic phase produced a stable water solution of fullerene with concentration 2×10^{-6} M that was stable for several months and consisted of 250 – 350-nm particles. Table 1 lists methods for preparing aqueous dispersions of fullerenes.

The ability to modify chemically the fullerene molecule allows the creation of particles with a given surface charge to which a drug can be bound using noncovalent or covalent bonds [35].

Figure 5 shows examples of this type of fullerenes.

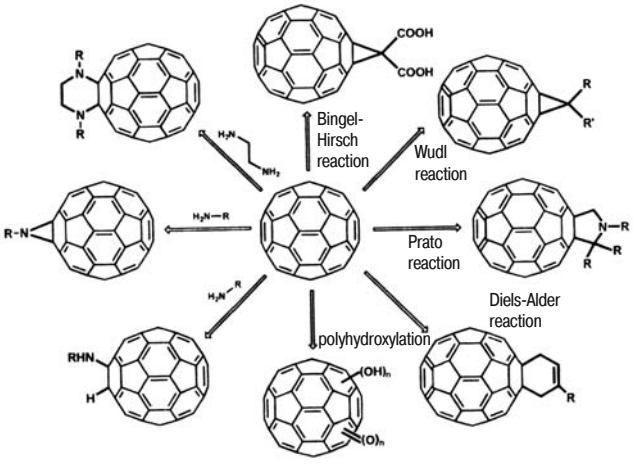


Fig. 5. Main reactions for chemical modification of fullerenes used to synthesize water-soluble derivatives suitable for biological research.

Several studies have shown the possibility in principle for practical application of fullerene derivatives in pharmaceutical practice [36 – 39].

Dendrimers. One of the most promising and rapidly growing research areas in modern chemistry over the last few years has been a new type of polymeric compounds. Their macromolecules have a specific (mainly spherical) shape and a three-dimensional densely packed framework consisting of concentric layers of branching elementary units.

The only approved name for this class of compounds is the term dendrimers or starburst dendrimers that was proposed by Donald Tomalia, who developed the first dendrimers as polyamidoamines [40]. The term arboroles that was proposed by Newkome, et al. [41] and the term cascade compounds are used less frequently. The choice of terms is based on the structure of the macrochain, which is reminiscent of the structure of tree crowns [dendron (Gr.), arbor (Lat.), tree] or a cascade of repeating structural elements and the average sizes of these compounds (1 – 100 nm).

This class of compounds is interesting because the number of branchings increases in a geometric progression with each unit step of growth. As a result, the shape and molecular rigidity changes as the molecular weight of such compounds increases. As a rule, this is accompanied by a change of physicochemical properties of the dendrimers such as the characteristic viscosity, solubility, density, etc. [42].

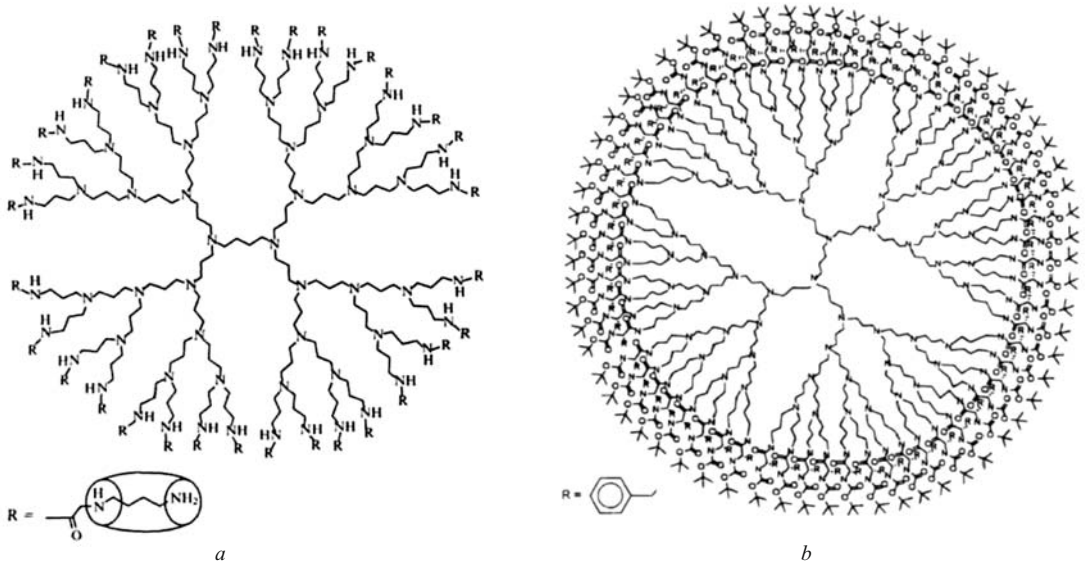
If circles passing through existing and potential points of dendrimer branching are envisioned around the center of the dendrite molecule and they are numbered starting with 0, it can be seen that all branching points fall on a circle owing to the symmetry of the molecule. The maximum number obtained in this manner is called the generation number G of the dendrimer.

The number of terminal (surface) groups in a dendrimer increases in a geometric progression in each generation. Also, the size of the molecule and, therefore, the surface available for locating surface groups in each generation increases only as a square law. This results in the packing density of surface groups of dendrimers also growing from lower generations to higher ones. As a result, the shape and rigidity of the dendrimers changes from loose structures to a shape reminiscent of starfish to rigid spheres. This property enables them to be viewed as promising materials with various practical uses [43].

Dendrimer macromolecules have been used successfully as agents for delivering chemotherapeutic drugs to cancer cells [44]. Thus, the completed experiments showed that the effectiveness of methotrexate was increased and the side effects and toxicity were decreased with dendrimer delivery of it into the organism. Further research in this area, according to the developers, will transform neoplastic diseases into a chronic controllable form.

TABLE 1. Preparation Methods and Characteristics of Aqueous Fullerene Dispersions

Ref.	Prepared by	To produce	Composition	Fullerene concentration
[26]	Grinding C_{60} fullerene powder	Suspension of C_{60} fullerene in water	Particles, 1 – 100 nm, 20% <1 nm, 60% 1 – 20 nm, 20% >20 nm	1 mg/mL
[27]	Prolonged milling of C_{60} fullerene with water	Stable suspension (2 months)	Particles, 7% 1650 – 1000 nm, 43% 500 – 1000 nm, 28% 250 – 450 nm, 22% < 200 nm	
[28]	Solution of C_{60} (0.15 mg) in benzene (100 μL) added to THF (10 mL), resulting solution added dropwise to acetone (100 mL). Resulting mostly acetone solution treated with water (150 mL), organic solvents distilled off	Stable colloidal suspension (up to 3 months)	Particles, 250 – 350 nm	2.1×10^{-6} M
[29]	Solution of C_{60} (and/or C_{70}) fullerene in toluene added to a large volume of another organic solvent (e.g., CH_3CN)	Stable colloidal suspension in organic solvent (several months)	Average particle size for C_{60}/C_{70} ~200 nm; C_{70}, ~300 nm, C_{70}, ~400 nm	10 mg/L, 10^{-5} M
[30]	Direct mixing of a saturated solution of C_{60} (and/or C_{70}) fullerene in THF and water with subsequent removal of THF by a stream of N_2	Stable colloidal suspension in water (up to 9 months)	Particles, ~60 nm	5.0×10^{-6} M
[31]	–	–	Particles, 25 – 500 nm	–
[32]	Ultrasound treatment of a mixture of C_{60} and C_{70} fullerenes in toluene and water	Stable colloidal suspension in water (up to 9 months)	Particles, 3.4 – 72 nm	up to 2.2 mM
[33]	Stirring of crystalline fullerene with water (2 weeks)	Stable colloidal suspension in water	Particles, 50 nm to 1 μm	–
[34]	Stirring of crystalline fullerene with water and illumination (2 months)	Stable colloidal suspension in water	Particles, 10 – 200 nm	5.0×10^{-6} M



a *b*

Fig. 6. Rigid dendrimer structure. Terminal groups are cucurbituryl (*a*) and benzyl (*b*).

Host-guest structures based on cucurbit(6)uryl and a dendrimer with terminal alkyldiammonium groups are known. Cucurbituryl [$C_{36}H_{36}N_{24}O_{12}$, cucurbit[6]uryl or Q6] is constructed of six bound methylenes of glycoluryl fragments. This compound is the first example of cucurbituryls. It was prepared by Berend in 1905 by condensation in acidic medium of formaldehyde and glycoluryl (the condensation product of urea and glyoxal). The synthesis of Berend was repeated in 1981 by Freeman, Mock, and Shih [45]. They obtained a colorless crystalline compound, a macrocyclic cavitand with a barrel shape.

Reaction of Q6 with a dendrimer having terminal alkylammonium groups conjugated to them the macrocycles and formed a dendrimer with terminal pseudorotaxane fragments (Fig. 6). The use of bulky molecules as a component part of the terminal groups imparted rigidity to the dendrimer structure. The resulting compound was globular and capable of holding guests within itself. The bulky group was removed upon increasing the solution pH. The dendrimer became conformationally flexible and released the guest molecules. This ability to incorporate molecules reversibly enables dendrimers to be used successfully for drug delivery [46, 47].

Capsules. Microencapsulation has been widely used in the production of various products and preparations [48], including those for medical purposes [49], as a principle for fabricating systems for targeted delivery and protection of compounds. These are pharmaceuticals of timed-release and prolonged action that provide protection from stomach acid upon oral administration of polypeptides, vaccines, and other preparations in addition to systems for parenteral administration in a biodegradable coating, agricultural agents (pesticides, pheromones), chemical products of various applications (dyes for carbonless paper copying, toners, antipyrenes for polymeric compositions, anaerobic hermetics, etc.), food and feed bioactive additives, components of cosmetics, etc. As a rule, microparticles of solid and liquid compounds that are encapsulated in the polymeric coating give entirely new properties to known chemical and pharmaceutical products and biologically active substances. Interest in microencapsulation remains high from a scientific and practical viewpoint. This can be seen from the expansive literature on this subject {e.g., monographs [50, 51], MML book series [52], and periodicals (*J. Microencapsulation*)} and regular international symposia organized by the International Society for Microencapsulation.

Gas-phase polymerization is one of the most common methods for encapsulation of compounds. A unique process consisting of selective thermal destruction of the *p*-xylylidene dimer with subsequent polymerization of the resulting radicals on the surface to be encapsulated has been used for

TABLE 2. Encapsulation Methods

Microcapsule preparation method	Main steps
Coacervation	Dispersion of drug in colloidal solution of coating material. Desolvation. Adsorption of resulting coacervated drops on encapsulated drug. Solidification of coacervate and isolation of capsules.
Pseudoliquef action	Transfer of nuclei of encapsulated material into pseudoliquefied state. Spraying of solution of coating material. Drying and precipitation of microcapsules.
Chemical method	Dispersion of drug in polymer solution to given sizes. Phase separation with formation at the phase boundary of solid coating. Isolation of microcapsules.

microencapsulation, in particular, of solid drugs by gas-phase polymerization.

The effectiveness of this microencapsulation method and the ability for rapid release of the drug from the microcapsule have been studied using crystalline piracetam (a nootropic preparation), the main fraction of which (83%) had particle size 200 μm. It was found that the release rate of piracetam from the microcapsules depended on the coating thickness. The release rate for a coating thickness >3 μm was significantly decreased. The polymeric coating could regulate the release rate of the drug from practically instantaneous (equivalent to the action of free piracetam) to insignificant in acidic and neutral media. The release rate of piracetam from microcapsules in alkaline medium (pH = 9.12) was faster than in acidic [53]. Thus, the different release rates of piracetam from microcapsules in various media enables the drug dose to be controlled in the organism. Less than 3.5% of the drug is released during the first hour after administration of microcapsules in a model medium with pH = 7 for a coating thickness up to 1.5 – 3.0 μm. In alkaline medium (pH = 9.1), about 60% of the compound is released in 5 h from these same microcapsules. Obviously, decreasing the coating thickness to 0.3 – 0.5 μm allows up to 100% of the drug to be released in the same time. Up to 10% of the compound can be released in the first hour. This example illustrates the promise of using microencapsulation to create new drug forms.

TABLE 2 shows the methods that are used most commonly to prepare drug nanocapsules.

The following problems can be resolved by using nanoencapsulation:

isolation of drugs from the environment;

transformation of liquids into pseudosolids;

prevention of dissolution or its regulation;

improvement of conditions for handling the product;

decreasing the volatility;

increasing the pharmacological activity;

controlled release of an encapsulated compound;

masking of odor, taste, and color;

ability to use microencapsulated products in mass production;

increased storage time;

most uniform distribution of very small coated particles in the gastrointestinal tract or other parts of the organism.

Drug nanocrystals. Nanosized forms of drugs are of definite interest. Many drugs with low bioavailability indicators are known today [54]. Therefore, preparation of water-soluble drug forms with properties suitable for clinical use is currently the main focus for drug development. The resolution of this problem is a critical task for applied pharmacology. Several approaches have been used to overcome these problems. One of them is the fabrication of suparmolecular complexes, in particular complexes of drugs with cyclodextrins [55, 56]. Studies of cyclodextrins have been concentrated on the fabrication of complexes with greater solubility and simultaneously decreased side effects [56]. An approach comprising smaller crystalline drugs and transformation of relatively crude drug particles into crystals of micrometer size with an average diameter in the range of 2 – 5 μm and a corresponding size distribution between about 0.1 and 20 μm was also proposed. In this instance the increased surface area results in increased drug solubility. However, there are currently many new drugs with unsatisfactory biodistribution indicators, even those with crystals of micrometer size. Thus, an approach that decreases the drug size to the nanometer level seems promising. For example, the total surface area increases 10 times if the size of a spherical drug particle is decreased from 50 to 5 μm. Decreasing the particle size to 500 nm increases the surface area by 100 times. This causes the drug to be more soluble. Nanosized drug particles can be prepared also for drugs that are very soluble in water. For example, an aqueous suspension of Paclitaxel prepared from nanocrystals was stable for 4 years at 4°C. On the other hand, the Paclitaxel concentration in an aqueous solution decreased to 80% within 25 min [57, 58]. Several nanosized drugs are currently available on the pharmaceutical market. In particular, coated Rapamune tablets are a more convenient form for practical use than its solution. The bioavailability of the tablet form is 27% greater than that of its solution [57].

It can also be proposed that nanosized drug forms themselves can act as agents for targeted delivery. In analogy with nanocapsules and nanoparticles for drug delivery, it can be concluded that treatment of drug surfaces with adjuvants will impart to them the required properties. In particular, treatment of a surface with polysorbate-80 should provide targeted delivery of a drug to the CNS.

Liposomes. The ability of liposomes to incorporate a variety of compounds without limitations on their chemical nature, properties, and sizes provides truly unique capabilities for solving several medical problems. Inclusion of drugs in liposomes can increase significantly their therapeutic effectiveness because, on one hand, the preparation located in the liposome is protected by its membrane from the effect of destructive factors and, on the other, this same membrane prevents a toxic preparation from exceeding the allowable concentration in biological fluids of the organism. In this instance, the liposome acts as a reservoir from which the preparation is gradually released in the necessary doses and in the required time interval [59 – 61].

From the viewpoint of biological compability, liposomes are ideal drug carriers. They are constructed from natural lipids. Therefore, they are nontoxic, do not elicit undesirable immune responses, and are biodegradable. However, the situation with therapeutic application of liposomes is not as simple as desired. Liposomes are not stable enough in blood and are rapidly eliminated from circulation by RES cells.

Thus, the natural function of macrophages on liposomes can be used to activate them. This is important in the battle with viral, bacterial, and fungal infections. The fact that

TABLE 3. Liposomal Drugs Allowed for Clinical Use [53]

Preparation name	Manufacturer	Active drug	Indication
AmBisome/AmBisome	Gilead (NeXstar)	Amphotericin B	Fungal infections
ABELCET/ABELCET	Gilead (NeXstar)	Amphotericin B	Fungal infections
DaunoXome	Gilead (NeXstar)	Daunorubicin	Kaposi's sarcoma
Doxil/Doxil	Alza (Sequus)	Doxorubicin	Kaposi's sarcoma, ovarian cancer, recurrent breast cancer
Caelyx/Caelyx	SCHERING-PLOUGH		
Myocet/Myocet	Elan (TLC)	Doxorubicin	Recurrent breast cancer
Altragen/Altragen	–	Retinoic acid	Acute myeloid leukemia, non-Hodgkins lymphoma, Kaposi's sarcoma, renal cell carcinoma
Nyotran/Nyotran	–	Nystatin	Fungal infections
NX211	–	Lurtotecan/Lurtotecan	Ovarian cancer
		Annamycin	Doxorubicin-resistant tumors

liposomes are not accumulated by organs such as the heart, kidneys, brain, and CNS cells can significantly decrease the cardiotoxicity, nephrotoxicity, and neurotoxicity of valuable preparations used for anticancer therapy by using liposomal drug forms. The problem of drug delivery to the required target can be solved by local administration of liposomal preparations, as was done for anti-arthritic preparations and preparations for treating respiratory syndrome of newborns and asthmatics. Furthermore, conjugation to liposome surfaces of molecules specific for target cells (for example, immunoglobulins) can in certain instances be effective for targeted delivery of anticancer, anti-infectious, and anti-inflammatory preparations.

Modification of the liposome surface, in particular, covalent conjugation of the synthetic polymer polyethyleneglycol (PEG), can increase significantly the circulation time of liposomes [62]. It was proposed that the strongly hydrated polymeric coating hinders adsorption of antibodies and other protective proteins on the surface of such liposomes. As a result, macrophages do not recognize them as foreign particles subject to removal. Animal experiments showed that the therapeutic action of antitumor preparations was unusually strengthened and in certain instances led to complete remission if they were incorporated into stealth liposomes. The size of metastases was greatly reduced in malignant tumors [59, 62]. Several liposomal preparations have been successfully used in the clinic (Table 3) [59].

Practical results that were achieved with liposomal forms of many drugs in the 1980s and 1990s prompted further clinical investigations. For example, life extension was observed after use of the antitumor preparation doxorubicin incorporated into pegylated liposomes to treat metastatic breast cancer patients. Positive results were obtained for combinational therapy consisting of doxil and cisplatin, mycet and paclitaxel, or kelix and carboplatin. Clinical investigations showed a significant result for therapy of skin T-cell lymphoma and sarcoma by DR in pegylated liposomes compared with the free drug [59].

Thus, it can be concluded that studies of nanosized drugs are timely and have definite practical significance for discovering new highly effective drugs using nanosized carriers that are structurally related to fullerenes, nanoparticles, dendrimers, and nano- and microcapsules.

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