

DOPPLER ECHOCARDIOGRAPHY. See
ECHOCARDIOGRAPHY AND DOPPLER ECHOCARDIOGRAPHY.

DOPPLER ULTRASOUND. See ULTRASONIC IMAGING.

DOPPLER VELOCIMETRY. See CUTANEOUS BLOOD
FLOW, DOPPLER MEASUREMENT OF.

DOSIMETRY, RADIOPHARMACEUTICAL. See
RADIOPHARMACEUTICAL DOSIMETRY.

DRUG DELIVERY SYSTEMS

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PRINCIPLES OF CONTROLLED DRUG DELIVERY

A perspective drug delivery systems can be defined as mechanisms to introduce therapeutic agents into the body. Chewing leaves and roots of medical plants and inhalation of soot from the burning of medical substances are examples of drug delivery from the earliest times. However, these primitive approaches of delivering drugs lacked a very basic need in drug delivery; that is, consistency and

uniformity (a required drug dose). This led to the development of different drug delivery methods in the later part of the eighteenth and early nineteenth century. Those methods included pills, syrups, capsules, tablets, elixirs, solutions, extracts, emulsions, suspension, cachets, troches, lozenges, nebulizers, and many other traditional delivery mechanisms. Many of these delivery mechanisms use the drugs derived from plant extracts.

The modern era of medicine development started with the discovery of vaccines in 1885 and techniques for purification of drugs from plant sources in the late nineteenth century, followed by the introduction of penicillin after its discovery in 1929, and a subsequent era of prolific drug discovery. The development and production of many pharmaceuticals involves the genetic modification of microorganisms to transform them into drug-producing factories. Examples are recombinant deoxyribonucleic acid (DNA), human insulin, interferon [for the treatment of acquired immunodeficiency syndrome (AIDS) related Kaposi's sarcoma, Hairy cell leukemia, Hepatitis B and C, etc.], interleukin-2 (Renal cell and other carcinomas), erythropoietin (for the treatment of anemia associated with chronic renal failure/AIDS/antiretroviral agents, chemotherapy-associated anemia in nonmyeloid malignancy patient), and tissue plasminogen activator (1). It is now possible to produce oligonucleotide, peptide, and protein drugs in large quantities, while gene therapies also appear to be clinically feasible. Each of these therapeutic agents, by virtue of size, stability, or the need for targeting, requires a specialized drug delivery system (2). While the conventional drug delivery forms are simple oral, topical, inhaled, or injections, more sophisticated delivery systems need to take into account pharmacokinetic principles, specific drug characteristics, and variability of response from one person to another and within the same person under different conditions.

The efficacy of many therapeutic agents depends on their action on target macromolecules located either within or on the surface of particular cells types. Many drugs interact with enzymes or other macromolecules that are shared by a large number of cell types, while most often a drug exerts its action on one cell type for the desired therapeutic effect. Certain hormones, for example, interact with receptor mechanisms that are present in only one or a few cell types. An ideal gene delivery system should allow the gene to find its target cell, penetrate the cell membrane, and enter into the nucleus. Further, genes should not be released until they find their target and one has to decide whether to release the genes only once or repeatedly through a predetermined way (2). Thus, the therapeutic efficacy of a drug can be improved and toxic effects can be reduced by augmenting the amount and persistence of drugs in the vicinity of the target cells, while reducing the drug exposure to the nontarget cells.

This basic rationale is behind controlled drug delivery. A controlled drug delivery system requires simultaneous consideration of several factors, such as the drug property, route of administration, nature of delivery vehicle, mechanism of drug release, ability of targeting, and biocompatibility. These have been summarized in Fig. 1.

It is not easy to achieve all these in one system because of extensive independency of these factors. Further,

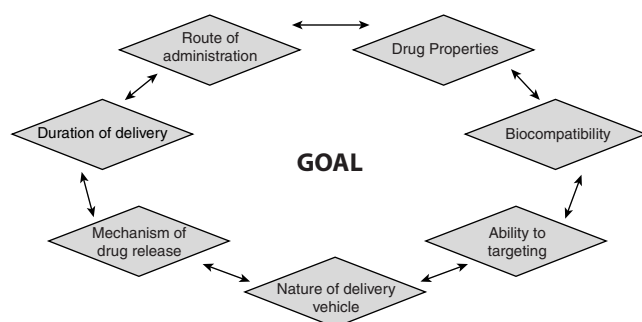


Figure 1. Design requirement for a drug delivery systems.

reliability and reproducibility of any drug delivery systems is the most important factor while designing such a system. The emphasis here is on the need for precision of control and to minimize any contribution to intraand intersubject variability associated with the drug delivery system. There are many different approaches for controlled drug delivery applications (3). They are summarized in the following section.

Overview of the Development of Drug Delivery Systems

To obtain a given therapeutic response, the suitable amount of the active drug must be absorbed and transported to the site of action at the right time and the rate of input can then be adjusted to produce the concentrations required to maintain the level of the effect for as long as necessary. The distribution of the drug-to-tissues other than the sites of action and organs of elimination is unnecessary, wasteful, and a potential cause of toxicity. The modification of the means of delivering the drug by projecting and preparing new advanced drug delivery devices can improve therapy. Since the 1960s, when silicone rubber was proposed as an implantable carrier for sustained delivery of low molecular weight drugs in animal tissues, various drug delivery systems have been developed.

At the beginning of the era of controlled drug delivery systems, a controlled release system utilizes a polymer matrix or pump as a rate-controlling device to deliver the drug in a fixed, predetermined pattern for a desired time period (4). These systems offered the following advantages compared to other methods of administration: (1) the possibility to maintain plasma drug levels a therapeutically desirable range, (2) the possibility to eliminate or reduce harmful side effects from systemic administration by local administration from a controlled release system, (3) drug administration may be improved and facilitated in underprivileged areas where good medical supervision is not available, (4) the administration of drugs with a short *in vivo* half-life may be greatly facilitated, (5) continuous small amounts of drug may be less painful than several large doses, (6) improvement of patient compliance, and (7) the use of drug delivery systems may result in a relatively less expensive product and less waste of the drug. The first generation of controlled delivery systems presented some disadvantages, that is possible toxicity, need for surgery to

implant the system, possible pain, and difficulty in shutting off release if necessary. Two types of diffusion-controlled systems have been developed. The reservoir is a core of drug surrounded with a polymer film. The matrix system is a polymeric bulk in which the drug is more or less uniformly distributed.

Pharmaceutical applications have been made in ocular disease with the Ocusert, a reservoir system for glaucoma therapy that is not widely used, and in contraception with four systems: (1) subdermal implants of nonbiodegradable polymers, such as Norplant (6 capsules of 36 mg levonorgestrel); (2) subdermal implant of biodegradable polymers; (3) steroid releasing intrauterine device (IUD); and (4) vaginal rings, which are silicone coated. Other applications have been made in the areas of dentistry, immunization, anticoagulation, cancer, narcotic antagonists, and insulin delivery. Transdermal delivery involves placing a polymeric system containing a contact adhesive on the skin.

Since the pioneering work in controlled drug delivery, it was demonstrated that when a pharmaceutical agent is encapsulated within, or attached to, a polymer or lipid, drug safety and efficacy may be greatly improved and new therapies are possible (5). This concept prompted active and intensive investigations for the design of degradable materials, intelligent delivery systems, and approaches for delivery through different portals in the body. Recent efforts have led to development of a new approach in the field of controlled drug delivery with the creation of responsive polymeric drug delivery systems (6). Such systems are capable of adjusting drug release rates in response to a physiological need. The release rate of these systems can be modulated by external stimuli or self-regulation process.

Different Approach for Controlled Drug Delivery

Localized Drug Delivery. In many cases, it would be desired to deliver drugs at a specific site inside the body to a particular diseased tissue or organ. This kind of regional therapy mechanism would reduce systemic toxicity and achieve peak drug level directly at the target site. A few examples of drugs that require this kind of therapy are anticancer drugs, antifertility agents, and antiinflammatory steroids. These drugs have many severe unintended side effects in addition to their therapeutic effects.

Targeted Drug Delivery. The best controlled mechanism would be delivery of drug exclusively to the targeted cells or cellular components. That means the development of delivery mechanisms that would equal or surpass the selectivity of naturally occurring effectors (e.g., peptide hormones). As in the case of hormone action, drug targeting would probably involve a recognition event between the drug carrier mechanism and specific receptors at the cell surface. The most obvious candidates for the targetable drug carriers are cell-type specific immunoglobulins. The concept of targeted drug delivery is different than localized drug delivery. The latter simply implies localization of the therapeutic agent at an organ or

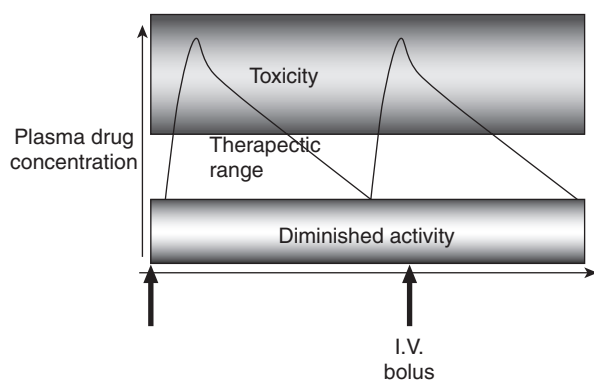


Figure 2. Plasma concentration versus time curve for intravenous (IV) drug administration showing first-order kinetics.

tissue site, while the former implies more subtle delivery to specific cell types.

Sustained Drug Delivery (Zero Order Release Profile).

Injected or ingested drugs follow first-order kinetics, with initial high blood levels of the drug after initial administration, followed by an exponential fall in blood concentration. Toxicity often occurs when blood levels peak, while efficacy of the drug diminishes as the drug levels fall below the therapeutic range. This profile is shown in Fig. 2. and the drug kinetics is undesirable, especially in the case where the margin between toxicity and required therapeutic concentration levels is small. The importance of controlled-release drug delivery systems may be argued with reference to the goal of achieving a continuous drug release profile consistent with zero-order kinetics, wherein blood levels of drugs would remain constant throughout the delivery period. The therapeutic advantages of continuous-release drug delivery systems are thus significant, and encompass: *in vivo* predictability of release rates on the basis of *in vitro* data; minimized peak plasma levels, and thereby reduced risk of toxic effects; predictable and extended duration of action; reduced inconvenience of frequent dosing, thereby improving patient compliance (7,8).

Figure 3 illustrates the constant plasma concentration that is desired for many therapeutic agents.

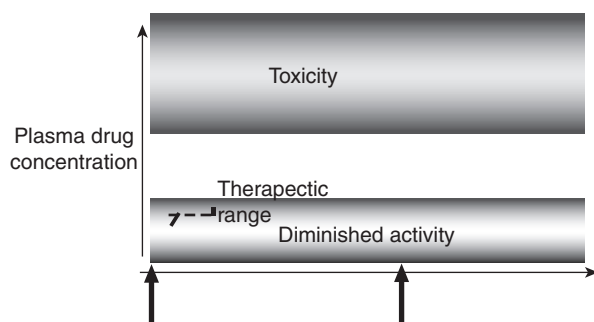


Figure 3. Plasma concentration versus time curve for sustained release profile of zero-order kinetics and pulsatile release profile.

The controlled release aspect of sustained drug delivery systems pertain to a reliable and reproducible system whose rate of drug delivery is independent of the environment in which it is placed. This requirement emphasizes the need for precision of control and elimination of undesired contribution associated with the drug delivery system.

Modulated Drug Delivery (Nonzero-Order Release Profile). A significant challenge in drug delivery is to create a delivery system that can achieve manipulable nonzero-order release profile. This could be pulsatile or ramp or some other pattern. In some cases it is also required that the release should be immediate. A pulsatile release profile within the therapeutic window is shown in Fig. 3.

Feedback Controlled Drug Delivery. The ideal drug delivery system is the feedback controlled drug delivery system that releases drug in response to a therapeutic marker. This can be classified into two classes: modulated and triggered device. A modulated device involves the ability to monitor the chemical environment and changes drug delivery rate continuously in response to the specific external marker, while in a triggered device no drug release takes place until it is triggered by a marker.

These different approaches of drug delivery can have different routes of administration. Some of the most preferred routes are oral, pulmonary inhalation, transdermal, transmucosal, and implantable systems.

Implantable Controlled Drug Delivery Devices.

Although most controlled drug delivery systems are designed for transdermal, subcutaneous, or intramuscular uses, implantable devices are very attractive for a number of classes of drugs, particularly those that cannot be delivered via the oral route or are irregularly absorbed via the gastrointestinal (GI) tract (9). Implantable systems are designed to deliver therapeutic agents into the bloodstream. This replaces the repeated insertion of IV catheters. The basic idea behind this device is simple: The treatment of certain diseases that require the chronic administration of drug could benefit from the presence of implantable devices. These systems can also be used to deliver drug to the optimum physiological site. These systems are particularly suited for drug delivery requirements of insulin, steroids, chemotherapeutics, antibiotics, analgesics, contraceptives, and heparin. Implantable systems are placed completely under the skin (usually in a convenient, but inconspicuous location). Benefits include the reduction of side effect (drug delivery rate within the therapeutic window) caused by traditional administration techniques, and better control. Ideally an implantable system will have a feedback controlled release mechanism and will be controlled by electronics with a long-life power source to achieve zero-order or manipulable nonzero-order release profiles in a manner similar to a physiological release profile.

The focus of this research is on two major requirements of an implantable controlled drug delivery device:

1. One of the major requirements for implantable drug delivery devices is to allow controlled-release of therapeutic agents, especially biological molecules, continuously over an extended period of time. The goal here is to achieve a continuous drug release profile consistent with zero-order kinetics where the concentration of drug in the blood remains constant throughout the delivery period. As mentioned earlier, the therapeutic advantages of continuous release of drug by implantable delivery devices are significant: minimized adverse reactions by reducing the peak levels, predictable and extended duration of action, reduced inconvenience of frequent dosing and thereby improved patient compliance.
2. The second, and more important requirement, is to achieve a manipulable nonzero-order release profile, such as pulsatile or any other pattern required for applications in therapeutic medicine. Vaccines and hormones are examples that require pulsatile delivery (10,11). Gonadotropin releasing hormone, for example, is most effective when delivered in a pulsatile manner to female patients undergoing treatment for infertility.

A sequence of two implantable systems was developed to achieve the above mentioned goals. The first device that addresses the first goal is named nanochannel delivery system 1 (or nDS1), while the device that addresses the second goal is called nanochannel delivery system 2 (or nDS2).

The Economics of Drug Delivery Devices

The fact that drug delivery technology can bring both therapeutic and commercial value to healthcare products cannot be neglected. Big pharmaceutical companies have recently started losing their market share to generic competitors after their patents expired, and therefore they have started recognizing the importance of drug delivery companies. Pharmaceutical companies are looking to extend their patents lifetimes by making strategic alliances with drug delivery technology companies, by presenting old drugs in new forms. Most of the drug delivery products therefore reach the market as a result of strategic alliance between drug delivery companies and pharmaceutical companies. Pharmaceutical companies provide the drug that may not be delivered efficaciously with a conventional delivery mechanism, while the drug delivery companies provide the cutting edge technology to administer the drug more effectively. The joint venture not only offers considerable advantages over the R&D efforts to bring new drug into the market as drug delivery systems provide means to reformulate existing products, but it also protects the drugs from erosion by generics in the case of patented drugs. As a result, drug delivery technology companies seem to enjoy a good return on their investments in the form of increased revenues and market share (9,12).

The global drug delivery market grew between 1998 and 2002, with a compound annual growth rate (CAGR) of 13.7%, increasing from \$39.6 billion to slightly > \$66 billion. The market is expected to grow at a slightly lower CAGR of 11.6% between 2002 and 2007 corresponding to a market value of \$114.3 billion by 2007. One of the contributing factors in this growth is the use of drug delivery systems as strategy to expand the shelf-life of products (particularly blockbusters), enabling pharmaceutical companies to sustain the revenue streams from their best sellers.

The largest market for drug delivery systems in the world is in the United States, having captured 47.9% of the global market's revenue generation in 2002. This figure is forecast to fall to 41.9% by 2007 although the U.S. market will retain its position as the leading market. The U.S. market for drug delivery systems was worth \$31.7 million in 2002, having experienced a CAGR of 12.6% during 1998–2002. Oral drug delivery systems had the largest market share, taking 47.7% of the total market share. Transmucosal, injectable, and implantable systems together had 8.8% of the market share in 2002. The U.S. market value for drug delivery systems is expected to grow at a rate of 8.5% annually, reaching a value of \$48 billion by 2007.

MICROELECTRO-MECHANICAL SYSTEMS

A number of devices have been developed to achieve controlled drug delivery. These devices utilize a different route of administration and different materials for device fabrication. Typically, each of these devices is targeted toward delivering one or a few of the therapeutics. The factors that need to be considered when designing a drug delivery device were previously discussed in great details (Fig. 1). This article begins with a brief history of implantable drug delivery devices. These include polymeric devices, osmotic pumps, micropumps, and microelectro-mechanical systems (MEMS) based devices. Since the drug delivery devices developed in this research are based upon MEMS technology, a good understanding of MEMS fabrication technology is needed, and therefore under the section MEMS for drug delivery devices, it is digressed from the topic implantable drug delivery devices and a more in-depth description on the use of MEMS for different drug delivery devices is presented. This includes MEMS for transdermal, oral, injectable, and *implantable* drug delivery. This article concludes with a critical analysis of implantable drug delivery devices.

A History of Implantable Drug Delivery Devices

The history of implantable devices goes back to May 1958 when the first implantable cardiac pacemaker was placed in an experimental animal (13). Later that year the first pacemaker was implanted in a human that operated for 3 h and then failed (14). The second unit operated for 8 h before failing, and the patient went unstimulated for 3 years before receiving a satisfactory implantable unit. The record shows that this patient was alive in 1991 and was using a pacemaker (15). The development of an implantable pacemaker revolutionized the field of biomedical science and

engineering over the last 30 years providing many different implantable biomedical devices to the medical professionals for therapeutic and diagnostic use. Today, implantable cardioverter-defibrillators, drug delivery systems, neurological stimulators, bone growth stimulators, and other implantable devices make possible the treatment, of a variety of diseases.

Extensive research has been done on implantable drug delivery devices over the last 30 years. Different technologies have been developed with many breakthroughs in clinical medicine. The first such device that saw extensive clinical use was reported in the 1970s (15–18). This system used a bellows-type pump activated by partially liquefied Freon. The Freon was reliquefied with each transcutaneous refill of the implantable device, and the administration was constant. Later, extensive research started to develop more sophisticated devices that could offer better control and more clinical options. Another device was developed by Medtronic Company that has a peristaltic pump to deliver the drugs (19). The device was controlled by electronics. Another system developed by MimiMed Technologies employs a solenoid pump, a reservoir, and advanced electronic control (20). The Infusaid Company developed an advanced programmable implantable pump that employed a bellows-type pump and a solenoid valve set to control drug flow (21). Other technologies developed to achieve this goal are summarized in the following sections.

Polymeric Implants. Polymers have been used extensively in controlled drug delivery systems. These can be classified as (1) nondegradable polymeric reservoirs and matrices, and (2) biodegradable polymeric devices. The first kind of polymeric devices are basically silicone elastomers. This kind of drug delivery system is based upon the research conducted in the 1960s, when researchers recognized that certain dye molecules could penetrate through the walls of silicone tubing (22–24). This led to the development of reservoir-based drug delivery system, which consisted of hollow polymer tubes filled with a drug suspension. The drug is released by dissolution into the polymer and then diffusion through the walls of the polymeric device. The two most commonly used nondegradable polymers are silicone and poly(ethylene-co-vinyl acetate) (EVAc). The Norplant 5 year contraceptive drug delivery system is based upon this technology. Some of the implantable reservoir systems are simple cylindrical reservoir surrounded by a polymeric membrane. The other variety in this first category is constructed of a solid matrix of nondegradable polymers. These systems are prepared by homogeneous dispersement of drug particles throughout the matrix (25). Drug release occurs by diffusion through the polymer matrix or by leaching or a combination of both (26). The matrix may be composed of either a lipophilic or hydrophilic polymer depending on the properties of the drug and the rate of release desired. However, it is difficult to achieve constant rates of drug release with nondegradable matrix systems, for example, the rate of release of carmustine from an EVAc matrix device drops continuously during incubation in buffered water (27). Constant release can sometimes be achieved by making the matrix as

a reservoir surrounded by a shell of rate-limiting polymeric membrane. In some cases, water soluble, cross-linked polymers can be used as matrices. Release is then activated by swelling of the polymer matrix after exposure to water (28). One other kind is a magnetically controlled system where magnetic beads are dispersed within the matrix (25). Drug is released by diffusion with a concentration gradient. The addition of an externally oscillating magnetic field causes the physical structure of the polymer to alter, creating new channels, and thus leading to further drug release.

Biodegradable polymeric devices are formed by physically entrapping drug molecules into matrices or microspheres. These polymers dissolve when implanted (injected) and release drugs. Examples of biodegradable polymers are poly(lactide-co-glycolide) (PLGA), and poly(*p*-carboxyphenoxypropane-co-sebacic acid) (PCPP-SA) (24). Some of the commercially available polymeric devices are Decapeptyl, Lupron Depot (microspheres), and Zoladex (cylindrical implants) for prostate cancer and Gliadel for recurrent malignant glioma. The half-life of therapeutics administered by microspheres is much longer than free drug injection. Polymers are also being investigated for treating brain tumors (29), and delivery of proteins and other macromolecules (30).

The above mentioned polymeric implants are utilized for sustained drug delivery. Methods have been developed to achieve controlled drug delivery profiles with implantable polymeric systems (31,32). These technologies include preprogrammed systems, as well as systems that are sensitive to (triggered or modulated by) modulated enzymatic or hydrolytic degradation, pH, magnetic fields, ultrasound, electric fields, temperature, light, and mechanical stimulation. Researchers are also exploring the use of nontraditional MEMS fabrication techniques and materials that could be used to form microwell- or microreservoir-based drug delivery devices. For example, microwells of varying sizes (as small as 3fL/well) have been fabricated by micromolding of poly(dimethylsiloxane) (PDMS) on a photoresist-coated silicon wafer that is photolithographically patterned (33).

Osmotic Pumps. Osmotic pumps are energy modulated devices (9). These are usually capsular in shape. When the system is exposed to an aqueous environment, such as that after subcutaneous implantation, water is drawn to the osmotically active agent through a semipermeable membrane and pressure is supplied to the collapsible drug reservoir and drug is released through an orifice with precise dimension. The delivery mechanism is dependent on the pressure created and is independent of drug properties. The ALZET pumps (only for investigational purpose at this time, not for humans) have been used in thousands of studies on the effects of controlled delivery of a wide range of experimental agents, including peptides, growth factors, cytokines, chemotherapeutic drugs, addictive drugs, hormones, steroids, and antibodies (34). The ALZA Corporation built the DUROS implant based upon the foundation of the ALZET osmotic pump, the system of choice for implant drug delivery in research laboratories around the world for > 20 years. Viadur, a once-yearly implant for the palliative treatment of advanced prostate cancer, is the first

approved product to incorporate ALZAs proprietary DUROS implant technology. A single Viadur implant continuously delivers precise levels of the peptide leuprolide for a period of 1 full year, providing an alternative to frequent leuprolide injections. Although most of the osmotic pumps are designed for sustained release profile, research is being conducted to modify this design for different patterns (9). Further, a catheter was attached to the exit port of an implantable osmotic pump to achieve site specific drug delivery at a location distant from site of implantation (35).

Micropumps. Micropumps have been actively investigated for drug delivery applications. Some micropumps are nonmechanical that utilizes electrohydrodynamic, electroosmotic, ultrasonic, or thermocapillary forces (36). However, most of the micropumps are mechanical, composed of mechanically moving membranes. A number of mechanical micropumps have been developed using various mechanisms, including piezoelectric (37), electrostatic (38), thermopneumatic (39), electromagnetic (40), bimetallic (41), shape memory alloy (SMA) (42), ionic conducting polymer films JCPF (43), and surface tension driven actuators (36). One example is the silicon piezoelectric micropump based on silicon bulk micromachining, silicon pyrex anodic bonding, and piezoelectric actuation (37). This can be used for application requiring low (typically $1 \mu\text{L} \cdot \text{min}^{-1}$), precisely controlled flow rate. The whole system includes the refillable reservoir, control, and telemetry electronics and battery. This can be implanted in the abdomen and a catheter can be brought to the specific site. The Synchro-Med pump is an implantable, programable, battery-powered device commercially available by Medtronic (44). A large number of other implantable drug delivery devices have been developed in last decade utilizing the silicon microfabrication technology that was developed in integrated circuits (ICs) industries.

MEMS for Drug Delivery

Since the invention of silicon microfabrication technology in early 1960s, the IC has changed our world. During last 40 years, the semiconductor industry has come up with a fastest growing industry in our history. From a modest beginning, which allowed few transistors on a chip, we have reached an integration level of tens of millions of components in a square centimeter of silicon. The minimum feature size on silicon is reducing and thus the number of devices per square centimeter is increasing. Since the observation made in 1965 by Gordon Moore (45), co-founder of Intel, the number of transistors per square inch on integrated circuits had doubled every year since the integrated circuit was invented. Moore predicted that this trend would continue for the foreseeable future. In subsequent years, the pace slowed down a bit, but data density has doubled approximately every 18 months, and this is the current definition of Moore's law.

This silicon fabrication technology was later extended to machining mechanical microdevices, which was later called MEMS. The pioneer work was done by Nathanson et al. in 1965 when they demonstrated the first micromachined structure to fabricate a free-standing gold beam electrode

used in a resonant gate transistor (46). By late 1970s, there was an immense interest in silicon as a mechanical material (47,48). During 1980s and 1990s, many MEMS devices were fabricated, for example, micrometers (49–51), deformable mirrors (52,53), accelerometers (54–58), and comb-drive actuators (59).

In recent years, this fabrication technology has been extensively used for the development of microfluidic devices for biological and biochemical applications (these are called bio-MEMS) (60,61). Further, the integration of microfluidic devices and integrated circuits over the last decade has revolutionized the chemical and biological analysis systems, and has opened the possibility of fabricating devices with increased functionality and complexity for these applications (62–64). These tiny devices hold promise for precision surgery with micrometer control, rapid screening of common diseases and genetic predispositions, and autonomous therapeutic management of allergies, pain and neurodegenerative diseases (7). The development of retinal implants to treat blindness (65), neural implants for stimulation and recording from the central nervous system (CNS) (66), and microneedles for painless vaccination (67), are examples in which MEMS technology has been used. With microfabrication technology it is also possible to produce the novel drug delivery modalities with capabilities not present in the current systems. A variety of microfabricated devices, such as microparticles, microneedles, microchips, nanoporous membranes, and micropumps, have been developed in recent years for drug delivery applications (68–71). This section reviews various microfabricated devices. These have been categorized and described below as microfabricated devices for transdermal, oral, IV, and implantable drug delivery devices.

Microneedles for Transdermal Drug Deliver. Transdermal drug delivery is probably the most favored way of drug delivery since it avoids any degradation of molecules in the GI tract and first-pass effects of the liver, both of which are associated with the oral drug delivery, and eliminates the pain associated with IV injection (72–76). However, the major barrier for the transdermal delivery is the stratum corneum, the outermost dead layer of the skin. In human, it is 10–20 μm thick. A number of different approaches have been studied with two common goals: first is to disrupt stratum corneum structure in order to create “holes” big enough for molecules to pass through and the second goal is to develop microneedles that are long enough to provide transport pathways across the stratum corneum and short enough to reach nerves found in deeper tissues. These approaches include chemical–lipid enhancers (77,78), electric fields employing iontophoresis and electroporation (79), and pressure waves generated by ultrasound or photoacoustic effects (80,81).

MEMS technology has provided an alternative approach to transdermal drug delivery. The development of microneedles for transdermal drug delivery enhances the poor permeability of the skin by creating microscale conduits for transport across the stratum corneum (69,76). Needles of micron dimensions can pierce into the skin surface to create holes large enough for molecules to enter, but small enough to avoid pain or significant damage.

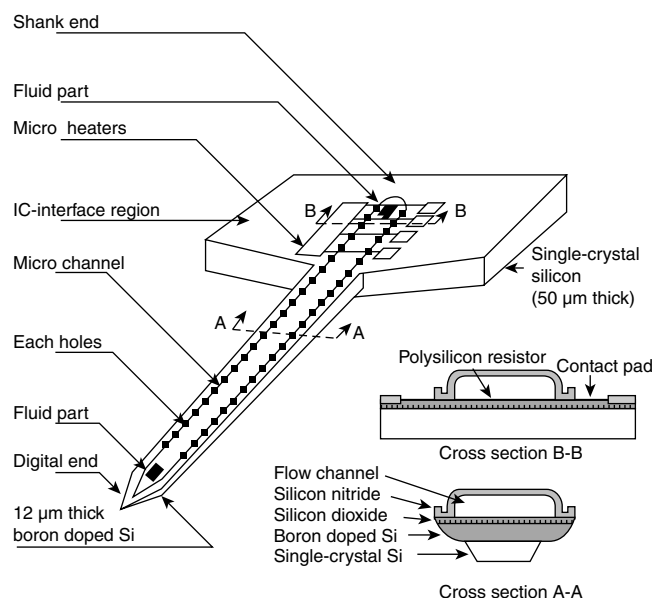


Figure 4. Schematic diagram of a silicon processed microneedles by Lin and Pisano (84).

Although the microneedles concept was proposed in the 1970s (82), it was not demonstrated experimentally until the 1990s (83). Since then, many different kinds of microneedles have been fabricated in several materials (e.g., silicon, glass, and metal). Further, these microneedles can be fabricated in-plane, where the needle lumen (flow channel) is parallel to the substrate surface, or out-of-plane, where the lumen is normal to the substrate. Some of these are summarized below.

Lin and Pisano (84) fabricated microneedles in silicon (Figs. 4 and 5). The primary structural material of these microneedles was silicon nitride, forming the top, and a bulk micromachined boron doped silicon base defined by etching the substrate in ethylenediamine pyrocatechol (EDP). This layer of silicon, which varied in thickness from $\sim 50\ \mu\text{m}$ at the shank to $12\ \mu\text{m}$ near the tip improved the structural strength. The lumen was defined by a sacrificial layer of phosphorous doped glass. These microneedles were 1–6 mm in length with lumens $9\ \mu\text{m}$ high and $30\text{--}50\ \mu\text{m}$ wide.

The proximal ends of the microstructures had integrated polycrystalline silicon heater strips. The heater could generate bubbles, which were useful in pumping fluid down the lumen. Authors suggested that electrodes could also be patterned along the length of the needle by a slight process modification for the measurement of neural activity.

Other microneedles made out of polysilicon molding process were reported by Talbot and Pisano (85) (Fig. 6). The two halves of the mold are produced by bulk micromachining of silicon wafers followed by deposition of a $2\ \mu\text{m}$ phosphosilicate glass (PSG) release layer. The two halves are temporarily bonded together under nitrogen ambience at 1000°C . After bonding, a $3\ \mu\text{m}$ layer of amorphous silicon is deposited by LPCVD through access holes in the top mold wafer. The mold along with the deposited film was then annealed at 1000°C . Deposition and annealing steps were repeated until the desired thickness of $12\text{--}18\ \mu\text{m}$ was

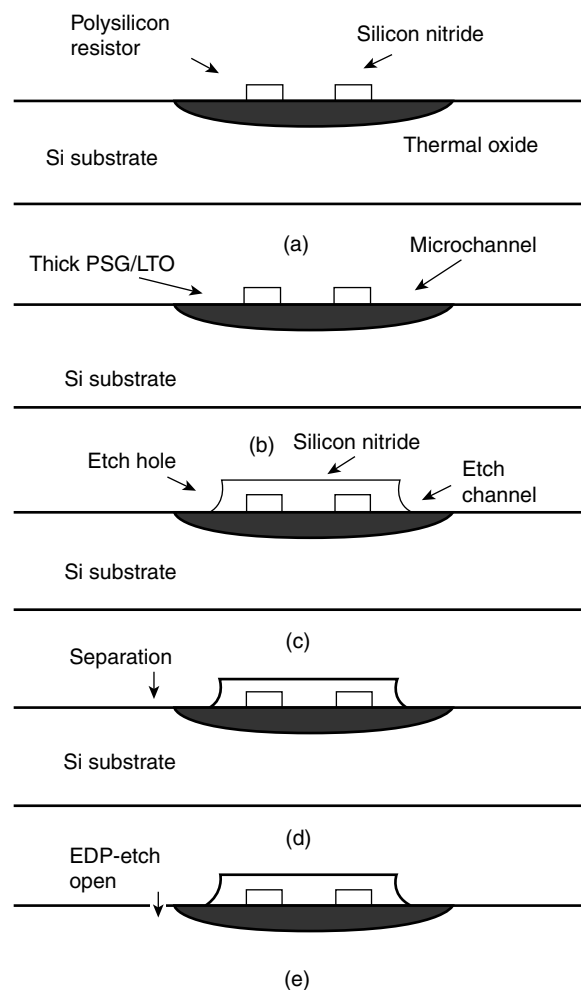


Figure 5. Process sequences of a silicon processed microneedles by Lin and Pissano (84).

obtained. Plasma etching was used to remove the polysilicon coating the funnel-shaped access holes in the top mold layer. The devices were released from the mold by etching in concentrated hydrofluoric acid, which selectively attacks the PSG. The mold could be used repeatedly by redepositing PSG, the release layer in order to minimize the cost. The resulting polysilicon microneedles are $1\text{--}7\ \mu\text{m}$ long, $110\text{--}200\ \mu\text{m}$ rectangular cross-section, and submicrometer tip radii.

Brazzle et al. (86–88) fabricated metal microneedles using a micromolding process. The fabrication process of the microneedles developed by Papautsky is shown in Fig. 7. AP+ etch stop layer was formed and backside anisotropic etching in KOH was performed to define a thin membrane. The lower wall of the microneedles consisted of deposited and patterned metal layers. A thick layer ($5\text{--}50\ \mu\text{m}$) of positive photoresist was then spin coated and lithographically patterned on the top of the lower metal walls.

The dimensions of this sacrificial layer precisely defined the cross-section of the lumen. After sputter deposition of a Pd seed layer, the thick metal structure walls and top of the microneedles were formed by electrodeposition. The sacrificial photoresist was removed with acetone and the P+

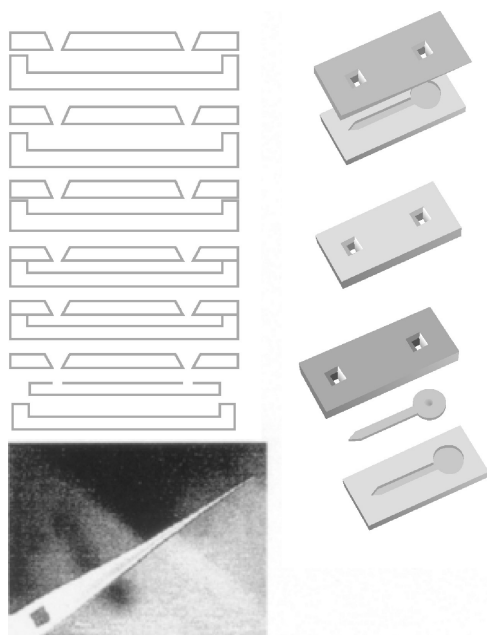


Figure 6. Microneedles fabricated from a polysilicon molding process using two silicon wafers (85).

membrane was etched away in an S176 plasma, resulting in a one-dimensional (1D) array of hollow microneedles released from the substrate.

Out-of-plane array of microneedles were fabricated by Stoeber and Liepmann (89,90). The fabrication process is summarized in Fig. 8. A double-sided polished wafer was oxidized. The lumen was etched through the wafer by plasma etching following a mask patterned at the backside. A silicon nitride film was then deposited across the backside and into the etched holes. Needle locations were photolithographically defined on the top surface on the wafer. The microneedle shaft was created by isotropic and etching on the silicon substrate. The isotropic etching forms a microneedle with a gradually increasing diameter along the shaft. By displacing the circular pattern for isotropic etching from the center of the lumen, a pointed needle shape was obtained. These microneedles were 200 μm tall, with a base diameter of 425 μm tapering to a 40 μm lumen. Individual needles were 750 μm apart. Fluid injection was demonstrated by delivering under the skin of a chicken thigh, a depth of $\sim 100 \mu\text{m}$.

Solid microneedles with no lumen were demonstrated by Henry et al. (76,91). The fabrication steps are shown in Fig. 9. A chrome mask was deposited on a silicon wafer and patterned into dots that have a diameter approximately equal to that of the base of the desired needles. A deep reactive ion etching was performed. Etching proceeded until the mask fell off from undercutting. The region protected by chromium remained and eventually became the microneedles. The tapering on the microneedles were controlled by adjusting the degree of anisotropy in the etch process. The resulting microneedles were 150 μm tall, and could be fabricated in dense arrays.

Gardeniers et al. (92) fabricated out-of-plane microneedles that employed reactive ion etching from both sides on

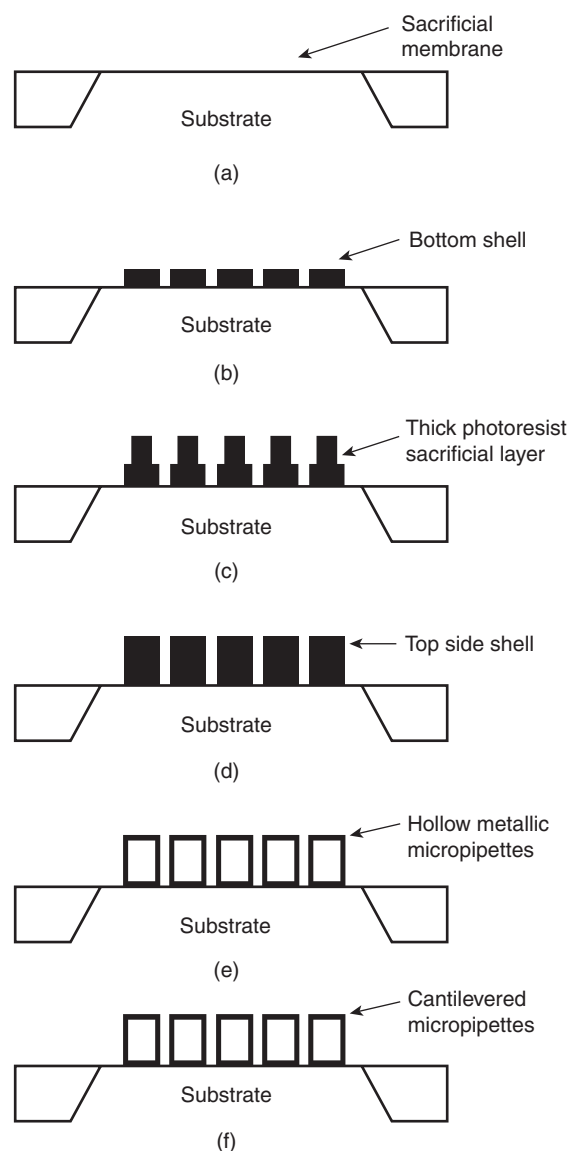


Figure 7. Fabrication process of a hollow in-plane microneedles (86).

a (100) oriented silicon wafer (Fig. 10). A hole (feature a in Fig. 10), which becomes lumen and a slot (feature b) that defines the position of the needles tip and needle sidewalls, was etched at the top surface. These structures were aligned to the crystallographic planes of silicon so that anisotropic etching performed later produces the slanted structure. The connecting lumen (feature c) was etched from the back side. The substrate, including the sidewalls of the etched features were coated with the chemically vapor deposited silicon nitride. The nitride was removed from the top surface of the wafer and etched in KOH. The etch left a structure defined by (111) plane in the areas where the nitride slot walls were concave, but where the mask was convex, the etch found all of the fast etching planes. The nitride mask was stripped at the end of the process.

Microneedles have also been developed for gene delivery. One such structure was fabricated by Dizon et al. (93).

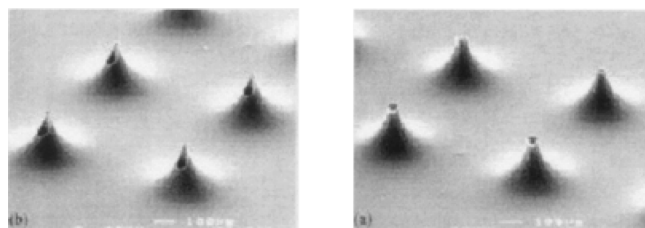
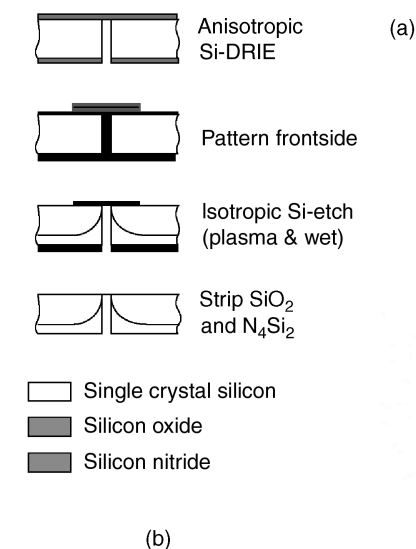


Figure 8. Out-of-plane array of microneedles. (a) Fabrication step, (b) Symmetric and asymmetric needles (90).

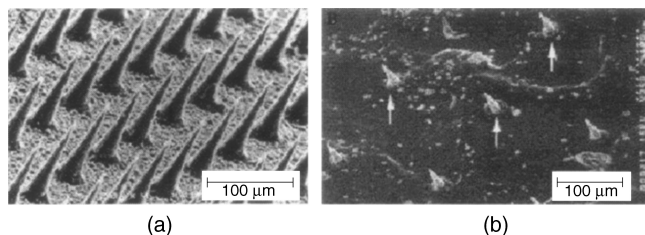


Figure 9. (a) Scanning electron micrograph (SEM) of microneedles made by reactive ion etching technique. (b) Micro-needle tips inserted across the epidermis. The underside of the epidermis is shown, indicating that the microneedles penetrated across the tissue and that the tips were not damaged. Arrows indicate some of the microneedle tips (91).

This structure was fabricated in dense array using a silicon bulk micromachining technique (Fig. 11), called Microprobes. The microprobes were $\sim 80 \mu\text{m}$ high topped by a wedge-shaped tip with a radius of curvature $< 0.1 \mu\text{m}$. The facets of the microstructure were fabricated utilizing fast etching (411) planes, produced by convex-corner undercutting in an anisotropic etching solution and a square mask. These microprobes can be coated with genes and pressed into cells or tissues. The sharp tips penetrate into cells and affect the transport of genetic material. Successful expression of foreign genes using this technique has been demonstrated in the nematode *Caenorhabditis elegans* (94), tobacco leaves (95), and mammals cells (96).

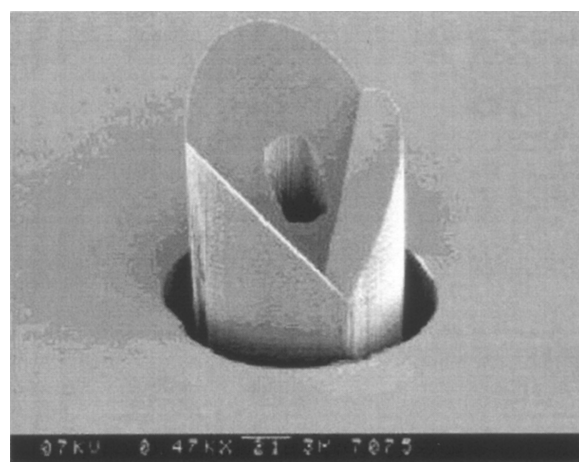


Figure 10. Out-of-plane microneedles were fabricated that employed reactive ion etching from both sides on a (100) silicon wafer (92).

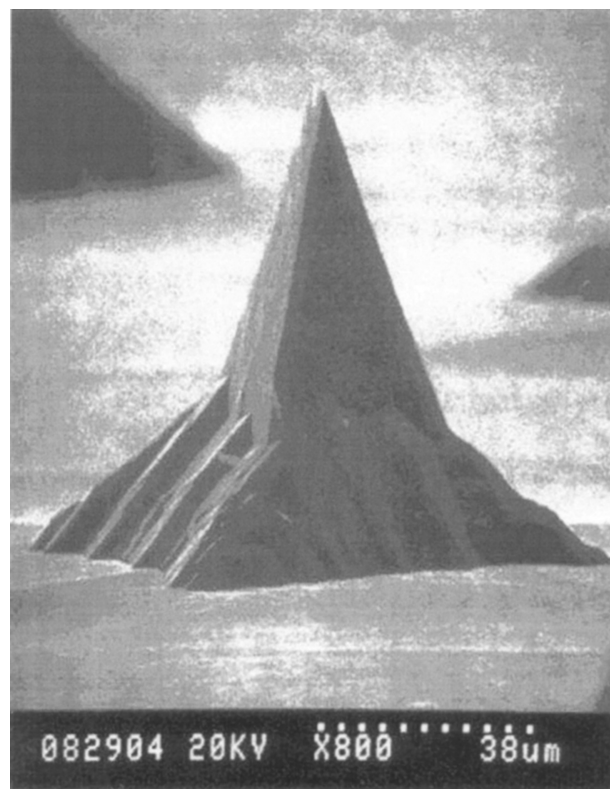


Figure 11. Solid silicon microprobe for gene delivery (93).

Miksza et al. (67) used silicon micromachining technology for DNA and vaccine delivery to the epidermis. Figure 12 shows the microstructure, which they call micro-enhancer arrays (MEAs), that was fabricated by isotropic chemical etching of silicon wafers.

On the whole, existing microneedle-based drug delivery devices offer several advantages, such as the ability to inject drugs directly through the stratum corneum at reproducible and accurate depth of penetration, minimal

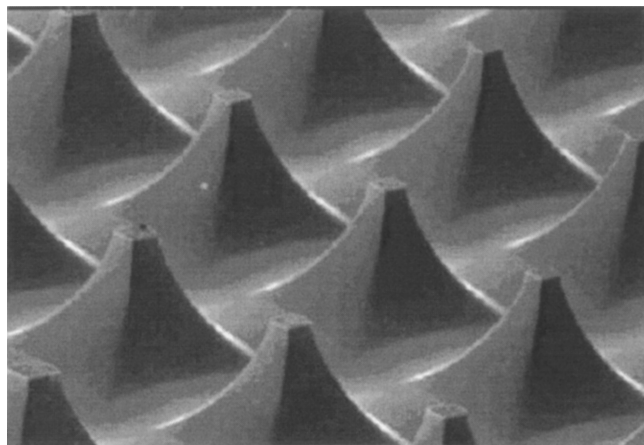


Figure 12. Silicon microenhancer arrays (MEAs) for DNA and vaccine delivery (67).

pain, and on-board ability to probe or sample the same device. Nevertheless, local irritation and low mechanical stability are some of the potential drawbacks that demand further investigation for alternate fabrication techniques and materials. Furthermore, improved fluid flow models that determine the most effective structural, fluidic, and biological design considerations for a given delivery application continue to be required.

Microparticles for Oral Drug Delivery. Oral route is a preferred method of drug delivery because of its ease of administration and better patient compliance. However, oral delivery of peptides and proteins has remained an illusive goal to date. The two main reasons why it is currently impossible are (1) destruction or inactivation due to enzymatic action, and the acidity of the upper GI tract; and (2) physiological permeation barrier, opposing penetration of large biological molecules through intestinal walls (71). These are mucosal layers and the tight junctions connecting intestinal epithelial cells, which restrict the possible passageways to be transcellular, and thus expose the diffusing biomolecule to enzymatic degradation. This method of drug delivery, therefore, leads to unacceptably low oral bioavailability. Consequently, various approaches based on the use of protective coatings (97), targeted delivery (98), permeation enhancers (99), protease inhibitors (100), and bioadhesive agents (101–103) have been explored in recent years. While all of these methods have been shown to increase the oral bioavailability of drug molecules, none of them offer a complete solution for adequate and safe oral delivery of peptides and proteins.

Microfabrication technology may address the shortcomings of the current oral drug delivery systems by combining the aforementioned approaches in a single drug delivery platform. Fabrication of microparticles of silicon and silicon dioxide has been conceptualized and demonstrated to achieve this (104–106). Unlike other spherical drug delivery particles, microfabricated devices may be designed to be flat, thin, and disk-shaped to maximize contact area with the intestinal lining and minimize the side areas exposed to the constant flow of liquids through the

intestines (107). The size of the particles (within thickness of 0.1–5 nm and diameters of 1–100 μm) can be selected to have good contact with the undulations of the intestinal wall and large enough to avoid endocytosis of the entire particle. Permeation enhancers, such as bile salts and metal chelating agents, can be added to loosen the tight junctions of the intestinal epithelium. Aprotinin, or other enzyme inhibitors, can also be added to protect the macromolecule from intestinal degradation. In addition, one can selectively attach bioadhesive agents onto the device surface using relatively simple surface chemical modification strategies. By replacing the specific markers attached to the microparticles, specific cell types and tissues can be targeted for therapy as well as imaging. This would allow for the high concentration of drug to be locally delivered while keeping the systemic concentration at a low level. Finally, these devices can have multiple reservoirs of desired size to contain not just one, but also many drugs–biomolecules of interest (108).

iMEDD Inc. in collaboration with Ferrari et al. (109) developed Oral MEDDS (Oral Micro-Engineered Delivery Devices), novel porous silicon particles that can be used as oral drug delivery vehicles. The microparticle dimensions ranged from $150 \times 150 \times 25$ – $240 \times 240 \times 25 \mu\text{m}$ with a pore distribution of 20–100 nm (Fig. 13). Once prepared, the particles could be loaded with a liquid drug formulation through simple capillary action. Interstitial air is removed by vacuum aspiration, and the formulation is dried completely using vacuum or freeze-drying. OralMEDDS particles have been designed to target intestinal epithelial cells, adhere to the apical cell surface, and deliver a drug formulation containing a permeation enhancer that would open the local tight junctions of the paracellular transport pathway. The absorption of macromolecules and hydrophilic drugs, which are unable to undergo transcellular transport across lipid membranes, is largely restricted to this paracellular route. Therefore, the intestinal absorption of orally administered water-soluble drugs can be greatly enhanced through the utilization of OralMEDDS particles (110).

Micromachined silicon dioxide and PMMA microparticles designed by Desai and co-workers (70,111) can be best described as microparticles with reservoirs (Figs. 14 and 15). These microparticles are adaptable for use as a bioadhesive controlled release oral drug delivery system. Silicon dioxide microparticles were created by growing a thermal oxide under wet conditions followed by low pressure chemical vapor deposition to deposit a sacrificial layer of

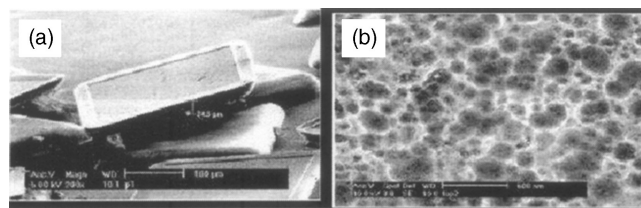


Figure 13. Scanning electron microscopy images of a porous silicon particle: (a) Demonstrating the thickness. (b) Particle demonstrating the pore size distribution of ~20–100 nm (110).

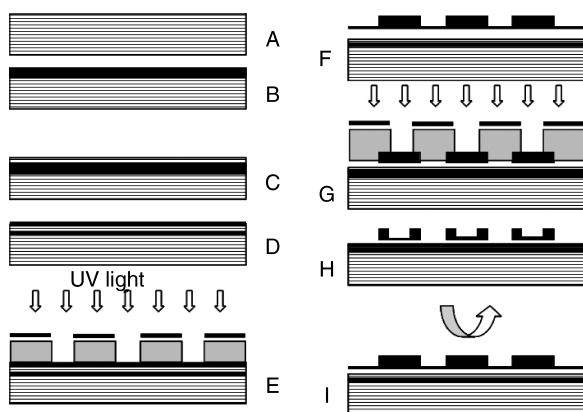
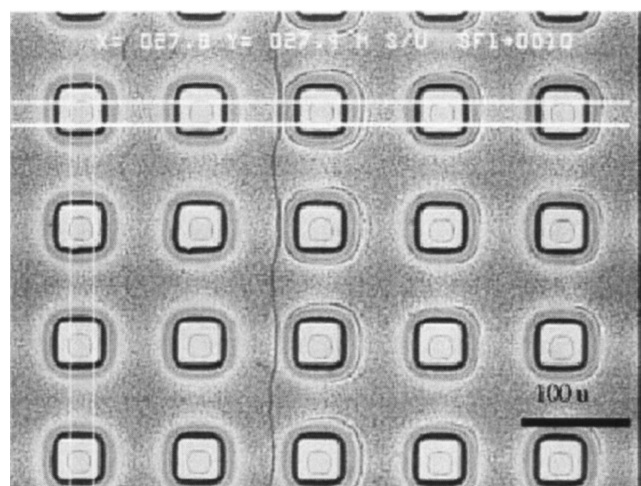


Figure 14. Process flow of the silicon dioxide microparticles (111).

(a)



(b)

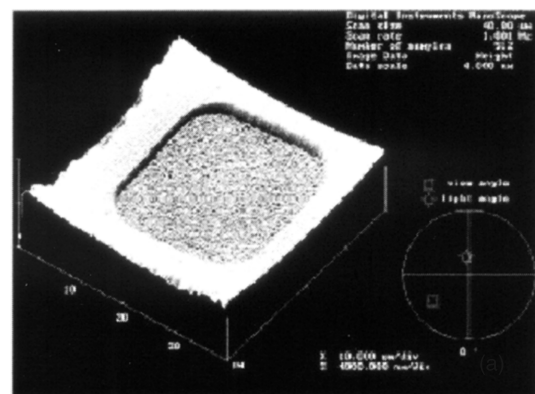


Figure 15. (a) 50 μ particles with 25 μ reservoirs. (b) AFM image of the particles (25 reservoir, 50 m particles) (111).

polycrystalline silicon (111). Next, a layer of low temperature silicon dioxide (LTO) was deposited to form the device layer. Positive lithography was carried out to define the shape of the device reservoir. A reactive ion etch (RIE) with S176 and O2 was used to fabricate the actual reservoir in the LTO device layer and any remaining photoresist was

then removed in negative photoresist remover. Negative lithography was carried out to define the device bodies. Reservoir features on the mask were aligned to the photo-mask features using front-side alignment. The unmasked area of the LTO layer was etched using RIE and the remaining photoresist was removed. These microdevices were then released into KOH solution by etching the sacrificial polysilicon layer. The particles were uniform and semitransparent due to their polycrystalline nature. Later, a lectin–biotin–avidin complex suited for binding these microparticles to the intestinal mucosa was developed. The Caco-2 cell line was used to examine the bioadhesive properties of microparticles *in vitro*. Bioadhesive silicon dioxide microparticles demonstrated greater adherence to Caco-2 cells as compared to unmodified particles.

Poly(methyl methacrylate) (PMMA) particles were fabricated by spinning PMMA (device layer) on to a clean silicon wafer (70). Positive lithography was carried out to define the device bodies followed by a reactive ion etching to carve the devices. Then a second mask positive photolithography was carried out to carve the device reservoir. The process flow of the device fabrication is shown in Fig. 16. The dimensions of the reservoir can be altered by changing the masked area and their depth can be modified by changing the time and/or flow rate of plasma in the RIE. By creating smaller reservoirs, a series of multiple reservoirs can be etched into the particles to create separate reservoirs for a combination of drugs or permeation enhancers. Since the PMMA is adherent to the surface of silicon by linkage to the native oxide layer, the wafer was soaked in basic solution to break this bonding and immediately release the particles. Bioadhesive properties were introduced to microfabricated PMMA microdevices by attachment of lectins, a group of proteins capable of specifically targeting cells in the GI tract. In this process, the PMMA microdevices were chemically modified by aminolysis to yield amine-terminated surfaces. Avidin molecules were covalently bound to the surface of the particles using a hydroxysuccinimide-catalyzed carbodiimide reagent and then incubated in an aqueous solution of biotinylated lectin. The bioadhesive characteristics of lectin-modified microdevices were successfully demonstrated *in vitro*.

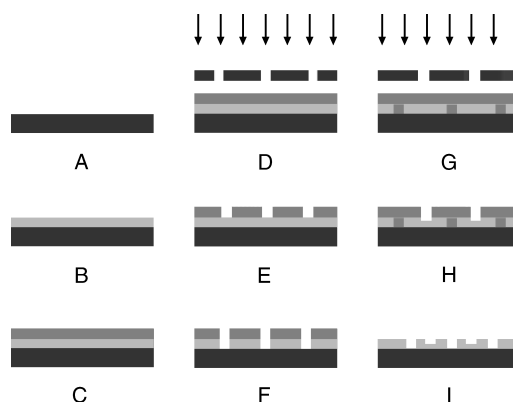


Figure 16. Process flow of PMMA microdevices with reservoir for oral drug delivery (70).

Microparticles for Intravenous Drug Delivery. The same microfabrication technology that has been used quite extensively for the fabrication of particles for oral drug delivery can be employed to develop precisely sized and shaped microparticles with high specific targeting abilities for IV delivery, especially for the treatment of diseases where oral and transdermal delivery are not effective. As an example, systemic chemotherapy using cytotoxic or biological treatment is the only treatment available for many patients with advanced metastatic cancer. While many tumors respond to initial courses of chemotherapy, after multiple courses and drugs, cancer cells become resistant to further therapy. In addition, growth of metastatic tumors is supported by factors, that are secreted by tumor cells themselves and cause angiogenic leaky vessels to grow. One strategy for preventing or treating metastatic tumors is to intervene in the process of angiogenesis by destroying the blood vessels that supply tumor cells rather than the tumor cells themselves (112). In such cases, precisely sized and shaped microparticles especially designed for IV delivery of cytotoxic biomolecules—drugs to the microvasculature of tumors with an improved safety profile could be employed. These have been described below.

Nonporous Microparticles. First generation of nonporous (solid) microparticles of silicon and silicon dioxide suitable for IV drug delivery (16,113), were rectangular shaped with thickness of $0.9\ \mu\text{m}$, and varied from 1 to $3\ \mu\text{m}$ in length and width (Fig. 17). These microparticles were treated with amino- and mercaptosilanes, followed by coupling to human antibody (IgG) by using the heterobifunctional cross-linker succinimidyl 4-(*N*-maleimidomethyl)-cyclohexane-1-carboxylate, to demonstrate their capability toward specific attachment of bioadhesive agents. These solid microparticles and their next generations are currently being explored for drug delivery and bioimaging applications (114).

Nanoporous Microparticles. Currently, porous silicon has begun to receive significant attention for biomedical usage. Nano- and microparticulates of this material have immense potential to be clinically and diagnostically significant both *in vivo* and *ex vivo* (115,116). Li et al. (113) demonstrated the incorporation, characterization, and release of cisplatin [*cis*-diammine dichloroplatinum(II)],

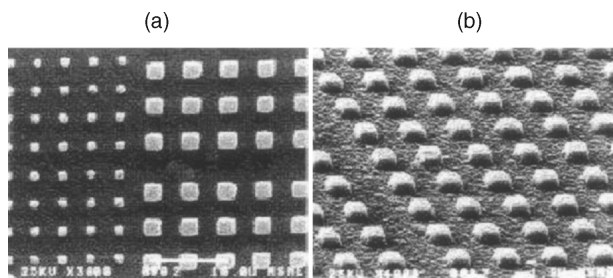


Figure 17. Scanning electron micrographs of microparticles. (a) Dimensions are $2.2 \times 2.1 (\pm 0.1)\ \text{mm}$ for the larger particles, and $1.2 \times 1.1 (\pm 0.05)\ \text{mm}$ for the smaller ones. (b) Shows tilted view of larger microparticles (104).

carboplatin [*cis*-diammine (cyclobutane-1,1-dicarboxylato) platinum(II)], and Pt(en)C12 [ethylenediamminedichloro platinum(II)] within layers of calcium phosphate on porous Si-Si substrates for bone cancer treatment.

Superior control over particle dimensions, pore size, pore shape, and loading capacity is critical for microparticles for IR drug delivery (17,117). iMEDD Inc. has developed nanoporous microparticles (called IV-MEDDS or NK-MEDDS, where NK denotes the fact that the particles mimic Natural Killer cells) to treat systemically accessible solid tumors, specifically the multiple lesion sites associated with metastatic disease (71). The approach here is to kill the circulatory accessible endothelial cells that support the existing tumor capillaries using micromachined asymmetrical particles, that is, the top face of the particle contains a pore loaded with cytotoxic drugs, which is plugged with an erodible gelatinous material and layered with chemically grafted ligand (including growth factors, e.g., FGF, EGF and VEGF to bind endothelial or tumor cell receptors or folate and tumor-targeting RGD peptides to bind $\alpha_v\beta_3$ with high affinity) for targeting and protection. Designed to mimic the behavior of NK cells, a potent cytolytic agent, such as bee venom-derived melittin, can be plugged with a material designed to erode in 1–48 h. After injection, the particles circulate within the bloodstream for several minutes to several hours after that they are removed from the body's immune system. Bound particles should release their contents in the vicinity of the tumors and cause lysis and death of the target endothelial cells. Melittin peptides released by particles elsewhere in the body and not bound to endothelial target, are inactivated by binding to albumin and thus are not toxic to normal cells (71).

Based on the above-mentioned concept, Cohen et al. (118) prepared micron-sized particles with nanometer-sized pores out of porous silicon and porous silicon dioxide. The fabrication steps are shown in Figs. 18–20. The particles were fabricated with precise shapes and sizes. The size and thickness of these particles could be altered by changing the dimensions of the photolithography mask, the anodization time, and the electropolishing time. The

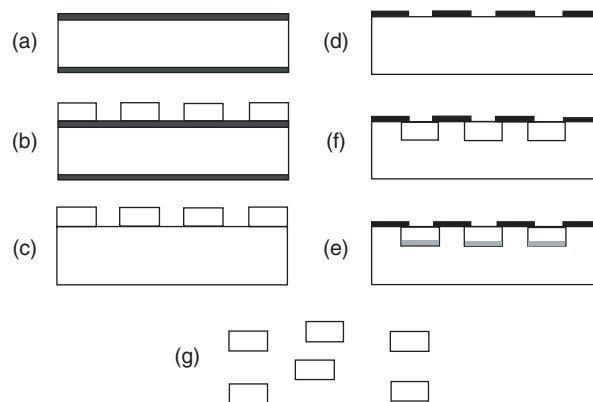


Figure 18. Fabrication details for porous silicon particles. (a) LPCVD silicon nitride deposition. (b) Photolithography. (c) Dry etch silicon nitride. (d) Piranha. (e) Anodization of silicon. (f) Electropolishing. (g) Particle release (118).

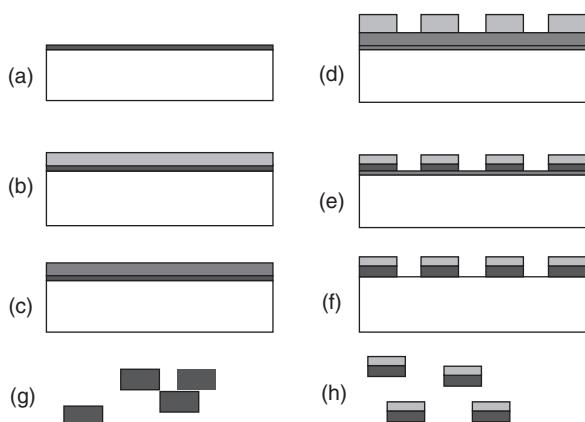


Figure 19. Process flow of porous SiO_2 particle fabrication. (a) Aluminum deposition. (b) Spun on mesoporous oxide film. (c) Baked mesoporous oxide film. (d) Photolithography. (f) Particle release in pirana. (g) Uncapped particles. (h) Particles capped with photoresist (118).

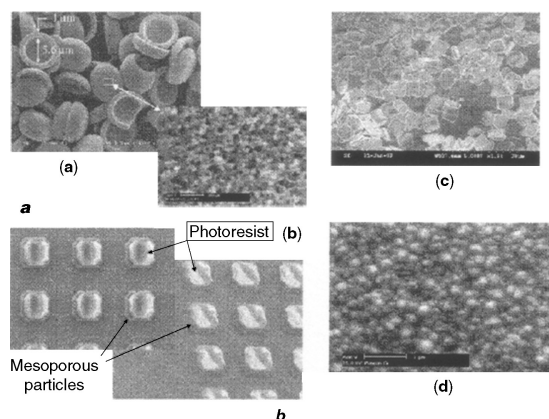


Figure 20. (a) SEM images of released porous silicon particles: Top image shows the shape and size of the particles. Bottom image demonstrates pores in the size range of 20–100 nm. (b) SEM images of mesoporous silicon oxide particles on wafer: (a) flat. (b) 45 tilt. (c) SEM images of released porous silicon dioxide particles. (d) SEM images of released porous silicon particles (118).

porous silicon dioxide particles were $4.7 \mu\text{m}$ squares with a thickness of $1.0 \mu\text{m}$. The porosity of silicon dioxide particles was 52.5%. In order to determine safe particle size and concentration for IV drug delivery, a safety study was performed using solid silicon particles with various shapes, squares and circles, and varying sizes, 2, 5, and $10 \mu\text{m}$. Results indicated that at concentrations of 1×10^7 particles per mouse, particles of size 2 and $5 \mu\text{m}$ safely circulate throughout the vasculature. No mice survived for any length of time when they were injected with $10 \mu\text{m}$ particles. Work is underway to demonstrate the coupling of EGF to porous dioxide particles that will allow for the particles to bind to the cells that express EGF receptors.

Smith et al. (114) prepared novel, controllably dual-sided, symmetric particulates of porous silicon from a polysilicon precursor. These particulates are precisely monodisperse on the scale of $1 \mu\text{m}$ (diameter and thickness) and may enable

unidirectional flow of transported drugs, proteins–peptides, nucleic acids, and so on. They may also facilitate controllably different intraparticle surface chemistries, and therefore potentially different types of antibodies, proteins, and so on, can be present on the same particle.

MEMS for Implantable Drug Delivery Devices. Implantable devices are preferred for the therapies that require many injections daily or weekly. The requirement and advantages of an implantable drug delivery device has been discussed above in greater detail. These devices can either be implanted into the human body or placed under the skin, consequently reducing the risk of infection by eliminating the need for frequent injections. Most of the implantable microsystems are expected not to cause pain or tissue trauma owing to their small size and are often virtually invisible. The advances in microfabricated implantable drug delivery device have been reviewed below.

Microreservoirs. Silicon microfabrication technology has been used to develop drug delivery device consisting of an array of microreservoirs (68,119,120) (Fig. 21). This device is currently being developed by MicroCHIPS, Inc., for use as external and implantable systems for the delivery of proteins, hormones, pain medications, and other pharmaceutical compounds (117). Each dosage is contained in a separate reservoir that is covered with a gold membrane. The membrane gets dissolved in the presence of chloride ions when anodic voltage is applied to the membrane of interest. This causes the membrane to weaken and rupture, allowing the drug within the reservoir to dissolve and diffuse into the surrounding tissues. This device allows the release of a potent substance in a pulsatile manner. Each microreservoir can be individually filled, so multiple substances can be delivered from a single MEMS device. Release of fluorescent dye and radiolabeled compounds has been demonstrated from these microreservoir devices *in vitro* in saline solution and serum (68).

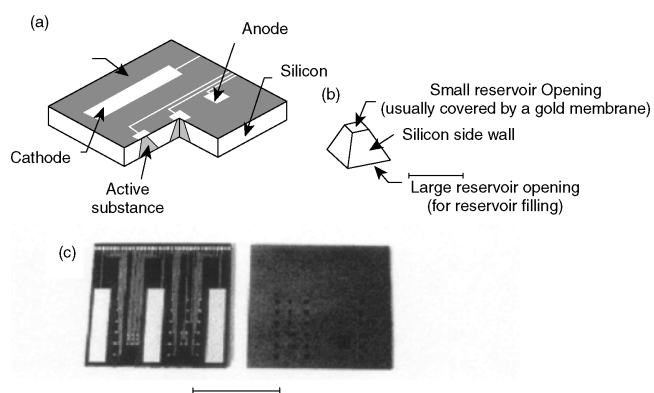


Figure 21. A schematic of a silicon microchip for controlled release. (a) Cut-away section showing anodes, cathodes, and reservoirs. (b) Shape of an individual reservoir. (c) Photograph of a prototype microchip: the electrode-containing frontside and the backside with openings for filling the reservoirs (15).

The release studies from this device demonstrated that the activation of each reservoir could be controlled individually, creating a possibility for achieving many complex release patterns. Varying amounts of chemical substances in solid, liquid, or gel form could be released into solution in either a pulsatile, a continuous, or a combination of both manners, either sequentially or simultaneously from a single device. Such a device has additional potential advantages including small size, quick response times, and low power consumption. In addition, all chemical substances to be released are stored in the reservoirs of the device itself, creating a possibility for the future development of autonomous devices. A microbattery, multiplexing circuitry, and memory could be integrated directly onto the device, allowing the entire device to be mounted onto the tip of a small probe, implanted, swallowed, integrated with microfluidic components to develop a laboratory-on-a-chip, or incorporated into a standard electronic package, depending on the particular application. Proper selection of biocompatible device materials may result in the development of an autonomous, controlled-release implant or a highly controllable tablet for drug delivery applications (68).

Nanoporous Silicon Membranes. Silicon nanopore membranes were developed by Ferrari and co-workers for application as immunoisolating biocapsules, and for molecular filtration (121–123). These membranes were shown to be sufficiently permeable to oxygen, insulin, and glucose, while at the same time impermeable to larger proteins, such as immunoglobulin G (IgG), which might lead to destruction of the transplanted cells (124). Since the diffusion through these membranes is linear, they can also be used for sustained drug delivery. This is currently being developed by iMEDD, Inc. (71,109). Over the years, nanopore technology has undergone continued improvements. Nevertheless, the basic structure and fabrication protocol for the nanopores has remained the same. The membrane area is made of thin layers of polysilicon, silicon dioxide, and/or single crystalline silicon depending on the design employed. The strategy used to make nano-size pores was based on the use of a sacrificial oxide layer sandwiched between two structural layers, for the definition of the pore pathways. The first design of nanoporous membranes consisted of a bilayer of polysilicon with L-shaped pore paths. The flow path of fluids and particles through the membrane is shown in (Fig. 22a) (125). As shown, fluid enters the pores through openings in the top polysilicon layer, travel laterally through the pores, make a 90° turn, and exit the pores through the bottom of the pore where both the top and bottom polysilicon layers lay on the etch stop layer. While this design performed well for preventing the diffusion of the larger, unwanted immune system molecules, its L-shaped path slowed down and, in some cases, prevented the diffusion of the smaller molecules of interest. The pores in this design were fairly long, which led to the slow diffusion of the desired molecules. Also, because of the large area per pore, it was difficult to increase the pore density and thus the diffusion rate. The next design had an improvement in the production of short, straight, vertical pores through a single-crystal base layer (Fig. 22b and c). This design had the advantage of direct flow paths. This

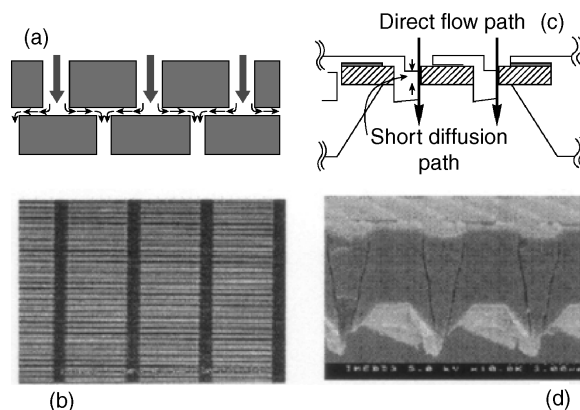


Figure 22. (a) Flow path through MI filters, with lateral diffusion through the nanopores defined by sacrificial oxide. (b) Cross-section of M2 design showing direct flow path. Scanning electron micrographs of microfabricated membrane: (c) top view detail; (d) side view detail (126).

direct path allows the smaller molecules of interest to diffuse much quicker through the membrane, while still size-separating the larger molecules. To further improve the reliability of the nanoporous membranes, several basic changes were made in the fabrication protocol from the previous membrane design to eliminate problems with the diffused etch stop layer (126). This design also incorporated a shorter diffusion path length, based on the thicknesses of the two structural layers. The design of a new membrane fabrication protocol incorporated several desired improvements: a well-defined etch stop layer, precise control of pore dimensions, and a lower stress state in the membrane. The new protocol also increased the exposed pore area of the membranes. The nanoporous membranes have been studied extensively for the use of drug delivery and the results are very encouraging.

Zero-Order Kinetics through Nanoporous Membrane.

In vitro bovine serum albumin (BSA) release data through 13 nm pore is shown in Fig. 23. The experimental results show zero-order release profile (zero-order kinetics). Note that the zero-order kinetics does not follow Fick's law. Fick's laws are usually adequate to describe diffusion kinetics of solutes from a region of higher concentration to a region of lower concentration through a thin, semi-permeable membrane. But, when the size of the membrane pores approaches that of the solute, an unexpected effect may occur, which deviate substantially from those predicted by Fick's laws. Diffusion of molecules in microporous media, such as zeolites, has led to experimental evidence of such unusual phenomena as molecular traffic control and single file diffusion (SFD) (127,128). Theoretical treatments and simulations suggest that in the case of SFD, solute molecules of equal size cannot pass each other in pores that approximate the dimensions of the molecule itself, regardless of the influence of concentration gradient, and thus their initial rate of movement (or flux) is underestimated by Fick's law (129–133).

The microfabricated nanopore channels are of molecular size in 1D, and therefore non-Fickian diffusion kinetics

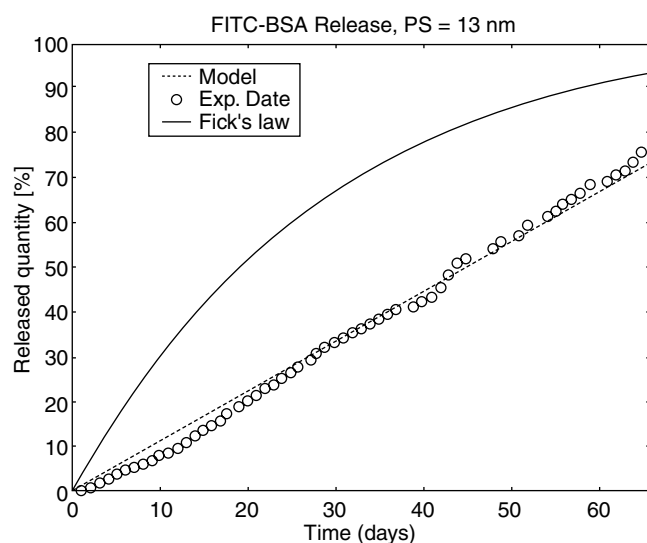


Figure 23. *In vitro* diffusion kinetics of fluorescein isothiocyanate (FITC) labeled BSA through 13 nm pore size: experimental data (o), Fick's law prediction (—), model-based simulation (---).

is observed. The observations are consistent with the diffusion reported for colloidal particles confined in closed 1D channels of micrometer scale where particle self-diffusion is non-Fickian for long time periods and the distribution of particle displacements is a Gaussian function (128). Zero-order flux is observed when a chamber filled with a solute is separated from a solute-free external medium by channels that are only several times wider than the hydrodynamic diameter of the individual molecules. The basic principle of diffusion as a mixing process with solutes free to undergo Brownian motion in three dimensions (3D) does not apply since in at least 1D solute movement within the nanopore is physically constrained by the channel walls. Experimental observations of colloidal particles in a density matched fluid confined between two flat plates reveal that particle diffusion becomes anisotropic near the interface; in this case leading to hindered diffusion as a consequence of constrained Brownian motion and hydrodynamic drag effects at distances close to the walls (134). In the case of nanoporous membranes, it is not entirely certain that the ordering of solutes imposed by the nanopore geometry will be as strict as true cylindrical pores, nor that the sequence of particles passing through the nanopores under the influence of the concentration gradient will remain unchanged over the time required to travel the 4 m length of the channel; particles could conceivably pass each other laterally. Whether a consequence of a SFD-like phenomenon or drag effects (or a combination of both), the nanopore membrane is rate limiting and, if properly tuned, restricts solute diffusion to a point that flux rates across the membrane are entirely independent of concentration gradient and follow zero-order kinetics.

In order to achieve further insight in the mechanisms involved in nanochannel diffusion, an experimental phenomenon in mathematical terms, thus yielding to the creation of a dynamical model, which makes it possible to simulate the diffusion experiments and fit the related data,

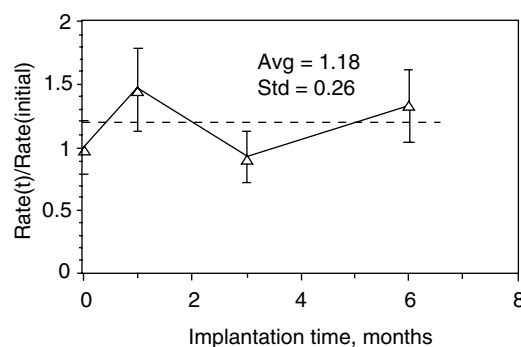


Figure 24. Ratio of post-preimplantation glucose diffusion rates.

is being investigated. A detailed description of such model is presented in Ref. 135.

Biocompatibility of Nanoporous Membranes. *In vivo* membrane biocompatibility was evaluated using glucose as a model molecule. Figure 24 shows the ratio of post-explantation glucose diffusion rate compared to its initial value. There was no noticeable change in glucose diffusion rates pre- and postimplantation illustrating that the silicon membranes did not foul over a 6-month implantation period. The membrane was placed on a titanium capsule and the entire assembly was placed subcutaneously in mice. The assembly was removed after 7 days and examined visually. There was no visible evidence of tissue binding to the surface. Figure 25 shows a photograph of the implant site after 30 days of implantation. As can be seen, only a thin vascular capsule forms around the implant as opposed to the avascular fibrous capsule. This minimal tissue response is supposed to be responsible for the comparable pre- and postimplantation glucose diffusion rates observed in this investigation.

Sandwich Design Filter. Nanochannels fabricated between two directly bonded silicon wafers were also developed for the applications as immunoisolating biocapsules, and molecular filtration (125,136–139). These devices possess high mechanical strength since the filtration occurs at the interface of two bonded silicon wafers instead of through a 1–10 μm thick membrane (in the case of silicon nanopores membrane). Well-developed bulk microfabrication



Figure 25. Photograph of implantation site after 30 days *in vivo*.

technology was used to fabricate these devices. With the use of a silicon dioxide sacrificial layer, pore sizes as small as 40 nm were fabricated with size variations < 4%. It was already established in the case of silicon nanopore membranes that the diffusion of molecules through nanopores is constant, and therefore the sandwich design filter can also be used for sustained drug delivery applications.

MOLECULAR DRUG DELIVERY SYSTEMS

This type of carrier, including cryptands, calixarenes (140), cyclophanes (141), spherands, cyclodextrins, and crown ethers, carry out chemical reactions that involve all intramolecular interactions where covalent bonds are not formed between interacting molecules, ions or radicals. Most of these reactions are of host–guest type.

Cyclodextrins: General Information

Between the several drug delivery systems, the molecular carrier have aroused great interest in the scientific world. Compared to all the molecular hosts mentioned above, cyclodextrins (CDs) are most important. As a result of molecular complexation phenomenon CDs are widely used in many industrial products, technologies, and analytical methods.

The CDs represent the more important molecular carrier today, in fact they are already strongly present in commerce for various types of drugs. Cyclodextrins have been discovered in the nineteenth century. They were produced for the first time by Villiers in 1881 by digesting the starch with *Bacillus amylobacter*, but only in 1903 was the cyclic structure of these compounds demonstrated by Schardinger.

Chemically, CDs are cyclic oligosaccharides, consisting of (α -1,4)-linked α -D-glucopyranose units. They are produced as a result of an intramolecular chain splitting reaction from degradation of starch by enzymes called cyclodextrins glucosyltransferases (CGTs) (142). In times past, only small amounts of CDs were generated and high production costs prevented their industrial application, but now most of the CGT genes have been cloned making the large scale production of this kind of carrier low cost.

The CDs are characterized by the presence of a lipophilic central cavity and a hydrophilic outer surface. The glucopyranose units are in the form of a chair and, for this reason, the CDs may be represented as a truncated cone. The OH groups are oriented with the primary hydroxyl groups of the various units of glucose on the narrow side of the cone and the secondary OH groups at the larger edge. The lipophilic character of the central cavity is determined by skeletal carbons and etheral oxygens.

The CDs may contain even >15–16 glucopyranose units, but the most abundant natural CDs are α -cyclodextrin (α -CD), β -cyclodextrin (β -CD), and γ -cyclodextrin (γ -CD) containing six, seven, and eight glucopyranose units, respectively.

The CDs are chemically stable in alkaline solutions, but are susceptible to hydrolytic cleavage under strong acidic conditions, however, they are more resistant toward

Table 1. The Principal Physical Chemical Characteristics of Natural CDs

	α -CDs	β -CDs	γ -CDs
Molecular weight	972	1135	1297
Unites of glucopyranose	6	7	8
Internal diameter, Å	5	6	8
Solubility, mg · 100 mL ⁻¹ , 25 °C	14.2	1.85	23.2
Melting point, °C	250–255	250–265	240–245

acid-catalyzed hydrolysis than linear dextrans and the hydrolytic rate decreases with decreasing cavity size (143). The rate of both the nonenzymatic and enzymatic hydrolysis is decreased when the cavity is occupied by drug molecule.

Table 1 reports the principal physical–chemical characteristics of natural CDs. Natural CDs, in particular β -CD, have aqueous solubility much lower with respect to comparable linear or branched dextrans. This is probably due to the relatively strong binding of the CDs molecules in the crystal state (i.e., relatively high crystal lattice energy). Moreover, β -CD form intramolecular hydrogen bonding between the secondary hydroxyl groups that reduces the number of hydroxyl groups capable of forming hydrogen bonds with the surrounding water molecules (142). This low aqueous solubility may cause precipitation of solid CDs complexes.

The most important characteristics of CDs is their ability to form inclusion complexes both in solution and in the solid state, in which the guest molecule places its self in the hydrophobic cavity hiding from the aqueous environment. This leads to a modification of physical, chemical, and biological properties of the guest molecules, but principally of the aqueous solubility.

The β -CD is the most useful pharmaceutical complexing agent principally because of its low cost and easy production. It contains 21 hydroxyls groups, of which 7 are primary and 14 are secondary (Fig. 26). All the OH groups are reactive enough to be used as points of reaction for structural modifications, allowing the introduction of several functional groups in to the natural macrocyclic

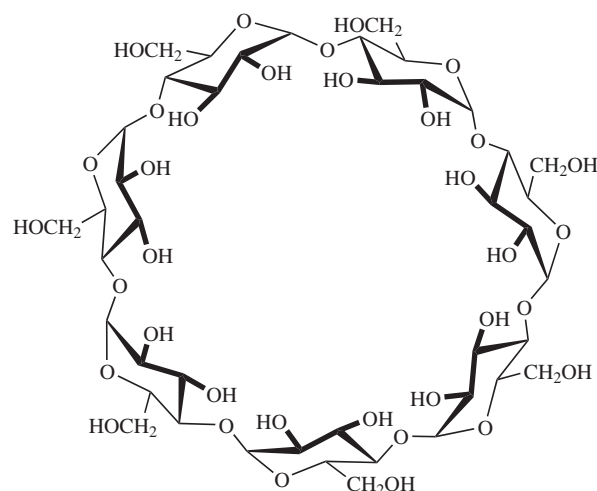


Figure 26. Structure of β -Cyclodextrin.

Table 2. Pharmaceutically Useful β -CD Derivatives^a

Derivative	Position of Substituent	Substituent
<i>Hydrophilic Derivatives</i>		
Methylated β -CD	2,6-; 2,3,6-	$-\text{O}-\text{CH}_3$
Hydroxyalkylated β -CD	Random	$-\text{O}-\text{CH}_3$ $-\text{CH}(\text{OH})-\text{CH}_3$
Branched β -CD	6-	$-\text{Glucosyl}$, $-\text{maltosyl}$
<i>Hydrophobic Derivatives</i>		
Ethylated β -CD	2,6-; 2,3,6-	$-\text{O}-\text{C}_2\text{H}_5$
Peracylated β -CD	2,3,6-	$-\text{O}-\text{CO}(\text{CH}_2)_n-\text{CH}_3$
<i>Ionizable Derivatives</i>		
Carboxyalkyl β -CD	Random	$-\text{O}-(\text{CH}_2)_n-\text{COONa}$
Carboxymethyl; ethyl	2,6-; 3-	$-\text{O}-\text{CH}_2-\text{COONa}$; $-\text{O}-\text{C}_2\text{H}_5$
Sulfates	Random	$-\text{O}-\text{SO}_3\text{Na}$
Alkylsulfonates	Random	$-\text{O}-(\text{CH}_2)_n-\text{SO}_3\text{Na}$

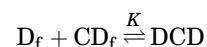
^aObtained by substitution of the OH groups located on the edge of the CD ring. From Ref. 144.

ring. In the past few years, research has led to a great a number of modified CDs having better characteristics with respect to natural CDs (Table 2).

To date, ~ 100 different CD derivatives are commercially available, but only few of those derivatives have gone through toxicological evaluations and are available as bulk chemicals for pharmaceutical use. In particular, CDs currently used in drug formulation, derived from natural CD, include 2-hydroxypropyl- β -CD (HP- β -CD), randomly methylated β CD (RM- β -CD), sulfobutylether β CD (SBE- β -CD), maltosyl β CD (ML- β -CD), and (hydroxyethyl) β CD (HE- β -CD). The aqueous solubility of all these cited derivatives is $>50 \text{ g} \cdot 100 \text{ mL}^{-1}$.

Inclusion Complex Formation. The most important feature of CDs is their ability to form solid inclusion complexes (of the host-guest type) with a very wide range of solid, liquid, and gaseous lipophilic compounds by a phenomenon of molecular complexation (145). In these complexes, a guest molecule is kept within the cavity of the CD. Complex formation is a dimensional fit between the host cavity and the guest molecule (146). The lipophilic cavity of CDs molecules supplies a microenvironment where an appropriately sized lipophilic moiety can enter to form an inclusion complex (147). The formation of the complex never involves the formation or the breaking of covalent bonds (148). The main driving force for this kind of process is the replacement of enthalpy-rich water molecules contained in the cavity with more hydrophobic guest molecules present in the solution to attain an apolar-apolar association and decrease of CD ring strain resulting in a more stable lower energy state (142). The binding of guest molecules within the host CD is not permanent and is characterized by a dynamic equilibrium, whose strength depends on how well the host-guest complex fits together and on specific local interactions between surface atoms. More specifically, in aqueous solution the CD-drug complexes are constantly being formed and broken.

In particular, considering a 1:1 complexation, the association is usually described by the following equilibrium:



The most important parameters that influence the inclusion process are the complexation strength or stability constant (K) defined by this equilibrium and equation 1, where (CD_f) and (D_f) are the concentrations of free CD and free drug, respectively; the other parameter is the lifetime (t) of the complex and equation 2, measured when the equilibrium is perturbed. The constants k_f and k_r are the forward and reverse rate constants, respectively, and k_{obs} is the observed rate constant for the reestablishment of the equilibrium after its perturbation.

$$K = \frac{k_f}{k_r} = \frac{[\text{DCD}]}{[\text{D}_f][\text{CD}_f]} \quad (1)$$

$$K_{\text{obs}} = \frac{l}{\tau} = k_f([\text{CD}_f] + [\text{D}_f]) + k_r \quad (2)$$

The CDs are able to complex the lipophilic substances both in solution, in this case water is the solvent of choice, or in the crystalline state. In some particular cases, the complexation may be performed also in the presence of any nonaqueous solvent, even if in this case a competition drug-solvent for the complexation may happen.

The inclusion of a drug in CDs lead to a profound change of its physicochemical properties as it is temporarily blocked within the host cavity giving rise to beneficial modifications of guest molecules, which are not achievable otherwise (149). In particular, the more influenced properties are enhanced solubility in water of highly insoluble guests, stabilization of labile guests against the degradative effects of oxidation, visible or ultraviolet (UV) light and heat, control of volatility and sublimation, physical isolation of incompatible compounds, chromatographic separations, taste modification by masking off flavors, unpleasant odors, and controlled release of drugs and flavors. Therefore, cyclodextrins may be used in several field: in food, pharmaceuticals, cosmetics, environment protection, bio-conversion, packing, and textile industry.

The substances that may be complexed in CDs are quite varied and includes such compounds as straight- or branched-chain aliphatics, aldehydes, ketones, alcohols, organic acids, fatty acids, aromatics, gases, and polar compounds (e.g., halogens, oxyacids, and amines) (149).

Main Methods of Preparation of Drug-Cyclodextrins Complex. The CD complexes may be prepared with various methods. In solution, the complexes are prepared by addition of an excess amount of the drug to an aqueous CD solution. The suspension formed is equilibrated (for periods of up to 1 week at the desired temperature) and then filtered or centrifuged to form a clear CD-drug complex solution. Then, the water is removed from this solution by evaporation or sublimation, for examples, spray or freeze drying to obtain a solid complex.

Other methods applied to prepare solid CD-drug complex include kneading and slurry methods, coprecipitation,

neutralization, and grinding techniques (150). In some cases, the complexation efficiency is not very high, and therefore relatively large amounts of CDs are needed to complex small amounts of a given drug. Moreover, various vehicle constituents, such as surfactants, lipids, organic solvents, buffer salts, and preservatives, often reduce the efficiency. However, it is possible to enhance the efficiency through formation of multicomponent complex systems (151). For example, recent research demonstrated that water-soluble polymers are able to enhance the complexation efficacy of a wide variety of guest molecules, through stabilization of the CD-drug complex, and to increase the aqueous solubility of the natural cyclodextrins (152).

Analytical Methods Used to Detect the Complex Formation. Following the preparation of a drug-CD complex, a fundamental step is to verify this complexation, the stoichiometry of the complex, and its stability constant. All these parameters may be clarified by mean of several technique: thin-layer chromatography (TLC) (153), high performance liquid chromatography (HPLC) (154); gas chromatography-mass spectrometry (GC-MS) for the appraisal of the pattern of substitution (155); nuclear magnetic resonance (NMR) (156); circular dichroism (CD) (157) differential scanning calorimetry, X-ray diffraction, ultraviolet (UV) spectrometry (158), capillary zone electrophoresis (159); electrokinetic chromatography (160); GC stationary phase (161); light scattering and cryoelectronic microscopy (162).

Toxicological Profile. An important limitation for the pharmaceutical application of a substance (both drug or excipient) is the appearance of toxicity after its administration. For this reason, a fervent field of research has been the evaluation of a toxicological profile of CDs. Recently, a review has been published showing the adverse effects from CDs (163).

In general, oral administration of CDs does not show any toxic effect, due to lack of absorption from the GI tract.

Natural CDs, α - and β -CDs, as well as many of their alkylated derivatives, show significant renal toxicity, and for this reason are not used for parenteral use (164). A number of safety evaluations have shown that HP- β -CD, SB-E- β CD, ML- β -CD, γ -CD, and HP- β -CD appear to be suitable in parenteral, as well as oral formulations (164,165). However, the lack of available toxicological data will, more than anything else, hinder pharmaceutical applications of CDs.

Cyclodextrins Elimination. Both HP- β -CD and SBE- β -CD are quantitatively cleared unmodified by renal filtration. Following IV administration, these cyclodextrins have an half-life of 1 h (for humans, this value is specie dependent) with the major amount present in the urine between 1 and 4 h after administration.

The elimination of an unmodified CD-drug complex may lead to an increase in renal clearance of unchanged drug. The drug elimination may occur also following another mechanism. Water reabsorption physiologically occurs in the proximal and distal tubules leading to about a 100-fold increase in the concentration of filtered molecules. In

this process, lipophilic drugs normally undergo passive reabsorption while polar molecules are only concentrated. This concentration, encourages the complex formation between the renal cleared cyclodextrins and any lipophilic molecule remaining in the kidney tubules. Since the complex is polar, the presence of the CDs is able to inhibit passive reabsorption of lipophilic drugs physiologically present resulting in greater renal clearance of lipophilic molecules.

Cyclodextrins as Drug Delivery Systems

The most classic application of CDs is in drug delivery. The CDs offer significant advantages over standard formulation. The CD-drug complexes can stabilize, enhance the solubility, bioavailability, and diminish the adverse effects of a drug. The bioadaptability and multifunctional characteristics of CDs make them able to minimize the undesirable properties of drugs in various routes of administration including oral, rectal, nasal, ocular, transdermal, and dermal. In Table 3, the role of CDs in drug formulation and delivery is reported in detail (166).

Cyclodextrins in Nasal Drug Delivery. Nasal administration of drugs is an important route of administration for several classes of drugs. Unfortunately, mucosa present both physical and metabolic barriers to drug permeation, restricting this therapeutically approach. The CDs may be used to overcome these obstacles. It has been demonstrated that CDs are able to reduce or to minimize the enzymatic activity of nasal mucosa (167).

Some morphological studies have shown that the methylated β -CDs are useful carriers for nasal drug delivery. Their effects on the mucosa are not significantly different to that of physiological saline and smaller than those of benzalkonium chloride, a worldwide used preservative for nasal drug formulations.

After nasal administration of a drug-CD formulation, only the drug permeates through the nasal epithelium, but not the highly water-soluble CD or its complex. In humans, DM- β -CD is hardly absorbed after nasal administration of a solution containing $\sim 5\%$ of dimethyl- β -CD. Four percent of the nasally administered dose was recovered in the urine (168). The fraction of the CD dose that is not absorbed from the nasal cavity is removed by the nasal mucociliary clearance system.

Moreover, studies of permeation of various lipophilic drugs complexed in CDs demonstrate that they can largely improve the permeation through nasal mucosa of these substances both in the case of polypeptides and proteins (169).

Cyclodextrins in Ophthalmic Drug Delivery. Tear fluid contains a large variety and amount of enzymes that influence the permeation of topical applied drugs. Numerous studies have shown that CDs are useful additives in ophthalmic formulations because they are able to increase the aqueous solubility, stability of ophthalmic drugs, and to decrease drug irritation (170).

The CD complexation of water-soluble drugs (in order to modify an adverse property, e.g., increase their chemical stability or to decrease ophthalmic drug irritation) generally

Table 3. Role of Cyclodextrins in Drug Delivery^a

Improved Drug Functions by CD Complexation	Example Drug	Type of CD
Increase in bioavailability (by increased solubility and stability)	Thalidomide	Natural CDs
As above	Nimuselide	β -CD, 2HP- β -CD
As above	Prednisolone	SBE-7- β -CD
As above	Oteprednol etabonate	γ -CD
As above	Sulfamethazole	β -CD and HP-CD
As above	Tacrolimus	Natural and hydrophilic CDs
As above	Artemisin	β - and γ -CD
As above	Prostaglandin E1	Sulfobutyl ether β -CD
Increase in solid-stability of amorphous drug	Quinapril	β -CDs
Increased absorption		
Oral delivery	Ketoconazole, testosterone	β -CD and HP β -CD
Rectal delivery	Flurbiprofen, carmafur biphenyl acetic acid	2 HP β -CD
Nasal delivery	Morphine, antiviral drug and insulin	2HP β -CD
Transdermal delivery	Prostaglandin E1	6-O-(carboxymethyl) O-ethyl β -CD
Ocular delivery	Dexamethasone, Carbonicanhydrase inhibitors	2HP β -CD β -CD
Protein and peptide delivery	Growth hormone, interleukin-2, aspartame, albumin and MABs	Different modified CDs
Reduction of local irritancy and toxicity	Pilocarpine, phenothiazine euroleptics, <i>all-trans</i> -retenoic acid	2 HP β -CD (2,6-diOmethyl) β -CD and β -CD

^aModified from Ref. 162.

decrease the ophthalmic bioavailability (171); in the case of water-poor soluble drugs, the bioavailability strongly depends on the amount of CDs present: It is fundamental to use only the minimum quantity to form a complex (to solubilize) of the drug (172). In fact, the amount of CDs in excess reduces the free drug and, as a result the ocular permeation will decrease. An example is given in the research of Jarho et al. 1996 (173), which measured the permeability of arachidonyl-ethanolamide through isolated rabbit cornea. As reported in Fig. 27 the maximum value of permeability was found when the minimum amount of CD was used to maintain the drug in solution.

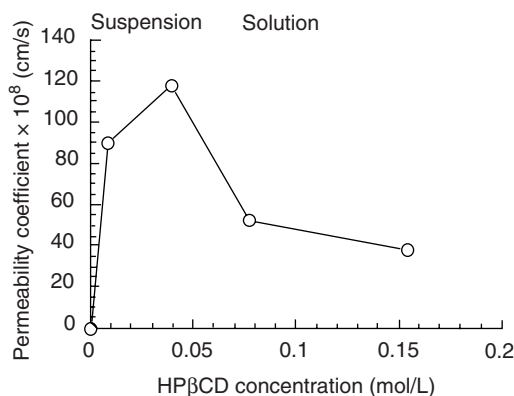


Figure 27. Permeability of arachidonyl-ethanolamide through isolated rabbit cornea as a function of HP- β -CD concentration. The vehicle consisted of 0.5 mg $\cdot$ mL⁻¹ suspension or solution of the drug in water containing 0–1.155 M HP- β -CD (173).

Moreover, this reduced bioavailability may be attributed to a too rapid ocular clearance (few minutes), and it has been demonstrated that by increasing the viscosity of the ophthalmic formulation, this obstacle may be reduced (174). In addition, a lot of substances used to increase the viscosity of aqueous solutions have been shown good ability to increase the complexation efficacy of CDs and, thus, the amount of CD needed to obtain adequate drug solubility can be decreased significantly when water-soluble polymer is present in the formulation (175).

Cyclodextrins in Dermal and Transdermal Drug Delivery.

The CDs are relatively large molecules, and consequently both they and their complexes are not able to permeate through intact skin easily. Only 0.02% of the applied dose of radiolabeled HP- β -CD permeated through human skin. The principal barrier to the permeation of CDs is represented by the stratum corneum, since by stripping it, it is possible to enhance the percutaneous permeation by 24% (176). Lipophilic CDs (as DM- β -CD and RM- β -CD) are absorbed to a greater extent, but this absorption is still of little significance (0.3% of the applied dose).

The CDs are able to interact with some components of skin lipids. In particular, it has been demonstrated that pure aqueous solution of β -CDs and RM- β -CDs and HP β CD are able to extract the lipids present in stratum corneum (177). Various studies (178,179) have shown that excess CDs, more than needed to complex the lipophilic drug, lead to a decrease of drug permeation through the skin (Fig. 28). When the drug (hydrocortisone) was in suspension, the increase of the cyclodextrin concentration lead to an increase of the flux through the skin. When all hydrocortisone was in solution,

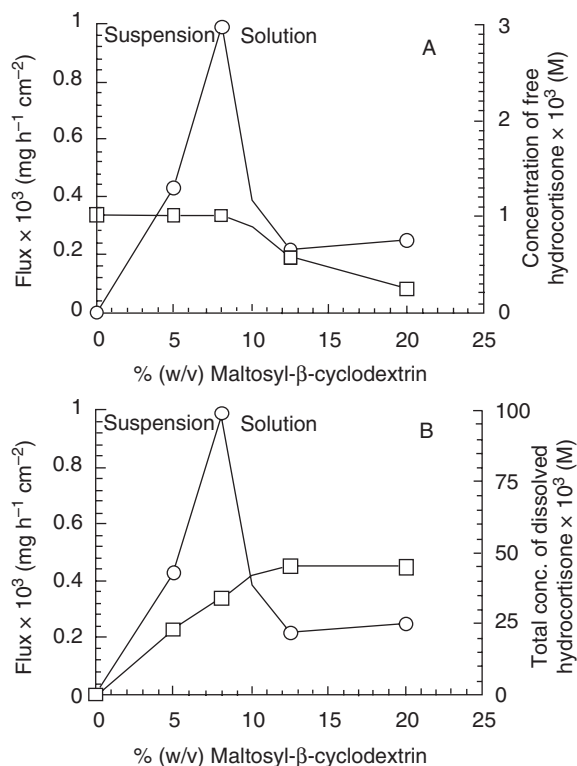


Figure 28. The effect of the maltosyl- β -cyclodextrin (ML- β -CD) concentration on the flux (○) of hydrocortisone through hairless mouse skin. The hydrocortisone concentration was kept constant at 0.045M. (a) The flux in relation to the amount of free hydrocortisone (□) in the donor phase. (b) The flux in relation to the total amount of dissolved hydrocortisone (□) in the donor phase. The donor phase consisted of aqueous hydrocortisone suspension at ML- β -CD concentrations < 8% (w/v), but hydrocortisone solution at higher ML- β -CD concentrations (175).

the increase of the cyclodextrin content led to a decrease in flux. In all cases, maximum flux through the skin was obtained when just enough cyclodextrin was added to the vehicle to keep all hydrocortisone in solution. The mechanism has been already elucidated in a previous section.

A great number of topical drugs have been complexed with CDs (Tables 4 and 5). In every case, it has been demonstrated that CDs can markedly enhance the dermal delivery of lipophilic drugs (e.g., corticoids and NSAIDs). In particular, from a comparative evaluation (181) among the compounds DM- β -CD, β -CD, and HP- β -CD, resulted in HP- β -CD being more able to increase the percutaneous permeation (Fig. 29).

The effects of CDs on the permeation rates of drugs through the skin may be determined by both the increase of thermodynamic activity of drugs in a vehicle (in particular it is referred to the escaping tendency of drugs, and it is supposed that increasing this activity will lead to the augmentation of the permeation rate of drugs through the skin; moreover, the thermodynamic activity is proportional to the solubility of drugs in its vehicle and is maximal just in the saturated solution), the extraction of skin component, and the partition coefficient of the drug between skin and vehicle.

Recently, it has been evaluated for transdermal use in peracylated CDs with medium alkyl chain length (C₄–C₆) and in particular 2,3,6-tri-*O*-valeryl- β -CD (TV- β -CD). This particular type of CD shows the property of forming a film, and for this reason is very promising in transdermal preparations.

Cyclodextrins in Rectal Drug Delivery. The CDs have been studied to optimize the rectal delivery of drugs for systemic use. Table 6 reports the CDs and drugs investigated for rectal application. The effects of CDs on the rectal delivery of drugs depends on vehicle type (hydrophilic or oleaginous), physicochemical properties of the complexes, and an existence of excipients, such as viscous polymer. The enhancement of rectal permeation of lipophilic drugs made by CDs is generally attributed to the improvement of the release from vehicles and the dissolution rates in rectal fluids (Fig. 30). In the case of inabsorbable drugs, such as antibiotics, peptide, and proteins, the rectal delivery is based on the direct action of CDs on the rectal epithelial cells.

On the other hand, the prolonging effects of CDs on the drug levels in blood are caused by several factors: sustained release from the vehicles, slower dissolution rates in the rectal fluid, and retardation in the rectal absorption of drugs by an inabsorbable complex formation.

Another important aspect that has been evaluated is the stabilization of drugs in rectal delivery. The complexation of drugs with CDs has been used to improve the chemical stability in suppository bases according to a stabilizing effects (principally of β -CD and DM- β -CD) attributable to a poor solubilization of drugs in the oleaginous suppository base; this may lead to a difficult interaction of drugs with the base.

The CDs may inhibit the bioconversion of drugs in the rectum (182) leading to the alleviation of the rectal irritancy of the some drugs as NSAIDs (Fig. 31).

Cyclodextrins in Oral Drug Delivery. An important parameter to be considered for the oral administration of a drug is its water solubility. For poorly aqueous soluble drugs, CDs are able to increase the aqueous solubility and thus enhance its dissolution rate and the biopharmaceutical parameters. An example was observed with the celecoxib (a nonsteroidal antiinflammatory drug). The complex celecoxib DM- β -CD showed an increased permeation respect to the free drug. This observed enhanced permeation was due to the fast dissolution rate of the included drug and to a destabilizing action exerted by the CD on the biomembrane (Fig. 32) (183).

The CD derivatives may be used in order to modify drug release of oral preparations. Table 6 reports some application of CDs.

The hydrophilic CDs are able to give an immediate release of the complexed drug, while hydrophobic CDs are useful for the prolonged release formulations. The use of *O*-carbrossimethyl-*O*-ethyl- β -CD (CM- β -CD) gives a delayed release formulation.

The immediate release formulation is required in an emergency situations and in a particular way in the administration of analgesics, coronaric antipyretics, and

Table 4. The Use of Parent Cyclodextrins in Transdermal Route^a

CDs	Abbreviation	Improvement	Drugs
α -Cyclodextrin	α -CD	Release and/or permeation Stability	Miconazole Tixoxortol 17-butyrate 21-propionate Betamethasone 4-biphenylacetic acid Chloramphenicol Ciprofloxacin Ethyl 4-biphenyl acetate Flurbiprofen Hydrocortisone
β -Cyclodextrin	β -CD	Release and/or permeation	Indomethacin Nitroglycerin Norfloxacin Piroxicam Prednisolone Prostaglandin E ₁ Sulfanilic acid Chlorpromazine hydrochloride Tretinoin
γ -Cyclodextrin	γ -CD	Release and/or permeation	Beclomethazone dipropionate Betamethasone Menadione Prednisolone

^aAdapted from Ref. 180.**Table 5. The Use of Cyclodextrin Derivatives in Transdermal Route^a**

CD Derivatives	Abbreviation	Improvement	Drugs
Dimethyl- β -cyclodextrin	DM- β -CD	Release and/or permeation	4-Biphenylacetic acid Ethyl 4-biphenyl acetate Indomethacin Prednisolone Sulfanilic acid
Random methyl- β -cyclodextrin	RM- β -CD	Local irritation Release and/or permeation	Chlorpromazine Acitretin Hydrocortisone Piribedil S-9977
Hydroxypropyl- β -cyclodextrin	HP- β -CD	Release and/or permeation	4-Biphenylacetic acid Dexamethasone 17 β -estradiol Ethyl 4-biphenyl acetate Hydrocortisone Liarozole Miconazole
Maltosyl- β -cyclodextrin	G ₂ - β -CD	Release and/or permeation	Hydrocortisone
β -Cyclodextrin polymer	β -CD polymer	Release and/or permeation	Tolnaftate Indomethacin
Diethyl- β -cyclodextrin	DE- β -CD	Release and/or permeation	Nitroglycerin
Carboxymethyl- β -cyclodextrin	CM- β -CD	Release and/or permeation	Hydrocortisone
Carboxymethyl-ethyl- β -cyclodextrin	CME- β -CD	Release and/or permeation	Prostaglandin

^aAdapted from Ref. 180.

vasodilators. Hydrophilic CDs have been used in order to improve the oral bioavailability of the previous mentioned drugs (184). The improvement is mainly dependent on the increase of solubility and wettability of drugs through the formation of inclusion complexes (185).

The oral bioavailability of a lipophilic drug from the CD complex may be optimized varying several factors,

that influence the equilibrium of dissociation of the complex (166,186). The maximum improvement of the absorption is obtained when a sufficient amount of CD is used to complex all the molecules of the drug present in suspension, and the further addition of CDs lead to a reduction the free fraction of the drug and, therefore, reduces the bioavailability of the drug. Moreover, drug

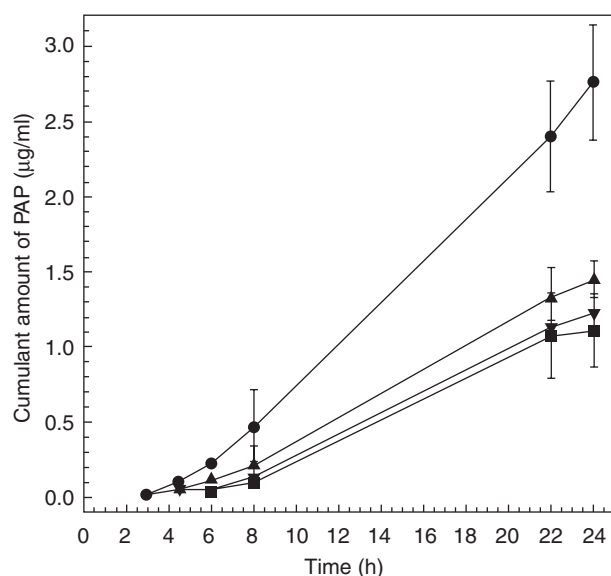


Figure 29. Total amount of free or complexed papaverine permeated through abdominal rat skin. Symbols: ν free PAP alone; $\blacktriangle$ PAP-HP- β -CyD; $\bullet$ PAP- β -CyD; λ PAP-DM- β -CyD (179).

formulations contain a certain amount of excipients that may compete with the drug for the cavity of the CD. Competition may also happen with the endogenous substances present in the absorption site. The replacement

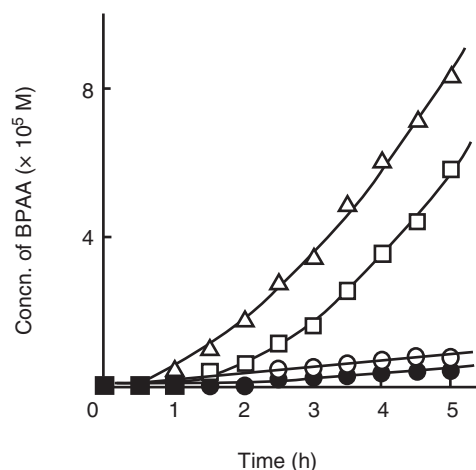


Figure 30. Profiles of BPAA permeation through isolated rat rectum after applications of suppositories containing EBA or its β -CD complexes in isotonic phosphate buffer (pH 7.4) at 37 °C. $\bullet$ without β -CDs; $\circ$ with β -CD; $\square$ with DM- β -CD; Δ with HP- β -CD (182).

of the drug from the cavity of the CD from both endogenous and exogenous substances lead to an acceleration of the absorption of the drug (187,188). Early studies showed that in some cases the improvement of oral bioavailability is principally due to a stabilizing effect of CDs on labile drugs (189).

Table 6. The Use of Cyclodextrins in Rectal Delivery^a

CDs	Improvement	Drugs
α -CD	Stability Release and/or permeation	Morphine hydrochloride Cefmetazole G-CSF Morphine hydrochloride AD1590 Carmoful
	Stability	Ethyl 4-biphenyl acetate 4-Biphenylacetic acid Ethyl 4-biphenyl acetate Naproxen Phenobarbital Piroxicum
β -CD	Release and/or permeation	Diazepam Flurbiprofen 4-Biphenylacetic acid Carmoful Diazepam
	Release and/or permeation	Ethyl 4-biphenyl acetate Flurbiprofen Insulin 4-Biphenylacetic acid Ethyl 4-biphenyl acetate
γ -CD	Release and/or permeation	Carmoful Diazepam Flurbiprofen
	Release and/or permeation	4-Biphenylacetic acid Diazepam Ethyl 4-biphenyl acetate
DM- β -CD	Release and/or permeation	Carmoful
	Local irritation	4-Biphenylacetic acid Ethyl 4-biphenyl acetate
TM- β -CD	Release and/or permeation	Carmoful Diazepam Flurbiprofen
	Release and/or permeation	4-Biphenylacetic acid Diazepam Ethyl 4-biphenyl acetate
HP- β -CD	Release and/or permeation	Carmofur
	Release and/or permeation	Carmofur
β -CD Polymer	Selective transfer into lymphatics	

^aAdapted from Ref. 180.

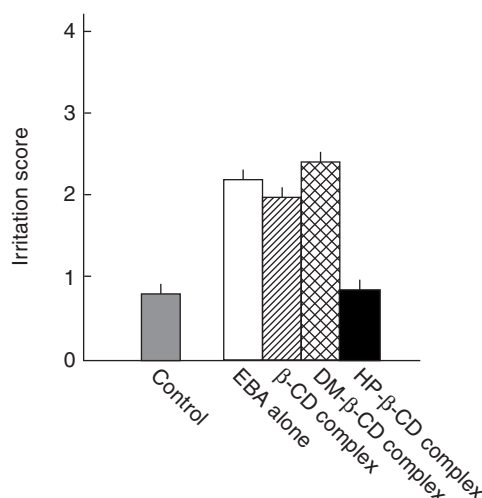


Figure 31. Irritation effect of suppositories containing EBA or its β -CD complexes (equivalent to BPAA $10 \text{ mg} \cdot \text{kg}^{-1}$) on rectal mucosa in rats 12 h after multiple (four times at 12 h intervals) administration (182).

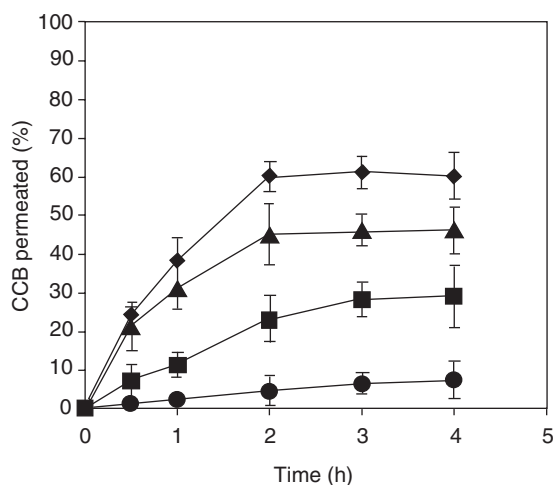


Figure 32. Permeation profiles of CCB alone or in the presence of the CCB-DM- β -CD complex in a different molar ratio. (●) CCB alone; (●) CCB-DM- β -CD 1:2 complex as suspension; (▲) CCB-DM- β -CD 1:5 complex as solution; (◆) CCB-DM- β -CD 1:10 complex as solution (181).

Others positive results may be obtained for the sublingual administration of drugs, complexed with CD (190,191). In this application, not only the drug permeates rapidly giving an immediate response, but it also avoid hepatic first pass metabolism.

The preparations characterized by a slow release are planned for having a zero-order release to guarantee a constant blood level for a long period of time. This type of formulation has many advantages, like the reduction of the administration frequency with an extension of the efficacy of the drug and the reduction of the toxicity associated with the administration of a simple dose. Hydrophobic CDs, as alkylated and acylated derivatives, are used as slow-release carriers for hydrophilic drugs. Between the

alkylated CDs, 2,6-di-*O*-ethyl- β -CD (DE- β -CD) and 2,3,6-tri-*O*-ethyl- β -CD (TE- β -CD) were the first used as slow-release carriers (192).

Another type of CD useful in oral formulation is 2,3,6-tri-*O*-butyryl- β -CD (TB- β -CD), whose bioadhesive property make it very advantageous in oral and transmucosal formulations.

Cyclodextrins in Parenteral Administration. Modern technology is trying to obtain semisynthetic CDs that have the following characteristics to use as parenteral drug delivery systems. For this application, the drug toxicity at high doses will need to be improved for chronic treatment; its inability to react with cholesterol, phospholipids, or others members of the cellular membrane, and its biodegradability in circulation and elimination of small molecular metabolites.

In general, for this kind of application only hydrophilic CDs, in particular HP- β CD are used. This has been, carefully studied by means of innumerable toxicological experiments and has been the object of numerous clinical tests on human. One formulation, based on the carrier Sporanox by Jassen (193) has been approved by the U.S. Food and Drug Administration (FDA). Another hydrophilic derivative is used for parenteral use is β -CD sulfobutylether. It is used under the name of Captisol.

The sulfate CD represent another class of soluble CD in water with a characteristic biological activity. It shows an antiangiogenic power that may be useful in new therapies against cancer. A few studies have demonstrated that the sulfate CD does not have any hemolytic properties at all, are not toxic, and protect against the nephrotoxicity induced by gentamicin without even reducing renal accumulation of this active principle (194).

Cyclodextrins in Anticancer Therapy. Cyclodextrins also play a vital role in the drug formulation design for cancer therapy. Bekers et al. (195) in 1991 studied the effect of cyclodextrins on the chemical stability of mitomycin C, a clinically useful anticancer drug able to generate severe dermatological problems after administration. The complexation of this drug with CD reduced the skin necrosis observed after the treatment with the free drug.

Real advantages were demonstrated in the delivery of paclitaxel, an anticancer agent used in breast, ovarian, lung, head and neck cancers, characterized by very low water solubility. For this reason, it must be formulated as a micellar solution made up of polyoxyethylated castor oil and 50% absolute ethanol. This formulation triggers severe acute adverse effects in both animals and humans. The complexation of paclitaxel in CDs, β -CD, DM- β -CD, and TM- β -CD, showed a modulation of the maintainance of the anticancer activity (196).

Cyclodextrins as Carrier for Biological Drugs. Besides drugs, different peptides and proteins (197), oligosaccharides, and oligonucleotides (198) are also delivered by the formation of inclusion complexes with cyclodextrins because of CDs ability of interacting with cellular membranes and giving rise to improved cellular uptake. The most recent usage of cyclodextrins lies in the ability of these agents to

deliver agents, such as plasmids, viral vectors, and antisense constructs. The *in vitro* stability of antisense molecules is increased by binding to CDs, such as hydroxypropyl β -CD. A two- to threefold increase in the cellular uptake of antisense constructs by hydroxyalkylated β -CD has been noted in human T-cell leukemia H9 cells (199). Certain CDs modulate the intracellular distribution or activity of antisense molecules and they may be used for reversal of atherosclerosis (200). Cyclodextrins are also used to formulate the enhancement of the physical stability of viral vectors for gene therapy by suspending the adenovirus and adeno-associated virus in blends of CD, complex carbohydrates, and various surfactants (201). Three native CDs (α , β , and γ) were observed to improve the antiviral effect of ganciclovir on two human cytomegalovirus strains (202). Use of CDs as carriers of antiviral drugs appears to be a good alternative to traditional treatments as it allows the administration of lower doses and reduces the toxic effect of drug molecules.

Cyclodextrins in Colon Targeting. Colon targeting may be classified as a delayed release with a fairly long time because the time required to reach the colon is ~ 8 h in humans (203). When a formulation is administered orally, it will dissociate in the GI fluid and for this reason CD complexes are not suitable for colon delivery. For this reason, it was proposed to use CD–drug conjugates (a prodrug) that were able to survive the passage through the stomach and small intestine. In particular, the linkage of CD to biphelylacetic acid (BPAA) has been investigated. It is interesting to note that the solubility of this type of prodrug is strictly related to the cavity size of the CD. Moreover, in the case of ester-type conjugates, drug release is the case of ester-type conjugates, drug release is triggered by the ring opening of CDs, which consequently provides site-specific drug delivery to the colon. On the other hand, the amide conjugates do not release the drug even in the cecum and colon, despite the ring opening of CDs. The amide linkage of the small saccharide–drug conjugates may be resistant to bacterial enzymes and poorly absorbable from the intestinal tract due to high hydrophilicity. Therefore, the ester-type conjugate is preferable as a delayed release-type prodrug that can release a parent drug selectively in the cecum and colon (204).

SUPRAMOLECULAR AGGREGATES FOR DRUG DELIVERY

General Characteristics of Surfactants

Surfactants are molecules characterized by a polar head and an apolar tail region, the latter occupies the larger molecular volume, in a particular way for ionic surfactants. When dispersed in water, surfactants self-associate into a variety of equilibrium phases, the nature of which stems directly from the interplay of the various (forces inter- and intermolecular), as well as entropy evaluations. Surfactants also self-associate in nonaqueous solvents, particularly apolar liquids, such as alkanes. In this case, the orientation of the surfactant molecules are reversed compared to those observed in aqueous solution. This reorientation lead to a lowering of the free energy of the system

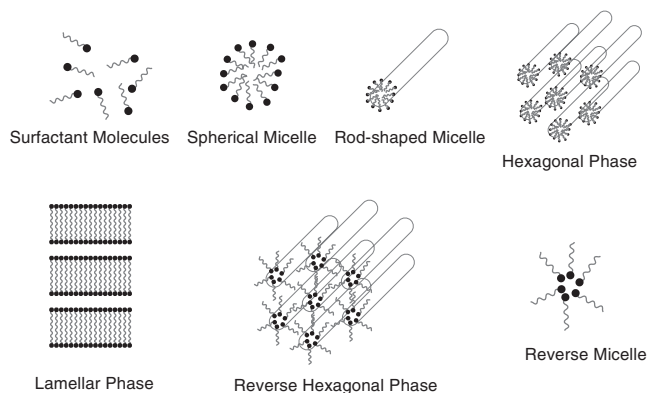


Figure 33. Schematic representation of the most commonly self association structures in water, oil or a combination thereof (205).

overall. When surfactants are incorporated into two immiscible solvents as oil and water, the surfactant molecules locate themselves at the oil–water interface. This arrangement is thermodynamically favorable.

Figure 33 reports a number of possible self-association structures that surfactant may form when placed in a oil and water (205).

Microemulsions

The microemulsion concept was introduced as early as the 1940s by Hoar and Schulman, who generated a clear single-phase solution by titrating a milky emulsion with hexanol (206). Later, in 1959, Schulman coined the term microemulsion (207). Today microemulsions are defined as A mixture of water, oil, and amphiphile substances forming a single optically, isotropic and thermodynamically stable liquid solution. The stability is the most important difference between emulsions and microemulsions. In fact, emulsions are fundamentally thermodynamically unstable and, even if they show an excellent kinetic stability, may undergo phase separation (208). Another important difference is related to their appearance. Emulsions are milky while microemulsions are clear or translucent. In addition, there is a noticeable difference in their method of preparation, since emulsions require a large input of energy while microemulsions do not. Microemulsions are dynamic systems in which the interface is continuously and spontaneously fluctuating (209).

Schematic representations of the three types of microemulsions are most likely formed are reported in Fig. 34. The structures shown are very different, but in each there is an interfacial surfactant monolayer separating the oil and water domains.

Three approaches have been proposed to explain the spontaneous microemulsion formation and their consequent stability: interfacial or mixed-film theories (210); solubilization theories (211); and thermodynamic treatments (212). In particular, the free energy of microemulsion formation reported in equation 3 is dependent on the extent to which surfactant is able to lower the surface tension of the oil–water interface and the change in

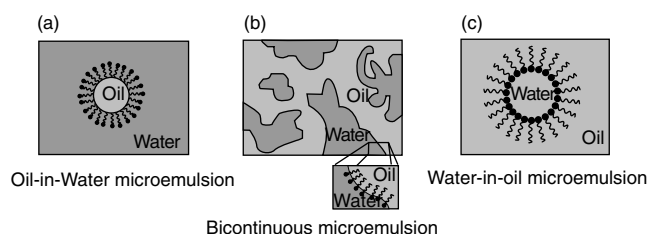


Figure 34. Schematic representation of three type of microemulsion microstructures: (a) oil-in-water, (b) bicontinuous, and (c) water-in-oil microemulsion (205).

entropy of the system such that,

$$\Delta G_f = \gamma \Delta A - T \Delta S \quad (3)$$

where ΔG is the free energy of formation, γ is the surface tension of the oil–water interface, ΔA is the change in interfacial area on microemulsification, ΔS is the change in entropy of the system, and T is the temperature.

When a microemulsion is formed, the change in ΔA is very large due to the formation of a great number of very small droplets generated. Originally, it was proposed that to form a microemulsion a negative value of γ was required. It is now accepted that this value of γ is always positive, but it is very small (of the order of fractions of $\text{mN} \cdot \text{m}^{-1}$), and is offset by the entropic component. The dominant favorable entropic contribution is the very large dispersion entropy arising from the mixing of one phase in the other in the form of large numbers of small droplets. Thus a negative free energy of formation is achieved when large reductions in surface tension are accompanied by significant favorable entropic change. In such cases, microemulsification is spontaneous and the resulting dispersion is thermodynamically stable.

The phase behavior of simple microemulsion systems comprising oil, water, and surfactant can be studied with the aid of a ternary phase diagram in which each corner of the diagram represents 100% of that particular component. More commonly, however, and in a special way in the case of microemulsions for pharmaceutical applications, the microemulsion contains additional components, such as a cosurfactant and/or drug. The cosurfactant is also amphiphilic with an affinity for both the oil and aqueous phases and partitions to an appreciable extent into the surfactant interfacial monolayer present at the oil–water interface. It has three functions: to provide very low interfacial tensions required for the formation of microemulsions and their thermodynamic stability; to modify the curvature of the interface based on the relative importance of their apolar groups; and to act on the fluidity of the interfacial film. If the film is too rigid, it prevents the formation of microemulsion and results in a more viscous phase. The existence of unsaturated bounds on the hydrocarbon chain of the surfactants equally increases the fluidity of the film. The cosurfactants used are small molecules, generally alcohols with the length of the carbon chain ranging from C2 and C10, or amines with short chains can also be used as cosurfactants. Moreover, a large number of drug molecules are themselves surface active and influence phase behavior.

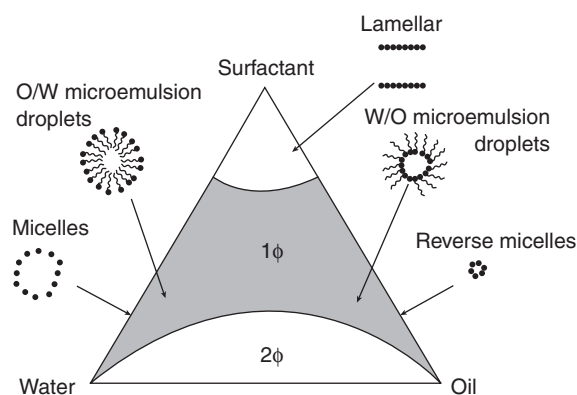


Figure 35. A hypothetical pseudo-ternary phase diagram of an oil–surfactant system with emphasis on microemulsion and emulsion phases. Within the phase diagram, existence fields are shown where conventional micelles or water–oil microemulsion, and oil–water microemulsions are formed along with the bicontinuous microemulsions. At very high surfactant concentrations two-phase systems are observed (205).

In the case where four or more components are present, pseudoternary phase diagrams are used where a corner will typically represent a binary mixture of two components, such as surfactant–cosurfactant, water–drug, or oil–drug. A highly schematic (pseudo) ternary-phase diagram illustrating various phases is presented in Fig. 35. Not every combination of various components produce microemulsions over the whole range of possible compositions, in some instances the extent of microemulsion formation may be very limited. The procedure most often employed to construct the phase diagrams is to prepare a series of (pseudo) binary compositions and titrate with the third component, evaluating the mixture after each addition. The temperature must be accurately controlled and the observations must not be made on metastable systems (213). Transitions between the various phases pictured in these phase diagrams can be driven by the further addition of one of the components, addition of a new component (drug or electrolyte), or by changing the temperature. Transitions from water/oil (w/o) to oil/water (o/w) microemulsions may occur via a number of different structural states including bicontinuous, lamellar, and also multiphase systems. In particular, microemulsions stabilized by nonionic surfactants are very susceptible to an increased temperature, leading to the phase inversion temperatures (PIT). The presence of PIT may cause problems especially when formulations are for parenteral application and must be sterilized by means of an autoclave. On the other hand, the presence of PIT may be useful for the drug delivery directed to a specific site.

Advantages of Microemulsions as Drug Delivery Systems.

Microemulsions present some important characteristics that make themselves very versatile carriers. In particular, they present a thermodynamic stability, optical clarity, and ease of preparation. The existence of microdomains of different polarity within the same single-phase solution allow the solubilization both water soluble and at the same time if this is so desired. Furthermore it is also possible to

incorporate amphiphilic drugs into the microemulsion. It must be emphasized that the use of o/w microemulsions in drug delivery is more straightforward than it is with w/o microemulsions. The reason is because the droplet structure of o/w microemulsions is not broken following the dilution by a biological aqueous phase; this aspect make possible the oral as well as parenteral administration. The process of dilution will result in the gradual desorption of surfactant present at the droplet interface. This process is thermodynamically driven by the requirement of surfactant to maintain an aqueous phase concentration equivalent to its critical micelle concentration while maintaining temperature, pH, and ionic strength. The use of w/o microemulsions for oral or parenteral drug delivery is complicated by the fact that they are destabilized when diluted by biological aqueous fluids.

Applicative Potentialities of Microemulsions

Transdermal Application. Microemulsions represent an ideal vehicle for the topical administration of drugs because they combine the emulsion properties with those of solution. It is well known that surfactants produce stratum corneum dehydration and barrier compromise (214,215), and consequently the high levels of surfactant-cosurfactant present in the microemulsions may cause a disruption of the stratum corneum. Consequently, there is an enhancement in the permeation of drugs. However, the choice of the component is very important to minimize the alteration of the stratum corneum and the appearance of toxic effects. The choice of biocompatible components can guarantee an increased skin tolerability. For this reason, the potential application of highly biocompatible o/w microemulsions as topical drug carrier systems for the percutaneous delivery of antiinflammatory drugs (i.e., ketoprofen) was investigated (216). The components were triglycerides as the oil phase, a mixture of lecithin, and *n*-butanol as a surfactant-cosurfactant system, and an aqueous solution as the external phase. The topical carrier potentialities of lecithin-based o/w microemulsions were compared with respect to conventional formulations (i.e., a w/o emulsion, a o/w emulsion, and a gel).

The percutaneous adsorption of ketoprofen, evaluated through healthy adult human skin, delivered with microemulsions, showed an enhancement with respect to conventional formulations. No significant percutaneous enhancer

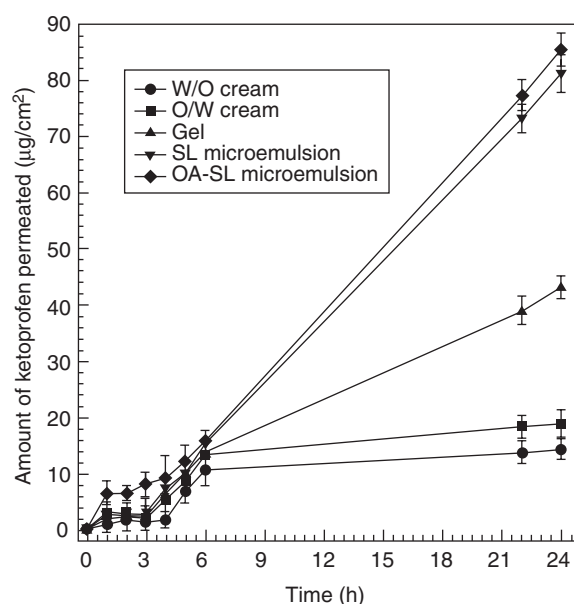


Figure 36. Permeation profiles of ketoprofen through human skin from various topical formulations. Each value is the mean value of three different experiments $\pm$ S.D (216).

effect was observed for ketoprofen-loaded oleic acid–lecithin microemulsions (Fig. 36). Moreover microemulsions showed a good human skin tolerability (Table 7).

Several reports have demonstrated that microemulsive vehicles may increase transdermal delivery of both lipophilic and hydrophilic drugs, compared to conventional formulations, depending on the constituents used for the microemulsive vehicle (217–219). These research papers suggested that microemulsion formulations may increase cutaneous drug delivery by means of the high solubility potential for both lipophilic and hydrophilic drugs, which creates an increased concentration gradient toward the skin and/or by using constituents with penetration enhancer activity (211). The incorporated ratio of the respective constituents influence in a significant way the percutaneous and transdermal drug delivery potential of the microemulsions. In every case, the enhancement of the drug delivery mechanism seems to be related to the drug mobility in the vehicle, and that measurement of self-diffusion

Table 7. Human Skin Irritancy Test of Various Topical Formulations After 24 h of Treatment

Sample	Irritation Evidence at 24 h								Score ^a
	Number of Cases ^b								
	Vesicles	Edema	Erythema	Flakiness	Dryness	Wrinkling	Glazing	No Visible Reaction	
OA 1%			3	7		2		18/30	10.17 ± 2.08
w/o						2	3	25/30	6.20 ± 2.77
O/W						2	1	27/30	4.67 ± 2.52
Gel						1	2	27/30	4.33 ± 1.15
SL-ME						1	1	28/30	3.50 ± 1.39
OA-SL-ME						2	1	27/30	4.67 ± 2.08

^aNonparametric variable Kruskal–Wallis test provided: $P < 0.001$ for OA (1% w/w) aqueous dispersion vs. all other samples; $P < 0.05$ for w/o cream versus all other samples; $P < 0.05$ for SL microemulsions versus o/w cream, gel, and OA-SL microemulsion.

^bThe value reported in each column represent the number of subjects who showed the skin reaction symptom.

coefficients is valuable to optimize the formulation of a given microemulsion vehicle, in order to maximize drug delivery.

Ophthalmic Application. The drug delivery system used in the ophthalmic field must overcome the disadvantages present in traditional formulations (e.g., a very low bioavailability, 1–10% of the drugs, and consequently frequent administrations are required during the day). Microemulsions represent an interesting alternative because their industrial production and sterilization are relatively simple and inexpensive; they are thermodynamically stable and permit us to solubilize both lipophilic and hydrophilic drugs.

With ophthalmic use, the choice of the various components is fundamental more than with any other topical application. The ionic surfactants are generally too toxic to be used for this application, therefore, nonionic surfactants are preferred (220). These surfactants are easily soluble in water due to the presence of either functional groups. The most used surfactants in the preparation of microemulsions are the poloxamers and polysorbates.

The choice of the oily phase is important because it conditions both the existence of the microemulsion and the solubilization of the drug. Polar oils, such as triglycerides with medium or long chains, are preferred instead of nonpolar oils, based on their solubility. The most often used consist of vegetable oils, such as soja oil, castor oil, or triglycerides, for which 95% of the fatty acids are made up of 8–10 carbon atoms, Myglyol 812s (triesters of glycerol, capric, and caprylic acids), isopropyl myristate, fatty acids, such as oleic acid, and esters of saccharose, such as mono-, di-, or tripalmitates of saccharose. As these excipients are well tolerated by the eye, their degree of purity must be high in order to prevent any contamination with potentially irritating substances.

Several additives, such as buffers, antibacterial, and isotonic agents, contained in the aqueous phase may affect the area of existence of the microemulsions, and therefore they must be studied in the presence of other constituents of the microemulsions. For example salinity influences the phase diagrams when ionic surfactants are added and decreases the phase inversion temperature (PIT) of the nonionic surfactants. Thiomersal and chlorobutanol are preservatives that are usually used in eye drop formulations, with concentrations of 0.01–0.2%, can be used without altering microemulsions structure (221).

The main advantage of the microemulsions is the increase in the solubilization of poorly soluble drugs. In a recent work, different o/w microemulsions containing indomethacin (an antiinflammatory drug), were evaluated *in vivo* by determining both the tolerability (Draize test) and the ocular drug bioavailability. This investigation showed that the colloidal carrier has a certain tolerability, eliciting only a slight irritation at the level of the conjunctiva. A positive effect regarding tolerability was exerted by hyaluronic acid. In fact, by increasing the concentration of hyaluronic acid present in the formulation up to 1% (w/v), an improved microemulsion ocular tolerability was observed with a substantial reduction of conjunctiva irritation (Table 8). *In vivo* ocular bioavailability of the microemulsion formulation containing indomethacin was evaluated

Table 8. Effect of Microemulsions on Ocular Structures^a

Ocular Structure	Without Hyaluronic Acid	With Hyaluronic Acid
Conjunctiva Irritation	1.8	0.4
Conjunctiva Edema	0.4	0.2
Fluorescein Adsorption	0.8	0.2

^aThe scores were calculated awarding a value on scales from 0 to 3 at each observed reaction. All the assigned values were added and divided for the number of subjects.

by means of the Draize test. At various time intervals, the rabbits were killed, aqueous humor samples were collected and indomethacin content was determined by high performance liquid chromatography (HPLC). Indomethacin-loaded microemulsion was compared with an aqueous dispersion of the drug, containing the same drug concentration. The microemulsion-encapsulated indomethacin formulation showed a significant ($P > 0.005$) increase of drug levels compared with the free drug (Fig. 37). High colloidal properties of microemulsions may achieve a better interaction with the corneal epithelium in terms of paracellular transport or passage, thus leading to a greater drug transport into the ocular tissues. The microemulsion controlled drug release showed by ocular pharmacokinetics was probably elicited by the colloidal carrier mucoadhesion on the cell surface, thus allowing a prolonged ocular permanence and a release of the content directly into the cell (222).

Lecithin Organogel

A particular type of self-aggregate is represented from lecithin organogel. They were seen for the first time in 1988 by Scartazzini and Luisi (223), who noted that an addition of trace amounts of water into nonaqueous solutions of lecithin caused a sudden increase in the viscosity (~ 100 times) producing a transition of the initial nonviscous solution into a jelly-like state. In succeeding years, it was demonstrated that lecithin, when dissolved in a nonpolar solvent, forms spherical reversed micelles. The addition of water induces an uniaxial growth of the micelles. As a result, at the end of the preparation one will find cylindrical aggregates instead of the initial spherical ones. After reaching threshold length, the extended micelles begin overlapping, forming a temporal 3D network. This

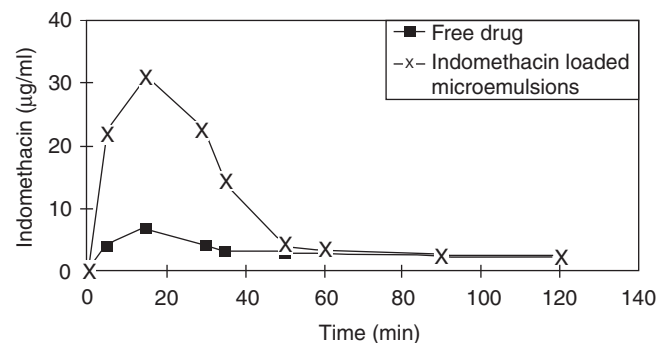


Figure 37. Bioavailability of free indomethacin or loaded microemulsions.

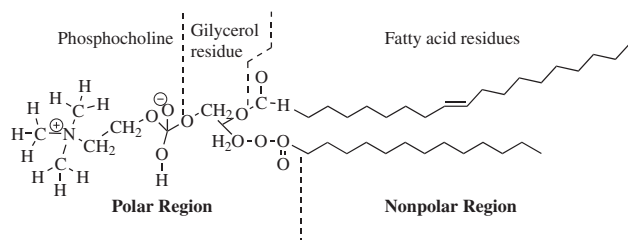


Figure 38. Structural formula of lecithin.

supramolecular structure from entangled micellar aggregates bears resemblance with that of uncrossed polymers in semidilute or concentrated solutions. For this reason, they are often called polymer-like micelles, wormlike, or threadlike micelles, or spaghetti-like structures.

The transition to polymer-like micelles is accompanied with a formation of hydrogen bonds between the phosphate group of a lecithin molecule (Fig. 38) and water.

The lecithin organogel is an optically transparent isotropic phase, appearing as the initial solution before the addition of water. The only difference between them is in the increased viscosity. This aspect is strictly dependent on oil, water, and lecithin concentrations, as well as on temperature (224). The amount of water needed to obtain the gel-like structure is a peculiar properties of any organic solvent (225). An important parameter for the organogel structure formation is the purity of the lecithin solution, in fact, commercial low purity lecithin is not able to form gels (226). The last component for the formation of lecithin organogel is water. This solvent can be substituted by polar organic solvents, such as glycerol, ethylene glycol, and formamide, or by a mix of ethanol–water in different ratios (227). The physical–chemical characteristics of the incorporated drug noticeably influence its release from organogel (144).

An important characteristic of this aggregate is its thermoreversibility, in fact, at 40 °C they become fluid, but by reducing the temperature they again reassemble a gel-like structure.

In this kind of carrier, it is possible to load hydrophilic drugs (localize themselves in aqueous, internal compartment), lipophilic drugs (in the hydrophobic environment), and amphiphilic substances (at the interface w/o).

The principal application of this carrier is its transdermal delivery, as first proposed in the early 1990s by Luisi's research group (225,226). Scopolamine, broxaterol, and propranolol were incorporated into lecithin organogels (containing cyclohexane, isooctane, or IPM as the oily phase). The permeation rates increased 10 fold compared to a solvent drug solution used as a control (180). The utility of lecithin organogels has been supported by *in vivo* human skin tolerability studies by means of a noninvasive technique as spectrophotometry of reflectance (228). *In vivo* percutaneous tolerability results showed no appearance of erythema even after 48 h of application. Certain amphiphilic lipids are characterized by lyotropic and thermotropic aggregation-phase transition. These supramolecular aggregates are under investigation to evaluate their potentialities as drug delivery systems (229).

COLLOIDAL DRUG DELIVERY DEVICES

The main scope of colloidal drug delivery systems is the modulation of the pharmacokinetics and/or the tissue distribution of a drug in a beneficial way. The properties of colloidal drug delivery systems to target specific sites of action (organs or tissues) are related to the physicochemical and morphological properties of the carriers, namely, these parameters determine the destination and the fate of the drug entrapped within the carrier system, provided that a drug is released from the system at a suitably controlled rate (230,231). By using colloidal carriers, drugs can be selectively directed to specific sites by applying passive or active strategies of delivery, rather than allowing a free drug diffusion throughout the body by using conventional dosage forms. The carrier physicochemical properties (i.e., size and surface properties) are the main determining factors in passive targeting of colloidal drug carriers. On the other hand, the possibility to achieve a colloidal carrier with active targeting capacity is related to the possibility of inserting specific ligands on the carrier surface so as to achieve a specific receptor-mediated interactions with target cells (232,233).

The potential use of colloidal drug carriers in clinical therapy is strongly related to their *in vivo* fate. In particular, the rapid uptake (following a phagocytosis mechanism) of these carriers by the reticulum endothelial systems (RES), that is abundantly present at the level of the liver, spleen bone marrow, and lungs, is the only fate after their IV administration, thus leading to rapid removal from blood circulation. The phenomenon of opsonization, that is based on binding of some plasma proteins (opsonines) onto the surface of colloidal carriers, is the first step allowing the carrier recognition and binding promotion by phagocytes (234). Therefore, the opportunity to avoid the carrier opsonization is often translated into a deep change of the carrier biodistribution patterns.

In this attempt, colloidal carriers with the ability to avoid RES uptake have been developed, thus achieving long circulating properties (235). The so-called Stealth colloidal carriers are obtained by grafting their surface with hydrophilic macromolecules, mainly poly(ethylene glycols), that hamper the opsonization.

Colloidal drug delivery systems are not able to extravasate, except in tissues and/or organs in which the endothelium is discontinuous (i.e., liver, spleen, and bone marrow) or defective, such as in the case of tumors or in the sites of infection and/or inflammation. Therefore, the therapeutic uses of IV administered colloidal drug delivery devices can be grouped into three cases (235,236): (1) drug accumulation in macrophages; (2) *in vivo* drug distribution away from the sites of toxicity; (3) circulating reservoirs of labile or short blood half-life drugs.

The use of colloidal drug delivery systems has the following advantages: protection, duration, direction, internalization, and amplification.

Protection. Drugs entrapped within colloidal carriers can be protected against both environmental factors (i.e., temperature, UV radiation, moisture) and the

action of detrimental factors of the host (i.e., degradative enzymes). Also, the patient can be protected against toxic effects of administered drugs.

Duration. These carriers can be suitably projected and prepared to achieve a perfectly controlled drug release to fulfill the therapeutic requirements, thus allowing the maintenances of therapeutic (but non-toxic) drug levels in the bloodstream or at the level of local administration sites for a prolonged time. This situation leads to a reduction of administration frequency, and hence to enhanced clinical safety and increased patient compliance.

Direction. As mentioned above, drugs may be passively or actively targeted to specific sites of action by colloidal delivery devices, thus providing an improvement of the drug therapeutic efficacy. These carriers can also provide a site-avoidance delivery, namely, the drug delivery away from sites of their toxicity.

Internalization. Colloidal carriers may be able to promote the intracellular delivery of drugs by ensuring different interaction pathways with target cells in comparison with the free drug that may not be able to reach the inner-cell due to unfavorable physicochemical parameters.

Amplification. In the case of antigen delivery, colloidal drug delivery systems can act as immunological adjuvant in vaccine formulations.

General Colloidal Carrier Classification

By considering the carrier features, colloidal drug delivery devices can be classified into conventional, long circulating and actively targeted systems (Fig. 39).

Conventional colloidal carriers (liposomes and nanoparticles) can be characterized by a wide differences both in terms of composition and physicochemical properties (i.e., size, size distribution, surface charge, number, and fluidity of phospholipid bilayers), in the case of liposomes, matrix compactness, in the case of nanoparticles. The modulation of these properties can influence technological properties, such as colloidal stability, drug loading, drug release rate, and to a certain extent the *in vivo* behavior of conventional colloidal carriers (i.e., blood stability, clearance, and distribution). However, some *in vivo* features are very consistent among different types of conventional colloidal carriers, by presenting a short blood circulation time when parenterally administered due to a rapid RES uptake. A consequential successful therapeutic use of conventional colloidal carriers characterized by the accumulation at the level of the mononuclear phagocyte system is the delivery of antimicrobial agents to infected macrophages (237,238). Conventional colloidal carriers are also very effective as vaccine adjuvants against viral, bacterial, and parasitic infections (239).

Long-circulating colloidal delivery systems allow the therapeutic treatment of a wide range of diseases involving tissues other than liver and spleen (240). A common characteristic of all long-circulating systems is the presence along the surface of the colloidal carrier of hydrophilic macromolecular moieties, such as polyethylene glycol

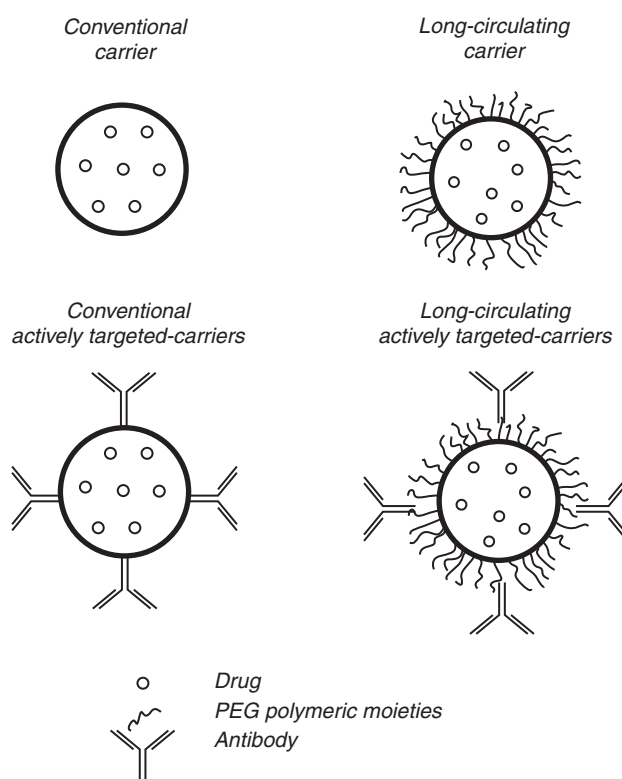


Figure 39. Schematic representation of the various kind of colloidal drug delivery devices. Conventional carriers are made up of a body matrix (phospholipid bilayers in vesicles or polymeric network in nanoparticles) with a hydrophilic colloidal surface (neutral, negatively, or positively charged). Long-circulating systems (the so-called Stealth devices) are coated by hydrophilic polymeric moieties (i.e., PEG) that provide a certain steric stability, and hence reduction of opsonization. Actively targeted carriers (i.e., antibody targeted) can be of conventional (targeting-agent conjugated directly to colloidal carrier surface) or sterically stabilized type (targeting-agent conjugated with a PEG moiety anchored to the surface of the colloidal carrier).

(PEG). Highly hydrated macromolecular moieties determine a steric barrier against interactions with molecular and cellular components in the biological environment, thus avoiding the opsonization phenomenon, and hence the RES organ uptake (235,240).

To obtain a certain specificity, actively targeted carriers can be obtained by conjugation of a colloidal drug delivery systems to specific antibodies, antibody fragments (e.g., Fab or single-chain antibodies), or small targeting agents (peptides, hormones, specific ligands), thus increasing target site binding and the delivery of the encapsulated drug. In the first generation of these kind of colloidal carriers, the active targeting agent was conjugated directly to their surface. This strategy led to a successful *in vitro* recognition and activity, but to a failure in *in vivo* applications due to the RES uptake. The last generation of actively targeted carriers is represented by long-circulating colloidal carriers with the PEG moieties conjugated with the targeting agent, thus presenting suitable *in vivo* features.

Colloidal Carrier Characterization

For routine measurements of particle sizes, two techniques are commonly used. Photon correlation spectroscopy (PCS) (also known as dynamic light scattering), which measures the fluctuation of the intensity of light when it is scattered by particles movements. The particle diameter range goes from a few nanometers to $\sim 3\ \mu\text{m}$, so PCS is not useful for lipid particles $>3\ \mu\text{m}$. In these cases, a laser diffraction (LD) technique is used. This method is based on the relation between the diffraction angle and the particle radius, so that smaller particles cause more intense scattering at high angles compared to the larger ones.

In general, it is recommended to use both techniques simultaneously in order to obtain precise data. However, it should be kept in mind that both PCS and LD do not measure particle sizes directly, they only correlate light scattering to particle size.

To obtain direct information on particle sizes and shapes, electron microscopy (EM) is used. Electron microscopy extracts structural information carried by the scattered electrons; the most commonly used EM techniques are transmission electron microscopy (TEM) and SEM. Atomic force microscopy (AFM) is another microscopic technique that is getting increasing attention. This method is based on the interactive forces between a surface and a probing tip that leads to the imaging of particles. This technique has the clear advantage of simplicity of sample preparation, so that it is possible to conduct analysis directly on the hydrated, solvent containing samples (241).

The field-flow fractionation (FFF) is a technique recently used for measurements of solid lipid nanoparticle sizes. It is based on the different effect of a perpendicular applied field on particles in a laminar flow (242); the characterization of particles is based on the different nature of perpendicular fields, for example, sedimentation size (cross-flow FFF) or charge (electric field FFF). All these principles can be used combined together in order to obtain unique resolution.

The determination of a zeta potential is predictive of the storage stability of colloidal dispersions (243). In general, the greater the zeta potential value of a nanoparticulate system, the better the colloidal suspension stability due to a repulsion effect between charged nanoparticles. Nanoparticle stability can also be obtained by the addition of some polymers, such as PEG, which adhere to the particle surface stabilizing it. Surface characteristics are also important for the *in vivo* fate and the interaction with biological systems of colloidal carriers.

The characterization of the physical state of colloidal carriers (particularly vesicles and lipid-based particles) can be efficiently carried out by two techniques, DSC and X ray. The DSC method is based on the fact that different material polymorphic forms possess different melting points and melting enthalpies (244) and that changes in thermotropic parameters of a systems are usually evidence of different spontaneous and/or induced arrangements. X-ray techniques allow the characterization of polymorphic forms and the determination of large and small spaces in an ordered matrix, such as the lipid grid of a solid lipid nanoparticle (245). The advantages of these two techniques

are the possibility of particle suspension analysis without drying the solvent, thus avoiding possible modifications of the carrier structure.

Also, NMR and electron spin resonance (ESR), are used for the investigation of dynamic phenomena in colloidal lipid dispersions. Nuclear magnetic resonance is based on the different proton relaxation times in the liquid and semisolid–solid state (246). The NMR technique can also be used to determine lamellarity in vesicular carriers (247). The ESR technique uses a paramagnetic spin probe to give a noninvasive characterization of the distribution of the spin probe between hydrophilic and hydrophobic phases. Both NMR and ESR are noninvasive methods and allow repeated measurements of the same sample.

Vesicular Drug Carriers

Drug delivery systems composed of lipidic compounds have gained great importance in medical, pharmaceutical, cosmetic, and alimentary fields. Formulations based on phospholipids and other excipients represent an interesting field of application in the novel research for delivery models.

Lipidic materials are characterized by their possibility to self-organize in different supramolecular arrangements as a function of some environmental factors (i.e., temperature, lipid concentration, type of medium, ionic strength, pH value, and presence of other compounds). Among the various supramolecular forms of aggregation, the bilayer structure, and hence the formation of vesicles (defined as a lipid bilayer surrounding an aqueous space) represents the most suitable device in terms of drug delivery. In fact, vesicles are boundary structures (Fig. 40), in which it is possible to have at the same time various microenvironments characterized by different physicochemical properties, namely, a highly hydrophilic region made up of the intravesicular aqueous compartment, a highly hydrophobic region of the bilayer core made up of the alkyl chains of the lipid constituent, and an amphipathic region at the level of the vesicular surface made up of the polar lipid headgroups. These peculiarities make vesicular systems a very versatile drug carrier being able to entrap and deliver hydrophilic (in the intravesicular aqueous compartment), hydrophobic (in the core of vesicular bilayers), and amphipathic (at the level of vesicular boundary zone) drugs.

An important feature that makes vesicles a unique drug delivery system is the biomimeticism of having the same supramolecular lipid organization of natural membrane living cells.

Therefore, the possibility to create a structure similar to the biological membrane for carrying out the delivery of drugs has represented an interesting challenge for a number of researchers. In particular, liposomes, ethosomes, transfersomes and niosomes have been extensively investigated and are up to now the main vesicular systems used in drug delivery.

Liposomal Carrier. The appearance of Bangham's vesicle in the mid-1960s, the so-called Liposome, represented a milestone in the field of innovative drug delivery. Liposomes are mostly made up of phospholipids, and for

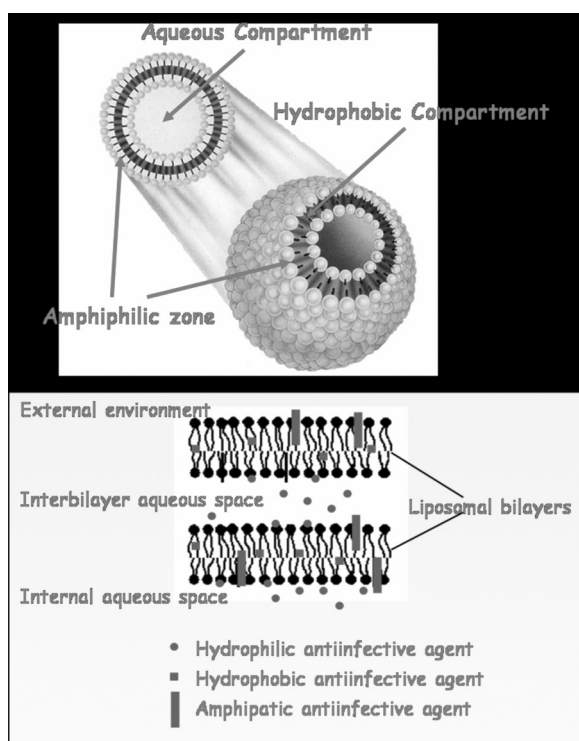


Figure 40. Schematic representation of a liposomal structure with the characteristic microenvironments.

this reason they are highly biocompatible and biodegradable. The liposomal carrier has the advantage of also being able to deliver macromolecules, such as enzymes, proteins, and genetic material (248).

From the morphological point of view (Table 9), liposome systems can be classified as a function of the number of bilayers and the mean size of the carrier in unilamellar, oligolamellar or multilamellar vesicles, and in small (<100 nm), medium (100–500 nm) and large (>1 μm) vesicles, respectively.

Lipid Component Used in Liposomal Formulations.

Lecithins and cholesterol (Chol) are the lipids most commonly used in the preparation of liposomes. Other components can be used in the liposome preparation, that is, steroid molecules, charged phospholipids, ganglioside,

and polymeric material to modulate the carrier properties as a function of the therapeutic requirements to be achieved (249). In fact, different components can modify the biodistribution, the surface charge, the release, and the clearance rate of the liposomal drug delivery system (249,250). The circulation lifetime of a liposome is also altered by the charge of the liposome surface that can influence the pharmacokinetic of the system (251).

It was demonstrated that the use of negatively charged lipids [i.e., phosphatic acid (PA), phosphatidylserine (PS), phosphatidylglycerol (PG)] are able to elicit a rapid clearance of the liposomal system from the blood stream mediated by the RES uptake (249,252).

Cholesterol plays a fundamental role in liposome formulations being, able to act as a vesicle membrane modulator as concern membrane fluidity. It has a stabilizing function on the liposome bilayers both *in vitro* and *in vivo*, allowing the protection of the vesicular structure by the action of blood high density lipoproteins (HDL) and hence the possibility of having a prolonged circulation of intact liposomes (253).

Similarly to cholesterol, some phospholipid components are also able to influence the physicochemical behaviors of liposomes to obtain a more rigid vesicular structure that is much more resistant to the phospholipid extraction effect mediated by blood HDL. In this attempt, both 1,2-distearoyl-3-*sn*-phosphatidylcholine (DSPC) and sphingomyelin (SM) have been used to maintain a certain vesicular carrier integrity following IV administration. A rigid vesicular structure of liposomes hampers an effective adsorption of opsonine and prolongs the plasmatic level of the drug carrier, that is, liposomes made-up of 1,2-distearoyl-3-*sn*-phosphatidylcholine-cholesterol (DSPE-Chol) showed higher half-time than liposomes prepared with phosphatidylcholine (PC) or 1,2-dipalmitoyl-3-*sn*-phosphatidylcholine (DPPC) (253). In particular, SM has an additional stabilizing effect on the liposome formulations when used together with Chol (254). In this case, SM can interact with cholesterol by forming intermolecular hydrogen bonds and eliciting an increased compactness of the liposomal bilayers that leads to an improved serum stability (249).

Since the appearance of liposomes, positively charged lipids were introduced in liposome composition to obtain a vesicular system characterized by a net positive charge

Table 9. Main Characteristics of the Various Liposome System

Liposome Type	Abbreviation	Properties
Multi-lamellar vesicles	MLVs	Vesicles constituted by 7–15 bilayers with a mean size > 1.5 μm
Multi-vesicular vesicles	MVVs	Vesicles constituted by 3–5 vesicles contained within a bigger one. The mean size is > 1.5 μm
Oligo lamellar vesicles	OLVs	Vesicles constituted by 2–5 bilayers with a mean size $\sim 1 \mu\text{m}$
Giant unilamellar vesicles	GUVs	Vesicles constituted by only one bilayers with mean size $\geq 1 \mu\text{m}$
Large unilamellar vesicles	LUVs	Vesicles constituted by only one bilayers with mean size ranging from 400 to 800 nm
Medium unilamellar vesicles	MUVs	Vesicles constituted by only one bilayers with mean size ranging from 200 to 400 nm
Small unilamellar vesicles	SUVs	Vesicles constituted by only one bilayers with mean size ranging from 30 to 100 nm

along the liposomal surface. In the last decade, positively charged liposomes have gained much more interest than in the past due to their potential application as carriers for genetic material delivery (255). In this attempt, the most frequently used cationic lipids are DMRIE, *N*-(2-hydroxyethyl)-*N,N*-dimethyl-2,3-bis(tetradecyloxy)-1-propanaminium bromide; dioctadecyl amino glycyl spermine (DOGS); dioleoylphosphatidylethanolamine (DOPE); 2,3-dioleoyloxy-*N*-[2(spermine carboxaminino)-ethyl]-*N,N*-dimethyl-1-propanaminium trifluoroacetate (DOSPA); 1,2-dioleoyl-3-trimethylammonium propane (DOTAP); 2,3-bis(oleyloxy)propyl-trimethylammonium chloride (DOTMA). Cationic liposomes composed of DOTMA and DOPE became commercially available as a transfection reagent designated Lipofectin.

The above mentioned cationic lipid components of this particular kind of liposomes are able to interact with, and neutralize, the negatively charged DNA or ribonucleic acid (RNA). This interaction leads to a genetic material condensation into a more compact structure. The resulting lipid-genetic material complexes (lipoplexes), rather than DNA or RNA encapsulation within liposomes, provide protection and promote cellular internalization and expression of the condensed plasmid (255).

Most recently, amphiphilic polymeric materials have been introduced in the composition of vesicles to cover their surface by inserting their hydrophobic domain in the liposomal bilayers (anchor moiety) and facing the hydrophilic domain toward the aqueous environment (shield moiety). This advance allowed a further modularity of the liposomal carrier by conjugating together the advances of colloidal drug delivery devices (carrier capacity) with those of macromolecules (fine chemical approach and infinite modulation potentiality) (249). The principal polymer used to cover the surface of liposome formulation was polyethylene glycol. This is a flexible-chained hydrophilic polymer of different molecular weight (i.e., PEG-750, PEG-2000, PEG-5000) conjugated to phosphatidylethanolamine (PE) or distearoylphosphatidylethanolamine (DSPE) (256). Liposomes containing PEG in their structure (the so-called pegylated liposomes) represented an important class of vesicular delivery systems that started the new generation of liposome carriers. The presence of this hydrophilic polymer on the surface of liposomes not only is able to reduce the RES uptake and to increase the blood circulation time (249), but it can also modulate some pattern of interaction with cultured cells, such as the intracellular drug delivery (257).

Another important aspect for drug delivery by liposomes is the possibility to achieve a triggered release of the encapsulated agent from the carrier following certain stimuli. Targeted drug delivery is based on the fact that upon attachment to the target site, or delivery into the target cell, the therapeutic agent must be released from the carrier to exert its action. When liposomes are taken up by the target cell through endocytosis, they come into contact with acidic conditions. For some drugs and biotechnological products (e.g., peptides and genetic material) it could be essential to escape from liposomes and endosomes, thus entering the cytosol before reaching the lysosomal structures with their highly efficient degradation machinery.

Liposome destabilization under acidic conditions and bilayer fusogenic properties are required to achieve lysosome escape. Besides the pH-dependent liposome release, other triggered releases may be accomplished for certain drug selectivity, namely, bilayer composition controlled release, destabilization by removal of bilayer components, complement-induced leakage, and temperature-induced destabilization of the liposomal bilayer structure. Therefore, to have a triggered liposomal carrier release, some compounds that are stimuli responsive must be introduced in the liposomal bilayer composition (e.g., DOPE, cholesteryl hemisuccinate, oleic acid, fusogenic peptides) (258,259).

Main Methods to Prepare Liposomes. As reported in Table 9, various types of liposomes exist, each of those with specific peculiarities that make them suitable for certain therapeutic applications. Although aqueous dispersions of phospholipids spontaneously lead to a self-aggregation into closed bilayers, vesicles, particular procedures must be carried out if a certain type of liposome has to be obtained. In fact, this type of liposome is mainly determined by the preparation procedure, and for these reason the main preparation methods are reported below.

Thin-Layer Evaporation (TLE). This method allows the formation of multilamellar vesicles. Basically, a mixture of lipid compounds is dissolved by an organic solvent (chloroform) or a mixture of two organic solvents (chloroform-methanol) in a round-bottomed flask. Other hydrophobic components (e.g., drugs) can be cosolubilized with the liposome-forming materials. The complete evaporation of the organic solvent by a rotavapor lead to the formation of a thin lipid film along the surface of the glass wall. This lipid film is then hydrated with an aqueous solution buffered to the desired pH value and solubilizing any hydrophilic component that should be entrapped within liposomes (e.g., water-soluble drugs). The hydration temperature is normally higher than the highest transition temperature (T_m) of lipids used in the film preparation. In some cases, to increase the surface of film deposition, and hence the surface undergoing buffer hydration, glass beads can be added during the TLE preparation procedures (260).

Reverse-Phase Evaporation Vesicles (REV's). This method allows us to obtain large unilamellar, oligolamellar, and multilamellar vesicles. A lipid film, formed as reported in the TLE method, is dissolved in an organic solvent (diethyl ether) and an aqueous solution is added. This two-phase mixture is energetically sonicated, thus obtaining an w/o emulsion. The organic solvent constituting the external phase of the w/o emulsion is gradually removed by a rotavapor up to the reversion of the phases with the appearance of an external hydrophilic phase. The total removal of the organic solvent leads to the formation of a gel-like highly concentrated liposome suspension that can be suitably diluted with a suitable aqueous buffer solution. This method represents the first approach used in the attempt to increase the amount of drug entrapped within vesicles (261,262).

Freeze and Thawed Multilamellar Vesicles (FAT-MLVs). A multilamellar liposome formulation obtained with the TLE method is subjected to a series of cycles of freezing in liquid nitrogen and thawing in warm water ($\sim 40^\circ\text{C}$). At the end of the procedure, liposomes are kept at room temperature to stabilize the bilayer. This procedure is carried out to obtain a multilamellar liposomes with a homogeneous distribution of solutes throughout the various multilamellar aqueous compartment (263,264).

Dehydration Rehydration Vesicles (DRVs). Multilamellar liposomes obtained with one of the previous methods are submitted to a freezing-drying process. The product of lyophilization is resuspended in an aqueous solution (265). This method leads to the formation of oligolamellar or multilamellar liposomes with an high drug entrapment efficiency.

Vesicles by Extrusion Technique (VET). The reduction of the mean size of a colloidal liposomal suspension characterized also by a narrow size distribution can be achieved with the extrusion of multilamellar liposomes through polycarbonate membranes of different sizes (from 400 to 50 nm). Usually, 10 cycles of extrusion are carried out to obtain an homogeneous formulation. Both LUV and SUV are obtained following the VET method (266).

pH Gradient Loading Method. This method is used to increase the loading capacity of liposomes in regard to ionizable drugs. This method is based on the formation of a pH gradient between the inner-liposomal aqueous phase and the external environment. This situation promotes the protonation or deprotonation of an entrapped drug thus favoring its accumulation within the vesicular carrier due to the incapability of a ionized molecule to freely diffuse through a lipid bilayer (Fig. 41) (267). Ammonium sulfate or ammonium citrate are used to obtain an acid pH environment while calcium acetate to have basic conditions (250,268). The efficiency of liposome

drug loading using the method of pH gradient is influenced by the drug partition coefficient between the aqueous phase and the lipid bilayer (269).

One of the most important parameters for an ideal drug delivery system is the drug loading capacity. The amount of drug encapsulated in liposome formulation is influenced by a series of parameters, such as the preparation method, the size of the liposome, and the type of lipid used to form the lipid film (263). Therefore, to have a colloidal liposome system with particular carrier properties, it is often necessary to carry out two or more preparation procedures. Namely, the DRV or FAT procedure can be carried out to improve the liposome encapsulation capacity, and then the VET method to obtain a small mean size with a narrow size distribution. These two aspects (carrier capacity and mean size) are very important for liposomes to be proposed for certain therapeutic application (i.e., antitumoral chemotherapy).

The removal of untrapped drug is the last step in the preparation of a drug-loaded liposome colloidal suspension. Many lipophilic drugs exhibit a high affinity to the bilayer and are completely liposome associated. For compounds with an encapsulation $< 100\%$, the nonencapsulated fraction of the drug may determine unacceptable side effects. The removal of the untrapped drug can be carried out by the following techniques: dialysis, ultracentrifugation, ultrafiltration, gel permeation chromatography, and ion exchange reactions.

Liposome Stability. An ideal drug delivery system should maintain its physicochemical characteristics during storage, that is, mean size, size distribution, thermotropic parameters, no lipid degradation (hydrolysis and/or peroxidation), no appearance of microbial flora, to be considered for practical applications. Liposomes are self-assembled colloidal carriers, and hence their stability can be strongly influenced by the component used for their preparation, considering that the presence of foreign molecules in the liposomal bilayers deeply influence their mode and strength of aggregation in a concentration-dependent manner. For this reason, in the case of drugs to be delivered by liposomes and characterized by liposomal bilayer localization, particular attention should be paid to the drug/lipid ratio. This is a very important parameter because the payload of the drug can be increased with a consequential reduction of the system stability. In some cases, the segregation of the lipid bilayer components in various microdomains can be observed (270).

The osmolarity of liposomes seems to be a very important factor to achieve a stable liposomal system. Some studies (271) showed that hypertonic conditions triggered a rapid drug release from Ara-C-loaded liposomes and that the release kinetic is characterized by a biphasic profile with a first step of very rapid and massive Ara-C release followed by a second phase of slow drug release (249).

The chemical stability of liposome formulations mainly depends on the chemical characteristics of both drugs and lipid component used for the carrier preparation (272). The

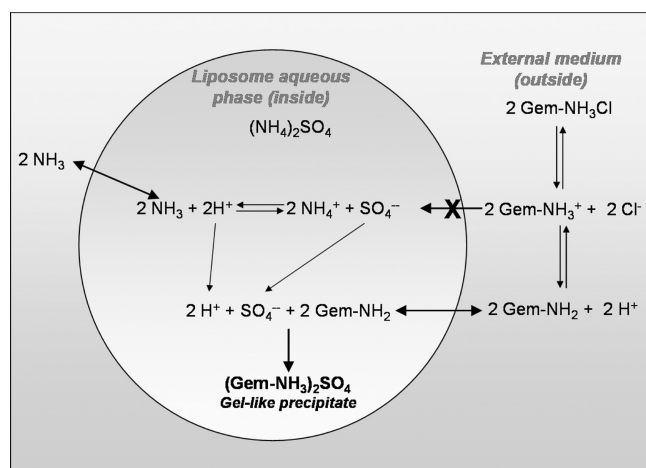


Figure 41. A schematic representation of the encapsulation of Gemcitabine in multilamellar liposomes by using a pH gradient method in the presence of ammonium sulfate 250 mM (267).

presence in the phospholipid bilayers of polyunsaturated fatty acid moieties, such as arachidonic, linoleic, or linolenic acid, can favor the occurrence of peroxidation processes at the level of single or conjugated double bonds. The membrane lipid peroxidation can destabilize liposomal bilayers due to the formation of secondary oxidation products that can change the integrity of the liposome structure (249).

Main Therapeutic Applications of Liposome. The following criteria should be taken into account to evaluate the possibility of delivering a drug by using the liposomal carrier: (1) the chosen drug should be sufficiently active; (2) the drug should be efficiently entrapped within liposomes; (3) the drug must be compatible with the liposomal carrier.

A basic concept for the success of liposome drug delivery is the fact that the encapsulated agent may be released at a suitable rate to become bioavailable upon arrival at the action site. Liposomes protect drugs from metabolism and inactivation in plasma and also allow a reduction of the drug accumulation in healthy tissues and/or organs, due to size restrictions in the transport of large macromolecules and carriers across healthy endothelium (271). A number of pathologies (i.e., cancer, stroke, infections, and some metabolic diseases) are characterized by direct or mediated inflammation, which elicits discontinuities in the endothelium vasculature of the diseased zone. This thus increases the extravasation of colloidal carriers and, in combination with an impaired lymphatics and a high value of interstitial pressure, the accumulation of the therapeutic agent-loaded liposomes at the level of the diseased site (passive targeting). This situation, referred to as enhanced permeation and retention (EPR) phenomenon, consequently elicits an increase of the drug therapeutic index (249).

A successful therapeutic approach of the liposomal passive targeting is the efficacious delivery both *in vitro* and *in vivo* of various anticancer drugs (249). As shown in Fig. 42, the use of pegylated liposomes (Stealth liposomes) with a mean size of ~ 100 nm allows the passage of the carrier in the tumor tissue and a local accumulation of the encapsulated drug. Furthermore, liposomal chemotherapeutic agents display distinctive pharmacokinetic charac-

teristics, because they possess longer elimination half-lives, reduced clearance, and smaller volume of distribution with respect to corresponding free drugs. Taken together, these features lead to the highest levels of cytotoxic agents in tumors, as demonstrated in preclinical models and clinical trials, whereas healthy tissues are spared from toxicity. Liposomal anticancer drugs lead to improved clinical effectiveness and better toxicity profile with respect to corresponding free drugs when they are used for the treatment of metastatic tumors (e.g., breast and ovarian cancers). A successful example of antitumoral agent-loaded long-circulating liposomes is Doxil, a doxorubicin-loaded pegylated liposomes with a 100 nm mean size.

This innovative liposomal formulation is currently approved for use in AIDS-related Kaposi's sarcoma and refractory ovarian cancer. It has also shown activity in other tumors, including metastatic breast cancer. A pre-clinical toxicology study of IV administered doxorubicin-loaded stealth liposomes compared to the free drug showed that the drug liposomal formulation was less toxic (LD_{50} $32 \text{ mg} \cdot \text{kg}^{-1}$) than the free doxorubicin (LD_{50} $17 \text{ mg} \cdot \text{kg}^{-1}$). The organ specific toxicities seen with Doxil were qualitatively similar to those of free doxorubicin, but less severe (273). In addition, Doxil accumulates in tumor tissues to a large extent with respect to the free drug due to its capacity to escape macrophagic uptake (274). Reduced toxicity and selectivity are the reasons of the improvement of doxorubicin therapeutic index.

A recent and very active field of research in the liposomal anticancer chemotherapy is the active targeting of long-circulating liposomes (249,275,276). A high density of the targeting moiety on the surface of liposomes is very important to have an efficient binding to the target site, a specific antigen or receptor expressed on the surface of target cell. This interaction increases the amount of drug in the target site and it decreases the systemic side effects (275).

Antibodies, particularly monoclonal, are the more versatile ligands that can be conjugated on the liposome surface (the so-called immunoliposomes). In the past, an obstacle in the use of immunoliposomes was the antigenicity of murine antibodies that were easily available, however, the more recent availability of humanized forms should contribute to overcome this problem. Important

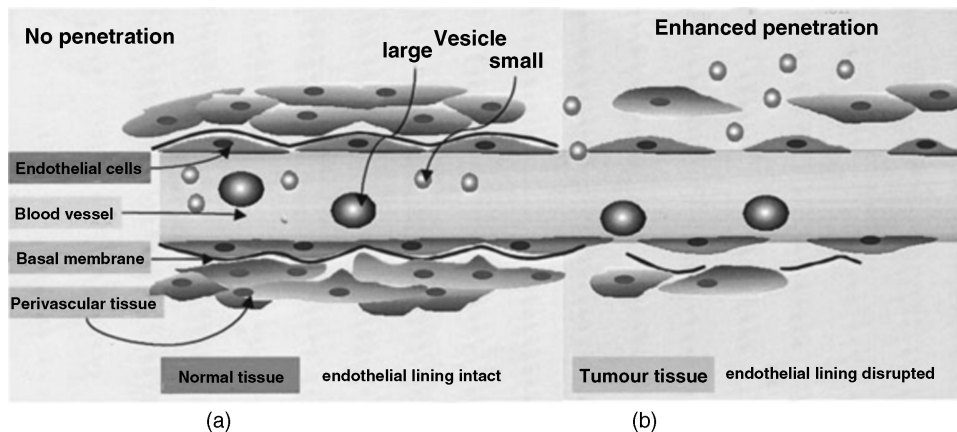


Figure 42. Schematization of the accumulation mechanism of long-circulating small unilamellar liposomes in solid tumor. Extravasation of liposomes through vascular endothelium of the tumor site (a); behaviour of liposomes in a normal tissue (b).

parameters for immunoliposomes are the ability to become selectively cell associated and the ability to deliver the loaded drug within target cells. In the case of immunoliposomes endocytosis seems to be the predominant mode of delivery to the cells, and hence has an efficient intracellular delivery. The mean size of immunoliposomes should be ≤ 100 nm.

Given a suitable antibody with high specificity and affinity for the target antigen, the critical factor is the *in vivo* accessibility of target cells to the immunoliposomes. To have an efficient target binding of the injected immunoliposomes, target cells should be located in the intravascular compartment and/or in accessible tissues and organs characterized by leaky vascular structures. Thus, in terms of targeting drug delivery by immunoliposomes, two anatomical compartments can be considered. One is a readily accessible intravascular site, such as the vascular endothelial surface, T cells, B cells, or a thrombus. The other is a much less accessible extravascular site, such as a solid tumor, an infection site, or an inflammation site, where the vascular structure is leaky (277).

Antibiotics encapsulation in liposomes is of great utility in the case of very potent drugs that can be administered intravenously and present a certain toxicity (i.e., nephro- and neurotoxicity). The toxicity of antibiotics limits their dosing, and hence the drug efficacy. Antimicrobial agent-loaded liposomes were used for the treatment of various obligate and facultative bacterial infections (i.e., *Salmonella*, *Listeria*, *Brucella*, *Mycobacterium*, *Staphylococcus* and *Escherichia coli*) (278). Obligate microbes are more difficult to eradicate due to the fact that they can multiply only within host cells, while facultative bacteria can be reached by the drug in the extracellular compartment. The conventional liposome biodistribution properties represent a noticeable advantage for treatment of infections in which bacteria are taken up and/or reside in the cells of the phagocytic systems. Another advantage of the liposome carrier is the capability to facilitate the entrance within infected cells of antimicrobial agents that are not able to cross cell membranes with a consequential intracellular drug accumulation (279) (Fig. 43). An intrabacterial antibiotic drug accumulation was also observed (280), thus showing that liposome formulations can contribute to overcome bacterial resistance phenomena due to drug impermeability (Fig. 44). In particular, in the case of intracellularly infected phagocytic cells (e.g., *Legionella pneumophila*, *Mycobacterium tuberculosis*, *Listeria monocytogenes*, and *Staphylococcus aureus*) a 10–100 times increased efficacy has been reported for the antibacterial agent-loaded liposome formulation compared to the free drug (278) both *in vitro* and *in vivo*. The specific macrophage targeting of liposomes can be further improved by grafting the surface of liposomes with carbohydrate moieties whose receptors are expressed along the surface of macrophages. This possibility may lead to an additional efficacy of the liposome delivery device in the *in vivo* treatment of intramacrophagic infections.

Long-circulating liposomes with a reduced size have the opportunity to accumulate in the infection site according to the mechanism reported in Fig. 42. In an experimental *in vivo* model, a large accumulation of long-circulating liposomes in the infected lung was observed; while no presence

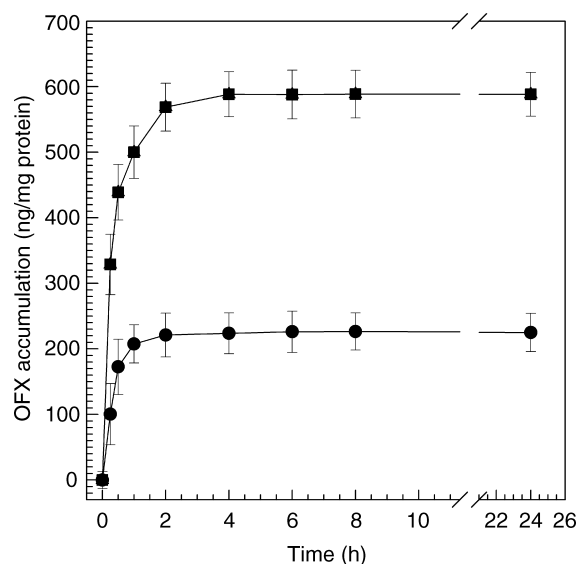


Figure 43. Accumulation profiles of ofloxacin into McCoy cells as a function of time. The biological assay was carried out at room temperature (20°C) by adding $5.7\ \mu\text{g}\cdot\text{mL}^{-1}$ of free (●) or liposome entrapped (■) ofloxacin into confluent McCoy cells. Each point represents the average of nine different experiments $\pm$ standard deviation. Data from Ref. 279.

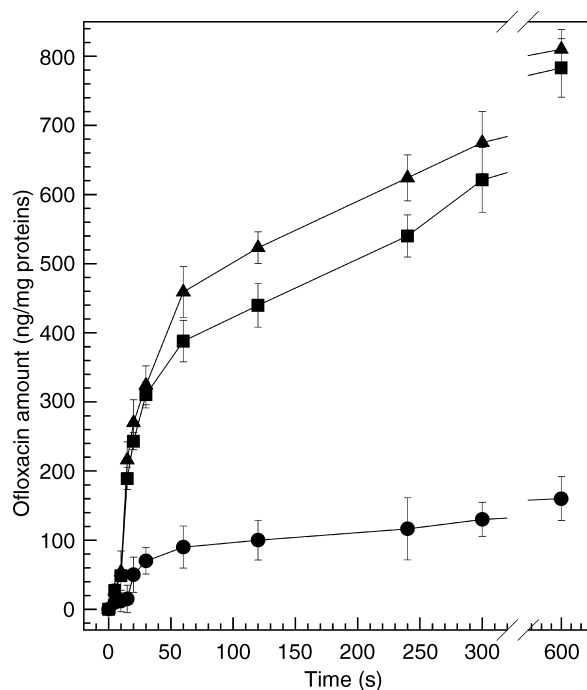


Figure 44. Intrabacterial accumulation of ofloxacin-loaded MC-Chol-DP (4:3:4 M ratio) unilamellar liposomes within *E. coli* ATCC 25922 (■) and *E. coli* ATCC 35218 (▲) versus the free drug (*E. coli* ATCC 35218 accumulation) (●) as a function of time. Free drug accumulation within both *E. coli* strains is very similar (data not reported). The experiments were carried out at 37°C . Each point represents the mean value of five different experiments $\pm$ S.D. Data from Ref. 280.

of long-circulating liposomes was noted in the noninfected lung. Interestingly, the accumulation extent seemed to be a function of the severity of the infection (281). Therefore, Stealth liposomes offer targeting to the deep tissues, which can harbor *Mycobacterium avium intercellulare*. The chance of using Stealth liposomes containing some new and potent antibacterial agents, (e.g., fluoroquinolones) can represent a real improvement in the therapy for the eradication of infections situated in organs and tissues other than the RES.

Liposomes can be suitable delivery devices in antiviral chemotherapy (282) due to their capability of delivering entrapped drugs across cell membranes (257,279). This aspect is of fundamental importance in antiviral chemotherapy, because the nature of virus action and proliferation is intracellular. In particular, the liposomal therapy of viral infections can be accomplished by two different approaches: (1) the encapsulation of the antiviral drug having a liposome-mediated antiviral activity; (2) the encapsulation of immunomodulators, such as lymphokines (macrophage activation factor, MAF), thus achieving an activation of the macrophages.

Liposomal antiviral chemotherapy can offer special targeting possibilities due to the natural ability of viruses to fuse with cellular membranes. In this case, various antiviral therapeutic approaches can be achieved by the following strategies: (1) the administration of drug-loaded long-circulating liposomes bearing cellular antigens that attract and destroy viruses; (2) the saturation of the cell receptor by binding other antigens delivered with liposomes; (3) the reconstitution of viral glycoproteins onto liposomes (the so-called virosomes), which are characterized by a very strong fusogenic activity depending also on the vesicle lipid composition. Such virosomes can bind to and fuse selectively with the infected cells. Therefore, this particular carrier can ensure a very effective and specific intracellular antiviral therapy.

Liposomal antiviral chemotherapy, for example, can be efficaciously used for the treatment of HIV infection. The encapsulation of gelonin (a plant toxin) allowed a selective killing of human immunodeficiency virus (HIV) infected cells (283). Another success with respect to HIV therapy was observed in the case of treatment with liposomes containing fragment A of diphtheria toxin, which was toxic to HIV infected cells, but not to uninfected cells (284).

Another application of liposomes in anti-infective chemotherapy can be the treatment of fungal infection. Invasive fungal infections are among the most important causes of morbidity and mortality in immunocompromised patients. Amphotericin B and nystatin are the most widely used drugs in the treatment of systemic fungal infections (285). These two drugs show some drawbacks when used *in vivo* in the treatment of mycosis (i.e., nephrotoxicity and side effects at the level of the CNS). In this case, liposomes are a suitable colloidal carriers for amphotericin B, not being able to accumulate in the kidneys (e.g., of the site avoidance mode of liposome action) and the nervous system and providing a smart system to efficaciously solubilize amphotericin B. As for other pathological situations, the most important advantage of the liposomal carrier is its ability to accumulate at the level of the same cells where

fungi are localized (mainly the RES). The improved selectivity and the reduced toxicity determined the noticeable increase of the amphotericin B therapeutic index. Considering the consistent therapeutic advantages of amphotericin B-loaded liposomes (286), a new liposomal formulation was produced and commercialized by Vestar, Inc., with the name of AmBisome. This pharmaceutical formulation is made up of phosphatidylcholine, cholesterol, distearoylglycerol, and amphotericin B (2:1:0.8:0.4 molar ration) with a 9.5 lipid/drug ratio. The mean size of these small unilamellar liposomes ranges from 45 to 80 nm.

Infective diseases caused by parasites are a great problem for developing countries. In these particular infections, especially for those pathologies where the infection agent is closely associated to the RES, the possibility of delivering already existing drugs by liposomes can represent a very attractive strategy. In fact, due to poor drug membrane penetration, *in vivo* treatments of these pathologies are often poorly effective, despite the *in vitro* effective activity of the drug. An interesting example of effective liposome treatment of protozoal diseases is leishmaniasis.

The parasites of leishmaniasis live almost exclusively in fixed macrophages at the level of the RES (liver, spleen, and the rest of the visceral). Antimonial derivatives (therapeutic index approaching 1) are the most effective drugs for this pathology. Liposomal formulations of these drugs can improve the therapeutic effectiveness up to a thousand times with respect to the free drug. Experiments showed that doses close to the lethal level of free potassium antimony tartrate were ineffective, but a single dose (40% of the previous dose; 20 mg·kg⁻¹) of drug-loaded liposomes completely eliminated the parasites (287).

Liposomes can also be used as immunoadjuvants for vaccines (288) and as macrophage activators against tumoral, viral, and microbial cells. For both applications, a substance is delivered to macrophages thus triggering immunization, immunomodulation, or activation by means of antigens. The presence in the liposome structure of a nonliposomal adjuvant, that is, muramyl tripeptide covalently coupled to phosphatidylethanolamine, can enhance the antibody response induced by liposome-associated antigens.

Liposomes can be efficaciously used to deliver to the CNS. Under some pathological conditions (i.e., tumors, ischemia, and traumatic shocks) a hypermeabilization of the blood-brain barrier can occur, thus allowing the passage of very small aggregates (< 100 nm). The CDP-choline loaded very small (50 nm) long circulating liposomes were used to treat successfully the cerebral ischemia (289,290). The drug-loaded liposome was able to increase the amount of drug that reached the brain and the survival rate of rats submitted to ischemia and reperfusion (Fig. 45). The liposomal formulation is also able to efficaciously antagonize the phenomenon of postischemic damage maturation that is the main reason of a poor neuronal recovery and hence of an enlargement of the damaged (291).

Liposome formulations resulted effective not only in systemic administration, but also in topical administration (e.g., dermal, mucosal, ocular, pulmonary).

The potential application of liposomes as dermal delivery systems has been extensively investigated, with regard

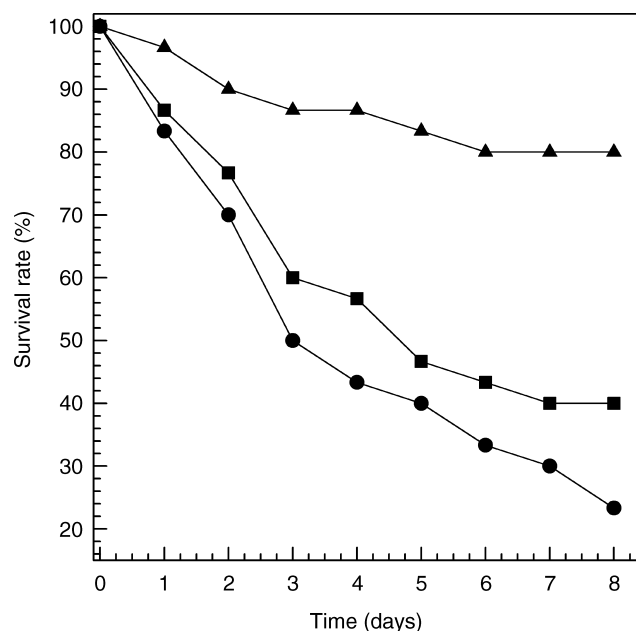


Figure 45. Survival rate of postischemic reperfusion Wistar rats (320–350 g). The duration of the ischemic event was 30 min. The rats were treated with saline ●, with the free drug ■, or with CDP-choline loaded liposomes ▲. Unloaded liposomes showed no effect on rat survival (data not reported). The results are expressed as the percentage of the total number of animals in each group which survived ischemia as a function of time. Data from Ref. 290.

to vesicle composition and size (292–294). It was proposed (295) that the main advantages of using liposomes as topical drug formulations were due to their demonstrated ability: (1) to reduce serious drawbacks and incompatibilities that may arise from undesirably high systemic absorption of drugs; (2) to enhance accumulation of drugs at administration sites due to the high substantivity of liposomes with biological membranes; and (3) to the possibility to incorporate both hydrophilic and hydrophobic drugs. In addition, liposomes can be readily prepared on a large scale. The requirement of smart drug delivery systems for skin application comes from the necessity to have a modulation of both the administration rate and the skin permeation properties, namely, a sustained drug release strictly confined at the level of the skin with no systemic absorption or an enhanced transdermal effect to deliver the drug to some inner structures (e.g., joints) or to achieve a systemic effect are required as a function of the disease to be treated (296). By the use of quantitative skin autoradiography, it was demonstrated that small liposomes allowed the localization of a greatest amount of caffeine (hydrophilic drug) in the epidermis and a lowest amount in the dermis and appendages (297). In this case, liposomes ensured a drug skin accumulation three times greater than that observed for an aqueous drug solution prepared in the presence of penetration enhancers.

The liposome lipid composition and the thermodynamic state of the liposomal bilayers play a crucial role in the effect of this vesicular carrier on drug transport rate across

the skin. In particular, incorporation of drugs in the liquid-state liposomes provides a higher skin permeation rate than that observed for drug-loaded gel-state (the so-called solid) liposomes (298). Liposomes made up of the same lipids usually present in the skin were prepared and referred to as skin-lipid liposomes (299). These kind of liposomes are able to provide a drug dermal delivery of the highest drug disposition within the deeper skin layers, that is, in the epidermis and dermis, while avoiding systemic drug adsorption (299). For example, skin-lipid liposomes can be a suitable topical carrier for chronic topical applications of corticosteroids by optimizing drug concentration at the site of action while minimizing systemic absorption and, as a consequence, possible side effects (300). In the case of transdermal drug delivery requirements, the high deformability of vesicular carriers seems to be a fundamental feature to achieve the intact vesicles penetration, thus also favoring the delivery of encapsulated drugs across the skin. Special liposomes characterized by an high bilayer elasticity have been developed, namely, ethosomes and transfersomes. Ethosomal systems are different from transfersomes by their structure and mechanism of action. As an example of different behavior, occlusion has no effect on skin permeation of molecules from ethosomes, while transfersomes are unable to enhance drug delivery under the occluded conditions. Ethosomal systems contain vesicles with fluid bilayers (soft vesicles) in a hydroethanolic milieu. Both components have a crucial role in the delivery of the active agent (301,302).

Liposomal colloidal carriers also can be applied as ophthalmic drug delivery devices to increase the bioavailability and the efficacy of drugs (303). Liposomes can enhance the ocular drug absorption and prolong the precorneal retention time (303), thus increasing drug effectiveness. In particular, the ocular application of positively charged small oligolamellar liposomes seems to be promising, considering that positively charged delivery devices may ensure a suitable bioadhesivity with the negatively charged corneal epithelium. As shown in Fig. 46, the acyclovir-loaded liposome showed a significant ($P < 0.005$) and noticeable increase of drug levels in the aqueous humor compared to the liposome-acyclovir physical mixture and the free drug (260). Several mechanisms can be proposed to elucidate the ocular effects of liposomes, but adsorption and/or lipid exchange seem to be most probably involved (303). Cornea permeability alteration due to liposomes may be discarded as a plausible explanation for enhanced drug penetration, since the presence of empty lipid vesicles added to drug solutions does not enhance the availability of the drug. Last, but not least, liposomes present a very good ocular tolerability showing no evidence of ocular inflammation or discomfort (260).

Niosomal Carrier. Niosomes are nonionic surfactant self-assembled vesicles that presents a structure similar to liposome (Fig. 47) and hence they can represent alternative vesicular systems with respect to liposomes, due to the niosome ability to encapsulate different type of drugs within their multienvironmental structure (304). The first application of niosomes was the cosmetic field followed by their use as drug delivery systems (305).

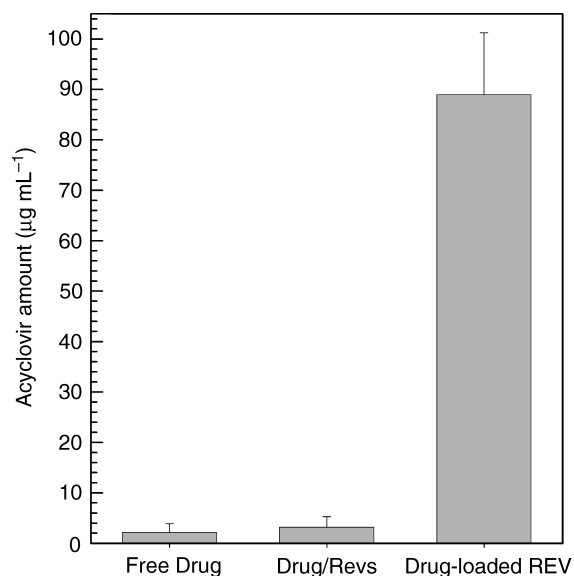


Figure 46. Aqueous humor concentrations of acyclovir at 30 min following topical instillation (50 µL) of acyclovir-loaded positively charged REV's (oligolamellar) liposomes (DPPC-Chol-DDAB 7:4:1 molar ratio), acyclovir–liposomes physical mixture and aqueous solution. Each bar represents mean values $\pm$ S.D. of four experiments. Data from Ref. 260.

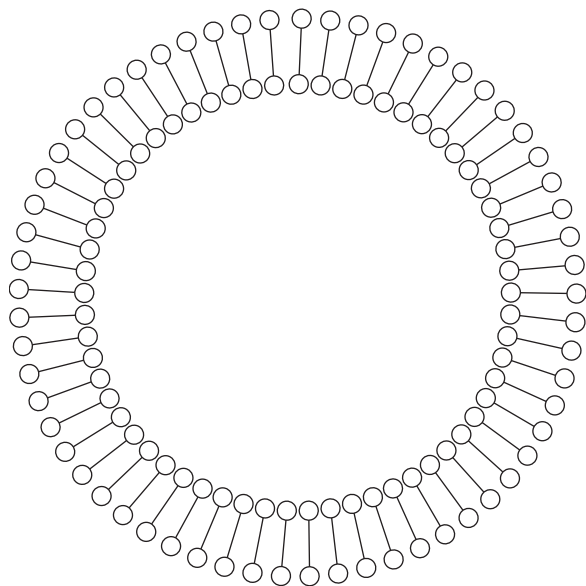


Figure 47. Schematic representation of a niosome structure. ○, hydrophilic head group; —, hydrophobic tail (305).

Components Used in Niosome Preparation. The main components of niosomes are nonionic surfactants. Different types of self-assembling nonionic surfactant were proposed as starting material to prepare niosomes (i.e., the SPAN and the Brij series). The type of surfactant can influence the stability of the vesicular system being able to influence the fluidity of bilayer structures. In particular, the nonionic surfactant can influence the leakiness of the entrapped drug from niosomes with the

following increasing order, SPAN80 < SPAN20 < SPAN40 < SPAN60.

High niosomal concentration of soluble surfactant agents can influence the solubility of this vesicular colloidal carrier and determine the formation of micelles or complex aggregates. This phenomenon is observed with the presence of actylglucoside in the niosome formulation. This compound can destabilize the niosome bilayer and start a micellization phenomenon (305).

Another fundamental component for the preparation of niosomes is cholesterol. This molecule is used as an additive compound both to reduce the temperature of the vesicular gel to the liquid-crystal phase transition (305) and to decrease the overall HLB value of the surfactant mixture used for the preparation (306,307). Thus, cholesterol allows a more efficient aggregation of the nonionic surfactant component into a closed bilayer structure, and then a higher stability of the niosomal vesicles. The inclusion of cholesterol into niosomal formulation can reduce the leakiness of the membrane. A 1:1 molar ratio of cholesterol and nonionic surfactant is generally used for niosome preparation.

A parameter that should be taken into account in the choice of the niosome component is the physicochemical property of the encapsulated drug, due to a series of possible interactions occurring with the nonionic surfactant component leading to the formation of homogeneous dispersion or aggregate structure (305).

Methods of Niosome Preparation. Niosomes are prepared through the hydration of a mixture of nonionic surfactant–helper lipid (cholesterol) (1:1 molar ratio) at a temperature ranging from 40 to 70 °C followed by a suitable sizing process to obtain the required colloidal dispersion characteristics. The methods used to reduce the niosome mean size and to achieve an homogenous size distribution are similar to those used for liposomes, that is, extrusion through decreasing pore size polycarbonate filters, cycles of sonication, and high pressure homogenization (305,308). Similarly to liposomes, the mean size of niosome formulations is very important to reduce the RES uptake (305).

As concern the hydration of the nonionic surfactant–helper lipid mixture, some procedures reported for liposomes also can be used (e.g., the TLE method). In addition, other specific preparation methods have been developed for niosomes (305):

1. Injection of an organic solution (ether) of surfactant and cholesterol in a drug aqueous solution and heating of this mixture above the boiling point of the organic solvent;
2. Formation of an o/w emulsion between a drug aqueous solution and an organic solution of surfactant–cholesterol. Then, the organic phase is evaporated off and an aqueous niosomal colloidal dispersion is obtained;
3. Injection of the melted surfactant–cholesterol mixture in an aqueous heated solution of the drug under continuous stirring or vice versa injection of a

warmed aqueous drug solution into the niosomal component mixture.

The niosomal formulations obtained with the previous mentioned methods are generally micro size.

Considering the importance of the drug loading parameter, some procedures can be carried out to increase the amount of the encapsulated drug within niosomes. There is evidence (305) that the DRV method, originally developed for the preparation of multilamellar liposomes with a high entrapment efficiency of water-soluble drugs (309), can also be used for niosomes with an increase of their loading capacity from 3.3 to 64.4%. Another method successfully used to increase the amount of drug entrapped in niosomes is based on the formation of a pH gradient (305).

At the end of the preparation procedures, the excess of nonencapsulated drug is removed by dialysis, centrifugation, or filtration.

Toxicological Aspects of Niosomes. Considering that niosomes are made up of at least 50% synthetic nonionic surfactant, the toxicological profile of this carrier is very important for its application as a drug delivery system. Unfortunately, there are not many studies on niosome toxicity. An *in vitro* investigation, made on a model of ciliotoxicity to evaluate the influence of alkyl polyoxyethylene moiety of niosomes on the nasal mucosa, showed that increasing of the alkyl chain length of the nonionic surfactant determined a reduction of toxicity while the increase of the polyoxyethylene chain length pronounced the carrier ciliotoxicity. These findings seem to be correlated with the thermotropic state of niosomes, considering that the longer the alkyl chain the higher the transition temperature from gel-to-liquid phase, while the longer the polyoxyethylene chains the lower the transition temperature. This finding concluded that gel-state niosomes are less ciliotoxic than the liquid-state vesicles. On the contrary experiments on human keratinocytes showed on toxic activity related to both the alkyl chain length and the length of polyoxyethylene chain (310).

For the parenteral administration of niosomes, usually through the IV route, the evaluation of the vesicular system hemocompatibility is very important. The incubation of C₁₆G₂ and Span 60 niosomes with rat erythrocytes showed <5% hemolysis after 5 h. This level of hemolysis is not significant, considering that <2% of an injected dose of C₁₆G₂ niosomes is still present in the blood stream 5 h after dosing (305).

In the case of niosomal soluble surfactant components, a dose-dependent effect was observed. When low concentrations are used, the soluble surfactant is totally incorporated in the niosome structure and a drastic reduction of its intrinsic toxicity is achieved. The situation changes when the amount of soluble surfactants (e.g., Solulan C₂₄) is increased, because the formation of micelles occurs, and then the free monomers and/or micelles may exert their toxic action on cultured cells (311). Therefore, the whole niosomal carrier should be investigated for potential toxicity rather than the single components.

The issue of niosome toxicity is quite complex due to the fact that the presence of a drug can change the toxicological

profiles of the unloaded carrier. For example, the inclusion of doxorubicin in C₁₆G₂ niosomes produce a severe dose-dependent inflammatory effect at the level of the lung within 24 h following intraperitoneal administration (305). After intraperitoneal administration of empty C₁₆G₂ niosomes or the free drug, such an effect on lungs is not observed. A possible explanation is the fact that doxorubicin-loaded niosomes are transported away from the peritoneum by the lymphatics via the thoracic duct allowing a higher dose in the main veins emptying into the heart. This hypothesis can be supported by the fact that 56% of a methotrexate-loaded niosome formulation is found in the thoracic lymph following intraperitoneal administration with respect to 12% observed for a free drug solution (312).

The modulation of drug toxicological effect is an important aim of the niosomal carrier. The encapsulation of vincristine in niosomes can reduce the free drug toxicological profile and improve the drug antitumoral activity in S-180 sarcoma and Erlich ascites mouse models (313).

Niosomes in Complex Systems. The need for a more precise controlled drug release prompted the research of new and more sophisticated delivery systems. For this reason, niosomes based on Span surfactants were used to prepare a v/w/o (vesicle in water in oil system) niosomal formulation (314). The release rate of carboxyfluorescein, a hydrophilic fluorescent probe, showed the following increasing trend: v/w/o < w/o emulsions < niosome dispersion. Also, the nature of surfactant can influence the release of the fluorescent probe according to the following decrease in order: Span 20 > Span 40 > Span 60. The presence of Span 80 in the v/w/o system can drastically increase the probe release from the system due to its unsaturation in the alkyl chain, which generate a more leaky bilayer structure. While, the crystallization of Span 60 in the oil phase elicit the formation of an oil gel phase that can noticeably reduce the release rate from this vesicular system (314). A temperature-dependent release can be obtained in Span 60 v/w/o by adding Span 20 as a stabilizer, thus providing a faster probe release at 37 °C (305).

Niosome colloidal dispersion can be easily viscosized by the addition of hydrocolloids.

The addition of Solulan C₂₄ in C₁₆G₂ niosomes determined the formation of the disome phase, that is a large vesicle (~60 µm) able to encapsulate hydrophilic compounds. These giant vesicles were found to be of two types: large vesicles that appear ellipsoid in shape and large vesicles that are truly discoid (305). The features of the disome structure prompt the use of this particular niosomal system as an ophthalmic drug delivery.

Therapeutic Applications of Niosomes. Niosomes can be used as a fine drug delivery system being able to confer a certain selectivity to the entrapped drug as a function of their composition and physicochemical properties. After IV administration, niosomes show a high liver tropism (304,305). However, a niosomal formulation containing doxorubicin, composed of palmitoyl muramic acid, cholesterol, Solulan C₂₄, can escape from the liver uptake (305).

At the same time, a iopromide-loaded niosomal formulation extruded through a 220 nm filter and with the presence of stearylamine in its composition is able to accumulate in the kidneys (315). These findings showed that the presence of a positive charge on the surface of the niosomes can improve the targeting to the kidneys. The intraperitoneal administration of niosomes with Span 80 in their formulation (312) can produce a lymphatics targeting, while $C_{16}G_2$ niosomes (305) after intraperitoneal administration can act as a depot system.

The presence in niosomal formulations of surfactant characterized by ester bonds can support the enzymatic degradation by esterases present in plasma, thus influencing the biodegradability, the residence time, and the stability of the system in the plasma. Moreover, the nature of the entrapped drug can influence the structure of the niosomal surface and the biodistribution of the system.

The first application of niosomes was as antiparasitic vesicular system for the treatment of leishmaniasis. The administration of a niosomal formulation containing stibogluconate was very useful to reduce the parasite disease because niosomes acted as a drug depot in the liver. In this case, the antiparasitic activity of niosomes regarding to the liver leishmania donovani can be correlated to the rapid uptake of the formulation in the liver after IV administration. However, this formulation cannot eradicate the parasite in the spleen and bone marrow. For this reason, different types of polyoxyethylene niosomes ($C_{16}EO_2$, $C_{16}EO_4$, $C_{16}EO_6$) are used to suppress the parasite in the spleen and bone marrow (305).

The IV administration of 100 nm of $C_{16}G_3$ niosomes containing methotrexate can improve the hepatic levels of the drug with serum levels of the drug higher than when it is administered in solution (316). In particular, a 23-fold increase in the area under the curve of methotrexate plasma level as a function of time is observed after IV administration of niosomes (4.5 μ m mean size) containing Span 60 to tumor bearing mice (317), this finding is probably due to the great size of this vesicular system. Span 60 niosomes can further increase the plasma level of methotrexate if they are administered following the macrophages activation with mramyl dipeptide-gelatin derivatives (317). The oral and IV administration of $C_{16}G_3$ niosomal formulation encapsulating methotrexate can cross the blood-brain barrier and provide a sustained release of this drug at the level of the CNS (316). However, the delivery of drug to the brain with niosomes has not been successful.

The administration of doxorubicin-loaded $C_{16}G_3$ niosomes (850 nm mean size) in tumor bearing mice determined a high drug level in the tumor site, serum, and lung, but not in the liver (305,318). While, doxorubicin-loaded 240 nm niosomes made up of Span 60 increased plasma, liver, and tumor levels. The reduction of proliferation of the S-180 sarcoma in NMRI mice after IV administration of niosomal formulation containing doxorubicin demonstrated an increased drug anticancer activity after encapsulation in niosomes (Fig. 48). At the same time the side effects, in particular cardiotoxic activity, are reduced following entrapment in niosomal formulations (314). Niosomes can improve the antitumoral effect of vincristine in S-180 sarcoma well as other anticancer drugs (313).

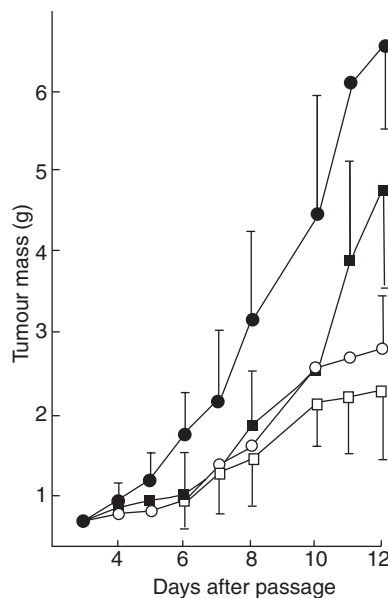


Figure 48. The growth in the mass of implanted tumor as a function of time after IV injection of (●) phosphate buffered saline pH 7.4, (■) doxorubicin solution (5 mg·kg⁻¹), (○) doxorubicin (5 mg·kg⁻¹) $C_{16}G_3$ niosomes, (□) doxorubicin (5 mg·kg⁻¹) $C_{16}G_3$:cholesterol (50:50) (305).

A diclofenac-loaded niosomal formulation composed by Span 60, cholesterol and DCP (22:73:5) produces a noticeable reduction of inflammatory processes in rat more efficaciously than the free drug. The improved activity of the drug can be determined by an increase in the area under the plasma time curve. Similar findings were obtained for niosomes-containing flurbiprofen, which showed an improved drug effect and bioavailability with a reduction of side effects produced by the free drug (305).

Niosomes can be used as agents for diagnostic imaging. Iopromide radiopaque agent encapsulated in niosomes made up of $C_{16}G_3$, cholesterol, and stearylamine, can be concentrated in the kidney after IV administration (315). As mentioned above, the kidney targeting action is mediated by the positive charge on niosome surface.

Niosomes also can be effectively used for the oral delivery of drugs. The first application in this field was carried out with methotrexate-loaded $C_{16}G_3$ niosomes characterized by a mean size of 100 nm (316). This investigation showed higher levels of methotrexate in serum, liver, and brain after oral delivery using the niosomal formulation with respect to the free drug. A certain interest is focused on the possibility of using niosomes as carrier for the oral delivery of peptides and proteins. For example, ovalbumine-loaded niosomes are able to increase the production of specific antibodies after oral administration (305).

Other successful applications of niosomes as delivery systems concern the topical administration of drugs and particularly the transdermal and ophthalmic delivery of drugs.

Niosomal formulations can increase the amount of drug permeated through the stratum corneum (319), even if the

exact mechanism involved in the drug and/or carrier passage has to be investigated and elucidated in a more detailed way. A hypothetical mechanism of skin penetration is related to a possible reorganization of the niosomal membrane at the level of the stratum corneum (320). *In vitro* data showed an efficacious transdermal delivery of oestradiol when it is entrapped in $C_{18}EO_7$ and $C_{12}EO_7$ niosomes. The improved drug passage through the outer skin layer seems to be mediated by the high flexibility of the bilayer structure of some niosomal formulations (319). Similarly, a niosomal formulation made-up of glyceryl dilauroates ($C_{16}EO_7$) and cholesterol can increase the passage through the stratum corneum and the penetration of cyclosporine A into the inner layer of the skin (305). Then, niosome can be used as a transdermal drug delivery system for both hydrophobic and hydrophilic drugs.

Niosomes were proposed as a potential ophthalmic drug delivery system. Cyclopentolate-loaded niosomes made-up of Span 20 and cholesterol can pass through the cornea in a pH dependant manner, that is, pH value 5.5 is optimal for the cyclopentolate penetration, while at pH 7.4 a decreased permeation was observed. However, the *in vivo* mydriatic response is irrespective of the pH of the niosomal formulation. The explanation of the increased corneal adsorption of cyclopentolate may be due to a niosome-induced modification of the permeability characteristics of the conjunctival and scleral membranes (321).

Similar to liposomes, niosomes can be used as a vaccine adjuvant. A niosomal formulation composed by 1-monopalmitoyl glycerol, cholesterol, diacetyl phosphate can be used to encapsulate antigenic compounds and this result is fundamental for the adjuvanticity (305). A v/w/o niosomal system containing Span 80 and cotton seed oil was evaluated as an immunological adjuvant using the antigen tetanus toxoid (314). An increased secondary response (level of IgG1) was observed when the v/w/o formulation was administered by the intramuscular route in comparison with the vesicle formulation and the free antigen.

Ethosomal Carrier. Ethosomes have been invented by Touitou (322–324). The low toxicity and the property of ethanol as a permeation enhancer (325) as well as the possibility to include ethanol in the liposomal formulation, has brought to the realization of a new vesicular system for transdermal delivery: ethosome (301).

Ethosomes presents interesting features correlated with its ability to permeate intact through the human skin due to its high deformability. In fact, ethosomes are soft, malleable vesicles tailored for enhanced delivery of active agents. It has been shown that the physicochemical characteristics of ethosomes allow this vesicular carrier to transport active substances more efficaciously through the stratum corneum into the deeper layers of the skin than conventional liposomes (326). This aspect is of great importance for the design of carriers to be applied topically both for topical and systemic drug administration. Furthermore, the ethosomal carrier is also able to provide an effective intracellular delivery of both hydrophilic and lipophilic molecules (327) and also the penetration of an antibiotic peptide (i.e., bacitracin) within fibroblast cells was facilitated (328).

Formulative Aspects of Ethosomes. Ethosomes are a vesicular system made up of a phospholipid component, ethanol, and water. Phospholipid is the lipid component that confers the shape of vesicle to the delivery system. Ethanol is an important component in ethosome due to its destabilizing action regarding the packed-ordered structure of conventional liposomes (326), thus conferring the characteristic elasticity and deformability to this vesicular carrier. There are a number of methods that can be used to prepare stable ethosomal formulations depending on drug and the target of drug delivery (322–324). Among these, a frequently used method to prepare ethosomes is based on the dissolution of phospholipids in ethanol (20–50% w/v). Then, an aqueous solution is added to the lipidic solution under stirring thus allowing the formation of ethosomes (327–329).

The ethanol/phospholipid ratio used for the preparation of ethosomes is a crucial factor influencing the mean size and size distribution of ethosomes (Fig. 49). Usually, ethosomes prepared with a great amount of ethanol ($\geq 40\%$ v/v) show a narrow vesicle size distribution. The size of ethosomes decreases with increasing ethanol concentration, while the concentration of phospholipid influenced the vesicle mean size in a different way, namely, the higher the phospholipid concentration the larger the ethosome mean size (301,330). The amount of ethanol used in the formulation can modify the superficial charge of ethosomes and the skin interaction (301). Normally, the presence of drugs have no significant influence on both mean size and size distribution. Ethosome composition can also influence the lamellarity as shown by electron transmission microscopy (Fig. 50), since the formation of either unilamellar or multilamellar ethosomes is a multifactor process.

Ethosomes can entrap hydrophobic and hydrophilic molecules in their structure. With respect to liposomes, where hydrophilic drugs are entrapped in the aqueous

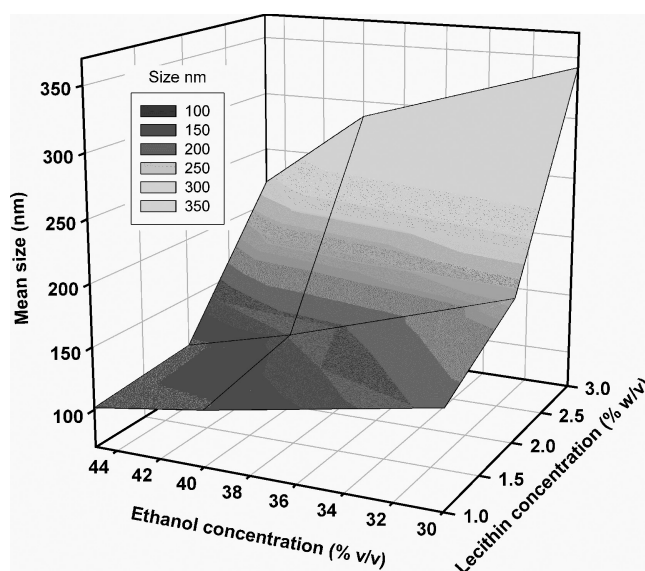


Figure 49. Influence of the amount of ethanol and lecithin used for the preparation of ethosomes on vesicle suspension mean size and colloidal polydispersity index. Data from Ref. 330.

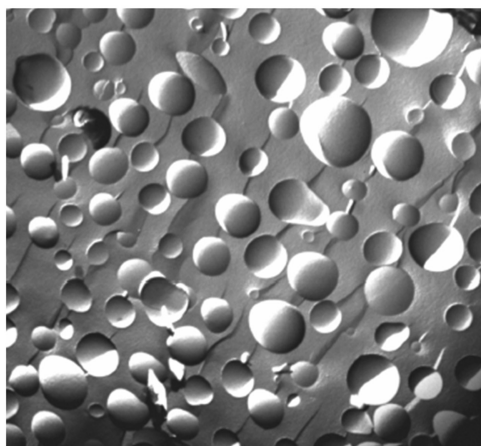
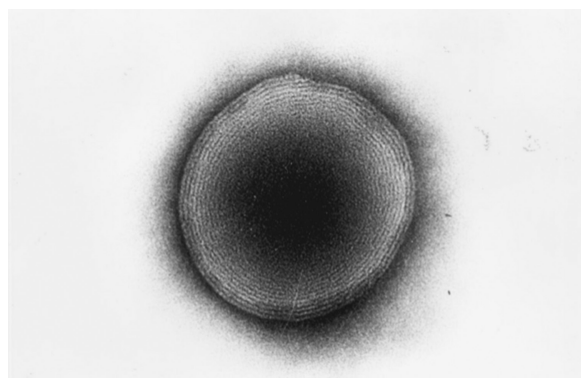


Figure 50. Transmission electron microscopy of ethosomal vesicles composed of 2% lecithin and 30% ethanol (a) (301). Freeze-fracture electron micrographs of ethosomes composed of 45% ethanol and 2% lecithin (b) (330).

compartment and hydrophobic drugs are in the lipid bilayer core, in ethosomal formulations drugs are homogeneously present in ethosome structures in spite of drug physicochemical properties (301,327) (Fig. 51). This finding can be explained by the multilamellarity of the ethosomal

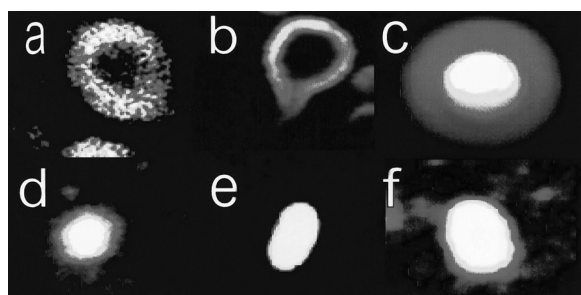


Figure 51. Entrapment of fluorescent probes by phospholipid vesicles determined by confocal scanning laser microscopy. Liposomes (a–c) or ethosomes (prepared with 2% lecithin and 30% ethanol) which (d–f) were prepared with one of three following fluorescent probes: rhodamine red, a highly lipophilic molecule (a,d); D-289, an amphiphilic molecule, (b,e); calceine, a hydrophilic molecule (c,f). White represents the highest concentration of a probe, followed by yellow, with red being the lowest probe concentration (301).

vesicles as well as by the presence of ethanol in the ethosome, which allows for better solubility of the lipophilic and amphiphilic probes (301). The ethosome composition can also influence the drug entrapment efficiency, that is, the amounts of ethanol and phospholipid used for ethosome preparation positively influence the loading capacity of the colloidal carrier. Namely, the higher the amount of ethanol and phospholipid the greater the drug entrapment within ethosomes (301,326,330), the values of drug entrapment efficiency are often higher than those expected for a conventional vesicle formulations (330). This fact can be explained by the presence of ethanol, which increases the drug solubility in the polar phase of ethosomes (301).

Therapeutic Potentialities of Ethosomes. The enhanced percutaneous permeation capability of ethosomes is due to the unique feature of this carrier that is able to interact with the stratum corneum and to elicit a reversible disorganization of the stratum corneum lipid packing order, thus increasing the skin permeability to drugs and vesicles (329).

An important characteristic to be evaluated before the proposal of a drug carrier as a potential topical drug delivery system is its *in vivo* skin tolerability on human subjects. *In vivo* reflectance spectrophotometry data (330) on volunteers showed that ethosomes elicit no induction of skin erythema, while a hydroethanolic solution with an equal water/ethanol ratio of ethosomes induces a remarkable skin erythema (Fig. 52). These results demonstrate that ethanol present in the ethosomal formulation is not

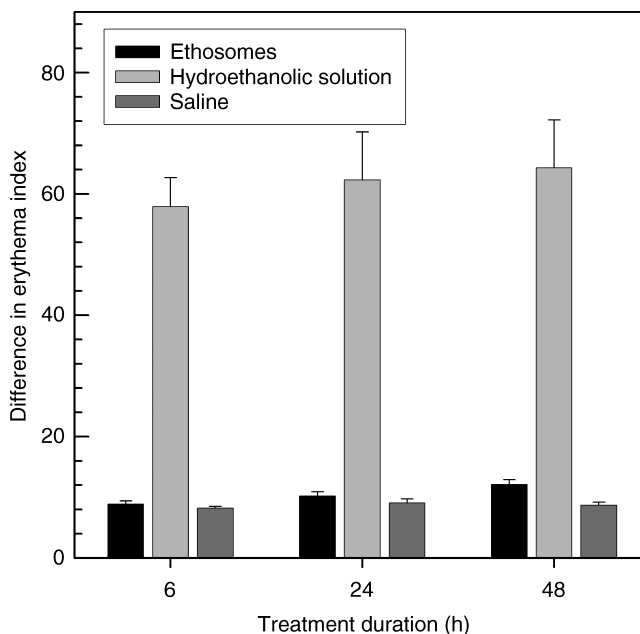


Figure 52. *In vivo* human skin tolerability of various topical formulations after 6, 24, or 48 h of treatment. Results are expressed as a mean value of the variation of the erythema index ($n = 6$) $\pm$ standard deviation. Legend keys: ethosomes, formulation containing 2% (w/v) Phospholipon 90 and 45% (v/v) ethanol; hydroethanolic solution, solution of water, and ethanol at a volume ratio of 55:45; saline, control saline (0.9% w/v NaCl in water) solution (330).

able to act as a skin erythema-inducing agent, even though it is present at a high concentration.

A wide range of drugs have been formulated in ethosomal carriers and tested *in vitro*, *in vivo*, and in clinical studies. These molecules comprise steroid hormones, antivirals, antibiotics, vitamins, peptides, and cosmeceutical agents. Moreover, ethosomes are very efficient carriers for targeting molecules to the pilosebaceous units and could be used for acne and alopecia treatment. Carrier consists of materials approved for pharmaceutical and cosmetic use (316,327–332).

An interesting example of the ethosome potential application as innovative topical carriers is represented by the transdermal delivery of cannabidiol (333), a new drug candidate for treatment of rheumatic diseases, that presents a number of drawbacks when administered orally. The ethosomal formulation is able to prevent the inflammation and edema induced by subplantar injection of carrageenan in ICR mice.

Often, the skin permeation enhancement observed for all ethosome-based formulations is much greater than that can be expected from ethanol alone. This behavior can be due to a synergistic mechanism between ethanol, phospholipid vesicles, and skin lipids (301).

Two different research groups reported an *in vivo* sustained release effect of ethosomes with a prolongation of the drug therapeutic activity, which can be related to an accumulation in the skin (330,333).

Then, recent findings on ethosomes are very encouraging and confirm that this carrier is very promising for the topical administration due to the enhanced delivery of drugs through the skin, thus prompting various opportunities for the development of suitable therapeutic strategies through the topical route.

Ultradeformable Vesicular Carrier. It is believed that liposomes, when administered on the skin, first disintegrate their structure, and then diffuse through the barrier in the form of small fragments or lipid monomers (334). Conventional rigid liposomes were shown to be unsuitable vesicular carriers to cross the skin barrier (335). Highly deformable vesicles were developed, the so-called transfersomes or ultradeformable liposomes were invented by Cevc (336). It was shown that the high deformability of vesicular carriers could allow them to penetrate intact skin if applied nonocclusively *in vivo* (336,337), thus favoring the delivery of encapsulated drugs across the skin (338,339). Ultradeformable vesicles seem to cross the skin without irreversible disruption, probably because they are much more elastic, and hence more deformable respect to classic liposomes. For the preparation of ultradeformable vesicles, the so-called edge activators were incorporated into the phospholipid bilayers at suitable amounts, namely, bile salts were often used for this purpose (340).

Formulative Aspects of Ultradeformable Liposomes. Lecithins and a bile salt at different molar ratios are the main components of ultradeformable liposomes that can be prepared with the TLE method or any other used for liposome preparation (the section main methods to prepare liposomes) (336–340). Small amounts of ethanol ($\leq 7\%$ v/v)

are normally used for the preparation of ultradeformable liposomes.

Similarly to ethosomes, the ratio between the various components is a crucial factor for the determination of the physicochemical and drug-loading capacity properties of ultradeformable liposomes.

The DSC studies have demonstrated that the amount of the edge activator is related to the increased fluidity of the vesicular bilayers up to a certain values, beyond this value the formation of mixed micelles and other kinds of colloidal aggregates were observed (341). For this reason, large amounts of edge activator beyond a certain value hinder the transdermal drug delivery; in fact, mixed micelles and aggregates are much less effective transdermal carriers than ultradeformable liposomes. The formation of a coexistence region characterized by various phospholipid/bile salt aggregates (i.e., mixed vesicles, opened vesicles, mixed micelles, and rod-like mixed micelles) is evidenced by a reduction of the mean size and a concomitant increase of the polydispersity index values, thus showing the presence of a wide size distribution (341). The presence of bile salts in the composition of ultradeformable liposomes leads to a negative zeta-potential due to the increase of negative charge (carboxylate group of bile salts) along the surface of vesicle bilayers.

The ratios between the components and the type of edge activator used to prepare ultradeformable liposomes can influence the amount of drug entrapped within this carrier. When nonionic surfactants (i.e. Span and Tween) are used instead of bile salts, a decrease of the drug entrapment efficiency of the carrier is observed. In any case, a high concentration of edge activators cause a drastic reduction of the drug loading capacity due to the presence of other forms of aggregation than vesicles. The above mentioned aggregates have a poor drug loading capacity. The higher hydrophilic form of ultradeformable liposomes than conventional liposomes and their high flexibility avoids the aggregation and fusion of the transfersomal system providing a stable vesicular structure (342).

Similarly to other vesicular carriers, ultradeformable liposomes can entrap different types of molecules (i.e., lipophilic, hydrophilic, and amphipatic drugs). The release rate of entrapped drug from ultradeformable liposomes is mainly influenced by the carrier composition and the drug physicochemical properties. Generally, the release of water soluble drugs from ultradeformable liposomes is modulated by the concentration gradient between the inner and the outer compartment. The water gradient through the skin can trigger the release of the entrapped drug. The release of hydrophobic drug is slower than that of the hydrophilic drug, and it is confined to the contact and lipid exchange between ultradeformable liposomes and biological membranes (342). The slower release of the hydrophobic drugs is due to a strong interaction between drugs and lipid bilayers (343). Amphipatic drugs have intermediate release characteristic between hydrophobic and hydrophilic drugs.

Therapeutic Potentialities of Ultradeformable Liposomes. Ultradeformable liposomes are characterized by a deformable structure that can pass intact through the skin using a

water active gradient, thus favoring the drug delivery through the skin without modifying the integrity of the cutaneous barrier (342,343).

In vitro and *in vivo* tests with ultradeformable liposomes showed that this vesicular system does not produce any toxic effects after topical application and it is well tolerated by the skin tissue (342).

Skin is a nanoporous barrier that permits only the passage of small (nanometer size molecule) compounds (334,342). The nonocclusive topical application of ultradeformable liposomes undergoes water evaporation from the formulation and the consequent drying out of the vesicles (343). The elastic and hydrophilic properties of ultradeformable liposomes determine the movement of the vesicle through the skin pores by following the transdermal water gradient. The topical application of ultradeformable liposomes can increase the size of skin nanopore (78,344). After ultradeformable liposome percutaneous permeation, these vesicles can distribute in cells and after the bypassing of cutaneous capillary they can reach the subcutaneous tissue.

The most important example of the application of ultradeformable liposomes as transdermal drug delivery is represented by Transfenac, formulation of diclofenac in ultradeformable liposomes (338). Transfenac mediates the agent transport through intact skin and into the target tissues. Therapeutically meaningful drug concentrations in the target tissue are reached even when the administered drug dose in Transfenac is $< 0.5 \text{ mg} \cdot \text{kg}^{-1}$ body weight. Diclofenac association with ultradeformable carriers permits it to have a longer effect and to reach 10 times higher concentrations in the tissues under the skin in comparison with the drug from a commercial hydrogel. The relative advantage of diclofenac delivery by means of ultradeformable liposomes increases with the treated muscle thickness and with decreasing drug dose, as seen in mice, rats, and pigs (338); this can be explained by assuming that the drug associated with carriers is cleared less efficiently by the dermal capillary plexus.

Transfenac, hence promises to be a useful formulation for the treatment of diseases of superficial tissues, such as muscles or joints, having the potentiality to replace combined oral-topical diclofenac administration in humans.

Particle Drug Carriers

Micro- and nanoparticles are solid colloidal suspensions in which the mean particle size is > 1 or $< 1 \mu\text{m}$, respectively. Under the morphological point of view, two different types of particles can be distinguished: capsules and spheres (Fig. 53). Sphere systems are usually characterized by a porous matrix in which drugs are contained, while capsule systems are formed by a core containing drugs surrounded by a shell.

Polymeric Particles. These colloidal carriers are prepared from natural or synthetic polymers and, in dependence of the preparation method and of the polymer used, micro- or nanocapsules and micro- or nanospheres can be distinguished. Polymeric particles have become very important because of their ability to deliver a variety of

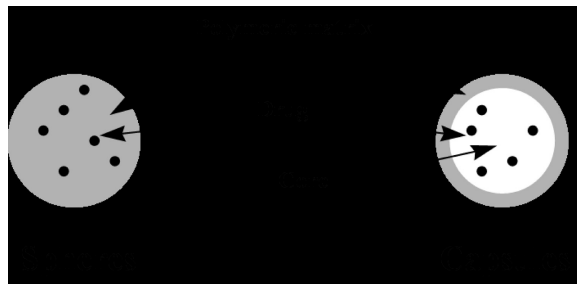


Figure 53. Schematic representation of spheres and capsules as potential drug delivery devices.

drugs to different areas of the body for sustained periods of time (345–347). Up to the end of 1980s, microparticles were extensively investigated. Now great interests are focused on the much smaller carriers (e.g., nanoparticle) that are able to ensure fine drug delivery opportunities both in terms of efficacy and selectivity. For this reason, this section will be mainly focused on polymeric nanosystems.

Concerning the materials used for the preparation of these colloidal carriers, natural polymers (i.e., proteins, polysaccharides, waxes) are not widely used because they present a huge variability in their purity and defined physicochemical properties. Furthermore, they often require a cross-linking procedure that may cause an alteration of the encapsulated drug. For these reasons, mainly synthetic polymers have received attention and have been largely investigated for potential use in drug delivery devices.

The use of a large series of polymers is restricted and limited by their bioacceptability, which is also influenced by colloidal particle mean size. In fact, the diameter of the smallest blood capillaries is $\sim 4 \mu\text{m}$, thus nanoparticles should have a smaller diameter than this to traverse all capillaries. As a general consideration, for the suitable choice of the appropriate macromolecular polymer to be used as a nanoparticle matrix, the colloidal particle size and the preparation method will first depend on the biocompatibility of the polymer, second on the physicochemical properties of the drug, as well as on the therapeutic goal to be reached. In this colloidal carrier, drugs can be adsorbed, attached, dissolved, entrapped, and/or encapsulated (348). Micro- and nanoparticles can be used to deliver both hydrophobic and hydrophilic molecules, proteins, vaccines, biological macromolecules. They can also be formulated for targeted delivery to all organs or made for long-term systemic circulation (235). Thus, a lot of synthesis procedures exists.

Preparation Methods and Formulative Aspects. Drugs can be incorporated into nanoparticles in a number of ways: (1) drug can be entrapped in the polymeric matrix; (2) it can be encapsulated in a nanoparticle core; (3) it can be chemically conjugated to the polymer; (4) it can be surrounded by a shell-like polymer membrane; (5) it can be adsorbed on particle surface.

Polymeric nanoparticles can be prepared using a lot of different techniques. One of the most used preparation methods is the emulsification–solvent evaporation technique. The polymer and the drug are solubilized in an organic

solvent and an emulsion is prepared by adding water and a surfactant. Liquid nanodroplets are produced by sonication or homogenization, and then the organic solvent is evaporated in order to achieve the nanoprecipitation of the polymeric material in solid nanoparticles (345,349). Obviously, this procedure can be used only for hydrophobic drugs. To allow the encapsulation of hydrophilic molecules, a modification of this procedure led to the multiple emulsion technique (350).

Another method is the phase-inversion nanoencapsulation (PIN), which has been used to encapsulate insulin for oral administration (351). A limitation of these two techniques is the use of toxic and fluorinated solvents, which may cause drug degradation. For these reasons, other techniques, that do not compromise drug stability have been developed.

One of these is the emulsification–diffusion method. In this case, the polymer and the active compound are dissolved in a partially water-soluble solvent. This organic solution is added, and then emulsified in an aqueous phase containing a surfactant. To favor the precipitation of nanoparticles, additional water is added to the emulsion under stirring. At the end of the process, the solvent can be removed by centrifugation or dialysis.

The nanoprecipitation method involves the dissolution of the polymer and the drug in a freely water-miscible organic solvent (e.g., acetone) and then the addition of this organic solution into a water phase containing a nonionic surfactant (e.g., Pluronic F68). The organic solvent is then removed under reduced pressure by a rotavapor (352). This procedure leads to the formation of nanospheres, but if a biocompatible oily component is added in the organic solution nanocapsules are formed (353).

The formation of nanospheres or nanocapsules also can be achieved by an *in situ* polymerization process. The emulsion or micellar polymerization is the most used approach to achieve nanocapsules and nanospheres by starting from the polymeric monomer, respectively. In the case of micellar polymerization, reactions take place in the solvent phase. The following polymers can be prepared as nanosphere colloidal suspensions following this preparation procedure: PMMA, poly(alkyl cyanoacrylate), and acrylic copolymer. When the polymerization is carried out at the interface (interfacial polymerization) between an oil phase and an aqueous solution, nanocapsules are formed (354).

The determination of the loading capacity of nanoparticle colloidal suspensions can be carried out by separation of the untrapped material with ultracentrifugation followed by the drug analysis after dissolution of the pelleted polymeric matrix. Other reliable separation methods are ultrafiltration and gel permeation chromatography (345,346). The drug loading capacity also can be calculated by determining the drug content in the supernatant or in the filtrate. In fact, the amount of drug entrapped in nanoparticle colloidal systems can be obtained by subtraction of the untrapped drug amount from the total amount of drug present in the suspension.

The mechanisms of drug release from nanoparticle colloidal suspensions depends on the characteristics of the colloidal suspension, as well as on physicochemical

properties of the drug. In particular, the release of a drug may occur by one of the following mechanisms or a cooperation of more than one of them: (1) drug desorption from the colloidal surface (both for nanospheres and nanocapsules); (2) drug diffusion through the polymeric network of the nanospheres; (3) drug diffusion through the polymeric shell of nanocapsules; (4) polymeric matrix erosion of nanoparticles. The drug release rate is dependent on the release mechanism, the diffusion coefficient, and polymer biodegradation rate. The nanoparticle drug release is also greatly influenced by the type of interaction with the biological substrate (345).

Besides the previous mentioned drug release mechanisms, it should be considered that the drug delivery function of nanoparticles also can be accomplished by a direct contact with the biological membranes, thus leading to an enhanced drug delivery through membranes with respect to a simple drug solution (355). As a consequence of this behavior, it may happen that the *in vitro* drug release profiles are poorly related to the *in vivo* drug delivery and release situation (356).

Size and zeta potential are important physicochemical parameters to be determined to achieve a suitable colloidal carrier. Nanoparticle size is influenced by the preparation technique and by the polymer used, that is, low molecular weight polymers form small-sized nanoparticles, but this fact reduces the amount of encapsulated drug. An increase of polymer concentration usually elicits an increase of both nanoparticle size and encapsulation efficiency (357,358).

The zeta potential is a measure of the surface electrical charge of the particles. As the zeta potential increases, the repulsion phenomenon between particles will be greater, thus leading to a more stable colloidal dispersion. The minimum zeta potential value to prevent particle aggregation and to have a stable nanosuspension was defined to be ± 30 mV (359).

Therapeutic Applications of Nanoparticles. The oral administration is one of the promising application of nanoparticles that have been administered either for achieving a systemic uptake or for having a local residence within the GI tract. The polymers used for peroral application are nondegradable polymers (cellulose, acrylate derivatives, etc.) and are designed not to be adsorbed (359).

Polymer nanoparticles for oral treatment may be formulated as an aqueous suspension or incorporated into traditional dosage forms. A lot of different nanoparticle formulations have been incorporated into tablets or capsules, and then compared with traditional dosage forms. In all cases, nanoparticles maintained the advantages of a colloidal carrier, such as an enhanced dissolution of lipophilic drugs and a prolonged and sustained release (360).

An innovative nanoparticle application in this field is oral chemotherapy, which can be a valid alternative, because it allows a continuous exposure of the cancer cells to anticancer drugs at a lower concentration, thus reducing or avoiding side effects. In addition, it is more convenient and better tolerated by the patients, especially for those with advanced metastatic cancers. Unfortunately, most anticancer drugs cannot be administered orally because of their poor solubility, stability, and permeability. It has

been found that anticancer drug encapsulation into an oral formulation of nanoparticles has been able to play a key role in drug adhesion and interaction with cancer cells. For example, PEG-coated nanoparticles are able to adhere to intestinal cells and subsequently to escape from the multidrug resistance pump proteins (361).

Chitosan-coated nanoparticles are used for colon targeted drug delivery of diclofenac. Chitosan nanoparticles are microencapsulated in Eudragit L-100 or S-100 to form a gastroresistant reservoir system in which the drug release is triggered only in the basic (pH 8) environment of colon (362).

For parenteral delivery, nanoparticles can be formulated as aqueous dispersions or they are converted in lyophilized powders to be resuspended just before their administrations (345,359).

Cancer therapy is one of the most important applications of polymeric nanoparticles. Nowadays, the aim of any anticancer research is to improve patient survival after chemo- or radiotherapy. Unfortunately, traditional anticancer therapy is affected by a lot of side effects that involve healthy cells leading to an unsuitable quality of life for cancer patients. So the effectiveness of a treatment is related to the ability to target the cancer cells while affecting as few healthy cells as possible. Nanoparticles can provide an alternative solution for the site-specific delivery of anticancer drugs due to their small size and the possibility to escape RES recognition and uptake, thus leading to a prolonged blood circulation time.

Biodegradable nanoparticles made of PLGA have been used to incorporate paclitaxel, a microtubule-stabilizing agent that causes cell death by promoting the polymerization of tubulin during cell division. Paclitaxel was encapsulated to a very large extent ($\sim 100\%$ encapsulation efficiency) and this paclitaxel-loaded colloidal system showed a 70% loss of viability of human small-cell lung cancer cells at a drug concentration as low as $0.025 \mu\text{g} \cdot \text{mL}$. Paclitaxel also has been incorporated in poly(ethylene oxide) modified poly(β -amino ester) nanoparticles to obtain a sustained release into most solid tumors (363). Also, Tamoxifen (364) and verteporfin (365) have been encapsulated into PLGA or poly(ϵ -caprolactone) particles for *in vivo* studies against breast cancer. Doxorubicin, a widely used anticancer drug, has been encapsulated into PLGA nanoparticles (366) that presented the ability to release the drug up to 1 month. In addition, this carrier system avoids a lot of the undesirable effects of doxorubicin (e.g., cardiotoxicity).

If a sustained release of the drug in the tumor site is required, then the nanoparticle surface must be modified in order to avoid RES macrophages, which recognize hydrophobic particles as foreign. To escape RES, the surface of nanoparticles is modified with hydrophilic molecules that form a steric barrier on the particle surface. Polyoxypropylene-polyoxyethylene (POP/POE) surfactants are suitable macromolecules to prevent nanoparticles from sticking to the blood vessel endothelium and to inhibit RES recognition. Indeed, among the various copolymer members, poloxamine and poloxamer have the best prolonged circulation time of nanoparticles (367). Unfortunately, poloxamer and poloxamine do not exhibit prolonged circulation times

when nanoparticles are made up of PLGA (368). Recently, the most common moiety used for nanoparticle surface coating to obtain the so-called Stealth nanoparticles is PEG and its derivatives (369,370). Attachment of PEG on the nanoparticle surface can be performed in different ways: (1) by adsorption, (2) by incorporation during the preparation process, (3) by covalent linkage with the nanoparticle polymeric matrix.

In the passive targeting, as with stealth liposomes, long circulating nanoparticles escape from the blood circulation through the fenestrations of capillaries perturbed by inflammatory processes or by tumors (235). Inflamed vessels present fenestration sized up to 700 nm, so improved colloidal nanoparticles ($< 200 \text{ nm}$) are able to pass across, thus accumulating and releasing the drug just in the site of action. These particular characteristic of nanoparticles leads to an increased therapeutic index of the incorporated drug. Nowadays, chitosan-based particulate systems are attracting the most attention as potential long-term drug delivery systems because of mucoadhesive and long circulating properties of chitosan. Doxorubicin-dextran conjugates were encapsulated into chitosan nanoparticles to minimize the cardiotoxicity of the drug. This system reduced not only the drug side effects, but also improved the therapeutic efficacy of doxorubicin in the case of solid tumors (371).

The strategy of nanoparticle passive targeting is widely used for cancer therapy, but it presents a limitation due to a high resistance factor of some solid tumors that cannot be circumvented by PEG-coated nanoparticles. Therefore, an alternative approach is the use of temperature-sensitive nanoparticles, which are able to release its drug content only in hyperthermic zones (235). Other approaches may involve the use of biochemical triggers, such as the pH-sensitive lipid-anchored copolymers, to generate fusogenic particles (249).

The strategy of active targeting increases the probability of having a selective direction of nanoparticles to a designed site. In this case, ligands that specifically bind to surface receptors of target sites are coupled to long-circulating particulates. Among polymers suitable for coupling to specific ligands, poloxamers and PEG have received the most attention. A derivatization is achieved between the end group of the poloxamer chains with pyridyl disulfide. After a disulfide exchange with a thiol-containing moiety on the peptide or antibody to be attached on the surface of the nanoparticle, long-circulating actively targeted particles are obtained (372).

The use of nanoparticles as drug delivery systems can overcome the barriers to the penetration of anti-infective drugs into cells, that is, strong protein binding, an unfavorable lipid-water distribution coefficient, an unfavorable pH gradient between different cellular compartments, and the existence of active transport pump mechanisms that prevent the accumulation of sufficient antibiotic concentrations in the interior of the infected cells (278). In particular, a suitable nanoparticle coating can promote the permeation of hydrophilic drugs through membranes (Fig. 54). In fact, the free drug, able to freely diffuse in the aqueous medium and to interact with the outer-hydrophilic zone of the membrane model, has to pass

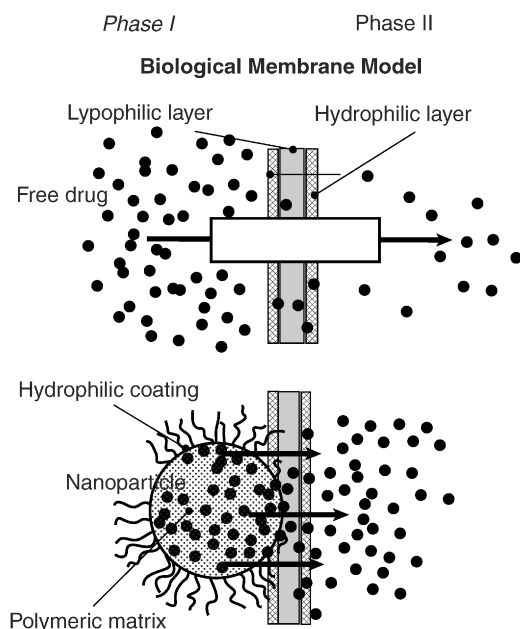


Figure 54. A model of the interaction between the aqueous phase containing a free hydrophilic antibiotic, or the drug-loaded PECA nanospheres and the biological membrane model. The permeation driving force is the different drug concentration between phases I and II (355).

through the lipophilic layer of the same membrane. This process could represent the limiting step in the diffusion through the membrane model of a hydrophilic molecule. In the case of nanoparticles, the outer hydrophilic shell of the particles (coated with nonionic surfactant) could ensure an interaction with the hydrophilic layer of the membrane, while the internal lipophilic core of the particle can ensure a close interaction with the hydrophobic layer of the membrane, providing a high permeation of the drug (354). The nanosphere-mediated increase of drug membrane permeation leads to an improvement of the antibacterial activity (373) and to an intrabacterial drug accumulation. The entrapment of antibiotics in nanospheres may prevent the bacterial resistance to drugs due to pleiotropic drug resistance and changes in the bacterial outer membrane leading to a decrease in OmpF porin, which probably causes decreased drug permeation.

Nanoparticles can be used for the treatment of various viral infection diseases localized at the level of the RES. For example, RES cells can be infected by both strains of the HIV, namely, HIV-1 and HIV-2. Monocytes and macrophages seem to have a fundamental role in the immunopathogenesis of the HIV infection, by behaving as virus reservoirs from which HIV can disseminate throughout the body and brain. Because nanoparticles can be easily phagocytosed by macrophages, they may represent a suitable and promising drug delivery system for the treatment of HIV infection persisting in these cells.

In vitro studies showed that human macrophages are able to phagocyte different types of polyacrylic and albumin nanoparticles (374). Interestingly, HIV-infected macrophages seem to have a higher phagocytotic activity concerning nanoparticle uptake than noninfected macrophages

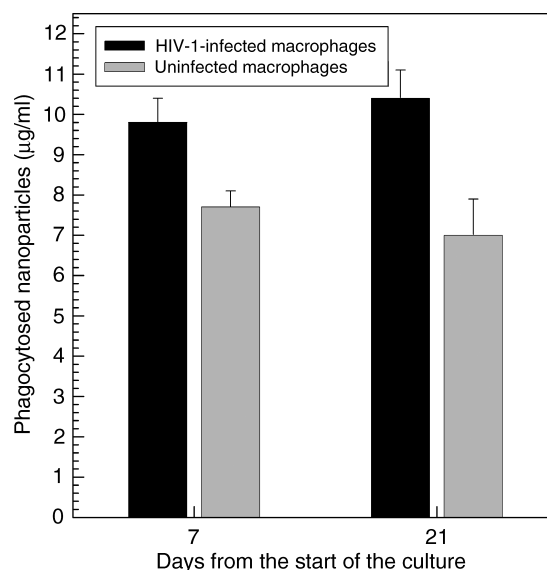


Figure 55. Influence of HIV infection on phagocytosis of poly(butylcyanoacrylate) nanoparticles by human macrophages. Cell cultures were infected with HIV-1 at day 1 after start of the culture. At day 7 or 21, the nanoparticles (200 nm diameter) were added at a final concentration of $0.5 \text{ mg} \cdot \text{ml}^{-1}$ to the infected cultures and incubated for 6 h. Data from Ref. 374.

(Fig. 55). This phenomenon can be due to an activated state of these infected cells, leading potentially to a preferential phagocytosis of drug-loaded nanoparticles, and hence to a targeted delivery of antiviral drugs to these cells.

The RES tropism of conventional nanoparticles also can be efficiently used for the treatment of protozoa infection (i.e., leishmaniasis) (348). Contrary to liposomes, empty poly(isobutylcyanoacrylate) nanospheres exhibit a certain *in vitro* and *in vivo* antiparasitic activity against (375). Probably, this action could be attributed to peroxide production following nanosphere phagocytosis, which led to a respiratory burst, more pronounced in infected than uninfected macrophages.

One of the major problems connected with ophthalmic therapy is drug loss after instillation of eyedrops. To improve ocular bioavailability, mainly nanoparticles have been used. Colloidal systems have the convenience of a drop and are able to maintain drug concentration and activity at its site of action, probably due to an improved ocular mucoadhesion. In fact, poly(butylcyanoacrylate) nanospheres were able to improve the amikacin ocular delivery (376) by increasing the corneal and aqueous humor concentration of amikacin with respect to the free drug and to other formulations. The surface coating of nanospheres can also be used for ophthalmic application and can be a crucial factor to achieve a well-tailored and efficient drug delivery. In spite of the nanoparticle polymer and the method of coating, the presence of PEG on the surface of nanoparticles improves the ocular drug permanence time and increases the drug level in various ocular structures compared with both conventional ocular formulations and uncoated nanoparticles (352,377). Ocular gene therapy is also possible with polymeric nanoparticles (378).

In recent years, mucosal surfaces (nasal, pulmonary, buccal, and ocular) have received much attention as alternative routes of systemic administration (379). Chitosan-coated nanoparticles present mucoadhesive properties that can be useful to enhance mucosal drug adsorption. An example is the enhanced nasal absorption of insulin-loaded chitosan nanoparticles that do not damage the biological system.

The buccal adsorption of vaccine encapsulated into nanoparticles is an alternative to the parenteral route of administration of vaccines. The oral or nasal delivery of ovalbumine from chitosan microparticles enhances the systemic and local immune response against diphtheria toxoid vaccine (380).

Lipid-Based Nanoparticles. Lipids instead of polymers can be used to obtain colloidal drug carriers. Solid lipid nanoparticles (SLN), nanostructured lipid carriers (NLC), and lipid drug conjugates (LDC) are nanoparticles with a solid lipid matrix and present an average diameter in the nanometer range. These innovative colloidal carriers have attracted increasing attention in recent years. They are regarded as an alternative carrier system to traditional colloidal systems (e.g., polymeric micro- and nanoparticles). General ingredients include solid lipids, emulsifiers, and water. Lipids include triglycerides, partial glycerides, fatty acids, steroids, and waxes. All excipients are generally recognized as safe (GRAS) substances, so a wide variety of compounds can be used for formulation purposes.

Solid Lipid Nanoparticles. These nanoparticles are colloidal systems made up of solid lipids and are stabilized by surfactants. During the 1950s, lipid nanoemulsions were introduced for the parenteral nutrition and later they were used as carriers for lipophilic drugs. The major problem with nanoemulsions was the loss of drugs related to their liquid form. The SLN were developed to overcome this problem. In fact, the use of solid lipids instead of liquid oils is a very attractive idea to achieve controlled drug release, because drug mobility is much slower into a solid lipid than in a liquid oil.

The SLN can be prepared using different procedures. The main preparation process is the high pressure homogenization (HPH) method, in which a dispersion of the drug in the melted lipid is constricted through a narrow gap (in the range of few microns) under very high pressure, thus disrupting lipid particles down to the submicron range. Other methods are the solvent emulsification–evaporation method and the microemulsion-based preparation (381). The first is a method to prepare nanoparticles by precipitation in o/w emulsions. The second production method is based on the preparation and the subsequent dilution in cold water (2–3 °C) of a microemulsion made up of a low melting lipid, an emulsifier, a coemulsifiers, and water. Following preparation, as for other colloidal carriers, the determination of the mean size, size distribution, and zeta potential of SLN is necessary to define their physicochemical properties.

Formulative Aspects of SLN. Evaluations on the degree of crystallinity and lipid modifications are very important for

SLN. In fact, the organization of lipids into a crystalline reticulate is fundamental for the determination of the drug encapsulation and release rates. In general, if the lipid matrix is made-up of pure molecules (i.e., tristearin or tripalmitin), a perfect crystal with few imperfections, is formed. As incorporated drugs are located between fatty acid chains, in crystal imperfections, and between the lipid layers, a highly ordered crystal form cannot accommodate large amounts of drug (382). Also, the lipid modification influences the encapsulation and release degree of a drug. In fact, glycerides exist in three different polymorphic forms: α , β' , and β . The degree of reticulate imperfection of the lipid decreases in this sequence: $\alpha < \beta' < \beta$. Lipid nanoparticles recrystallize at least partially in the α -form, but with increasing formation of the more stable β'/β modifications, the lattice is becoming perfected, the number of imperfections decreases, and drug is expelled from nanoparticles.

The coexistence of additional colloidal structures, such as micelles, liposomes, and supercooled melts, has to be taken into account after the preparation of SLN; the quantification of these additional structures is a serious challenge because of their size similarities with those of the SLN. Therefore, it would be desirable to use methods that are sensitive to the simultaneous presence of different colloidal species. Both NMR and ESR techniques meet these requirements.

Physical stability of SLN dispersions is generally >1 year (383), up to 3 years in the case of SLN made of glyceryl palmitate. The storage stability of SLN depends on two factors: (1) the physical modification of lipid structure ($\alpha \rightarrow \beta'/\beta$); (2) the presence of additional colloidal structures (liposomes, micelles, drug nanoparticles).

Gelation phenomena, that is, increase of particle sizes and drug expulsion from the lipid carrier, are the major problems of storage stability. Gelation is the transformation of a low viscosity SLN colloidal dispersion into a viscous gel. It occurs when SLN is put in contact with other surfaces and shear forces, and it is connected with crystallization processes. This destabilizing phenomenon can be retarded or prevented by the addition of coemulsifying surfactants with high dynamic mobility, such as glycocholate (381).

The increase of particle sizes is a consequence of particle aggregation and it is less significant when SLN have a zeta potential value of -25 mV, while it becomes an important phenomenon when the zeta potential is -15 mV or less (381).

Drug expulsion is related to crystallization of lipids and their modification to the β'/β form, in which the lipid lattice is packed in a more ordered way with a reduction of imperfections.

To have an optimal storage conditions, SLN can be lyophilized or spray-dried. During lyophilization, a colloidal lipid dispersion is deprived of its solvent to guarantee a better chemical and physical stability. However, two transformations in the formulation occur during and after this process, which might be the source of additional stability problems: (1) passage of SLN from an aqueous dispersion to a powder with possible changes of osmolarity and pH; (2) resolubilization that favors particle aggregation. To overcome these problems, a cryoprotector (e.g., trehalose,

sorbitol, mannose, and glucose) is added to the SLN dispersion before lyophilizing. These protective agents are used in a 10–15% (w/v) concentration and act to decrease the osmotic activity of water and favour a glassy state of the frozen sample (384).

Spray drying is an alternative method to transform an aqueous SLN dispersion into a dry product. It is cheaper and simpler than lyophilization, but has the disadvantage of needing high temperatures, which can cause particle aggregation. Therefore, it is recommended to use lipids with melting points $>70^{\circ}\text{C}$ for spray drying. Also, in this case the addition of cryoprotective agents may be useful to prevent particle aggregation.

Drug incorporation into SLN is related to crystalline modification of the lipids and is inversely proportional to the β'/β modification of lipids. Depending on the drug/lipid ratio and solubility, the drug is located in the core of the particles, in the shell, or dispersed throughout the matrix, so that drug loading capacity of conventional SLNs is generally from 25 up to 50% (381). The drug-loading capacity is higher for lipid mixtures with different acyl chain lengths than for lipids that form a perfect crystal with few imperfections and cannot accommodate large amount of drug.

The release profiles could be modulated showing a burst release followed by a prolonged release, or generating systems without any burst release at all. The release kinetic can be controlled by modification of the preparation procedures and the type of surfactant and lipid material.

Therapeutic Applications of SLN. Due to their small sizes, SLN may be administered through every route: oral, transdermal, and IV administration can be possible.

The SLN for oral administration may include aqueous dispersions or conventional dosage forms (e.g., tablets, capsules, and pellets). Camptothecin-loaded particles are an example of orally administered SLN. This is a stearic acid/Poloxamer 188 formulation of SLN that present a zeta potential value of -69 mV and an encapsulation efficiency of 99.6%. The incorporation of camptothecin into SLN provided drug protection from hydrolysis (381). A better bioavailability, prolonged plasma levels, and lack of nephrotoxicity are observed for orally administered SLN encapsulating drugs, thus leading to the conclusion that SLN are a promising sustained release system for the oral administration of lipophilic drugs (381,385).

The SLN are formulated into creams, hydrogels, or ointments before their application onto the skin and form an adhesive film upon the skin, which is able to restore the protective action of the naturally occurring hydrolipidic skin film when it is damaged. Many different cosmetic ingredients have been encapsulated into SLN (i.e., coenzyme Q_{10} , vitamin E, and retinal) (386). A modern approach to an intelligent release of the drug from SLN to the skin is related to lipid modification from the α to the β form. An intelligent drug-loaded SLN is a colloidal system that maintains itself into the more energetic α -form during storage, while transforming into the β -form after application onto the skin, thus releasing its incorporated drug by expulsion from the lipid crystalline reticule. The SLN per se also have a sun protective effect (387) that is due to their particulate nature and their ability to scatter UV light. In

this case, SLN show a synergistic effect if formulated with a molecular sunscreen, also showing better skin protection and a reduction in side effects.

It is possible to use SLN for pulmonary drug delivery since they maintain their particle size and polydispersion index after nebulization. Only very little aggregation could be detected, which is of no significance for pulmonary administration (388). In addition, SLN may be used as a powder for inhalation. The use of SLN instead of polymeric nanoparticles has many advantages, such as high tolerability, faster degradation, and passive targeting toward lung macrophages.

The SLN can be injected intravenously and used to target drugs to particular organs. They also can be administered intramuscularly or subcutaneously. When administered subcutaneously, SLN act as a depot of the drug, while they are cleared from the circulation by RES (liver and spleen) when administered intravenously. The incorporated drug is released upon erosion by diffusion from the particles or by enzyme degradation. Obviously, in the case of IV administration, the SLN size must be $<5\text{ }\mu\text{m}$ to avoid the possibility of embolism into the fine capillaries. Similar to any colloidal drug with a small mean size ($\leq 200\text{ nm}$), SLN have been coated by polyoxyethylene in order to achieve long circulating colloidal lipid particles (389). Stealth SLN can be prepared by using Pluronic F188, a block poly(oxyethylene/polyoxypropylene) copolymer that anchors its hydrophobic portion in the SLN lipid matrix, while the hydrophilic portion forms the hydrophilic coating of SLN. Stealth SLN can be used in tumor and antibacterial therapy. Paclitaxel-loaded SLN provided a higher and prolonged plasma level of the drug with respect to the cremophor EL-based commercial formulation with a consistent reduction of side effects (381). In all the investigated cases, SLN containing an anticancer drug showed higher blood levels with respect to the relative commercial drug formulations after IV injection.

The therapy at the level of the brain is always difficult due to the presence of the blood–brain barrier, and hence the possibility of having a suitable drug delivery system that is able to reach the brain can be extremely useful. Stealth SLN allow brain delivery of drugs that are not capable of passing through the blood–brain barrier (390).

The potential SLN toxicity has to be considered as a function of the administration route. Topical and oral administration are absolutely nonproblematic because all excipients used in SLN formulations are those currently employed for the formulation of traditional dosage forms or cosmetic products. The situation is slightly different for parenteral administration. In this case, only surfactants accepted for parenteral administration can be used (e.g., lecithin, Tween 80, PVP, Poloxamer 188). Up to now, there is no SLN product for parenteral use on the market. However, SLN show a very good tolerability both *in vitro* and *in vivo* (381).

Nanostructured Lipid Carrier. The NLC were introduced at the end of the 1990s in order to overcome some limitations of SLN: (1) too low payload for a number of drugs, (2) drug expulsion during storage, (3) high water content of SLN dispersions.

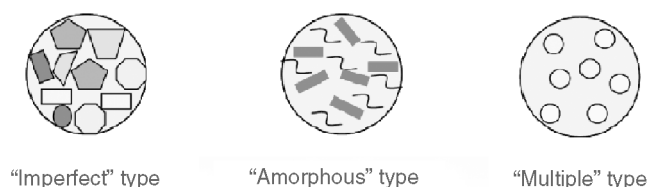


Figure 56. Schematization of the three types of NLCs.

The NLC are made up of very different lipid molecules mixed together, that is, a blend of solid with liquid lipids (oils). The resulting matrix of the lipid particles shows a melting point depression compared to the original solid lipid, but the matrix is still solid at body temperature. There are three different models of NLC, depending on the way of production and the composition of the lipid blend (Fig. 56). In the first model, called the imperfect type, spatially different lipids lead to larger distances between the fatty acid chains of the glycerides and to general imperfections in the crystal structure, thus allowing a greater extent of drug entrapment. The second type of NLC, the so-called amorphous type, contains liquid oil nanocompartments within the lipid particle matrix. In this way, crystallization can be avoided and the solid character of NLC can be maintained as shown by NMR and DSC measurements (391). The third type of NLC, the so-called multiple type, are produced by mixing a solid lipid with a high amount of oil. In this way, a phase separation occurs between solid lipids and oil molecules thus forming nanocompartments. This phenomenon occurs during the cooling process after the hot homogenisation method.

The NLC are produced successfully by the high pressure homogenization method and it is possible to obtain particle dispersions with a solid content of 50 or 60% (392). The particle dispersions thus produced have a high consistency with a cream-like or almost solid appearance.

Because of their high particle concentrations, NCL can be used for granulation or as wetting agents in pellet production. In addition, NLC can easier be processed to traditional oral dosage forms, such as tablets or capsules (393).

These carriers have been used for dermal delivery. Similarly to SLN, they can be incorporated in to existing products or formulated in a final product containing only NLC. When incorporated into an o/w emulsion saturated with the drug, the NLC disordered structure is preserved and the drug remains inside the particles. Following skin application, and then increased temperature, water loss and NLC lipid transition to a more stable polymorphic form triggers drug expulsion. The drug expelled from NLC is supersaturated with the drug already present in the emulsion. The supersaturation phenomenon can be used to increase the skin drug permeation as observed for cyclosporine (394).

In the future, an area of particular interest can be the prolonged release of drugs after subcutaneous or intramuscular injection (e.g. erythropoietin). Also, NLC dispersions for IV injection appear feasible (395).

To overcome the important limitation of SLN and NLC to incorporate only lipophilic drugs or very low concentrations of highly potent hydrophilic drugs, lipid drug

conjugates were recently developed with drug loading capacities of up to 33% (396). In this type of lipid nanoparticle, an insoluble drug-lipid conjugate bulk is prepared using two different methods: (1) salt formation with a hydrophobic moiety (e.g., with a fatty acid) and (2) covalent linking (e.g., to ethers or ester).

FUTURE PERSPECTIVES

Drug delivery technology has had considerable advances, bringing many clinical products to the market. However, the major needs for drug delivery devices are still unmet and important classes of drugs have yet to benefit from these technological successes. The central focus of any controlled delivery devices is "control." This can be achieved if: (1) the size of the device can be modulated as accurately as possible; (2) the device can be produced with a certain reproducibility; (3) the device is stable enough for administration purpose; (4) the device is biocompatible; (5) the rate of drug delivery should be independent of the surrounding environment. To date, it is possible to have recourse to a lot of innovative approaches characterized by different features as a function of the physicochemical properties of the delivered drug, the administration route, and therapeutic aims.

Many aspects of these drug delivery devices require an improvement to be applied to clinical use, but considering the *in vitro* and *in vivo* results, they seem to be very interesting.

From the appearance of the first drug delivery device to date, a constant improvement has been made, that is, from microtechnology we have passed to nanotechnology, and from an aspecific drug delivery, we have passed to a selective drug delivery. New challenges for the future are the feasibility of scaling-up processes to bring to the market quickly innovative therapeutic entities and the possibility of obtaining multifunctional devices that will be able to fulfill the different biological and therapeutic requirements.

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