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Drug Vectors

Drug Delivery by Soft Matter: Matrix and Vesicular Carriers

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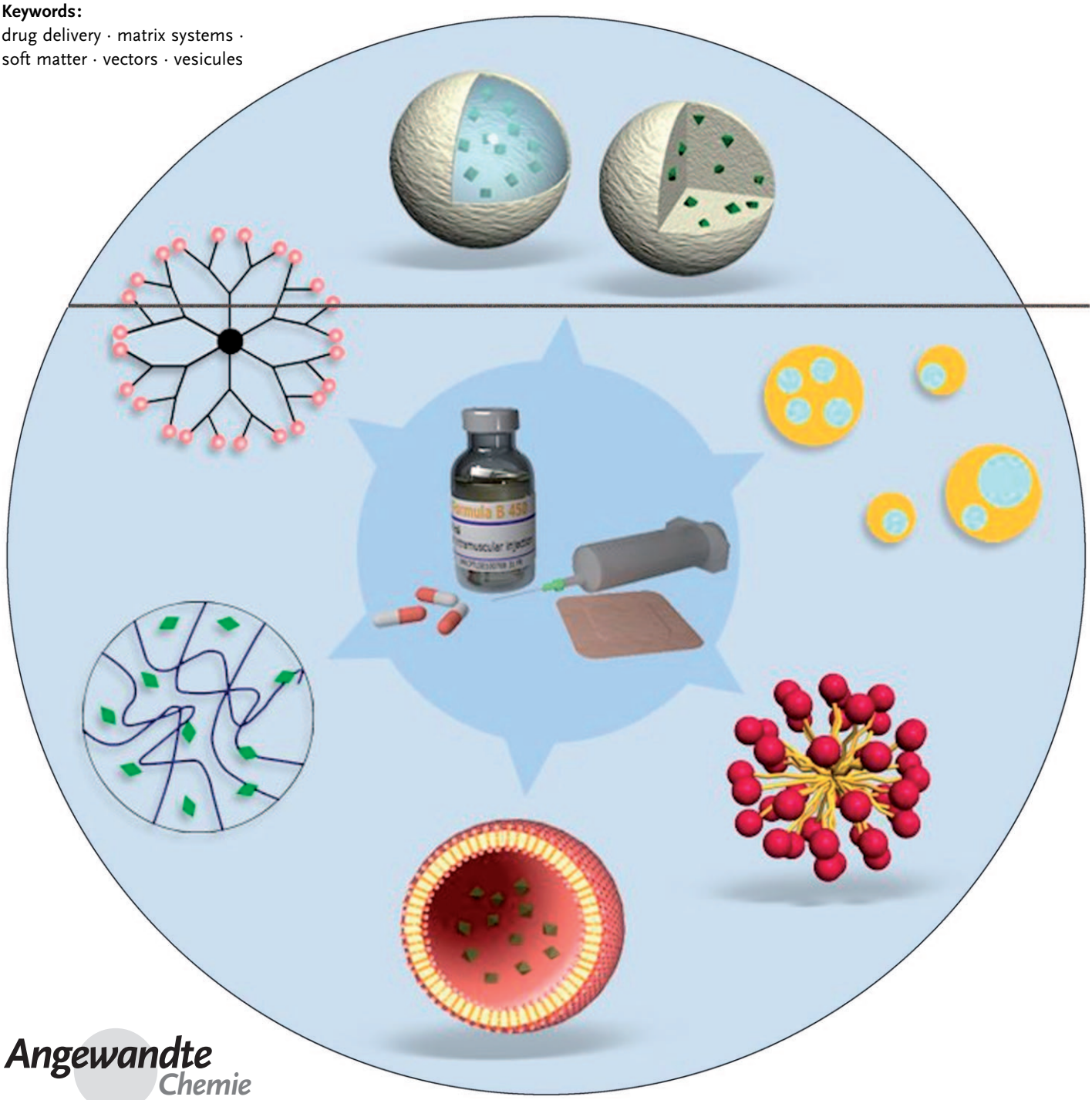

The increasing need for drug delivery systems that improve specificity and activity and at the same time reduce toxicity to ensure maximum treatment safety has led to the development of a great variety of drug vectors. Carriers based on soft matter have particularly interesting characteristics. Herein we present the current standing of the research in this area, and focus on two main families, namely matrix systems and vesicles. We outline the structure, properties, and potential applications of these vectors, and discuss their main advantages and drawbacks in their synthesis.

From the Contents

1. Introduction

Vectorization, or the specific delivery of active principles, which are the therapeutic constituents of a drug to an organ, a tissue, or unhealthy cells by carriers, is one of the major challenges in therapeutic research.[1] Many drugs have physicochemical characteristics that are not favorable to transit through the biological barriers that separate the administration site from the site of action. Some drugs also run up against enzymatic barriers, which lead to their degradation and fast metabolization. The distribution of these active molecules to the diseased target zones can therefore be difficult. Moreover, the accumulation of drugs in healthy tissues can cause unacceptable toxic effects, leading to the abandonment of treatment despite its effectiveness.

Two of the most important aspects of vectorization are therapeutic efficiency and treatment safety. These aspects are achieved by controlling the concentration of the drug released and its specific delivery to the desired action site. The development of carriers for drug delivery has seen considerable expansion in the last twenty years, with the development of new medicines delivered by matrix or vesicular carriers, such as doxil, in which the drug is transported by liposomes used in cancer treatment, or superfect, which uses a dendritic vector for transfection.

Herein we present the current state of progress that has been made in this field. We present two broad families of vectors, namely matrix systems and vesicles, both of which have been and still are the subject of many studies. We will not discuss the interaction between vectors and cells, as the mechanism has not been fully elucidated in all cases. Finally, we will develop the particular case of drug delivery by vesicles formed from catanionic surfactants, and will present advances made by our group in this domain.

2. Matrix Systems

Matrix systems are three-dimensional networks that are formed by polymers, surfactants, or dendrimers, in which active principles are trapped. This section concentrates on the study of micelles, emulsions, hydrogels, dendrimers, nanospheres, and solid lipid particles.

2.1. Micelles

2.1.1. Structure and Properties

Micelles are aggregates of amphiphilic molecules in which the polar headgroups are in contact with water and the hydrophobic moieties are gathered in the core to minimize their contact with water (Figure 1).

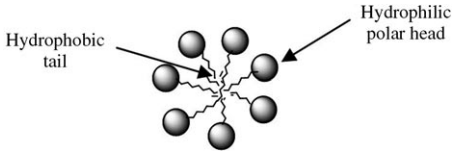

Figure 1. Representation of a surfactant micelle.

The main driving force in the autoassociation process of these surfactants is their hydrophobicity. The micelles form above a certain concentration, known as the critical micelle concentration (CMC). The mean size of these objects usually varies from 1 nm to 100 nm. It should be noted that these objects are dynamic, because the surfactants can exchange freely and rapidly between the micellar structure and the aqueous solution.

In addition to surfactants, block copolymers (having both a hydrophilic and a hydrophobic part) or triblock copolymers (with one hydrophobic and two hydrophilic parts or one hydrophilic and two hydrophobic parts) can also self-assemble to form polymeric micelles (Figure 2). These polymeric micelles have a mean diameter of 20 to 50 nm and are practically monodisperse.[2] Polymeric micelles are generally more stable than surfactant micelles, and form at markedly lower CMCs. These objects are also much less dynamic than those formed from surfactants. One surprising result obtained by separating the non-micellized or free polymers from the

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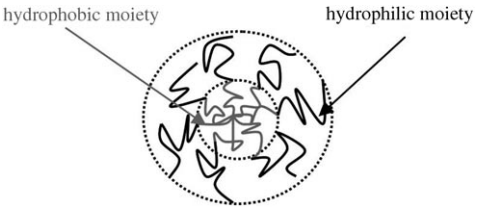

Figure 2. Representation of a block copolymer micelle.

polymers forming the micelles using size exclusion chromatography is that the objects are not destroyed, even if the resulting free polymer concentration is below the CMC.[3] Polymeric micelles therefore have numerous advantages over surfactant micelles.

2.1.2. *Application in Vectorization*

At the end of the 1960s, micelles attracted growing interest for application in drug vectorization because of both the ease with which their properties could be controlled and for their pharmaceutical characteristics.[4,5]

The anisotropic water molecule distribution in the structure of these objects (water concentration decreases from the surface to the core of the aggregates, from which water is excluded) allows the solubilization of hydrophobic active principles[6] in the micelles and enhances their bioavailability. In addition, active molecules are protected from enzymes that could degrade them and lead to their metabolization in biological media.[7–9]

According to the molecule to be transported, the size, charge, and surface properties can easily be modified by the addition of new co-surfactants to the original mixture of micelle-forming surfactants. Moreover, large quantities of micelles can be easily prepared in a reproducible manner by usual methods.[10]

Polymeric micelles are more frequently used in vectorization than surfactant micelles. The slow degradation kinetics of polymeric micelles has contributed to their success in vectorization applications, usually for anticancer hydrophobic drug delivery (such as paclitaxel) to tumors.

Numerous studies are based on micelles formed from a polyethyleneoxide polymer (PEO) for the hydrophilic moiety because of its high hydration level and the existence of strong repulsive forces between chains. These forces lead to large-volume micelles and participate in their stabilization.[10] Moreover, PEO polymer chains prevent micelle recognition by the reticulo-endothelial system, and therefore minimize the elimination of these vectors in the blood. Active principles can then be delivered over extended periods of time.[11]

New hydrophilic polymers, such as sugar-derived polymers[12] and polyglycerol polymers,[13,14] have also been developed in the last few years for their considerable biocompatibility.

Studies concerning the hydrophobic moiety of the copolymers used for the formation of vectors have been oriented towards the development of biodegradable polymers, such as poly(lactates).[15] Micelles obtained from these polymers and having a biodegradable hydrophobic moiety offer new perspectives in the field of vectorization because their degradation allows the kinetics of active principle delivery to be controlled, and because the elimination of the polymer in vivo is made easier.

Polymeric micelles also have the advantage of being able to deliver an active principle to its specific site of action if the polymer structure is tuned to make them sensitive to the

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Elodie Soussan studied chemistry at the Ecole Supérieure de Chimie Organique et Minérale (Cergy, France) in 2003. She obtained her MSc (2004) and PhD (2007) from the University of Paul Sabatier under the supervision of Dr. I. Rico-Lattes on the conception of new catanionic vectors. She currently holds a postdoctoral position at the Max Planck Institute for Colloids and Interfaces (Potsdam, Germany) in the group of G. Brezesinski.

Isabelle Rico Lattes was a CNRS researcher in Paris, where she prepared a PhD thesis on fluorine chemistry in collaboration with industry. She then started a research group at the IMRCP laboratory in Toulouse working on molecular organized systems. Several important discoveries, such as oxane HD, a fluorinated organized molecular system designed to treat the serious cases of retinal detachment, and selectiose, a ramnose derived amphiphile active against eczema, are credited to her. She was recognized by the CNRS with a silver medal in 2006.

Stéphanie Cassel studied chemistry at the University of Orléans. She received her PhD in 2000 on carbohydrate chemistry and the valorization of glycerol. She then worked as a research associate with Prof. D. A. Leigh at the Universities of Warwick and Edinburgh on supramolecular assemblies, and after a temporary lecturer position at the University of Versailles, she has been lecturer at the University of Toulouse since 2004. Her research interests focus on the self-organization of surfactants in non-aqueous media.

medium in which they are found. An example is the development of pH-sensitive copolymers by inclusion of amine[16] or acid[17] functional groups into the copolymer skeleton, which changes the solubility of the polymer and therefore the stability of the vectors according to the pH. The active principles can then be delivered by micelle destabilization at a site of action possessing a specific pH.

In general, micelles are used to solubilize hydrophobic active principles, make them bioavailable, and increase the bioavailability timescale in the biological medium. The same is true for reverse micelles, which allow hydrophilic active principles to be solubilized.

The major drawback of micellar vectors, and in particular surfactant vectors, is their tendency to break up upon dilution. This is not the case for polymeric micelles, but their synthesis can sometimes prove difficult for use in biological applications, which have specific requirements, such as nontoxicity, biocompatibility, degradability, and accurate molecular weight.

The development of pH-sensitive therapeutic micelles is often problematic because the variations of pH are small in the biological environment; for example, tumor tissue has a pH of 5 to 7, and normal tissues have a pH of 7.4.

2.2. Emulsions

2.2.1. Structure and Properties

Emulsions are heterogeneous dispersions of two immiscible liquids, such as oil in water (O/W) or water in oil (W/O), and are susceptible to rapid destabilization by aggregation, coalescence, or flocculation, leading to phase segregation. Nevertheless, the stability of these emulsions can be improved by adding surfactants that are able to form a monolayer or a multilayer around the droplets of dispersed liquid. This lowers the interfacial tension between the immiscible liquids and increases repulsion between the droplets.

According to the concentration of the three components (water, oil, and surfactant) and the method used for the preparation of the emulsion (which determines the droplet size), the mixture obtained can be a standard emulsion (droplet size from 100 nm to 10 μm), a nanoemulsion (droplet size from 10 nm to 100 nm), or even a multiple emulsion (O/W/O or W/O/W)[18] (Figure 3).

Unlike standard emulsions and multiple emulsions, only microemulsions are transparent and thermodynamically stable systems.[3]

2.2.2. Application in Vectorization

Emulsions are flexible drug formulation systems. The active molecule can be either hydrophilic, in which case a W/O emulsion is used, or hydrophobic, for which an O/W emulsion is employed. Their characteristics can be easily tuned by adjusting parameters such as volume fraction of the dispersed phase, droplet size, or osmotic gradient.[19] Moreover, emulsions can be produced in very large quantities by means of suitable methods.[20]

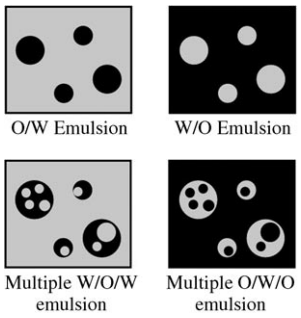


Figure 3. Representation of different types of emulsions (gray: aqueous phase, black: oil phase).

As early as 1960, A. Wretlind et al. developed the first intravenously injectable O/W emulsion as a source of nutriments for patients unable to feed themselves orally or unable to metabolize food. Since then, many hydrophobic drugs, such as diazepam, a barbiturate, have been formulated using O/W emulsions to enhance their bioavailability.[21]

Microemulsions[22] are also frequently used in vectorization for their high stability. Numerous studies have been carried out in the field of anticancer drug delivery; for example, the solubilization of vincristin in an oil phase consisting of vitamin E and oleic acid. The O/W emulsion obtained by mixing this oil phase with water, polyethyleneglycol, and cholesterol is very stable, and only 7.5% of vincristin decomposed after one year of storage. Moreover, the drug biodistribution around tumors was higher whereas toxicity was considerably reduced.[23]

Multiple emulsions, and in particular W/O/W emulsions, have proved to be excellent candidates for the vectorization of hydrophilic active principles thanks to the presence of the intermediate oily phase that acts as a membrane and allows the controlled release of active substances. The effectiveness of double emulsions was demonstrated in the case of the encapsulation and delivery of antibiotics,[24] proteins,[25] and anticancer drugs.[26]

Emulsions offer many possibilities for drug delivery, but some difficulties restrict their use. In particular, the high surfactant concentration (mostly in microemulsions) can lead to toxicity risks. In the case of formulations for intravenous administration, some precautions are required, because under dilution, a phase separation of the emulsion can occur, which can induce a risk of embolism for the patient.

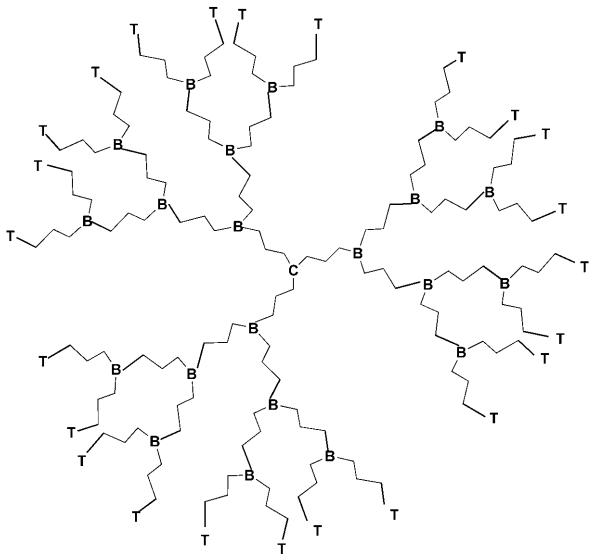
2.3. Dendrimers

2.3.1. Structure and Properties

The term dendrimer is formed from two separate words, dendrite and polymer. The first evokes the branched structure of the molecules (the Greek "dendron" means tree), and the second refers to the repetitive pattern of the structure ("meros" meaning unit in Greek).

A dendrimer is a molecule composed of monomers that associate according to a tree-like process around a central plurifunctional core. The structure of these highly ordered

and regularly branched globular macromolecules can be divided into three distinct parts (Scheme 1): a core (C), repetitive layers emanating from the core, or branches (B), and terminal groups on the outer layer of the repeating units (T). Dendrimers are classified according to their generation, which corresponds to the number of repeating layers.



Scheme 1. A generation-three dendrimer.

Dendrimers can be synthesized using a divergent method.[27] In this case, the dendrimer is built outwards from the core by repetition of a sequence of reactions, which allows fast growth of the dendrimer in both size and in number of terminal groups. Another method is the convergent method,[28] in which the core is incorporated in the final step of elaboration of the dendrimer. The latter method is widely used when the core functions are sensitive to the dendrimer-growing synthetic conditions.

Whatever the synthetic method, the product obtained is a macromolecule that possesses a well-defined surface-function number.

2.3.2. *Application in Vectorization*

Owing to their large number of surface groups, dendrimers have the ability to create multivalent interactions. Collectively, these polyvalent interactions can be stronger and more specific than the corresponding total number of monovalent interactions.[29] Dendrimers are therefore more competitive for vectorization than monomers; this is the so-called "dendritic effect".[30] This property has led many research groups to study the possible applications of dendrimers in vectorization[31] by tuning the dendrimer surface functional groups to induce an electrostatic-type interaction with active molecules. For example, negatively charged DNA chains can be complexed to positively charged dendrimers. Several research groups have demonstrated that dendrimer/DNA complexes, which are very compact, easily penetrate cells by endocytosis and therefore improve transfection.[32–36] Dendritic vectors, such as superfect, are marketed by Qiagen for this particular application.

Dendrimers can also be structured to encapsulate a drug inside their internal cavities. This method helps to improve the stability and therefore the bioavailability of some active substances. The dendrimer structure (apolar core and polar surface) can be compared to "unimolecular micelles", with the dendritic structure having the great asset of being independent of the dendrimer concentration. Thus, molecules such as indomethacin, an anti-inflammatory drug, have been encapsulated into hydrophobic 4,4′-bis(4′-hydroxyphenyl)-pentanol-derived dendrimers. The peripheral functions of this dendrimer were covalently linked to poly(ethyleneglycol) to make the whole structure more water soluble and therefore more biocompatible.[37,38]

Dendritic boxes can allow the encapsulation of active substances. Meijer et al. have synthesized poly(propyleneimine)-based dendrimers that form such structures. Active compounds can be encapsulated in the cavities of the dendritic boxes during the synthesis of the dendrimer. At the end of the reaction, the dendrimer possesses a very dense external layer that prevents leaking of the active principle before the expected hydrolysis of this layer in a biological medium.[39–42]

Dendrimers offer numerous possibilities for the vectorization of either hydrophilic or hydrophobic drugs[43] thanks to their globular structure and their high number of surface functions. However, dendrimer synthesis is difficult and can be quite expensive. Another drawback of this type of vector lies in the release of the active principle in the biological medium. In some cases, the bulkiness of the dendrimer and the density of its structure make the cleavage of the water-soluble and biodegradable bonds of the peripheral layer quite difficult. Delivery of active principles is therefore not so straightforward.[41] In other cases, the encapsulated molecules are not well trapped and may be released prematurely.[38] Nevertheless, the functional groups of dendrimers can be easily tuned and therefore make versatile drug vectors.

An alternative to the difficult and expensive synthesis of dendritic vectors is the use of hyperbranched polymers, as suggested by Haag.[44] Biocompatible hyperbranched polyglycerol and poly(ethyleneimine) can be used to encapsulate cytostatic molecules that could be specifically delivered to tumor tissue thanks to their sensitivity to pH.

2.4. *Hydrogels*

2.4.1. *Structure and Properties*

Hydrogels are three-dimensional networks composed of hydrophilic polymer chains. These structures have the ability to swell in water without dissolving.[45] There are many different types of hydrogels, which can be classified according to their physicochemical properties and their preparation methods.[46]

Hydrogels can be formed from either natural or synthetic polymers. Natural hydrogels include proteins, polysaccharides, and deoxyribonucleotide (DNA). Hydrogels based on synthetic polymers are prepared by polymerization of syn-

thetic monomers. There are also biohybrid hydrogels that result from a mixture of synthetic polymers (for their functionality) and natural polymers (for their biocompatibility).[47,48]

The type of cross-linking, which allows the polymer chains to form a three-dimensional network, can be either chemical or physical. Chemical gels are networks of chains connected by covalent bonds, whereas physical gels result from the spontaneous self-assembly of polymer chains into a disordered three-dimensional network[49] through weak interactions, such as hydrogen bonds or hydrophobic interactions. Physical hydrogels are therefore formed by a reversible process that can be solvent-, pH-, or temperature-dependent. These three-dimensional networks have a great affinity for water, which can penetrate in between the polymer chains, thus inducing a swelling of the material and the formation of a hydrogel.

2.4.2. *Application in Vectorization*

Hydrogels play an important role in specific drug delivery. Their high water content makes them highly biocompatible.[50] Moreover, their physicochemical, mechanical, and biological properties can be controlled by the type of polymer used and the preparation method employed.

Since the first study by Wichterle and Lim[51] in 1960, which was devoted to the biomedical application of a poly(2-hydroxyethyl methacrylate)-based hydrogel, a large number of hydrogels have been developed for therapeutic purposes.

The encapsulation of hydrophilic active molecules can be easily achieved, either by mixing the drug with the monomers, followed by polymerization, or by swelling the gel in a drug-containing aqueous medium. The active principles can be released later in the biological medium by diffusion according to Fick's law, by hydrogel dissolution, by osmotic force modification, or by ion exchange.[46]

Like polymeric micelles, there are also hydrogels that are sensitive to medium parameters, such as pH, temperature, and light). Modification of these parameters leads to compression or swelling of the hydrogel, resulting in the release of the encapsulated molecule (Figure 4). For example, poly(*N*-isopropylacrylamide) networks are often used to form

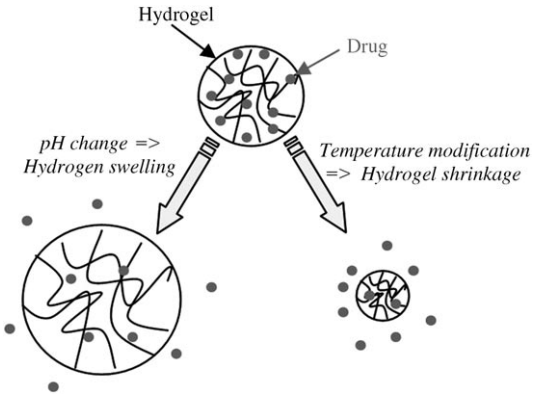


Figure 4. Drug release from a hydrogel that is sensitive to parameters of the medium.

temperature-sensitive hydrogels because of the drastic variation of their water solubility with temperature.[52] This effect is due to competition between hydrogen bond formation and hydrophobic interactions. The formation of hydrogen bonds between the polar parts of the polymer is predominant at low temperature, which allows the hydrogel to swell. When the temperature rises, hydrophobic interactions prevail and the hydrogel shrinks. This behavior has been used for the elaboration of so-called "on/off" vectors.[53,54]

Hydrogels have been synthesized that are sensitive to blood-sugar levels. These hydrogels are able to deliver insulin when the glucose concentration becomes too high, which is of great interest for people suffering from diabetes (Figure 5). A glucose oxidase enzyme is added to pH-sensitive hydrogels; when the glucose present is metabolized into gluconic acid by the enzyme within the hydrogel, the pH decreases, causing a swelling of the gel and the release of a large quantity of insulin.[55]

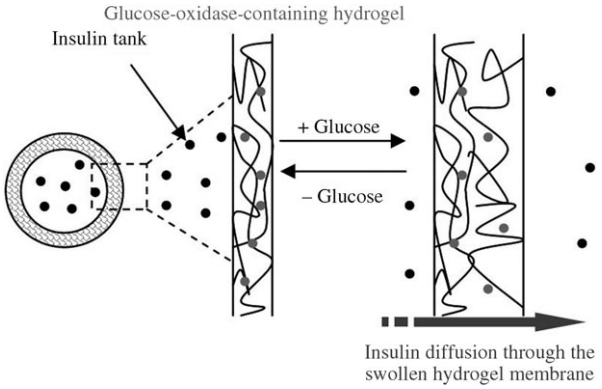


Figure 5. Insulin delivery regulation by hydrogels that are sensitive to glucose concentration.

The synthesis of new polymers has given rise to hydrogels with various new properties, such as bioadhesives[56] and recognition using molecular imprinting.[57] These vectors should play an important role in the field of specific drug delivery. The hydration ratio of hydrogels is one of the most important parameters to be taken into account for applications in vectorization, and its level has to be controlled. Indeed, for a certain type of polymer, it depends on the chain length, molecular mass, and density of reticulation.[58]

Despite all the straightforward advantages of hydrogels, owing to their sensitivity to some biological parameters, they have to be improved for use in applications. Notably, insulin release by hydrogels in response to an elevation of blood sugar is not yet fast enough.[46]

2.5. *Nanospheres*

2.5.1. *Structure and Properties*

Nanospheres are solid colloidal particles with diameters between 100 and 200 nm, and they are formed by a polymeric

matrix. Several types of polymers can be used for the preparation of these nanospheres: natural polymers (biopolymers) and degradable or non-degradable synthetic polymers. Nanospheres can be elaborated using two different methods,[59] depending on the polymer of which they are composed.

If polymerization of monomers is necessary, for instance in the case of poly(methylmethacrylate) or poly(ethylcyanoacrylate), the preparation generally requires a preliminary emulsification or dispersion step before polymerization.

If the polymer has been preformed, nanoprecipitation is employed. This method consists of dissolving the polymer in a water-miscible organic solvent, such as acetone, ethanol, or DMSO, followed by dropwise addition of this solution to an aqueous solution, which may contain surfactants. The organic solvent diffuses into the whole aqueous solution, which leads to polymer precipitation and then to the formation of nanospheres. This technique, which allows nanospheres to be obtained easily without emulsion preparation, is one of the most widely used among the existing techniques for forming objects from a preformed polymer. Other methods include emulsion–evaporation, the salt effect, and emulsion–diffusion.

The nanosphere elaboration is often followed by surface functionalization, depending on the desired application.

2.5.2. Application in Vectorization

The main interest of nanospheres for vectorization lies in the solid nature of the polymeric matrix, which gives these objects great stability.[60] In this kind of solid polymeric system, active principles can be dispersed into the core of the nanospheres or adsorbed on their surface. Nanosphere drug delivery generally operates by diffusion of the drug through the matrix or by direct matrix degradation. Nanospheres are thus continuous delivery systems with kinetics that are dependent on the type of polymer used, the fabrication process (and therefore the porosity of the polymeric matrix), and the nature of the drug.

The use of solid nanospheres is however limited by their massive capture by macrophages in vivo. Recently, second-generation particles that are invisible to macrophages have been developed to overcome this problem and to increase their blood circulation time.[61] It has been demonstrated that lowering the particle size (100 nm) and/or increasing the surface hydrophilicity (the surfaces are generally rather hydrophobic) can prevent their capture by macrophages. These second-generation nanospheres have been particularly studied for the vectorisation of anticancer drugs.[62–64] For instance, taxol incorporated into 50–60 nm diameter poly(vinylpyrrolidone) nanospheres has greater efficiency (enhancement of blood circulation time) and lower toxicity than its free form.[65] The same conclusions can be drawn about the efficiency of a dextran (hydrophilic polymer)–doxorubicin (hydrophilic drug) complex incorporated into chitosan nanospheres of 100 nm diameter.[66]

Specific ligands can be subsequently associated with these hydrophilic surface nanospheres to target a particular site of action. In this manner, in 2007, Jain et al. developed dextran nanospheres that have surfaces functionalized with vitamin B12. These vectors are able to deliver insulin incorporated into the nanospheres by oral administration to the systemic circulation using vitamin B_{12}, limiting the degradation induced by the hostile gastrointestinal environment. The activity is prolonged even if lower insulin doses are administered.[67]

Polymer nanospheres therefore have great potential for application in drug vectorization. The principal limitation of these vectors remains their preparation, which can be unwieldy on the industrial scale. Moreover, the formation of these systems requires the use of solvents and monomers that can sometimes prove toxic and hard to eliminate.

2.6. Solid Lipid Nanoparticles

2.6.1. Structure and Properties

Solid lipid nanoparticles (SLN) are usually glycerides with a diameter between 50 and 1000 nm. These nanoparticles can be obtained by different methods:

- High pressure homogenization:[68] The lipids are heated at a temperature approximately 5 to 10 °C above their melting point, and are then dispersed by stirring in an aqueous solution of surfactants at the same temperature. The pre-emulsion obtained is homogenized at high pressure, then cooled down, and the lipids crystallize to form solid nanoparticles. An adaptation of this process allows homogenization at lower temperatures to obtain the particles.
- Microemulsion:[69] A lipid microemulsion is prepared by dispersion above the melting point in an aqueous surfactant continuous phase. The thermodynamically stable, transparent system is then mixed mechanically with a cold (2–3 °C) aqueous solution, and the precipitation of the dispersed lipid phase leads to the formation of solid nanoparticles.
- Nanoprecipitation: this method is identical to that used to form polymer nanospheres, but requires the use of an organic solvent.

The degree of crystallinity of the lipid can be modified according to the SLN preparation method used. Crystalline polymorphism, being directly correlated with the density and the colloidal stability of these systems, is an important parameter to be taken into consideration for the use of SLN.

2.6.2. Application in Vectorization

Since 1990, several research groups have focused their interest on the use of SLN as an interesting alternative to polymer nanospheres for vectorization.[70,71] Generally, lipids such as triglycerides are well tolerated by the organism. Moreover, the production of these nanoparticles is much simpler than that of the nanospheres and can be transposed to the industrial scale at lower cost.

The active substance required for the desired application is dissolved or dispersed into the melted lipid phase, and then one of the methods for SLN preparation is applied to obtain

the drug-containing nanocarriers. Following fast cooling of the glycerides, an α crystalline structure is obtained that is unstable and not well ordered.[72] Active molecules then preferentially gather in the amorphous areas of the matrix. However, the α crystalline structure adopted by the lipids alters during standing to a β crystalline structure, which is more stable and better ordered.[73] During this rearrangement, the increase in the ordering of the lipid phase leads to an expulsion of the active substances into the amorphous regions.[74] Control of the lipid matrix transformation from the α form to the β form (for example, by temperature control) should therefore allow an on-command release of the drug.[68] However, to date, these SLN with controlled crystalline transformation have not been fully mastered.

As the drug loading capacity of the particles relies essentially on the structure and the polymorphism of the lipid forming the nanoparticles, some new types of lipid particles exhibiting amorphous zones have been developed.[75–77] These lipid particles, which are partially crystalline, can be composed of a mixture of glycerides with different fatty acids possessing various chain length and degree of unsaturation, leading to an imperfect material, and therefore offering a better drug-loading rate. A second type of lipid particle, called multiple lipid particles, is obtained by mixing liquid lipids with solid lipids when preparing the nanoparticles. The active substances become localized in the oily compartments contained in the solid lipid particles. Finally, an amorphous system can be obtained with a particular mixture of lipids. The incorporation of active molecules into this kind of solid nanoparticles is one of the most efficient.

The use of these solid nanoparticles in drug vectorization is now under development, as both in vitro and in vivo studies have proved that these carriers are well tolerated. However, the polymorphism of these lipid matrixes and possible crystal rearrangements has to be controlled to avoid stability problems in these structures (gelification problems[78]). Moreover, the release of the active molecules incorporated into these solid nanoparticles is not always well controlled, which limits their applications in vectorization.

3. Vesicles[79]

Vesicles are colloidal systems with a size of less than a micrometer. They can be formed from polymers, surfactants, or lipids. In these systems, the active principle is trapped in an oil or water cavity surrounded by a membrane. Vesicles therefore enable the encapsulation of larger amounts of drugs than matrix systems, which means that much smaller amounts of vectors can be administered. Most of these systems have versatile transport properties, and can vectorize hydrophilic and lipophilic substances.

3.1. Nanocapsules and Polymersomes

3.1.1. Structure and Properties

Nanocapsules[80] and polymersomes[81,82] are tank-like systems consisting of a liquid central core enclosed in a thin polymer wall not more than a few nanometres thick.[59] The main differences between these two carriers are the structure of the polymer membrane and the hydrophilicity of their internal cavity (Figure 6).

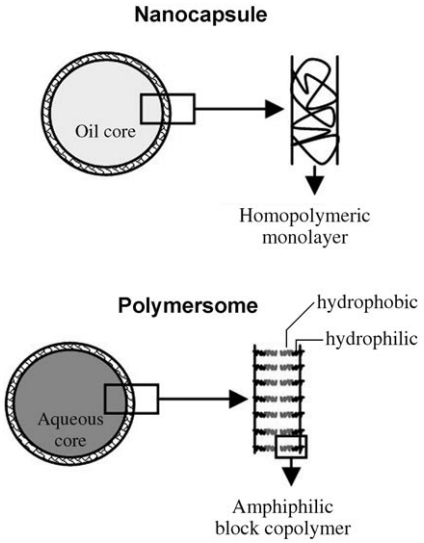


Figure 6. Structures of nanocapsules and polymersomes.

The nanocapsule membrane is a thin monolayer formed by a homopolymer or a block copolymer, with its hydrophilic moiety turned to the outside of the membrane. The core of these nanocapsules is oily in most cases, although but nanocapsules with a water cavity have also been formed.[62]

In a similar fashion to nanospheres, nanocapsules can be obtained from preformed polymers or by polymerizing monomers. The interfacial deposition technique can be applied in both cases. The procedure consists of mixing an oil that contains a water-miscible organic solvent in which the polymer or the monomers are dissolved with a surfactant aqueous solution. After dispersion of the oily phase in water, the polymer or the monomer aggregates around the oil droplets, whereas the organic solvent diffuses into the water, leading to the formation of nanocapsules (after the polymerization step in the case of monomers).[83]

The polymersome membrane is formed from a block copolymer that is organized in a bilayer, in a similar fashion to those of the liposomes. These polymersomes have an aqueous internal cavity.

The technique used to obtain polymersomes is based on the rehydration film method. The copolymer is dissolved in a volatile organic solvent, which is evaporated to obtain a polymer film. This film is then rehydrated with an aqueous solution and redispersed by stirring, sonication, or extrusion to obtain polymersomes.

3.1.2. Application in Vectorization

In vectorization, nanocapsules are generally used to retain lipophilic drugs in their oily internal cavity, and polymersomes to encapsulate hydrophilic drugs in their aqueous core.

The encapsulation of active substances is achieved during the elaboration process of these vectors: the hydrophobic drug is solubilized in the oil before the formation of nanocapsule by interfacial deposition, whereas a so-called passive encapsulation, in which the polymer film is rehydrated in the presence of the hydrophilic drug, is used for encapsulation by polymersomes.

With these two kinds of vesicles based on polymers, an active substance can be protected and its toxicity lowered. The substance is delivered by diffusion or by degradation of the polymer membrane in a biological medium.

Halofantrine nanoencapsulation was recently described by Mosqueira et al.[84] Halofantrine is a very active drug for the treatment of malaria, and is used when usual drugs, such as chloroquinine and quinine, are no longer effective. However, halofantrine is hydrophobic and thus has low bioavailability; above all, is very toxic (it can lead to cardiac arrest in the patient). Encapsulation of this drug in poly-ε-caprolactone nanocapsules has enabled cardiac risks to be considerably reduced, and the lethal dose is increased from 200 $mg\,kg^{-1}$ to 300 $mg\,kg^{-1}$. The results prove that delivery is modified by poly-ε-caprolactone nanoencapsulation, thanks notably to the low in vivo degradation of the polymer compared with polymers usually employed to form nanocapsules, such as PLA-PEG (poly(lactic acid)–poly(ethylene glycol).

As for nanospheres, the nanocapsule surface can be functionalized by ligands such as PEG, allowing a longer circulation time in biological media or by specific ligands to target a particular site of action.

In contrast with oily core nanocapsules, polymersomes exhibit versatile transport properties, as hydrophobic drugs can be enclosed in the membrane of the carrier, whereas hydrophilic drugs are encapsulated in their aqueous cavity. This system has been used for delivery of anticancer drugs, such as paclitaxel (hydrophobic) and doxorubicin (hydrophilic).[85] Doxorubicin was encapsulated in the internal cavity of the polymersome, whereas paclitaxel was incorporated into the polymer bilayer during polymer film formation to maximize the anticancer drug efficiency with a cocktail of active substances. Polymersomes were obtained by mixing two block copolymers, namely biodegradable PLA-PEG and inert poly(ethylene glycol)–poly(butadiene) (PEG-PBD). Hydrolysis of PLA-PEG then forms pores in the membrane, which allows the delivery of both drugs to be controlled. Twice as much apoptosis was induced in the tumors by the polymersome–drug cocktail after two days than by the two drugs taken separately.

Despite their efficiency, the major drawback of polymersomes is their instability, leading to leakage of the encapsulated drugs. Moreover, passive encapsulation used in the case of polymersomes requires a high amount of active substances, as the encapsulated concentration is identical to the concentration of the aqueous solution used to rehydrate the polymer film.

Methods for the preparation of nanocapsules and polymersomes are restricted at the industrial scale, and the side products resulting from their synthesis are toxic and difficult to eliminate completely.

3.2. Liposomes[86]

3.2.1. Structure and Properties

Liposomes are vesicles formed by the auto-association of one or several phospholipid bilayers that enclose an aqueous compartment. They have attracted the attention of a number of research groups in various fields, such as physical chemistry, biophysics, and pharmaceutics because of their structure, which is comparable to the phospholipid membranes of living cells.

Liposome structure depends on their composition and also on the preparation method. In most cases, the different steps to obtain liposomes are as follows:[87]

- dissolution of the lipids in an organic solvent
- solvent evaporation
- dispersion of the dried lipids in an aqueous solution

The main differences arise from the method used to disperse the dried lipids. The dispersion can be induced by hydration of the phospholipid film, sonication, microfluidification, extrusion, reverse-phase evaporation, ether infusion, injection of an ethanol solution, freeze-drying/rehydration, freezing/thawing, surfactant removal, or electroformation.

The liposome characteristics depend on the preparation technique. In particular, the mean size of these supramolecular objects can vary from tens to a hundred micrometers. They can also be made of one or several lipid bilayer(s). The way they are named[3] takes these two parameters into account (Figure 7).

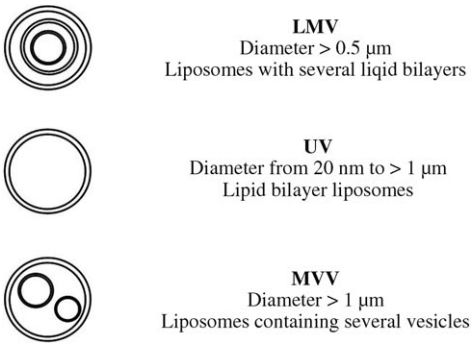


Figure 7. Nomenclature of structure-based liposomes.

Unilamellar vesicles are divided into three subclasses:

- SUV (small unilamellar vesicles), with a diameter between 20 and 100 nm
- LUV (large unilamellar vesicles), with diameters above 100 nm
- GUV (giant unilamellar vesicles), with diameters above 1 μm

GUVs are frequently used, as their structure and size are very close to those of cells.

3.2.2. Application in Vectorization

Because of the innocuousness of liposome phospholipidic components, this kind of reservoir system rapidly became the ideal candidate for drug vectorization in biological media. Like polymersomes, liposomes are able to transport both hydrophobic substances anchored into the bilayer and hydrophilic substances encapsulated in their cavity.

Numerous drug encapsulation techniques that are dependent on the properties of the molecule that has to be internalized in the aqueous compartment and on the type of formulation of the liposome have been described in the literature. The simplest method is passive encapsulation, which is identical to that used for polymersomes, in which the lipid film is rehydrated in the presence of the active substance to be encapsulated.[88] Active encapsulation methods[89,90] are applied after liposome formation using a concentration or pH gradient. Hydrophobic drugs are mixed with phospholipids before the formation of the lipid film.

Encapsulated drug delivery mechanisms can be set up according to the liposome membrane composition, which can be made sensitive to various environmental parameters. Therefore, pH-sensitive liposomes have been developed that are stable at a pH value of 7.4 (physiological pH). They destabilize under acidic conditions (for example, into tumors or endosomial cells), leading to the release of their contents.[91] These liposomes are made up of either charged phospholipids or neutral phospholipids that can be hydrolyzed within a certain pH range.

Phospholipid liposomes containing disulfur bridges are sensitive to the redox potential of the medium. The reduction of these bonds leads to liposome destruction, thus delivering the encapsulated drugs.[92]

Temperature-sensitive liposomes have also been elaborated using lipids such as 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine, which has a phase-transition temperature between 41 and 43 °C. These liposomes could be used in association with hyperthermia treatments, for example in the delivery of drugs into solid tumors.[93]

Ligands can be anchored onto the liposome surface to deliver encapsulated drugs for specific action sites.[94] These ligands can be antibodies which bind to specific cell receptors, or less-specific ligands, such as folate or selectin.

Virosomes are an example of liposomes that are surface-functionalized (virus mimes) towards specific targets. They are generally composed of phosphatidylcholine and of some virus-derived proteins, such as hemagglutinin (HA) together with neuraminidase in its biologically active conformation. The presence of HA allows recognition between the virosome and the immune cells. Virosomes have been widely used for vaccination by the delivery of antigens.[95]

Attachment of PEG to liposomes[96] can also protect them from detection by monocytes and macrophages in the liver and spleen, which allows a prolonged circulation time within the bloodstream. The liposomes utilized in doxil, which is marketed as a chemotherapy drug, are formulated with surface-bound methoxypolyethylene glycol (MPEG).

Liposomes are thus versatile reservoir systems. The more they develop, the more sophisticated their compositions become, allowing very specific targeting and completely controlled drug delivery. However, these rather complex systems have to be systematically tuned according to the drug to be encapsulated and the desired application.

The physical and chemical stability of liposomes also limits their use in vectorization. Chemically, their poor stability can be attributed to lipid ester bond hydrolysis and, physically, the aggregation or the fusion of several liposomes can lead to the formation of large-sized objects that are therefore no longer usable in vectorization. Moreover, these objects may be subject to leakage, releasing the encapsulated drugs before they reach their site of action.

It should be mentioned that their preparation procedure also requires the use of an organic solvent, which can leave toxic residual traces.

3.3. Niosomes

3.3.1. Structure and Properties

Niosomes[80] are made of nonionic surfactants that are organized into spherical bilayers enclosing an aqueous compartment, and have an identical structure to liposomes and polymersomes. Several preparation methods for niosomes have been described in the literature.[97]

In a similar fashion to liposome formation, the surfactant rehydration film technique can also be used for niosomes. The formation of an O/W emulsion, using an organic solvent in which the surfactants are dissolved on one side and an aqueous solution on the other, followed by evaporation of the organic solvent, also leads to the formation of niosomes. Other techniques that do not require the use of organic solvents, which are difficult to completely eliminate and sometimes toxic, have been developed; for example, melted surfactants can be injected into a vigorously stirred warm aqueous solution.

The niosomes obtained by these different techniques have sizes in the micrometer range. Some procedures have been set up to reduce their diameter to approximately 300 nm. In general, niosome size reduction is induced by sonication, microfluidization, extrusion, by a combination of sonication and filtration, or by high pressure homogenization.

In most cases, niosome formation requires the addition of molecules such as cholesterol to stabilize the bilayer and molecules that prevent the formation of niosome aggregates by steric or electrostatic repulsion.

3.3.2. Application in Vectorization

In an analogous fashion to liposomes, niosomes are able to vectorize hydrophobic drugs enclosed in their bilayer and hydrophilic substances encapsulated in their aqueous cavity. Unlike phospholipidic liposomes, niosomes, which are made of surfactants, are not sensitive to hydrolysis or oxidation. This is an advantage for their use in biological media. Moreover, surfactants are cheaper and easier to store than phospholipids. A further advantage of niosomes relative to liposomes lies in their formulation, as these vectors can be elaborated from a wide variety of surfactants, the hydrophilic

heads of which can be chosen according to the application and the desired site of action.[97] Notably, surfactant niosomes have been obtained with glycerol,[98] ethylene oxide,[99] crown ethers,[100] and polyhydroxylated[101] or sugar-based[102] polar headgroups.

Hydrophilic drug encapsulation is achieved during the elaboration step of the objects by the passive method, in which the active principle is dissolved in the aqueous phase. Hydrophobic drugs are simply mixed with surfactants prior to niosome formation.

The encapsulation of active substances in niosomes can reduce their toxicity, increase their absorption through cell membranes, and allow them to target organs or specific tissues. Recently, antibody surface-functionalized niosomes were developed[103] in a similar way to virosomes. Tests performed on model cells have demonstrated very specific targeting using these new "immunocarriers".

Niosomes have been developed to reach the same specific drug delivery objectives as liposomes, thus overcoming the drawbacks of phospholipid use. However, niosome membranes are permeable to low-molecular-weight molecules, and a leakage of drugs encapsulated in the aqueous cavity of niosomes over time has been observed. Moreover, few niosome toxicity studies have been performed to date, despite the fact that the toxicological profile is an essential element for the development of vectors for pharmaceutical applications.

3.4. Catanionic Vesicles

3.4.1. Structure and Properties

Catanionic amphiphiles are generally bicatenar systems that result from mixing oppositely charged surfactants in water (Figure 8).

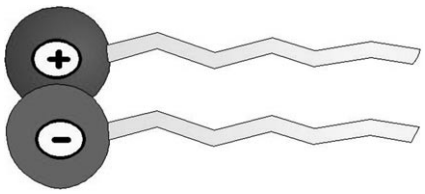


Figure 8. A bicatenary catanionic amphiphile.

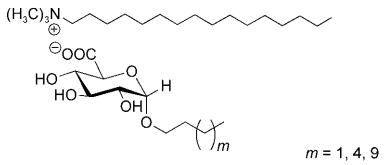
When discussing catanionic amphiphile preparation methods, it is necessary to distinguish between catanionic mixtures and catanionic surfactants (pure ion pairs).[104] In the latter category, the inorganic counterions associated with the amphiphiles are eliminated, whereas in catanionic mixtures, counterions remain in solution. For use in vectorization, the presence of residual salts is to be avoided, as some salts are toxic.

Four catanionic surfactant preparation methods (without residual salts) have been described in the literature:[105–107]

- The extraction method[105] consists of an equimolar mixing of oppositely charged surfactants that are dissolved in water beforehand. Addition of a suitable organic solvent allows the extraction of the catanionic surfactant that is obtained, and the salts remaining in the aqueous phase.
- The precipitation method can be processed in two ways:[105] The first is precipitation of the potassium, sodium, or lithium salt of the anionic surfactant with silver ions in the aqueous phase. After purification of the precipitate, it is dissolved in a mixture of water and organic solvent, and one equivalent of the cationic surfactant with a chloride or bromide counterion is then added to the solution. The pure catanionic surfactant is obtained after filtration of the precipitated silver halide salt. The second method is based on the preparation of a supersaturated aqueous solution of equimolar quantities of two oppositely charged surfactants. The ion pair precipitates and is removed by filtation, and residual salts remain in solution.
- The often-employed ion-exchange method[105] consists of converting the cationic surfactant into its hydroxide form and the anionic surfactant into its protonated form by elution on a suitable ion-exchange resin. The two surfactant solutions are then mixed to produce the pure catanionic surfactant by an acid–base reaction. For example, Zemb et al. obtained catanionic surfactants by mixing myristic acid with cetyltrimethylammonium hydroxide (CTAOH) after exchange of the chloride counterion of cetyltrimethylammonium chloride with hydroxide.[106–108]
- A proton exchange method developed in our laboratory[109,110] consists of mixing an equimolar quantity of an aminosugar-derived surfactant with an amphiphile bearing an acidic function in water. By a simple acid–base reaction, equimolar catanionic amphiphiles are obtained without residual salts.

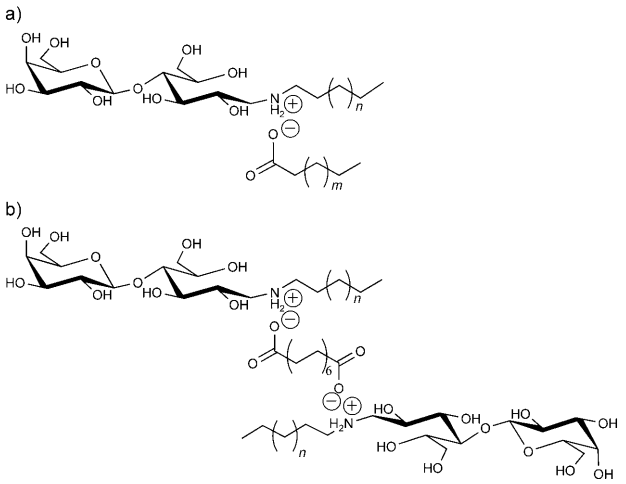
Catanionic surfactants can spontaneously form vesicles in water.[111,112] However, it should be noted that the catanionic surfactants usually precipitate when the two oppositely charged amphiphiles are mixed in equimolar quantities.[111] In fact, the electrostatic interaction between charges leads to a shrinking of the polar head and therefore a decrease in the hydrophilicity of the system. The weakened solvation sphere makes the solubilization more difficult. Thus catanionic vesicles are generally formed with an excess of either positive or negative charge.

In 1997, Menger et al.[113] obtained the first example of a water-soluble catanionic surfactant based on a glycosidic amphiphile (Scheme 2). In our laboratory, several sugar-



Scheme 2. Structure of the first sugar-derived catanionic surfactant.

derived catanionic surfactant families have also been made[109,110,114–116] that have hydrophilicity high enough to make them water soluble (Scheme 3).



Scheme 3. a) Bicatenary and b) gemini catanionic surfactant structures.

Several research groups have evaluated the encapsulation ability of hydrophilic probes of vesicles obtained by the equimolar mixing of oppositely charged surfactants free from residual salts. However, in these studies, some energy supply by means of sonication was necessary to solubilize the catanionic surfactants at equimolarity.

Fukuda et al. prepared a catanionic surfactant by mixing trimethyl-*n*-hexadecylammonium hydroxide with one equivalent of palmitic acid. Sonication of a dispersion of this surfactant in water led to the formation of vesicles that were able to encapsulate 0.9% riboflavin.[117]

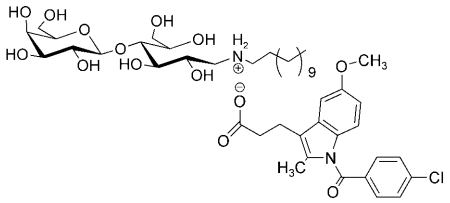
Bhattacharya et al. studied the riboflavin encapsulation ability of catanionic vesicles formed with different mixtures of bolaamphiphilic surfactants bearing two acidic groups and two equivalents of CTAOH. Using these catanionic surfactants, vesicles able to encapsulate up to 2.28% riboflavin were formed by sonication in water.[118]

Tondre et al. studied the encapsulation efficiency of catanionic vesicles formed by an equimolar mixture of dodecylbenzenesulphonic acid and CTAOH. The vesicles formed with this catanionic surfactant were able to encapsulate about 2.5% glucose.[119] However, a glucose retention stability study in the aqueous core of the vesicles showed that after 24 h, only 8% of the initially encapsulated glucose was still present in the internal cavity of the catanionic vesicles, which indicates the high permeability of this system.

To date, few studies have been performed on the encapsulation efficiency of hydrophilic molecules by catanionic vesicles formed at equimolarity and without residual salts. Nevertheless, these preliminary studies show that the association of catanionic surfactants in a solution containing probe molecules leads to the formation of vesicles enclosing a certain ratio of the probe in their aqueous core.

3.4.2. ***Application in Vectorization***

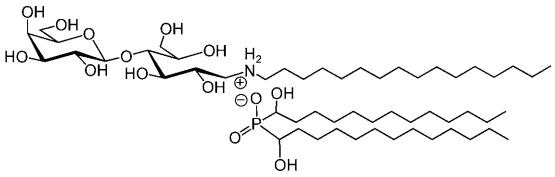
Catanionic vesicles have not yet been widely used in vectorization.[112] A new concept has been developed that involves the direct association of a potentially ionizable drug with a surfactant that gives rise to a catanionic active entity.[120,121] To the best of our knowledge, only one study, which was carried out in our laboratory, is in the process of industrial development. This study involves the cutaneous delivery of an anti-inflammatory drug by direct association with a sugar-derived amphiphile forming a catanionic surfactant (Scheme 4).



Scheme 4. Structure of a catanionic surfactant from the association of an anti-inflammatory drug and a sugar-derived surfactant.

The catanionic assembly, which spontaneously forms vesicles, ensures an increased anti-inflammatory activity of the active principle together with a controlled and prolonged release through the skin. It also protects the drug from harmful irradiation effects.[122,123]

It should also be noted that studies, carried out by Lindman et al., are underway in the field of transfection using catanionic surfactants.[124] For that purpose, a sugar-derived tricatenar (three-chain) catanionic surfactant (Scheme 5) has



Scheme 5. Tricatenar 1-*N*-hexadecylammonium 1-deoxylactitol bis-(α-hydroxydodecyl)phosphinate.

been designed and synthesized to obtain stable vesicles that could be exploited for drug vectorization. The aggregation properties and encapsulation efficiency of this catanionic surfactant have been studied in pure water and in phosphate buffer.[125,126] The vesicles form by self-aggregation, regardless of the medium used. Moreover, a hydrophilic probe (arbutin) entrapment of 8% has been determined that is independent of the conditions of vesicle formation (in water or in buffer solution). This encapsulation efficiency is one of the highest obtained with catanionic vesicles at surfactant equimolarity.[118,119,127] Moreover, the tricatenar catanionic surfactant is able to retain drugs in the aqueous cavity of the vesicles for at least 30 h, which is among the highest probe entrapment stabilities for catanionic systems at equimolar conditions. This tricatenar catanionic surfactant could be a promising new kind of delivery system in which the drug is easily entrapped during spontaneous surfactant self-assembly in water.

The studies dealing with drug vectorization by catanionic vesicles thus remain limited. These vectors would however bring together the advantages of several previously developed systems:

As in the case of liposomes, polymersomes, nanocapsules, and niosomes, vesicles are reservoir systems, which are able to encapsulate active substances and therefore protect them from the biological environment, reduce their toxicity, and improve their bioavailability.

As for liposomes, polymersomes, and niosomes, their transport properties are versatile; they can deliver hydrophobic drugs enclosed in the membrane of the catanionic vesicles and hydrophilic drugs encapsulated in the aqueous internal cavity. Like niosomes, these vectors can be built from a great variety of surfactants with polar headgroups chosen according to the application and the desired site of action.

A major asset from the industrial point of view is that, like micelles, the formation of vesicles in water is spontaneous. Moreover, their formation does not require the use of organic solvents or other products that could be toxic.

Because these systems are multiply charged and are likely to form microdomains of either positive or negative charge, strong electrostatic interactions can be expected, which could induce potential massive membrane fusion processes with cells, thus creating an efficient means of vectorization.

Thanks to their properties, these vectors have great potential in vectorisation, but to date they are underexploited.

4. Conclusions

Drug vectorization has undergone rapid development since 1906, when Paul Ehrlich dreamt of "magic bullets" to specifically transport a drug towards its site of action.

All vectorization systems developed to date, whether matrix systems or vesicular systems, have remarkable properties. These vectors have helped to improve the therapeutic efficiency of some active principles; for example, anticancer drugs with physicochemical characteristics that are not favorable for the crossing of biological barriers. Drug delivery to a particular site of action has also been optimized by the addition of specific ligands to the vectors. Finally, all these efforts have also allowed the toxicity of some drugs, such as halofantrin, to be considerably reduced.

However, some drawbacks limit the applications of some of these vectors. Notably, the often complex systems have to be tuned according to the drug to be encapsulated, and they are consequently difficult to transpose to the industrial scale. Moreover, the use of organic solvents or toxic reagents, which are hard to completely remove, could also generate toxicity risks.

Catanionic vesicles that spontaneously form in water combine the advantages of several previously developed vectors, and could potentially overcome some of the problems encountered when setting up an application in vectorization on the industrial scale. These reservoir systems, which have versatile transport properties, can be formed from a great variety of surfactants and can be tuned according to the site of action where the drug has to be delivered by choosing an appropriate surfactant.

A good drug is able to reach its target at the right place and at the right concentration. The considerable progress made in vectorization has allowed these parameters to be better controlled, and has therefore increased the therapeutic efficiency of drugs and minimized their toxicity. However, it is necessary to develop less complex carriers that are more easily tuneable according to the target and therefore simpler to set up on the industrial scale so as to broaden the possible applications in the domain of specific delivery.

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