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Review article

Physicochemical characterization of colloidal drug delivery systems such as reverse micelles, vesicles, liquid crystals and nanoparticles for topical administration

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Abstract

Topical administration of cosmetics and pharmaceuticals involves a variety of different formulations of which colloidal drug carrier systems are currently of particular interest. After a short introduction of reverse micellar solutions, liquid crystals, vesicles and nanoparticles, appropriate methods of physicochemical characterization are introduced including X-ray diffraction, laser light scattering, electron microscopy, and differential scanning calorimetry. Emphasis is laid on topical applications of the colloidal drug delivery systems (DDS) covered, with the main objective of both sustained drug release and improved stability of DDS.

Keywords: Colloidal drug delivery system; Reverse micelles; Liquid crystals; Vesicles; Topical; Physicochemical characterization; Review

1. Introduction

Colloidal drug carrier systems such as micellar solutions, vesicle and liquid crystal dispersions, as well as nanoparticle dispersions consist of small particles of 10–400 nm diameter. They show promise as drug delivery systems (DDS). When developing these formulations, the goal is to obtain systems with optimized drug loading and release properties, long shelf life and low toxicity. Since the properties of colloidally dispersed materials may differ significantly from those in the bulk, comprehensive structural investigations are necessary to achieve this goal [1]. The incorporated drug participates in the microstructure of the system, and may even influence it due to molecular interactions, especially if the drug possesses amphiphilic and/or mesogenic properties. These properties are met by a variety of compounds such as some nonsteroidal anti-inflammatory drugs (NSAID) [2,3], which are widely used for dermal application. The present contribution mainly deals with colloidal systems for topical administration. In this review, different colloidal carriers are introduced, followed by a discussion of the respective drug delivery systems.

1.1. Reverse micelles

Amphiphilic molecules such as surfactants associate to form micelles beyond the critical micelle concentration (CMC) of the compound in an aqueous solution. Micellar solutions not only exist in aqueous systems but also form in oily systems. In this case reverse micelles are formed with the lipophilic part of the surfactant molecule facing the oily medium and the hydrophilic part representing the inner core of the associate. A widely used excipient in drug development is lecithin, which is able to form reverse micelles in different oily media. Normal micelles of lecithin do not exist, instead liposomes are formed when phospholipids are dispersed in aqueous media.

Solubilization of drug molecules is possible both in normal micelles and in reverse micelles. While the solubilization within normal micelles improves the bioavailability of poorly soluble drugs [4], the use of reverse micelles allows sustained drug release. Upon contact of a reverse micellar solution with aqueous body fluids the reverse micellar solution transforms into a liquid crystalline

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phase or vesicle dispersion which reduces the release rate of the solubilized drug molecules [5].

1.2. Liquid crystals

The liquid crystalline state combines properties of both liquid and solid states. The liquid state is associated with the ability to flow whereas solids have an ordered, crystalline structure [6]. Liquid crystals show at least orientational long-range order and may show short-range order whereas the positional long-range order as characteristic in real crystals has disappeared [7]. Therefore liquid crystalline phases represent intermediate states and are also called mesophases. A prerequirement for the formation of liquid crystalline phases is an anisometric molecular shape, which is generally associated with a marked anisotropy of the polarizability. Molecules that can form mesophases are called mesogens. The latter are often excipients of drugs e.g. surfactants. Even drug compounds themselves, e.g. the salts of organic acids or bases with anisometric molecular shape, may fulfill the requirements for the liquid crystal formation [8].

Starting with the crystalline state, the mesophase is reached either by increasing the temperature or by adding a solvent. Accordingly thermotropic or lyotropic liquid crystals form. As with thermotropic liquid crystals, variation in temperature can also cause a phase transformation between different mesophases of lyotropic liquid crystals.

Lyotropic liquid crystals arise from mesogens that are not the molecules themselves but their hydrates or solvates as well as associates of hydrated or solvated molecules. Water or a mixture of water and organic solvent are the most important solvents for drug molecules, and the degree of hydration or solvation depends on the amphiphilic properties of a drug molecule. Hydration or solvation of a mostly rod-shaped molecule results in different geometries, i.e. cone or cylinder [9].

Cylinders arrange in layers. This results in a lamellar phase with alternating polar and nonpolar layers. Water and aqueous drug solutions can be included in the polar layers, resulting in an increase in layer thickness. Analogously, molecules with appropriate affinity can be included in the nonpolar layers. In addition to the increased layer thickness of the lamellar phase, lateral inclusion between molecules is also possible with an increase in the solvent concentration, which transforms the rod shape of the solvated molecules to a cone shape. This leads to a phase change. Depending on the polarity of the solvating agent and the molecule itself, the transition results in a hexagonal or inverse hexagonal phase.

The hexagonal phase is named after the hexagonally packed rod micelles of solvated molecules, whereby their polar functional groups either point to the outside or inside of the structure. In the hexagonal phase, the additional amount of water or nonpolar solvent that can be included is limited. As the molecular geometry changes further during solvation, a cubic (type I) or inverse cubic form (type IV) develops, consisting of spherical or ellipsoidal micelles and/or inverse micelles.

In addition to the cubic and/or inverse cubic forms described above, further transitional forms exist between the lamellar phase and hexagonal (cubic, type II) or inverse hexagonal mesophases (cubic, type III). In contrast to the discontinuous type I and IV phases, type II and III cubic mesophases form bicontinuous phases. A range of lyotropic mesophases is possible depending on the mesogen concentration, the lipophilic or hydrophilic characteristics of the solvent and the molecule itself [10]. However, not all theoretically possible mesophases may occur in practice.

1.3. Vesicles

With some molecules, a high concentration results in a lamellar phase but no additional mesophases are formed if the concentration is reduced. The lamellar phase is dispersed as concentric layered particles in an excess of solvent (water or aqueous solution). This results in a vesicular dispersion. If the mesogenic material consists of phospholipids, the vesicular dispersion is called a liposomal dispersion [11]. In principle, the liposomes may be dispersed in oily continuous media, too. However, the latter systems are of minor interest in drug formulation.

Liposomes consist either of many, a few or just one phospholipid bilayer. Therefore multilamellar vesicles (MLV), oligolamellar vesicles (OLV), small unilamellar (SUV) and large unilamellar vesicles (LUV) have to be distinguished. Furthermore multivesicular liposomes (MVL) may be formed. The polar character of the liposomal core enables polar drug molecules to be encapsulated. Amphiphilic and lipophilic molecules are solubilized within the phospholipid bilayer according to their affinity towards the phospholipids. Participation of nonionic surfactants instead of phospholipids in the bilayer formation results in niosomes. The term sphingosome is suggested for vesicles from sphingolipids. However, nomenclature is not consistent, i.e. the term liposome is used as a general term, although vesicles would be the better choice.

The standard manufacturing procedure of liposomes is the film-forming method. Alternatively, solvent injection and reverse phase dialysis are appropriate procedures for the formation of SUV and LUV. Freeze thaw procedures enable drug loading of the liposomes and also offer an evaluation of the stability of the vesicular dispersion. Further information is available elsewhere [11,12].

1.4. Nanoparticles

Nanoparticles are in the solid state, and either amorphous or crystalline. They are able to adsorb and/or encapsulate a drug, thus protecting it against chemical and enzymatic degradation. Furthermore the encapsulated drug may be

prevented from crystallization, thus forming a solid solution. However, it has been shown that the drug, as a foreign material, is only incorporated to a limited extent in the different crystal lattice of the nanoparticle carrier material, due to a limited solubility of the respective drug in the stable crystal modification. The loading capacity of solid lipid nanoparticles is lower than that of an equally concentrated nanoemulsion [13,14]. Depending on drug solubility in the carrier, a drug load varying from only a few percent up to 50% as in the case of ubidecarenone has been reported [14]. In this context, drug nanodispersions and nanoparticles of supercooled drug melts consisting of pure drug in either a crystalline or amorphous state are gaining increasing interest [15].

Nanoparticles as drug carriers can be formed from different materials. In addition to solid lipids, both biodegradable polymers and nonbiodegradable polymers have been used as carrier materials [16–19]. Polymer nanoparticles are most commonly prepared by precipitation via solvent displacement, salting-out, and pH variation, and also polymerization from microemulsions and mixed micelles [20,21]. Preparation techniques for lipid nanoparticles involve high-pressure homogenization, precipitation from microemulsions and solvent evaporation [19]. Recently, it has been reported that lipid nanoparticles were successfully prepared by solvent injection, which is also appropriate for the preparation of vesicle dispersions [22].

2. Methods for the physicochemical characterization of colloidal DDS

Methods appropriate for investigation and characterization of colloids are frequently used in drug development and thus may be found in pharmaceutical laboratories. Both macroscopic and microscopic techniques are used.

2.1. Particle size determination by laser light scattering

The particle size is an important parameter in in-process control and particularly in quality assurance, because the physical stability of vesicle dispersions depends on particle size and particle size distribution. An appropriate and particularly quick method is laser light scattering or diffraction. Laser light diffraction can be applied for particles >1 μm and refers to the proportionality between the intensity of diffraction and the square of the particle diameter according to the diffraction theory of Fraunhofer.

For particles below 200 nm Rayleigh's theory holds, which considers the scattering intensity to be proportional to the sixth potency of the particle diameter. Both, Fraunhofer's and Rayleigh's theories, are only approximations of Mie's theory which claims that the scattering intensity depends on the scattering angle, the absorption and the size of the particles as well as the refractive indices of both the particles and the dispersion medium.

Unfortunately, the latter parameters are difficult to determine. Furthermore, most colloidal dispersions consist of particles from below 200 nm up to 1 μm. Therefore photon correlation spectroscopy (PCS) based on laser light scattering provides an appropriate method of investigation [23].

Dynamic processes in the dispersion such as Brownian molecular motion, cause variations in the intensities of the scattered light and hence PCS results with time. These fluctuations increase as particle size decreases due to greater Brownian motion. Thus a correlation between the different intensities measured is only possible for short time intervals. Consequently, in the case of a monodisperse system following first order kinetics, the autocorrelation function decreases rather fast. In a half-logarithmic plot of the autocorrelation function the slope of the graph enables the hydrodynamic radius to be calculated according to the Stokes–Einstein equation. With commercial PCS devices the z-average is determined, which corresponds to the hydrodynamic radius.

In the case of a polydisperse system, calculation of the particle size distribution using special transformation algorithms is also possible. For this purpose certain requirements need to be fulfilled, e.g. a spherical particle shape, sufficient dilution and a large difference between the refractive indices of the inner and the outer phase. Since usually not all requirements can be fulfilled, the z-average as a directly accessible parameter is preferred to the distribution function.

2.2. Microscopy

Polarized light microscopy (PLM) is suitable for detection of lyotropic liquid crystals (except cubic mesophases) because liquid crystals show birefringence just like real crystals. Each liquid crystal shows typical black and white textures. In the case of an additional λ-plate with strong birefringent properties, colour effects of the textures can also be observed [24]. Hexagonal mesophases can be recognized by their typical fan shape texture. Lamellar mesophases typically show oily streaks with inserted maltese crosses. The latter result from defect structures, called confocal domains that arise from concentric rearrangement of plane layers. In some lamellar mesophases these defects prevail. Hence no oily streaks occur but maltese crosses are the dominant texture. Further information is available elsewhere [24]. A drawback of PLM is that it is restricted to particle dimensions in the micron or submicron range whereas colloidal dimensions of liquid crystals are only resolved by transmission electron microscopy (TEM).

2.3. Transmission electron microscopy (TEM)

The microstructure of colloidal systems can be visualized with the high-magnification power of the electron microscope. However, aqueous samples do not survive the high vacuum of an electron microscope and water loss occurs leading to microstructure changes. Therefore, special techniques of sample preparation are necessary prior to electron microscopy. Freeze fracture has proven to be successful at overcoming these problems [8].

Fig. 1a–c represents transmission electron micrographs of different lyotropic liquid crystals after freeze fracture without etching. One can clearly see the layered structure of the lamellar mesophase including confocal domains,

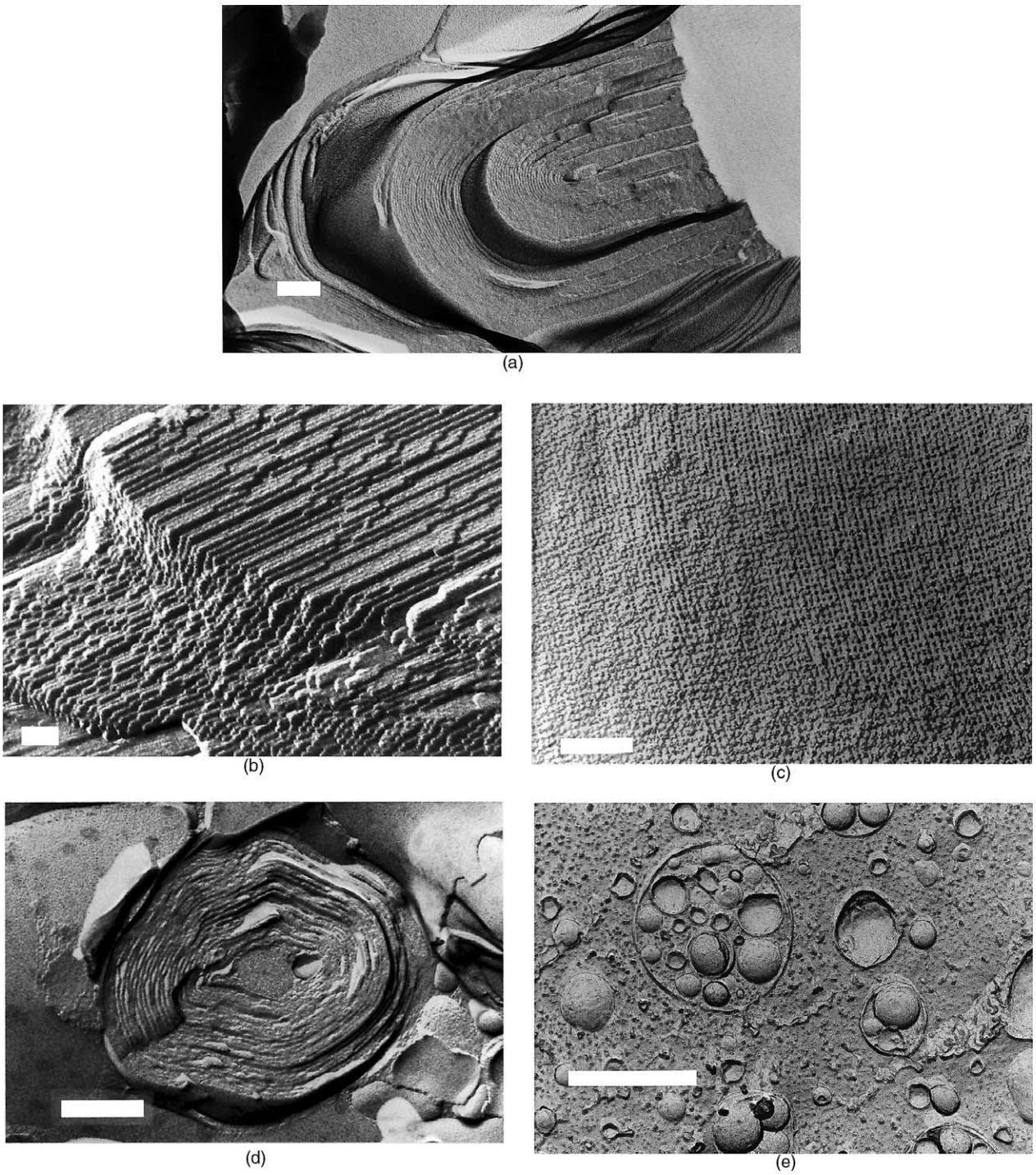


Fig. 1. Transmission electron micrographs of freeze fractured liquid crystals: (a) lamellar with confocal defects, bar 100 nm, (b) hexagonal, bar 100 nm, (c) cubic type I, bar 100 nm, (d) multilamellar vesicle consisting of dodecyl-PEG-23-ether, cholesterol and water, bar 200 nm and (e) multivesicular vesicle, bar 1 μm (a, b from Ref. [25], c from Ref. [26] and d, e from Ref. [8]).

the hexagonal arrangement of rod-like micelles within the hexagonal mesophase as well as close packed spherical micelles within the cubic liquid crystal.

Fig. 1d and e show aqueous dispersions of vesicles. The smaller vesicles are less likely to cross-fracture, and therefore the question as to whether the vesicle is uni- or multilamellar is difficult to answer. For fluid vesicle dispersions it is possible to solve the problem using cryo-TEM.

Sufficient contrast can be given to a thin film of the frozen sample by use of osmium tetroxide. This allows the sample to be viewed directly in the TEM (at a temperature of $-196\,^{\circ}C$). The adjustment of the temperature to $-196\,^{\circ}C$ leads to a very low vapor pressure, especially of water, so that the examination of the sample is possible by preservation of the microstructure despite the high vacuum. A disadvantage of cryo-TEM is the difficulty in establishing size classification of vesicles. Due to the fluid property of the vesicle dispersion prior to freezing, the sample film thickness increases from the center to the outside. Hence the smaller vesicles stay in the center, where the film is thin, while the larger ones linger at the outside margin in the thicker part of the film. In this outer part the vesicles are out of the field of view. Therefore the resulting distribution does not represent the true size distribution.

2.4. X-ray scattering

With X-ray scattering experiments characteristic interferences are generated from an ordered microstructure [27]. A typical interference pattern arises due to specific repeat distances of the associated interlayer spacings d. According to Bragg's equation (Eq. (1)) d can be calculated:

$$d = n\lambda/2 \sin \gamma \quad (1)$$

where λ is the wavelength of the X-ray being used, n is an integer and nominates the order of the interference, and γ is the angle under which the interference occurs (reflection conditions are fulfilled).

From Bragg's equation it can be seen that the interlayer spacing d is inversely proportional to the angle of reflection γ. Large terms for d in the region of long-range order can be measured by small-angle X-ray diffraction (SAXD), while small terms for d in the region of short-range order can be investigated by wide-angle X-ray diffraction (WAXD). SAXD is the most appropriate technique for the exact determination of the distances of interlayer spacings of liquid crystalline systems. The short-range order of crystalline nanosuspensions can be detected by WAXD.

Interferences can be detected by using either film detection, or scintillation counters or position-sensitive detectors.

However, in addition to interference detection, and subsequent calculation of interlayer spacings of the crystalline material, the SAXD method enables the sequence of the interferences and thus the type of ordering to be detected [28,29].

A further option involving X-ray scattering is the diffuse SAXS technique, which is especially useful for colloidal dispersions because information about size and shape of the particles can be obtained at the same time [30].

2.5. Differential scanning calorimetry (DSC)

Phase transitions are accompanied by free energy changes, and are due to either an alteration in the enthalpy (ΔH) or entropy (ΔS) of the system. Enthalpy changes result in either endothermic or exothermic signals, depending on whether the transition is due to consumption of energy, e.g. melting of a solid, or a release of energy, e.g. recrystallization of an isotropic melt.

It should be mentioned that the transition from the crystalline to amorphous phase requires a high energy input. This is in contrast to crystalline to liquid crystalline and liquid crystalline to amorphous transitions as well as changes between different liquid crystalline phases, which all consume low amounts of energy. Therefore care has to be taken to ensure that the measuring device is sensitive enough to give a sufficiently low detection limit [31].

Entropically caused phase transitions may be recognized by a change in baseline slope due to a change in the specific heat capacity. Liquid crystalline polymer phase transitions are entropically related and are thus considered second order transitions such as those from glass to rubber. These are usually called glass transitions. They may be accompanied by an enthalpic effect, therefore, complicating their detection.

2.6. Rheology

Different types of colloidal carriers exhibit different rheological properties [32,33]. With increased organization of the liquid crystal microstructure, viscosity increases. The coefficient of dynamic viscosity η describes the viscosity of ideal flow behavior (Newtonian systems), and is rather high for cubic and hexagonal liquid crystals but fairly low for lamellar systems. However, it should be kept in mind that these systems exhibit flow characteristics that are not Newtonian but plastic and pseudoplastic, respectively.

The high viscosity of lyotropic liquid crystals such as cubic and hexagonal mesophases is due to their three-dimensional and two-dimensional order, respectively. Lamellar mesophases with one-dimensional long-range order have a fairly low viscosity. Due to their gel character, cubic and hexagonal mesophases exhibit a yield value after which flow occurs. Unlike the corresponding inverse liquid crystals, the gel character is much more pronounced, resulting from interactions between polar functional groups located at the surface of the associates. The associates may form strong networks with each other through polar interactions such as hydrogen bonds. In contrast, the surface

of inverse mesophase associates consists of nonpolar groups. This results in interactions that are weaker and the gel deforms more easily.

A mechanical oscillation measurement is the method of choice for determining liquid crystalline gel elasticity. Since shear strain is not applied, the viscoelastic properties of such systems may be studied without a change in network microstructure. This is in contrast to rheological investigations where mechanical deformation usually occurs and changes the character to be determined. The oscillation experiments allow determination of the viscoelastic character of cubic, hexagonal and lamellar mesophases, in addition to highly concentrated vesicle dispersions showing viscoelastic behavior. A vesicle dispersion with a low inner phase content, however, exhibits Newtonian flow. According to the Einstein equation (Eq. (2)) the dynamic viscosity coefficient η is larger than η_0 of the continuous phase (usually pure water or solvent) by factor 2.5 × volume ratio of the dispersed phase φ.

$$\eta = \eta_0(1 + 2.5\varphi) \quad (2)$$

where η_0 is the viscosity of the pure solvent (continuous phase) and φ is the volume ratio of the inner phase.

3. Applications of colloidal carriers as DDS

3.1. Reverse micellar solutions

The therapy of a chronic disease requires repeated drug administration. In the case of a short biological half-life, the drug has to be administered within short intervals up to several times daily. To reduce application frequency sustained formulations have been developed. Liquid crystalline excipients are appropriate candidates for this purpose, because in a liquid crystalline vehicle the drug diffusion is reduced by factor 10–1000 in comparison with a liquid vehicle such as a solution [5,34,35]. The factor depends on which kind of liquid crystal is used.

A further possibility is the formation of liquid crystals upon contact with body fluids at the site of application. The drug solution interacts with body fluids such as plasma, tear fluid or skin lipids and undergoes a phase transition into a mono- or multiphasic system of liquid crystals (Fig. 2). An example of this is oily solutions of reverse micellar phospholipids, which solubilize additional drug and transform into liquid crystalline lamellar phases by absorbing water upon mucosal application. Drug release is controlled by the liquid crystals because the diffusion within the liquid crystalline phase is slowest and is thus the rate-controlling step [35]. This principle can be used for ophthalmological administration as well as for nasal, buccal, rectal, vaginal or even parenteral subcutaneous application [36]. The peroral administration of such reverse micellar solutions either directly or encapsulated within soft gelatin capsules is not recommended, however [37], because the sustained release effect is limited by interindividual variations in digestion. This results from differences in the amount and composition of the gastric fluid as well as the ability of the gastric fluid to emulsify and dissolve the dose prior to absorption.

Paradontitis of infected gum pockets has been successfully treated by metronidazole administration. The crystalline prodrug metronidazole benzoate, which dissolves and hydrolyses to form the active metronidazole has been used suspended in an oleogel (Elyzol Dentalgel). The oleogel consists of glycerol monooleate and sesame oil, which is immobilized within the matrix structure of the surfactant. The base melts at body temperature and spreads evenly over the inner surface of the gum pockets. The molten system absorbs water and transforms into a reverse hexagonal phase. This liquid crystalline structure has a high viscosity. The resulting system adheres well to the surface of the mucosa and slowly releases the active ingredient [38].

3.2. Liquid crystalline drug substances

Some drug substances are able to form mesophases either together with a solvent (lyotropic liquid crystals) or alone

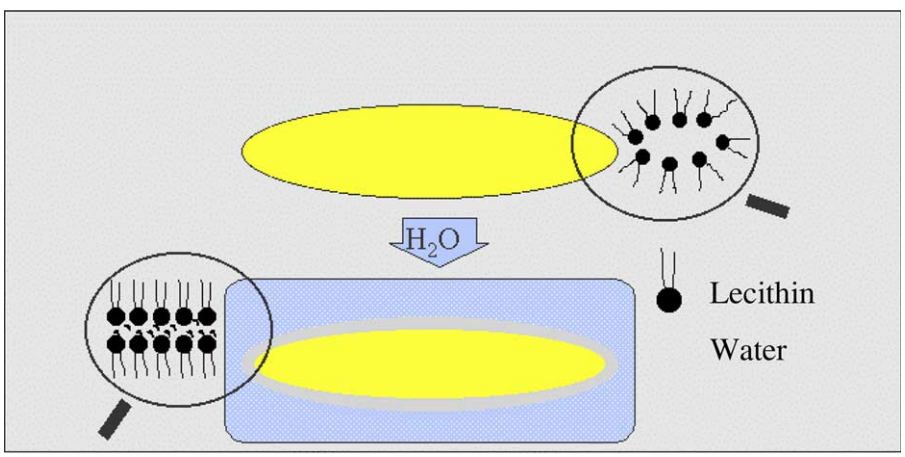


Fig. 2. Application induced transformation of a reverse micellar solution into a liquid crystal upon contact with aqueous media (from Ref. [38]).

Table 1
Liquid crystalline drug substances

Drug	Type of liquid crystal	Reference
Arsphenamine	Nematic	[39]
Disodium cromoglicinate	Nematic, hexagonal	[40]
Nafoxidin-HCl	Hexagonal, cubic, lamellar	[41]
Diethylammonium flufenamate	Lamellar	[42]
NSAID salts		
Fenoprofen	Lamellar	[1]
Ketoprofen	Lamellar	[1]
Ibuprofen	Lamellar	[1]
Flurbiprofen	Lamellar	[1]
Pirprofen	Lamellar	[1]
Diclofenac	Lamellar	[2]
Peptide hormone LH-RH analogue		[43]

(thermotropic liquid crystals) [1,2,39–44]. Thermotropic and/or lyotropic liquid crystalline mesophases of drug substances may interact with mesomorphous vehicles as well as with liquid crystalline structures present in humans. Table 1 presents drug substances that have been proven to exhibit either thermotropic or lyotropic mesomorphism.

The molecular structure of arsphenamine is a typical representative of a thermotropic mesogen [39]. With its symmetrical arrangement of the atoms the same holds for disodium cromoglicinate DNCG [40] which forms both thermotropic liquid crystals and additionally lyotropic mesophases with water. If micronized DNCG powder is applied to nasal or bronchial mucosa the powder will absorb water due to the high relative humidity of the respiratory tract. It will then transform into a lyotropic mesophase, followed by solution formation, depending on the amount of water available.

For therapeutic purposes, a frequently used group of drug compounds are the nonsteroidal anti-inflammatory drugs (NSAID). One of the best-known representatives of the aryl acetic acid derivatives is diclofenac, and ibuprofen is an aryl propionic acid derivative. As both have acidic properties they dissociate while being dissolved and may form salts with amphiphilic properties. Together with appropriate counter ions these amphiphilic organic acids may form lyotropic mesophases with water at room or body temperature, e.g. diclofenac diethylamine or ibuprofen lysinate [1,2]. Furthermore, some NSAID anhydrates exhibit thermotropic mesomorphism after thermal dehydration of the crystalline salt, e.g. fenoprofen calcium [44].

All the other drug substances listed in Table 1 have not yet been used for therapeutic purposes.

3.3. Liquid crystalline formulations for dermal application

Since drug molecules with amphiphilic character may form lyotropic mesophases, amphiphilic excipients in drug formulations also form lyotropic liquid crystals. This is particularly so for surfactants, which are commonly used as emulsifiers in dermal formulations, and associate to form micelles after dissolving in a solvent. With increasing concentration the probability of interaction between the micelles increases and thus liquid crystals form.

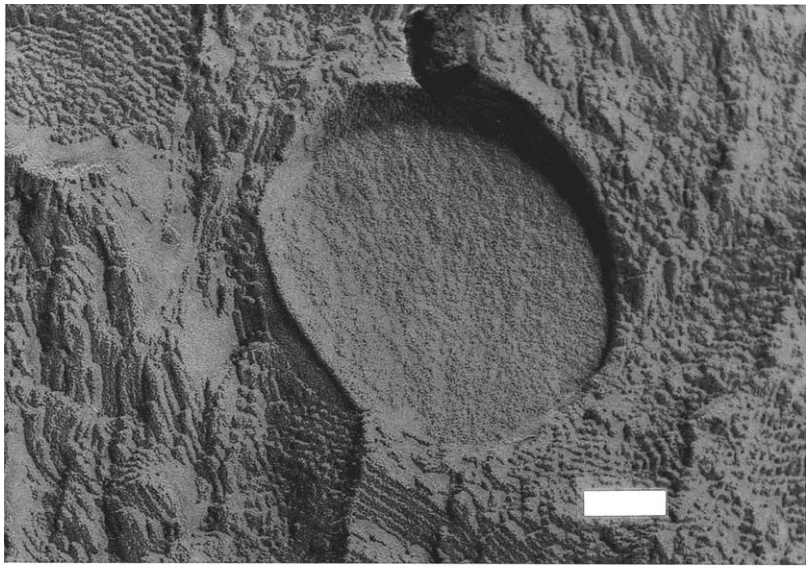
3.3.1. Surfactant gels

The use of monophasic systems of lyotropic liquid crystals is relatively seldom and is limited to gels. A variety of polar surfactants (e.g. ethoxylated fatty alcohols) hydrate in the presence of water and form spherical or ellipsoidal micelles. At high surfactant concentrations these associates are densely packed and are identified as cubic liquid crystals [26].

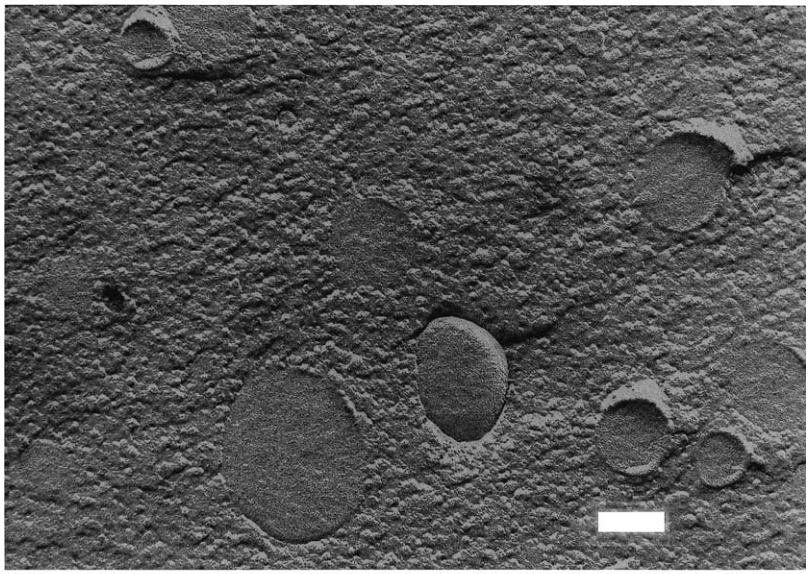
Fig. 1c represents a transmission electron micrograph of a cubic liquid crystalline surfactant gel. Such gels are optically transparent. If agitated mechanically, their elastic properties become evident. Due to resonance effects in the audible range, they are also called ringing gels. These gels are composed of the lipophilic components solubilized together with the active ingredients in hydrated associates of the surfactants. However, the solubilization capacity of lipophilic components is generally limited, and any excess disperses dropwise in the liquid crystalline phase (Fig. 3b). Such systems exhibit a white appearance according to the change in refractive index at the interface between continuous liquid crystalline and dispersed oil phase. The dispersed drops are mechanically stabilized because the liquid crystalline phase of either hexagonal (Fig. 3a) or cubic character (Fig. 3b) has a high yield value.

Ringing gels with cubic liquid crystalline microstructure are marketed as commercial drug formulations especially for topical NSAID formulations. Examples on the German Market include Contrheuma Gel Forte N, Trauma-Dolgit Gel and Dolgit Mikrogel. The latter was introduced in 1996 and contains ibuprofen as the active ingredient. The high surfactant concentration of such gels on one hand is necessary to verify the liquid crystalline microstructure, and on the other hand influences the microstructure of the stratum corneum lipids increasing permeability. Increased permeability is also achieved by alcohol, which is also solubilized in the formulation. In permeation tests with excised human stratum corneum, the amount of ibuprofen permeating a specific surface area over time was much higher for Dolgit Mikrogel than for an aqueous mixed micellar solution of the drug [8]. Although relatively high permeation rates are possible for the liquid preparation, the commercial formulation is significantly more effective since the high surfactant content and the alcohol lead to a high permeability. Comparisons between different commercial formulations revealed the superiority of the ringing gel [45].

A ringing surfactant gel of liquid crystalline microstructure containing the anti-mycotic bifonazole was introduced to the German Market in 1995 (Bifomyk Gel). Like surfactant gels containing NSAIDs, improved penetration



(a)



(b)

Fig. 3. Transmission electron micrographs of freeze fractured oily droplets dispersed (a) in a hexagonal and (b) in a cubic liquid crystalline phase, bar represents 100 nm (from Ref. [25]).

of the active ingredient is desired in anti-fungal therapy of the dermis. However, since the liquid crystal structure only forms with a relatively high surfactant concentration, the positive effect of improved penetration must be considered together with the potential for irritation. The objective is to improve penetration as much as possible via a change in skin structure, while minimizing irritation. Since hyphae of fungi (mycelium) can penetrate deep into the epidermal layers by sliding past the corneocytes of the horny layer, improvement of the anti-mycotic therapy is of particular importance. The same holds for the penetration of NSAIDs through several epidermal layers, because they have to reach the muscle and joint tissue located more deeply.

3.3.2. Ointments and creams

Commonly, the surfactant concentration in ointments and creams is significantly lower than in surfactant gels. Ointments are nonaqueous preparations, whereas creams result from adding water to ointments. If a liquid crystalline network or matrix is formed by amphiphilic molecules, the microstructure of ointments or creams may be liquid crystalline. In this situation, the system is more easily deformed by shear stress. Such formulations show plastic and thixotropic flow behavior. Systems with a liquid crystalline matrix exhibit a short regeneration time after shearing. In comparison, a crystalline matrix is usually destroyed irreversibly by shear. To obtain a liquid

crystalline matrix amphiphilic surfactants that form lyotropic liquid crystals at room temperature must be selected. It is preferred if lamellar liquid crystals are formed, which are able to solubilize high amounts of other ingredients and spread through the whole formulation as a network-forming cross-linked matrix. In contrast, ointments which contain long-chain fatty alcohols such as cetyl and/or stearyl alcohol, have a crystalline structure at room temperature [46].

Although the α-phase of the fatty alcohols, a thermotropic smectic-B liquid crystal with hexagonal arrangement of the molecules within double layers, is initially formed from the melt during the manufacturing process, it normally transforms into a crystalline modification as it cools. However, crystallization of the gel matrix can be avoided if the α-phase is kept stable as it cools to room temperature. This can be achieved by combining appropriate surfactants such as myristyl or lauryl alcohol and cholesterol, which mix to form a lamellar liquid crystal at room temperature [47].

The polar character of a surfactant molecule enables the addition of water to form creams. Depending on whether the surfactant or the surfactant mixture has a strong or weak polar character, an o/w or w/o cream will form, respectively. Creams of w/o type are produced from systems which are solely stabilized with weakly polar surfactants such as fatty alcohols, cholesterol, glycerol monostearate, or sorbitan fatty acid esters. The surfactants or surfactant mixtures are adsorbed at the interface between the dispersed aqueous and continuous lipophilic phase. Even multiple layers of the surfactants are adsorbed if the concentration of mesogenic molecules is high enough to form their own liquid crystalline phase (Fig. 4). Apart from the reduction of the surface tension and/or surface energy, the liquid crystalline interface also has a mechanically stabilizing effect on the emulsion drops.

Surfactants such as sulfated fatty alcohols may be hydrated to a higher extent than the fatty alcohols alone and thus stabilize o/w emulsions. The combination of an anionic and a nonionic surfactant has proven to be particularly effective, since the electrostatic repulsion forces between the ionic surfactant molecules at the interface are reduced by the incorporation of nonionic molecules, thus improving the emulsion stability. The combination of cetyl/stearyl sulfate (Lanette E) and cetyl/stearyl alcohol (Lanette O) to yield an emulsifying cetyl/stearyl alcohol (Lanette N) is an example of this approach. The polar properties of this surfactant mixture are dominant, and therefore o/w creams are formed. In contrast to w/o systems, the stabilizing effect of the surfactant mixture is only partly due to adsorption at the interface. Instead, the mixed surfactants are highly hydrated and form a lamellar network, that is dispersed throughout the continuous aqueous phase, and the dispersed lipophilic components are immobilized within the gel network. However, this hydrated gel matrix is not crystalline at room temperature as is the case for corresponding w/o creams with cetyl/stearyl alcohol, but is in its α-phase, which belongs to the thermotropic smectic liquid crystals and exhibits a strong similarity to lyotropic lamellar liquid crystals.

Analogous gel matrices of liquid crystalline lamellar phases can also be formed with nonionic mesogens, e.g. with the combination of cetyl/stearyl alcohol and ethoxylated fatty alcohol, provided that the hydrophilic and lipophilic properties of the surfactant molecules are more or less balanced to favor the formation of lamellar structures. New possibilities for the development of controlled drug delivery systems of lamellar lyotropic

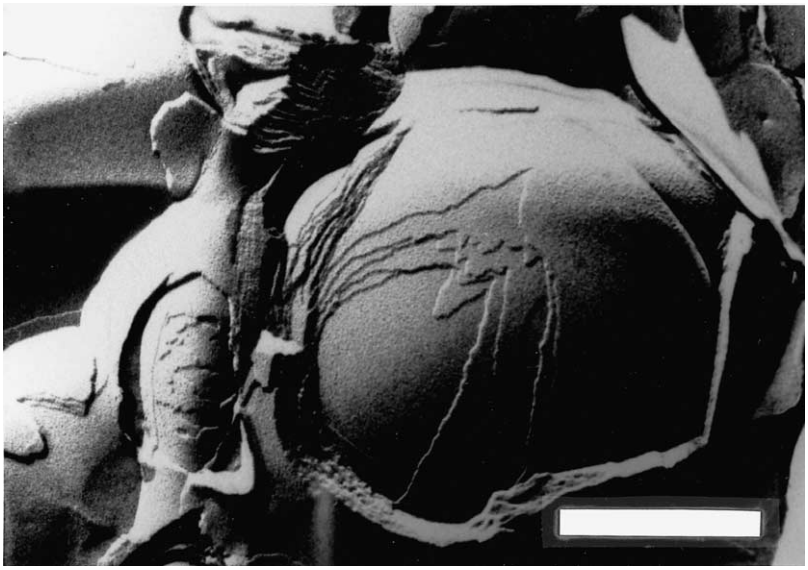


Fig. 4. Transmission electron micrograph of a freeze fractured w/o cream. The aqueous phase is dispersed as droplets within the continuous lipophilic phase; the interface consists of multiple bilayers of hydrated surfactant molecules; bar 500 nm (from Ref. [47]).

liquid-crystalline systems are introduced due to their stability and special, skin-friendly structure [48].

3.4. Vesicle dispersions for topical application

Although liposomes have been studied intensely since 1970, only a few commercial drug formulations contain liposomes as drug carriers [49,50]. The first commercial drug formulation with liposomes for topical administration was registered in Italy. The anti-mycotic econazole was encapsulated in liposomes dispersed in a hydrogel (Ecosom Liposomengel, formerly Pevaryl Lipogel). A highly hydrated gel network of the hydrophilic polymers forms, and liposomes are immobilized within the gel network and thus mechanically stabilized. This stabilization via gelation of the continuous aqueous phase can also be applied to other disperse systems, e.g. suspensions or emulsions. An example of such an emulsion/hydrogel combination that contains heparin sodium as the active ingredient and, since 1995, liposomes as an additional dispersed phase is Hepaplus Liposom. Voltaren Emulgel is a formulation with an analogous emulsion/hydrogel combination but without additional liposomes. The transmission electron micrograph in Fig. 5 reveals an adsorption of lamellar liquid crystals at the interface between dispersed oil drops and the aqueous continuous phase. The aqueous continuous phase is again a hydrogel derived from polyacrylate, in which the lipophilic phase is immobilized. The interface consists of multilamellar layers consisting of both surfactant and drug molecules. Thus the hydrogel is not only stabilized by the hydrogel network itself but also by the liquid crystalline interface. The active ingredient, diclofenac diethylamine, diffuses slowly from the dispersed phase via the multilamellar interface into the continuous phase from where it penetrates into the epidermis.

Similar to Voltaren Emulgel, oily droplets of eutectic mixture of lidocaine and prilocaine are dispersed in a hydrogel to provide local anaesthesia of the skin for injections and surgical treatment (Emla cream). A further possibility is the dermal administration of a liposome dispersion as a spray (Heparin PUR ratiopharm Sprühgel). After administration, water and isopropyl alcohol evaporate partially increasing the liposome concentration, and a transition from the initial liposome dispersion into a lamellar liquid crystal occurs [51]. The therapeutic effect thus appears to be influenced favorably by the presence of lecithins alone, rather than by the degree of dispersion of the liposomes.

3.4.1. Vesicle dispersions for parenteral administration yielding local effects

Depending on their size and surface charge, parenterally administered liposomes interact with the reticulo-endothelial system (RES) and provoke an immunological response. After adsorption of certain serum proteins, so called opsonines, the liposomes are identified as a foreign invader and are then destroyed by specific immune cells, mainly in the liver, spleen and bone marrow.

This passive drug targeting enables an efficient therapy of diseases of these organs or their affected cells. Clinical tests in the therapy of parasitic diseases, especially concerning the liver and spleen, have proven most efficient with liposomal encapsulation of the drug substance.

In addition to passive drug targeting, drug encapsulation within liposomes can change the intensity and duration of the therapeutic effect, and also minimize undesired side effects. For this purpose, the liposomes have to circulate as long as possible in the vascular system while remaining unrecognized by phagocytic cells.

Amphotericin, an anti-mycotic, is encapsulated in liposomes and marketed as AmBisome against severe

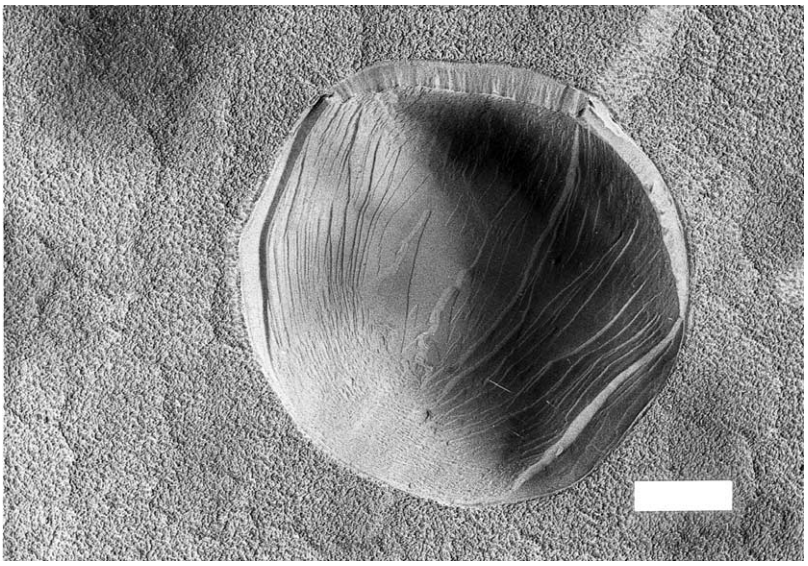


Fig. 5. Transmission electron micrograph of Voltaren Emulgel; the interface between the continuous hydrogel and the dispersed emulsion droplets consists of multiple bilayers of hydrated surfactant molecules; bar 500 nm (from Ref. [52]).

systemic mycosis. The liposomal encapsulation reduces the toxicity of amphotericin while increasing half-life of the drug and plasma level peaks [50]. To increase stability, the parenteral formulation is a lyophilized powder that has to be reconstituted by adding the solvent just before administration.

The cytostatic daunorubicin, which is administered in the later stages of Kaposi's sarcoma of AIDS patients, is encapsulated in liposomes of about 45 nm in size [50]. The liposomal dispersion is marketed as a sterile, pyrogen-free concentrate (DaunoXome) and has to be diluted with a 5% glucose solution just before being administered as an infusion. Although daunorubicin itself is cardiotoxic, the liposomal formulation prevents significant attack on the cardiac tissue, but strongly targets the tumor cells by being taken up preferentially. It is postulated that small unilamellar vesicles (SUV) may pass endothelial gaps in recently formed capillaries of the tumor thus entering the tumor tissue. The drug is released from the liposomal carrier at this site and inhibits proliferation of the tumor cells.

The photosensitizing agent verteporfin (Visudyne) was introduced into photodynamic therapy in 2000, to treat subfoveal choroidal neovascularization in age-related macular degeneration [53]. The benzoporphyrin derivative is encapsulated in liposomes and freeze-dried for stabilization. Prior to intravenous infusion the liposomal powder is dissolved in water for injection and glucose solution. The liposomally encapsulated drug is bound to the LDL fraction of the blood and is selectively concentrated in rapidly proliferating cells such as endothelium of subfoveal choroidal neovascularization. The uptake is triggered by LDL receptors. Radiation at 689 ± 3 nm for 83 s induces activation of the concentrated drug and finally cell death via singulet oxygen.

3.4.2. Vesicle dispersions for administration to the lung

A liposomal formulation consisting of surfactant, which usually coats the mucosa of the bronchi and prevents collapse of the alveolar vesicles of the lung, has been developed for patients who suffer either from infant respiratory distress syndrome (IRDS) or adult/acquired respiratory distress syndrome (ARDS). IRDS often affects premature babies who have not developed a functional lung surfactant and therefore develop a failure in pulmonal gas exchange. ARDS is also a life-threatening failure/loss of the lung function and is usually acquired by illness or accident. Clinical trials with liposomal surfactant have proven to be effective in prophylactic treatment of IRDS and ARDS. AlveofactTM contains all relevant components of the lung surfactant for pulmonal gas exchange [50].

3.5. Nanoparticle dispersions for topical administration

Solid lipid nanoparticles (SLN) have gained increasing interest as pharmaceutical and cosmetic formulations recently [54–78]. Due to their small particle size and consequent high surface area they possess strong adhesive properties. This leads to film formation on the skin, which may help to restore a previously damaged protective lipid film on the skin and increase the moisturizing effect via occlusion [54–56,59,70]. Furthermore SLN have been proposed as novel carriers for sunscreens [57–61].

Incorporation of active ingredients into the solid lipid matrix offers protection against chemical degradation of the active compound [74], as well as allowing either immediate or sustained release, depending on the polymorphic transitions of the lipid matrix [69]. Sustained release is important with active ingredients that are irritating at high concentrations or when supply of a drug to the skin over a prolonged period of time is desired, whereas immediate release can be useful to improve drug penetration. According to Jenning et al. [69] sustained release is often related to the metastable beta′ polymorph of the lipid matrix (glyceryl behenate in the present study). Drug expulsion is explained by a reduction of amorphous regions in the carrier lattice due to a beta′ to beta(i) polymorphic transition. This transformation can be controlled with surfactant mixtures or, in the case of a hydrogel or an oil/water cream, with gelling agents or humectants. Thus, the release rate for the topical route of application can be adjusted.

Stability enhancement was reported for vitamin E [68] and retinol [74] by encapsulation of retinoids in solid lipid nanoparticles. Different lipids were compared, and glyceryl behenate gave superior entrapment compared to tripalmitate, cetyl palmitate and solid paraffin [74]. Three drugs were compared, and entrapment increased with decreasing polarity of the molecule (tretinoin < retinol < retinyl palmitate). Encapsulation efficacy was enhanced by formulating SLN from mixtures of liquid and solid lipids. These particles were solid and provided better protection for sensitive drugs than an emulsion. X-ray investigations revealed that good encapsulation was correlated with a low degree of crystallinity and lattice defects. With highly ordered crystals, as in the case of cetyl palmitate, drug expulsion from the carrier was more pronounced.

Based on the experiences with solid lipid nanoparticles, a new type of solid lipid nanoparticle has been developed by incorporating triglyceride containing oils in the solid shell of the particle [72,73]. A medium chain triglyceride oil was successfully incorporated into a solid long chain glyceride matrix. The crystal order was greatly disturbed, but the carrier remained solid. The oil inside the particle remained in a liquid state and induced a slight shift from the beta′ polymorph to the beta(i) form of the crystalline lipid. Long spacings varied within 0.1 nm with increasing oil loads. There was a linear relationship between oil supplementation and melting point depression of the glyceryl behenate. From ^{1}H-NMR measurements it was found [73] that the mobility of the oil molecules inside the particles was considerably lower than that of the emulsified oil. Moreover, two different chemical shifts for each of the lipid signals were observed indicating two different chemical environments.

The experimental data are in line with a model describing uniform distribution of the oil molecules in the glyceryl behenate for low oil loads. However, at higher oil loads oil clusters appear to form within the solid nanoparticle. Nanoparticles with low oil concentrations showed sustained release properties. Improved drug load levels were achieved by lipid particles supplemented with oily constituents.

In a recent publication the term nanostructured lipid carrier (NLC) has been suggested for the oil-loaded SLN with superior drug-loading capacity and controlled-release characteristics [78]. Proton nuclear magnetic resonance spectroscopy and electron spin resonance experiments were performed to investigate component mobility and the molecular environment of model drugs. NLC nanoparticles differ from nanoemulsions and SLN by forming a liquid compartment that strongly interacts with the solid lipid. The electron spin resonance model drug was found to be accommodated either on the particle surface with close water contact (SLN) or additionally in the oil (NLC). From these data the authors suggested that NLC are not spherical solid lipid particles with embedded liquid droplets, but they are rather solid platelets with oil present between the solid platelet and the surfactant layer. In contrast to previous results, it was found that neither SLN nor NLC lipid nanoparticles showed any advantage with respect to incorporation rate or retarded accessibility of the drug compared with conventional nanoemulsions.

While most published data deal with glyceride SLN, little knowledge is reported on wax carriers. A comparison of the two substances was made with respect to retinol encapsulation efficacy, particle size distribution after production and storage, and crystal packing [71]. Glyceride SLN showed good drug encapsulation, while physical stability was poor. In contrast, wax SLN possessed good physical stability but lacked sufficient drug encapsulation in the solidified state. These differences were attributed in part to different crystal packing. Less ordered crystal lattices favor successful drug inclusion, as in the case of glyceryl monosterate and glyceryl behenate SLN. The highly ordered crystal packing of wax SLN, comprised of beeswax or cetyl palmitate, for instance, leads to drug expulsion, but also to superior physical stability.

Besides dermal application, delivery to the eye of nanoparticles is possible and has demonstrated promising results over the last 10 years. A recent review on cyclosporin A delivery to the eye summarizes a variety of different carrier systems including nanoparticles and other colloidal carriers [79].

4. Conclusion

A broad knowledge of physicochemical characteristics of a variety of different colloidal carriers relevant for topical delivery is available, providing a better understanding of action of the respective systems in many cases. A drug delivery system has to be optimally developed, depending on the requirements of therapy. Colloidal drug delivery systems often offer superior properties in this context and thus may be the carriers of choice.

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