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ORIGINAL CONTRIBUTION

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Novel lipid-based colloidal dispersions as potential drug administration systems – expectations and reality

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Abstract Colloidal drug carriers offer a number of potential advantages as delivery systems for, for example, poorly soluble compounds. The first generation of colloidal carriers, in particular liposomes and submicron-sized lipid emulsions, are, however, associated with several drawbacks which so far have prevented the extensive use of these carriers in drug delivery. As an alternative colloidal delivery system melt-emulsified nanoparticles based on solid lipids have been proposed. Careful physicochemical characterization has demonstrated that these lipid-based nanosuspensions (solid lipid nanoparticles) are not just "emulsions with solidified droplets". During the development process of these systems interesting phenomena have been observed, such as gel formation on solidification and upon storage, unexpected dynamics of polymorphic transitions, extensive annealing of nanocrystals over significant periods of time, stepwise melting of particle fractions in the lower-nanometer-size range, drug expulsion from the carrier particles on crystallization and upon storage, and extensive supercooling. These phenomena can be related to the crystalline nature of the carrier matrix in combination with its colloidal state. Observation of the supercooling effect has led to the development of a second new type of carrier system: nanospheres of supercooled melts. This novel type of colloidal lipidic carrier represents an intermediate state between emulsions and suspensions. Moreover, these dispersions are particularly suited to the study of the basic differences between colloidal triglyceride emulsions and suspensions. For many decades drug carriers have represented the only group of colloidal drug administration systems. Nowadays a fundamentally different group of dispersions is also under investigation: drug nanodispersions. They overcome a number of carrier-related drawbacks, such as limitations in drug load as well as side effects due to the matrix material of the carrier particles. Utilizing this concept virtually insoluble drugs can be formulated as colloidal particles, of solid or supercooled nature. For example, coenzyme Q_{10} (Q_{10}) has been successfully processed into a dispersion of a supercooled melt. Droplet sizes in the lower nanometer range and shelf lives of more than 3 years can easily be achieved for Q_{10} dispersions. The drug load of the emulsion particles reaches nearly 100%.

Key words Nanosuspensions · Solid lipid nanoparticles · Nanospheres of supercooled melt · Drug nanodispersions

Introduction

Many drugs, among them very promising candidates, are poorly soluble in aqueous media. The formulation of these drug substances into effective and safe pharmaceuticals has always been a challenge, in particular if the drugs have to be administered into the bloodstream. Solubilization of lipophilic drugs with the aid of colloidal particles, such as (mixed) micelles, liposomes, or emulsion droplets, is one approach to overcome the problem of bringing a hydrophobic substance into the aqueous blood compartment. Other beneficial effects expected for colloidal carrier systems compared to free drug are the protection of sensitive drugs against degradation in biological fluids, protection of the patient against irritative side effects of the drug, or prolonged drug action due to sustained release. Another aspect of colloidal particles as drug carrier systems has gained much research interest in pharmaceutical and related fields. Since the distribution of colloidal particles within the body after administration into the bloodstream is different from that of dissolved substances, the colloidal particles may serve as carriers to specifically transport drugs to certain body sites. This so-called drug targeting would enormously increase the effectiveness and safety of drug therapy, since the drug level at the site of action would be increased while at the same time the level at other sites – where undesired side effects could be caused – would decrease. This concept is particularly interesting for very potent but unspecifically acting drugs (e.g., anticancer drugs), which beside their desired beneficial effects cause a number of severe side effects due to their unspecific mode of action. The most specific targeting could be reached with colloids coupled to a "homing device" which would guide the particle exactly to the body structure where the drug is needed. Such active targeting could be achieved, for example, by specific interaction with receptors or immunologic (antigen–antibody) reactions.

A broad variety of colloidal drug carrier systems have been developed for drug solublization and as an approach to realize the intriguing concept of drug targeting (Table 1). Such colloids may not only be used for intravenous administration but also for other different administration routes, such as dermal, peroral, nasal, ocular, or pulmonal routes. Probably most research has been and is being performed in the field of lipidic carriers, such as liposomes, lipid emulsions, etc. These systems are often derived from physiological structures and have compositions similar to their physiological counterparts; therefore, a high physiological acceptance is expected for these types of carriers. Lipids are expected to be metabolized rapidly, resulting exclusively in nontoxic breakdown products. There are also a large number of reports concerning polymer-based colloids [1], and investigations on colloidal dispersions of pure drugs – either in solid or liquid from – have recently also been described [2–5]. In spite of intensive research on colloidal drug carrier systems – in some cases for several decades (e.g., with lipid emulsions or liposomes) – remarkably few have been introduced onto the market, indicating that there seem to be problems either with the underlying concepts or with the formulation of adequate colloidal carriers, or

Table 1 Examples for colloidal drug administration systems

Systems	Product examples
Lipid-based carrier systems	
Liposomes	AmBisome, DaunoXome, Doxil, Pevaryl Lipogel
Lipid emulsions	Diazepam Lipuro, Etomidat Lipuro, Disoprivan, Lipotalon
Phospholipid/glycocholate mixed micelles	Valium MM Konakion MM
Microemulsions	Sandimmun Optoral
Lipid complexes (with low-molecular-weight compounds or DNA)	Abelcet, Amphotec
Solid lipid nanoparticles	
Emulsions of supercooled melts	
Dispersions of lyotropic liquid-crystalline phases (cubosomes, hexosomes)	
Low-density lipoproteins, artificial low-density lipoproteins	
Polymer-based carrier systems	
Polymer nanoparticles (nanocapsules, nanospheres)	
Polymer complexes (DNA)	
Colloidal drug particles	
Drug suspensions	
Particles of supercooled melts	

both. In this context, it has, of course, to be taken into account that the development and registration of a new type of administration system may take as long as for a new drug – that means up to 10 years or even more [6]; therefore, products will appear on the market only a considerable period of time after the concept has been successfully realized.

One major problem with the administration of colloidal particles is their interaction with the reticuloendothelial system (RES). This system, which belongs to the unspecific immune system of the organism, consists of phagocytic cells, which rapidly and effectively remove foreign particles from the bloodstream [7]. This also holds true for most lipid-based colloidal formulations which have been introduced to the market so far. Intensive research has, however, led to techniques to reduce or overcome this severe problem, for example, by coating the particles with hydrophilic macromolecules to "hide" the colloids [8]. Unfortunately, most coating materials exhibit only a limited (toxicological) acceptance.

Apart from problems arising from the interaction of colloidal systems with their biological environment after administration, the formulation of the colloidal carriers themselves is a difficult task due to many problems that arise from their colloidal state and specific pharmaceutical demands on such formulations (Table 2). One major point is colloid stability. "Stability" in a pharmaceutical sense refers to a shelf life of usually 3–5 years. Shorter shelf lives will only be accepted in very special cases; however, for most systems of pharmaceutical interest the colloidal state is at its best metastable. This is valid, for example, for oil-in-water emulsions and common liposome formulations. Thermodynamically stable colloids such as micelles and mixed micelles may also exhibit a fairly limited shelf life due to chemical degradation of micelle-forming components. The colloidal state may cause several additional instabilities, for example, due to the presence of large interfaces (adsorption–desorption processes, interactions in the stabilizer layer, higher risk of chemical instabilities, etc.). Since many colloidal administration systems are intended for intravenous use, stability is an even more crucial point. For intravenous administration systems, which are most promising with respect to drug targeting, particles of colloidal size are an absolute prerequisite. The particle dimensions must not exceed a critical size since large particles may lead to the blockage of small blood vessels, thereby causing potentially lethal side effects, such as thrombosis or embolism. An important instability phenomenon often associated with colloidal systems is, however, particle growth; therefore, this process must be absolutely controlled in carrier systems intended for intravenous administration. Since intravenous products have to be sterile, the colloidal dispersions should withstand autoclaving, for example, at 121 °C and 2 bar for 15 min [9]. In the case of colloidal dispersions containing particle fractions with dimensions above 100–200 nm, such as commercial intravenous emusions, the alternative, pharmaceutically less favored sterile filtration process is simply impossible. Autoclaving however, represents a tremendous stress for the system, easily causing particle growth especially if the stabilizers exhibit a temperature-dependent phase behaviour.

Table 2 Pharmaceutical demands on colloidal dispersions with special regard to intravenous products

1. Biodegradable, nontoxic components
2. Low or no reticuloendothelial system activity
3. No significant fraction of microparticles
4. Sterile
5. Shelf life 3–5 years

The colloidal state thus offers interesting potential for drug administration systems. Colloidal administration systems must, however, be very carefully designed and characterized since, in particular with respect to instability phenomena, they also pose clear limitations. This has been outlined extensively in the literature for conventional emulsion and liposome formulations since both types of systems have been under investigation for more than 30 years. During the development process of colloidal drug carrier systems unexpected phenomena may be observed which are due to the colloidal state. In some cases, these phenomena may form the basis for the development of modified, novel systems. This situation shall be illustrated using a few examples that have been encountered during the work of the author's group concerning colloidal lipidic drug carrier systems intended mainly for intravenous administration. The examples shall illustrate the specific features of colloidal drug carrier systems prepared via (melt) homogenization and the specific possibilities and drawbacks which are due to them.

Gel formation observed during the development process of solid lipid nanoparticles

Colloidal triglyceride emulsions have been used for parenteral nutrition for more than 30 years [10, 11]. Since the beginning of the 1970s they have also been investigated as drug carrier systems and some of these drug-loaded emulsions have been introduced into the therapeutic field [12, 13] (Table 1). Colloidal lipid emulsions are thus one of the oldest commercialized colloidal drug carrier systems. The number of drug emulsions on the market is, however, still fairly limited. This is due to the fact that for most poorly water soluble drugs there are also solubility limitations in vegetable oils and medium-chain triglycerides. In addition, drug-

loaded triglyceride emulsions pose a lot of stability problems and, due to the fluid state of their lipid matrix, they are not very promising with respect to sustained drug release. In most cases the carrier particles release drug molecules within seconds after intravenous administration [14, 15]; afterwards, they circulate as empty carriers in the blood stream. For drug targeting, sustained release is, however, a prerequisite since the drug has to remain associated with the colloidal carrier until it has reached the target.

Compared to emulsion droplets, a solid core of the dispersed particles could bind incorporated drug molecules more strongly and would offer a higher potential for sustained release and targeting. A simple concept would be to replace the liquid core of triglyceride emulsions by solid lipids. The resulting suspension particles should have modified properties, not only with respect to sustained release of drug molecules but, potentially, also with respect to stability aspects such as drug leakage from the carrier upon storage or coalescence. This concept has been worked on for almost as long as that of colloidal triglyceride emulsions. In spite of these efforts, no reports concerning the successful development of such systems can be found in the literature – neither in the pharmaceutical nor in the chemical field – until the late 1980s and early 1990s [16–21].

The reason for this surprising situation immediately becomes evident when one tries to transfer the established compositions and preparation procedures for parenteral triglyceride emulsions to the preparation of triglyceride suspensions. Triglyceride emulsions for parenteral administration consist of a vegetable oil, such as soy bean oil, as the lipophilic phase, phospholipids as stabilizers, and glycerol-containing water as the aqueous phase. Dispersion of the oil into colloidal particles is accomplished by high-pressure homogenization. Reasonably stable products with droplet sizes of about 150–500 nm can be obtained. An appropriate determination and specification of the average particle size and size distribution is, however, still a problem and this is even so far spherical particles if they have a broad (nonmonomodal) size distribution. Solid triglycerides, such as tripalmitin can be processed the same way as liquid oils if homogenization is carried out above the melting temperature of the lipid. The resulting hot colloidal tripalmitin emulsions, however, form water-rich, semisolid gels upon cooling to room temperature if pharmaceutically well established nontoxic phosphatidylcholine-rich phospholipid mixtures are used as stabilizers (Table 3). Depending on their composition the gels may be stable on storage for many years. Electron microscopic studies indicate that the gels consist of a three-dimensional network of crystalline triglyceride, immobilizing easily up to 90 wt% aqueous phase. The crystalline network can be described by a ''card-house'' structure with ''cards'' of an average thickness of only a few triglyceride layers [22]. Obviously, the formation of crystalline particles dramatically alters the demands of the dispersion with respect to the stabilizers. Colloidal triglyceride suspensions should thus not be regarded as ''lipid emulsions with solidified droplets''. Their tendency toward gel formation has prevented the development of stable triglyceride suspensions for decades.

Table 3 Model compositions. The bidistilled water contained 2.2 wt% glycerol and 0.01% wt/V (weight per volume) thiomersal

Soy bean oil		100 mg	
Phospholipids		12 mg	
Bidistilled water	to	1000 mg	
			⇒ emulsion
Tripalmitin		100 mg	
Phospholipids		12 mg	
Bidistilled water	to	1000 mg	
			⇒ gel
Tripalmitin		100 mg	
Phospholipids		12 mg/24 mg	
Sodium glycocholate		4 mg/6 mg	
Bidistilled water	to	1000 mg	
			⇒ suspension

Gel formation can, however, be avoided if a second, more mobile stabilizer, such as sodium glycocholate, is introduced into the compositions in sufficiently high concentrations (Table 3). The resulting dispersions of solid triglycerides are stable for pharmaceutically relevant periods of time [16, 22]. Electron micrographs of the suspensions show layered particles with a plateletlike shape resembling triglyceride single crystals (Fig. 1) [16, 22]. This result, combined with the macroscopic observation of gel formation, leads to the conclusion that destabilization is correlated with the transformation of spherical emulsion droplets into platelets upon crystallization. The particle surfaces freshly created upon crystallization have to be stabilized immediately on formation. Phospholipids, which are present in triglyceride emulsions as monolayers in the interface of emulsion droplets and in vesicular structures in the aqueous phase, are comparatively immobile stabilizers, exhibiting no significant solubility in aqueous media; therefore, the addition of a mobile stabilizer, for example, a micelle-forming surfactant with a high critical micelle concentration, is required to immediately stabilize the new surfaces.

Phospholipid/glycocholate mixtures are not the only blend to stabilize triglyceride suspensions: a number of other stabilizers have also been used [23, 24]. In the pharmaceutical field, however, the choice of stabilizers is very limited since physiological acceptance must be strictly observed. In particular, for parenteral administration very few stabilizers have been approved, among them phospholipids and bile salts. Moreover, the dispersions have to survive autoclaving. From the

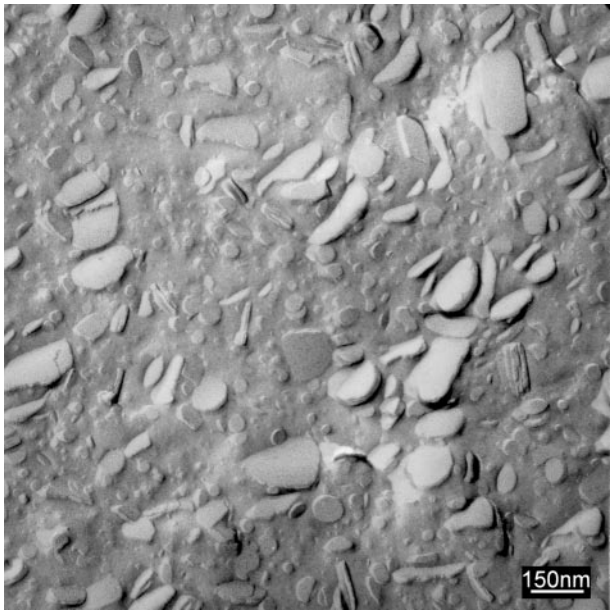

Fig. 1 Transmission electron micrograph of a freeze fractured trimyristin suspension (10% trimyristin, 3.2% phospholipid (Lipoid S100), 0.8% sodium glycocholate)

toxicological point of view, mixtures of phospholipids with other stabilizers, for example, nonionic surfactants, may be alternatives to the phospholipid/bile salt combinations. In such types of mixtures however, gel formation – potentially of a different type – has also been observed [23, 24].

Supercooling

Different types of solid triglycerides and other lipidic matrix materials with a melting temperature above room temperature have been described as matrix materials for melt-homogenized colloidal suspensions [16, 25–31]. Upon storage, some of these systems display pronounced differences in their behavior, depending on the triglyceride type and the storage temperature. For example, trimyristin and different hard fat dispersions can incorporate 2% menadione as a model drug when stored at room temperature but the drug phase-separates from the dispersions if the samples are stored in a refrigerator. In contrast to tripalmitin dispersions, trimyristin dispersions can be stabilized exclusively with phospholipids for several days at least, if they are stored at room temperature. Cooling of these trimyristin dispersions to refrigerator temperatures, however, again results in gel formation [27].

These examples illustrate that there must be basic structural differences between the differently behaving dispersions. Detailed structural investigations intended to confirm the crystalline state of the nanoparticles after preparation revealed that processing of solid triglycerides by melt-emulsification does not necessarily lead to the formation of solid nanoparticles upon cooling to room or refrigerator temperature. Dispersions with lower melting points in particular monoacid triglycerides such as trilaurin or trimyristin, do not display melting transitions upon heating in the differential scanning calorimeter or X-ray reflections due to crystalline material after storage at room temperature [26, 27]. As confirmed by studies with quantitative ^{1}H NMR spectroscopy, the matrix of the dispersed particles consists of liquid triglyceride in such particles [27].

Obviously, the substances under investigation have a high tendency toward supercooling. The critical crystallization temperature is mainly dependent on the composition of the triglyceride matrix and can also be modified by incorporated drugs. According to results from differential scanning calorimetry (DSC) studies the degree of supercooling is much higher in the nanoparticles than for the bulk triglyceride (Fig. 2). This phenomenon results in the fact that, for example, melt-homogenized trimyristin nanoparticles (DSC bulk crystallization temperature of about 30 °C) do not crystallize if stored at room temperature and that nanoparticles of trilaurin (DSC bulk crystallization temperature of about 13 °C) require subzero temperatures for crystallization [26, 32]. For triglycerides, pronounced supercooling in the dispersed state has been known for decades and has been confirmed by several

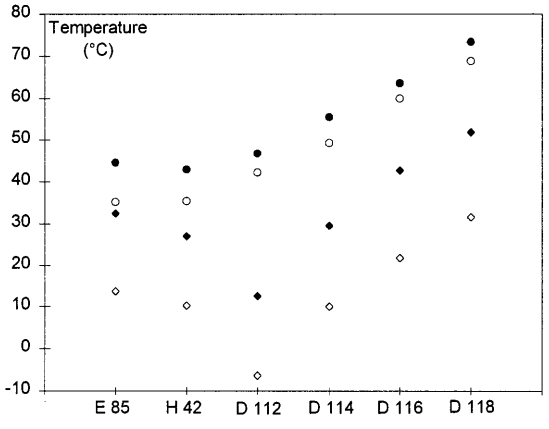

Fig. 2 Differential scanning calorimetry (*DSC*) melting (●) and crystallization (◆) temperatures of different pharmaceutical triglycerides in bulk (*full symbols*) and dispersion (*open symbols*). *E 85*, *H 42*: Witepsol E 85, Witepsol H 42 (hard fat suppository masses); *D 112*: Dynasan 112 (trilaurin), *D 114*: Dynasan 114 (trimyristin), *D 116*: Dynasan 116 (tripalmitin), *D 118*: Dynasan 118 (tristearin). All dispersions (10%) were stabilized with 2% phospholipid (Lipoid S100) and 2% tyloxapol. Melting temperatures are given as peak temperatures, crystallization temperatures as onset temperatures. Data were recorded at a scan rate of 5 °C/min. Under isothermal conditions, crystallization may occur at higher temperatures, in particular in bulk material

studies [33–36]. In contrast to result of studies performed on bulk phases and (presumably) microparticulate dispersions, however, the supercooled state in nanoparticles is stable for a considerable, pharmaceutically relevant period of time (i.e., several months to years) [37].

The possibility to create stable colloidal dispersions of supercooled melts by melt-homogenization is of major importance for several reasons. Emulsions of supercooled melts are, in many aspects, structurally equivalent to conventional triglyceride emulsions, for example, with respect to stabilizer requirements and drug loading capacity. A major difference is the fact that the matrix is, in principle, able to crystallize. The development of such systems thus requires very careful evaluation of potential instabilities due to crystallization of the matrix material (e.g., gel formation, drug expulsion) which may be highly retarded. Although the viscosity of the supercooled triglyceride may be higher than in conventional vegetable oil emulsions, the state of the supercooled matrix and corresponding effects concerning drug release are much more related to emulsions than to solid triglyceride particles such that, for example, drug release is expected to be rapider than from solid particles. Dispersions of supercooled melts must be strictly discriminated from solid lipid nanoparticles in spite of the fact that they may be obtained from the same matrix material! Unfortunately, there has been – and still is – confusion concerning this fact in the pharmaceutical literature [38–42]. After processing of lipophilic substances by melt-emulsification the physical state of the resulting colloidal particles has to be checked by utilizing techniques that allow the investigation of the native dispersions since preparation procedures which destroy the colloidal state of the particles may lead to artificial results.

In addition to triglyceride emulsions and suspensions, dispersions of supercooled triglycerides may be developed into alternative drug administration systems. Moreover, dispersions of certain triglycerides, which can be obtained as suspensions or emulsions of supercooled melts under convenient laboratory conditions (e.g., trimyristin dispersions), can serve as model systems for the study of the differences between triglyceride emulsions and suspensions, for example, with respect to drug incorporation or stabilizer requirements and distribution [26, 27].

High supercooling and the formation of stable dispersions of supercooled melts have not only been observed in melt-emulsified triglyceride dispersions but also in similarly processed dispersions of the extremely poorly water soluble, heart-protecting drug ubidecarenone (coenzyme Q_{10}, Q_{10}) [5]. Q_{10} is an endogenous quinone with a long side chain consisting of ten isoprenoid units (Fig. 3). Its solubility in aqueous media is less than 4 ng/ml. Colloidal Q_{10} has an even

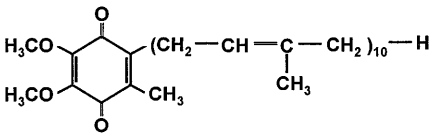

Fig. 3 Structural formula of ubidecarenone (coenzyme Q_{10}, Q_{10})

lower crystallization tendency than colloidal triglycerides with comparable melting temperature. The overall particle shape and the internal structure of the supercooled particles can be derived from transmission electron microscopy (TEM) micrographs of freeze-fractured specimens. In contrast to the colloidal suspension particles of monoacid triglycerides discussed previously, the particles are of spherical shape and have an amorphous core (Fig. 4). Cryogenic TEM studies indicate that phospholipid-containing dispersions contain numerous liposomal structures beside the desired drug particles (Fig. 5). The TEM as well as the ^{1}H NMR data confirm the emulsion-like character of these systems.

The preparation of stable colloidal dispersions of pure drugs is a very interesting alternative to conventional colloidal drug administration systems based on carrier particles. All drug carrier systems have a specific – usually very limited – drug loading capacity. In addition, the matrix materials of the carrier particles have to be metabolized and/or excreted. In contrast, particles in drug dispersions consist of almost 100% drug and so the loading capacity in this type of dispersion is limited only by the achievable concentration of the dispersed phase. With Q_{10}, colloidal dispersions containing 3–10% supercooled drug can easily be prepared (Table 4). For extremely poorly water soluble drugs, such as Q_{10}, processing into a colloidal supercooled state does not only solve the problem of bringing the drug into an aqueous medium for intravenous administration. The virtual insolubility of Q_{10} in water results in a very poor peroral bioavailability from conventional dosage forms. Processing into nanoparticles of supercooled melts should lead to improved bioavailability since a higher dissolution rate can be expected for several reasons:

- Improved wetting with aqueous media due to hydrophilic particle surfaces.
- Very high specific surface area.
- Shorter diffusional pathways.
- Higher apparent equilibrium solubility of colloidal substances compared to the bulk material.

Moreover, no lattice energy is required for dissolution. Another advantage of supercooled liquid drug particles compared to nanocrystals is that they require less emulsifier due to their spherical shape and that they are able to spread on surfaces.

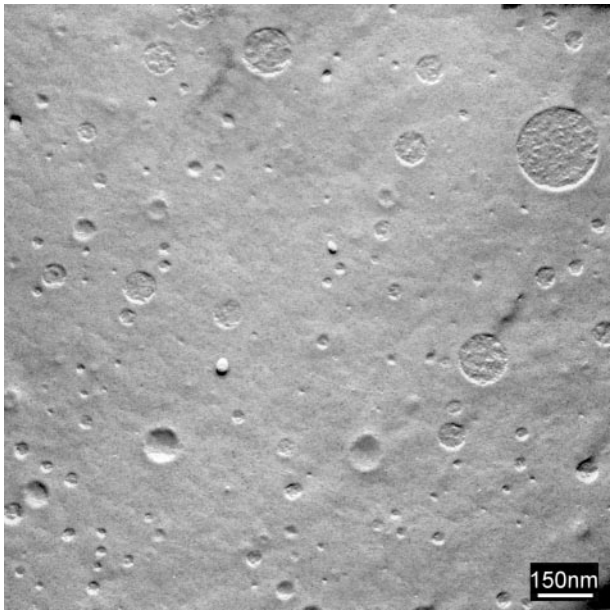

Fig. 4 Transmission electron micrograph of a freeze fractured dispersion of supercooled Q_{10}: 5% Q_{10}, 2.5% phospholipid (Lipoid S75)

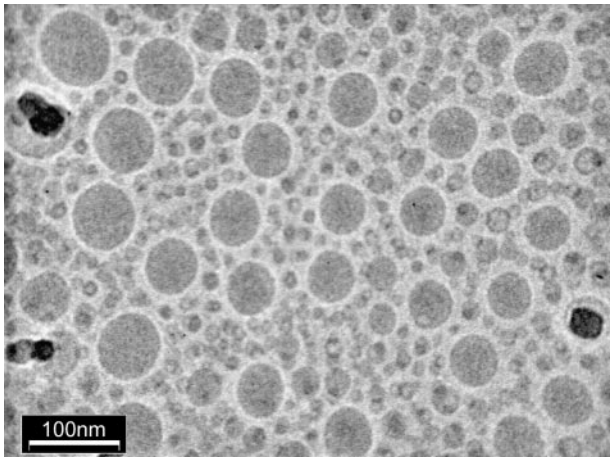

Fig. 5 Cryogenic electron micrograph of a dispersion of supercooled Q_{10}: 10% Q_{10}, 5% phospholipid (Lipoid S75)

In general, aqueous dispersions of colloidal drugs are safer and easier to handle than (ultra)fine drug powders, for example, in an industrial environment (no risk of dust explosions, reduced risk of crosscontamination and inhalation). Moreover, the particle sizes achievable with the melt-emulsification technique are much smaller than usually obtained with powders. Colloidal size is hardly achievable (ineffectively, expensively) and in some cases is impossible to achieve with conventional methods such as milling or grinding.

Dynamics of colloidal solid triglycerides

The basic significance of the crystallization process for the stability of triglyceride dispersions has already been outlined. Triglycerides may exist in different crystal modifications and they usually undergo a complex process of monotropic polymorphic transitions after crystallization. Crystallization usually occurs in the α modification, which transforms via the β' modification into the stable β polymorph. The different crystal polymorphs exhibit different density and molecular order and the polymorphic transitions are associated with a complex molecular reorganization process within the crystal lattice. Moreover, ageing processes that lead to a higher crystalline order within the triglyceride cannot be excluded after crystallization of these complex molecules. Concerning the development process of triglyceride nanoparticles it is important to know if and to what extent these processes also occur within the nanoparticles since they may have important consequences for the dispersions, in particular with regard to stability aspects. For example, it has been observed that expulsion of an incorporated model drug occurs at different rates from different types of triglyceride matrices [27].

X-ray diffraction experiments have shown that tripalmitin nanoparticles crystallize in the α modification and transform into the stable β form upon storage, as does the bulk material [26]. Transformation in dispersions stabilized with a phospholipid/bile salt blend is, however, much faster in the nanoparticles than in the bulk material, i.e., the α modification is far less stable in the colloidal state [25]. The type of stabilizer strongly influences the time course of the polymorphic transitions of triglyceride nanoparticles. While, for example, almost no occurrence of the α modification is observed when using polyoxyl 35 castor oil (Cremophor™ EL), the α modification is stable upon recrystallization for more than 60 min, even in usually rapidly transforming trimyristin dispersions when sodium glycocholate is employed as the only stabilizer. For tripalmitin dispersions stabilized with sodium glycocholate only different

Table 4 Examples for the composition of colloidal emulsions of supercooled coenzyme Q_{10} (Q_{10}) containing soy bean lecithin (Phospholipon 100) (*PL*) and sodium glycocholate (*SGC*) [5] Composition (wt% in bidistilled water) PCS particle size (nm)[a] Storage stability (months)[b]

3% Q_{10}, 1.8% PL	103	30
3% Q_{10}, 1.5% PL, 0.3% SGC	69	30
10% Q_{10}, 6% PL, 1.25% SGC	144	21
10% Q_{10}, 1.2% PL, 0.4% SGC	259	21

[a] PCS particle size is given as the mean of the number distribution determined with photon correlation spectroscopy (PCS)
[b] "Storage stability" denotes the period of time for which no crystalline material could be detected by differential scanning calorimetry

types of the α form are obtained when crystallization is carried out at different rates. Slow cooling leads to the formation of the usual α form displaying strong small-angle and wide-angle X-ray reflections. Upon rapid cooling, however, the resulting α modification leads to the occurrence of a strong wide-angle X-ray reflection but only a very weak small-angle reflection is observed [23, 24]. These results indicate that the stabilizers do not influence the colloidal state of the dispersion but may have pronounced effects on the internal structure of the particles.

The polymorphic behavior of particles prepared from triglyceride-rich hard fat suppository masses which are complex (tri)glyceride mixtures is different from that of particles of the monoacid triglycerides. Upon crystallization of these particles, the α modification is retained longer than in the bulk, where it transforms into the β' modification almost immediately after crystallization. For the hard fats under investigation, the β' form is very stable in the bulk and can be observed in the raw materials even after several years of storage at room temperature. In colloidal dispersions, however, these hard fats obtain a crystalline form displaying an X-ray diffraction pattern similar to that of the stable form of monoacid triglycerides, albeit with weaker and more diffuse reflections. This modification was not observed for the bulk materials. The nanoparticles thus seem to transform into a more stable polymorph than the bulk material, indicating higher dynamics in the colloidally dispersed state in agreement with the behavior of colloidal monoacid triglycerides. The higher stability of the metastable α form in the hard fat nanoparticles compared to in the bulk material is probably due to the low crystallization temperature in the dispersed state [27]. Polymorphic transitions are, however, not the only dynamic process that can be observed in triglyceride nanoparticles. The determination of the degree of crystallinity by DSC indicates that the degree of crystalline order may increase for a considerably period of time after complete transformation of the dispersions into the β modification before a stable value is reached [25].

These results lead to the conclusion that nanosuspensions of triglycerides are highly dynamic systems not only during the process of crystallization but also after solidification due to polymorphic transformation and ageing processes. The type of crystal polymorph formed after a given period of storage cannot be deduced from the situation in the bulk material since the kinetics of polymorphic transitions may be altered dramatically due to dispersion into the colloidal state. Moreover, the type of crystal polymorph finally obtained in the dispersion may even be completely different from that expected from the behavior of the bulk material.

Drug incorporation into solid lipid nanoparticles

A more rigid association between drug and carrier is expected for solid compared to liquid triglyceride nanoparticles. This hypothesis could be verified for several drugs with high-resolution ^{1}H NMR spectroscopy on colloidal trimyristin emulsions of supercooled melt and suspensions. In general, however, drug incorporation into triglyceride nanosuspensions is not without problems, particularly with respect to loading capacity. In most cases, only comparatively low amounts of drug can be loaded into the dispersions and the drug load of the suspensions is lower than for the carriers with a liquid matrix (cf., ''supercooling''). Higher drug loads tend to phase-separate upon crystallization of the triglyceride matrix or even cannot be incorporated into the corresponding emulsion system because their solubility in the molten triglyceride is too low [27].

Only for very special systems, for example, with Q_{10}, could high drug loads be achieved. Q_{10} can be incorporated into the dispersions in concentrations up to above 50% of the lipophilic phase. A concentration-dependent association of the drug with the nanoparticles can be deduced from the influence of drug load on the melting and recrystallization temperatures as well as on the kinetics of the polymorphic transitions of the triglyceride. The observed crystallization-point depression favors the formation of colloidally dispersed supercooled melts (instead of solid particles) which may be stable on storage for a considerable period of time. The absence of characteristic X-ray reflections and DSC melting endotherms of Q_{10} points to a noncrystalline state of Q_{10} within the triglyceride dispersions. Studies using ^{1}H NMR spectroscopy reveal that in highly loaded (50% of the dispersed phase) suspensions most of the drug is present in a liquid state. At concentrations of 5 and 10% Q_{10}, the almost complete disappearance of Q_{10} signals from the NMR spectra of triglyceride suspensions points to a comparatively rigid association between drug and carrier matrix. From results of investigations using freeze-fracture and cryogenic TEM it becomes obvious that dispersions loaded with 30 and 50% Q_{10} contain nanoparticles consisting of a plateletlike basis similar to unloaded triglyceride nanoparticles and a cap containing the liquid drug. In particles loaded with only 10% Q_{10} no caps are observed on the surface of the platelets, suggesting that no or only little drug is present in excess. Obviously, only minor amounts of Q_{10} are associated with the triglyceride matrix in such a way that the mobility of the drug molecules is restricted. Excess drug is expelled from the particle matrix upon crystallization of the triglyceride but remains associated with the lipid nanoparticles [23, 43].

Localization of the incorporated drug in the carrier particles should be carefully confirmed during the development process of drug-loaded dispersions. It has to be considered that pharmaceutical colloidal dispersions may be very complex systems. For example, conventional parenteral emulsions do not only contain emulsion droplets but also a large number of vesicular structures – predominantly small unilamellar vesicles – due to their tremendous excess of phospholipids [44, 45]. Liposomes have also been observed in dispersions of supercooled Q_{10} (Fig. 5). Drugs may partition between different colloidal structures within the dispersion; however, the distribution of drugs between the coexisting types of colloidal structures has not been studied in detail so far for the novel types of dispersions.

Size effects in triglyceride nanoparticles

For several reasons, the preparation of very small (e.g., smaller than 100–150 nm) triglyceride nanoparticles may be advantageous from the pharmaceutical point of view. For example, the biodistribution of colloidal, intravenously administered particles is influenced by their size. Small particles are usually less rapidly removed from the circulation by the RES and thus have a longer circulation time. Moreover, if the particles are much smaller than 150 nm they may be able to leave the bloodstream through openings which are located at certain sites of the vascular system and may thus be useful for drug targeting to extravascular body sites.

When comparing the properties of small triglyceride nanoparticles, in particular from monoacid triglycerides, to those of larger nanoparticles, an unusual phenomenon is observed upon heating in the differential scanning calorimeter: with decreasing particle size, the melting transition of the β polymorph broadens and shifts to lower temperatures. While in coarser colloidal particles the particles melt in a single event, additional sharp transitions at lower temperatures appear in the melting curve of smaller triglyceride nanoparticles (Fig. 6). Although a decrease in the melting temperature with particle size is expected for colloidally dispersed material the occurrence of several discrete melting transitions is surprising, at least at first glance. Since the triglyceride nanoparticles are prepared via the emulsified state, a broad melting transition reflecting melting of particles with the size distribution of the former emulsion particles would be expected rather than the occurrence of a number of discrete transitions. Intensive investigations of this phenomenon have led to the conclusion that the structured melting event results from the plateletlike shape and the layered structure of crystalline triglyceride nanoparticles (Fig. 1). The shape of the nanoparticles prepared from saturated monoacid triglycerides, such as tripalmitin or trimyristin, resembles those of triglyceride

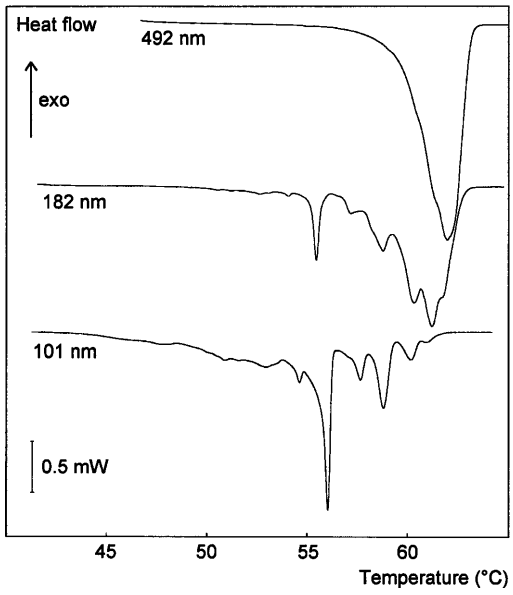

Fig. 6 DSC melting curves (scan rate 0.02 °C/min) of tripalmitin dispersions (stabilized with tyloxapol [23] with the denoted PSC *z*-average diameters

single crystals. The triglyceride molecules are arranged in layers parallel to the large (0 0 1) face of the plateletlike crystals. The thickness of the nanoplatelets can only change in steps corresponding to the thickness of one triglyceride layer. With the help of X-ray lineshape analysis it has been shown that the different melting transitions correspond to the melting of particle fractions with different platelet thickness [23, 46].

From the pharmaceutical point of view, the complex melting behavior of triglyceride nanoparticles in the lower colloidal size range is of great importance for several reasons. If comparatively low-melting-point triglycerides are processed into very small colloidal particles the melting temperature may be depressed below body temperature and the particles will lose the advantages of a solid matrix upon administration. Moreover, melting of a fraction of the nanoparticles during storage may lead to stability problems since repeated melting and recrystallization could result in instabilities such as particle growth, gel formation, and expulsion of incorporated drugs. On the other hand, the structured melting behavior may allow the development of carrier systems with specific drug release properties by utilizing the different drug release rates expected for solid and liquid (molten) particles. Particles with melting temperatures slightly above body temperature could be forced to release their drug load at certain body sites if the temperature is raised above the melting temperature of the particles at these sites, for example, by external warming. While the use of particles with one sharp melting transition would lead to a comparatively sudden

release of the whole drug load in the vicinity of the heated body site, administration of a dispersion with several discrete melting transitions could provide extended drug release when several warming steps are applied.

Conclusions

A variety of unexpected phenomena are observed during the development process of colloidal drug carrier dispersions based on solid triglycerides. They arise from the colloidal size of the triglycerides in combination with their crystalline state and are expressed, for example, in terms of stability, melting, and crystallization behavior. Typical instability phenomena are gel formation (spontaneously or by shear forces; time- and temperature-dependent with certain emulsifier compositions), the formation of coarse particles, or expulsion of the drug from the carrier. All instability phenomena can be highly retarded and are specific for a certain composition. The examples given here illustrate that it is very risky and often impossible to predict the behavior of the colloidal state from that of the bulk material. Even within the colloidal size range, the properties of triglyceride nanoparticles may vary distinctly depending on the particle size. The examples presented here demonstrate that the transfer of experiences with one colloidal system (e.g., a triglyceride emulsion) to another, apparently closely related one (e.g., a triglyceride suspension) may not be as obvious as it seems and has thus to be done very carefully.

The estimation of drug distribution is difficult for dispersions consisting of more than one type of colloidal particle. Depending on the type of stabilizer and on the concentration ratio of stabilizer to matrix material significant numbers of particles such as liposomes and/or (mixed) micelles may coexist with the expected type of particles.

During the development process of colloidal carriers a detailed evaluation of the colloid properties is required to avoid complications. The increase in basic knowledge that can be obtained by careful physicochemical characterization can provide the basis for the scientific forecast of perspectives and potentials of novel drug delivery systems, since the specific properties of colloidally dispersed materials may not only pose difficulties during the development process but may also offer fascinating, unexpected new possibilities for the development of novel types of drug administration systems, such as nanospheres of supercooled melts.

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