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Lecithin-based nanoemulsions

V. Klang, C. Valenta*

Department of Pharmaceutical Technology and Biopharmaceutics, Faculty of Life Sciences, Althanstraße 14, 1090 Vienna, Austria
*Correspondence: claudia.valenta@univie.ac.at

Lipid-based emulsions with particle sizes in the submicron range have been extensively investigated as vehicles for various active agents during the last decades. This review summarizes the current knowledge about nanoemulsions with a special focus on nanoemulsions comprising lecithin as the main emulsifier. A short introduction is given on the origin of lecithin-based nanoscale emulsions and the properties of these metastable systems. The differences between nanoemulsions and microemulsion phases are highlighted and recent discrepancies in terminology are discussed. Furthermore, the peculiarities of lecithin as emulsifying agent are presented. The production and optimization of nanoemulsions as well as the controversy concerning the different proposed production methods is another important aspect that is covered in detail. Moreover, general information about the stability and characterization of lecithin-based nanoemulsions is included. The last part of the review deals with current applications and research focuses with emphasis on the application of lecithin-based nanoemulsions on skin.

Key words: Nanoemulsion – Submicron emulsion – Lecithin – Phospholipids – Lipid-based drug delivery.

I. INTRODUCTION

1. Overview

Nanotechnology is increasingly employed for pharmaceutical or cosmetic applications. The design of lipid-based drug delivery systems aims at optimizing specific strategies in the treatment, prevention or diagnosis of diseases. These strategies include efficient solubilization of active substances, protection and enhanced delivery of sensitive compounds, drug targeting and controlled release of active ingredients [1]. However, the commercialization of nanosized lipid drug delivery vehicles is confronted with certain obstacles. First of all, the preparation of many nano-carriers involves the use of large amounts of surfactants or solvents which might be harmful upon administration. Secondly, an important pre-requisite for the successful marketing of a new carrier system is ease of production and the potential for up-scaling. For colloidal emulsion systems, another factor affecting their commercialization is their potential lack of stability [2]. All of these requirements are met by lecithin-based nanoemulsions which can be created from well-tolerated excipients. Moreover, they can be produced on a large scale in reproducible quality. However, the number of marketed lecithin-based nanoemulsions is limited, mostly to the fields of parenteral nutrition or drug delivery, dermal drug delivery and cosmetics.

This review aims to give a consistent overview about the development of these formulations during the last decades. A thorough introduction on the origin of lecithin-based nanoemulsions is given. The characteristics of these systems are summarized and a comparison with microemulsion systems serves to explain the difficulties in terminology of nano-sized colloidal systems. In addition, different methods of nanoemulsion production are explained and the current dissent about the validity of low-energy emulsification methods in this context is demonstrated. Subsequently, the adaptation of these submicron-sized carriers for various routes of drug delivery is summarized. Since the dermal application of nanoemulsions has been intensively investigated throughout the last years, an emphasis is placed on this field of application. Overall, this article aims to inform the reader about the basics of nanoemulsion technology and thus stimulate further research in this area. The general focus is on practical aspects which are essential for the successful production of lecithin-based nanoemulsions. The principles of lecithin self-assembly and nanoemulsion formation are only briefly presented as comprehensive reviews on these topics can be found in the literature [3, 4].

2. Historical development, terminology and use of submicron-sized emulsions

Emulsification of two immiscible liquids with the help of emulsifying agents can lead to various emulsion droplet sizes according to the components and processing conditions [5]. Coarse macroemulsions have been known for a long time and have found various applications in fields such as pharmaceutical technology, cosmetics and the food industry. The development of new technologies in emulsion processing and new insights into the basic principles of emulsion formation have led to remarkable progress in this field. Finely dispersed emulsions of oil in water with mean droplet diameters in the submicron range were first introduced to parenteral nutrition during the 1920s [6-8]. These early lipid-based formulations consisted of vegetable oils which were dispersed in a mixture of water and glycerol with the help of phospholipids [6]. Optimized formulations devoid of any adverse reactions have been available for clinical nutrition since the 1970s [9].

It was soon discovered that these submicron-sized emulsions were suitable as parenteral delivery systems for lipophilic active substances, which led to the development of numerous formulations [10-13]. Therefore, submicron emulsions were soon investigated in other potential fields of application, such as dermal, ocular, oral or rectal administration of drugs [14-24]. The term nanoemulsion must have emerged during this intensive research period and was originally applied for emulsions with droplet sizes below 100 nm, in analogy to nanoparticles with the same size. This term has gained in popularity ever since and has been applied to a myriad of submicron-sized formulations although not all of these systems fulfill the requirements of the original convention. Nanoemulsions in the original sense of the word were almost per definition stabilized by natural lecithin mixtures [25]. However, this specific understanding of mostly dermatological and cosmetic formulations did not prevail. Today, the term nanoemulsion is frequently applied to all kinds of metastable colloidal dispersions which exhibit droplet sizes in the submicron range.

Overall, the terminology of submicron-sized emulsions is a controversial field. A remarkable amount of research deals with the development and investigation of nanoemulsion or submicron emulsion systems [15, 18, 26-32]. A thorough literature research, however, represents a certain challenge as the general terminology is not consistent among authors [3, 33]. This review attempts to clarify established expressions in context with submicron-sized emulsions and microemulsions and to find a solution that is generally acceptable. Emulsions are inherently unstable dispersions of at least one liquid phase in another immiscible liquid phase. Such systems are produced through energy input by mechanical shear and are kinetically stabilized with the help of surfactants, polymers or other stabilizing agents. The addition of amphiphilic surface-active molecules serves to lower the interfacial tension between the immiscible liquid phases, which usually consist of oil and water. However, the thermodynamical instability will eventually lead to complete separation of the two phases [3, 14, 34]. Nanoemulsions, being conventional emulsions with droplet sizes in the lower submicron range, are no exception.

According to the components used, different types of emulsion systems can be produced. Emulsions of oil droplets that are surrounded by an aqueous bulk phase are called oil-in-water emulsions (o/w emulsions). Conversely, if water droplets are surrounded by an oily bulk phase, the system is termed a water-in-oil emulsion (w/o emulsions) [5, 14]. The factors that determine the emulsion type and properties include its composition, the chemical structures of both oil and surfactants as well as processing conditions [5].

The selection of appropriate surfactants is essential for emulsion formation and stability. Various studies have shown that combinations of surfactants can lead to emulsions with enhanced stability, possibly due to tighter molecular packing at the oil/water interface [5]. In order to identify suitable surfactant combinations the hydrophilic/lipophilic balance (HLB) values of individual emulsifiers can be employed. The HLB scale has been created to classify nonionic surfactants according to their emulsifying properties, which are related to their molecular structure. Different ratios of hydrophilic and lipophilic molecule parts of amphiphiles lead to different solubility and different behaviour at the oil/water interface [5]. Although the classification of lecithins in the HLB system is scientifically problematic, it is still a valuable support for the choice of phospholipid mixtures to obtain satisfying emulsion stability [35].

The stability of the produced emulsion is dependent on several factors, including its composition as well as its droplet size. Since the droplet size of conventional macroemulsions is larger than one micron, the droplets are susceptible to gravity forces [34]. In this context, the droplet size distribution is of great importance, which can, along with other factors,be influenced by different methods of preparation [34]. Emulsions which exhibit droplet diameters below one micron, but usually above the size range of microemulsion phases are conventionally developed by high-energy emulsification methods. These emulsions are termed submicron emulsions [27, 36], mini-emulsions [37], ultrafine emulsions [20, 34], translucent emulsions [34] and nanoemulsions [26, 38, 39]. If the droplet size of these emulsions is small enough, they may appear translucent (*Figure 1*). These nanoscale droplets can be kept suspended for long periods of time since Brownian motion largely prevents gravitationally driven sedimentation or creaming [3, 34]. Therefore, such nanoemulsions exhibit enhanced physico-chemical stability, often cited as approaching thermodynamic stability [40-42]. Conventional o/w nanoemulsions are well-investigated systems and have been studied and reviewed thoroughly [3, 12, 20, 36, 40, 43, 44], while inverse w/o nanoemulsions are less frequently investigated [45-47]. Since o/w nanoemulsions are by far more frequently employed for most routes of delivery, the focus of this review will remain with them. From a strictly technical viewpoint, nanoemulsions are emulsions of two immiscible liquids with a mean droplet diameter below 100 nm [3, 39, 43, 46, 48, 49]. Considering the present convention for nanoscale materials, which describes them as materials having lenght scales in the range from 1 to 100 nm, this appears reasonable [3]. However, the prefix nano is an appealing term that is frequently used in publications. Over the course of time, the expression nanoemulsion has widely replaced the term submicron emulsion, even though these expressions are not forcibly synonyms. In exact terms, nanoemulsions represent the extreme lower limit of the submicron scale and are therefore a special form of submicron emulsions [3].

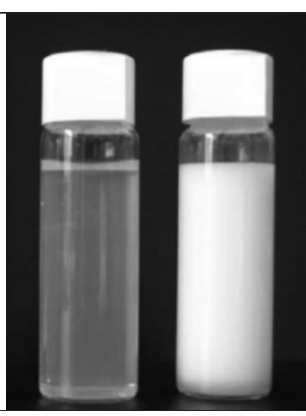

Figure 1 - Optical appearance of a nanoemulsion (on the left, droplet diameters around 35 nm) and a macroemulsion (on the right, droplet size around 1,000 nm). Picture reprinted from reference [20] with permission from Elsevier.

Therefore, metastable emulsions with particle sizes below one micron can generally be referred to as submicron emulsions. If an emulsion exhibits particle sizes below 100 nm, the term nanoemulsion would be appropriate. However, since this term has been adopted by the scientific community for a large number of emulsion formulations which do not reach particle sizes below 100 nm, it is safe to assume that it will prevail over time. For reasons of clarity, lecithin-based submicron emulsions will be further referred to as nanoemulsions within the scope of this review, even if their particle size exceeds 100 nm.

Nanoemulsions as colloidal drug delivery systems are stabilized by surface active agents; however, the amount of surfactant is usually very low. Successful formation of nanoemulsions with appropriate lecithin mixtures is possible with surfactant concentrations as little as 1.5 % [50]. Although nanoemulsions can be regarded as normal emulsion systems in a nanoscale size range, they differ from their microscale counterparts in several distinct properties which will be briefly summarized for lecithin-based nanoemulsions at a later point. Nanoemulsions offer distinct advantages as drug delivery systems, such as a high solubilizing capacity for lipophilic drugs [6]. From a practical viewpoint, nanoemulsions are advantageous formulations because they can be produced in large scales with good reproducibility [51]. The final emulsions can often be sterilized. Moreover, drugs which are susceptible to hydrolysis can be stabilized by incorporation in nanoemulsions [6] and co-administration of drugs is possible [51]. The use of nanoemulsions for controlled and retarded drug delivery is under constant investigation. In this respect, classical nanoemulsions with small particles and low viscosity may not be ideal candidates for all routes of application [6]. However, the use of viscosity enhancers such as polymers which might slow the release of drugs might be future approaches to deal with this shortcome. Parenteral o/w nanoemulsions have already successfully been used to achieve prolonged activity of incorporated drugs as compared to drugs in solution [52]. For many investigated drugs however, a rapid release can be observed [53-55]. It is assumed that a retarded release can be achieved for very lipophilic drugs with high affinity to the oil phase [6].

The main limiting factor for the practical use of lecithin-based nanoemulsions is their potential physico-chemical instability upon admixture of drugs. Nevertheless, several drugs such as diazepam, propofol, fat soluble vitamins or amphotericin B have been successfully marketed as nanoemulsion formulations [27]. In general, nanoemulsions have been found to be rather sensitive to modifications in their composition [56]. In terms of stability and drug solubilization nanoemulsions seem inferior when compared to microemulsions. However, it should be kept in mind that each of these systems has been designed with focus on specific properties, such as superior biocompatibility in the case of lecithin-based nanoemulsions. The following chapter serves to highlight the differences between nanoemulsions and microemulsions, which each have their validity in specific fields of application.

3. Nanoemulsions versus microemulsions

As described above, nanoemulsions are metastable emulsions which can be kinetically stabilized by applying mechanical shear. The term microemulsion, however, represents a complex mixture of completely different equilibrium systems [3]. Lyotropic liquid crystalline microemulsion phases form spontaneously upon addition of a surfactant to a mixture of oil and water without applying any siginifcant shear. Usually, the presence of an additional co-surfactant or co-solvent is required [57, 58]. This spontaneous emulsification is related to the formation of equilibrium lyotropic liquid crystalline phases, in which the surface tension effectively vanishes. Droplets are formed by thermodynamic molecular self-assembly [3].

These lyotropic phases are also referred to as microemulsions, mesophases or swollen micelles [59]. They are not emulsions in the classical sense, but true self-assembled thermodynamic phases. The morphologies of such phases can be variable; planar stacks of lamellae, hexagonally packed tubes and spherical droplets have been observed [3]. These microemulsion phases can consist of spherical “droplets” or micelles of the same size range as nanoemulsions, which might lead to confusion when categorising new formulations made of oil, water and surfactant. However, a thermodynamic phase must not be confused with an emulsion system [3]. *Table I* gives an overview of the properties of nanoemulsions and microemulsions.

The historical choice of the word microemulsion to describe self-assembled phases in the nanoscale range up to 100 nm seems unfortunate, since nanoemulsion droplets are in the same size range [3]. In many cases, microemulsions exhibit even lower droplet sizes of between 10 and 50 nm [49]. A myriad of recent publications deals with new nanoemulsion systems for the delivery of lipophilic drugs. However, some researchers fail to recognize the difference between nanoemulsions and microemulsions while others use terminology irrespective of historical and conventional terms. Some authors are determined to establish the term nanoemulsion instead of microemulsion for thermodynamically stable, spontaneously formed transparent mixtures of oil, water and surfactant/co-surfactant mixtures in large amounts of 20 to 50 % [33, 60]. In terms of the droplet size range, their claim to use the expression nanoemulsion for droplet sizes below 100 nm rather than microemulsion may be justified. However, they fail to acknowledge that the lyotropic liquid crystalline mesophases in question are not real emulsion systems. Therefore, the expression nanoemulsion for these stable systems would be just as misleading. A compromise which might assist in solving this issue would be to abolish the term microemulsion in favor of mesophases or other appropriate terminology. In any case, the differences between emulsions and thermodynamically stable mesophases should be respected in the nomenclature of semisolid lipid-based drug delivery systems. Otherwise, the inconsistencies in terminology will render the literature inquiry for nanoemulsions an increasingly time-consuming quest.

It must again be pointed out that the distinguishing difference between equilibrium microemulsions and metastable emulsions is not one of composition, but rather of thermodynamics. The surface tension between oil and water phases in normal metastable emulsions is generally quite high. In nanoemulsions, this surface tension is reduced by means of energy input. In microemulsions, however, surface tension does not play a significant role [3]. These points are of importance when considering the large number of recent publications dealing with low-energy emulsification methods to produce nanoemulsions [20, 34, 47, 61-65]. Although these methods offer interesting possibilities for formulation development without the use of possibility cost-intensive equipment, the argumentation in terms of the thermodynamic forces governing these emulsification processes appears slightly inconsistent. While both interface instability [64] or changes in surfactant solubility [20] are mentioned to be involved in the emulsification process, the formed systems cannot definitely be differentiated from microemulsion phases. Moreover, lipid-based systems created by low-energy emulsification methods are increasingly investigated with the aid of ternary or pseudo-ternary phase diagrams in order to identify different regions of produced formulations [47, 61, 66]. This strategy was formerly reserved for investigations of microemulsion systems. The exact mechanisms leading to spontaneous emulsification are often poorly defined, as is the nature and extent of spontaneous emulsification itself and the obtained formulations [42].

Different opinions on this issue can be found [3, 20]. Mason *et al.* [3] state that it is inappropriate to use a ternary phase diagram which comprises both nanoemulsions and microemulsions, since their formation is an entirely different one. The authors argue that due to the thermodynamic distinctions between the two systems, a lyotropic liquid crystalline phase cannot be converted into a nanoemulsion system just by varying the temperature. According to these authors, a reduction of the surface tension in the range from 10^{-2} to 10^{-5} mN m^{-1} cannot be achieved without applying shear. Systems exhibiting such low surface tensions can basically be considered microemulsion phases according to the original conventional definition [67] and will eventually convert back into other types of microemulsion phases [3]. Indeed, recent findings indicate that the interfacial tension at nanosized o/w emulsion droplets is hardly influenced by addition of surfactant at all [68]. Microemulsions, on the contrary, exhibit ultra-low interfacial tension [69]. The originally high surface tension of nanoemulsions can only be slightly reduced by applying extreme shear to rupture

Table I - Overview of the differences between nanoemulsions and microemulsions (adapted from [6]).

Properties	Nanoemulsion	Microemulsion
Alternative terminology in literature	submicron emulsion, miniemulsion, translucent emulsion, ultrafine emulsion, lipid emulsion	mesophases, swollen micelles or micellar solutions, lyotropic liquid crystalline phase, critical solution, “true” nanoemulsion
Classical terminology	submicron emulsion	swollen micellar solution
Popular terminology	nanoemulsion	microemulsion
Particle size	150-300 nm (< 1,000 nm)	10-100 nm
Optical appearance	milky white to translucent	transparent
Formation	high-energy input	spontaneous
Stabilisation	kinetically stable	thermodynamically stable
Surfactant concentration	low (1.2-5 %)	high (20-50 %)
Co-surfactant/co-solvent	not compulsory	usually compulsory
Microstructure	static	dynamic
Optical isotropy	anisotropic	isotropic
Interfacial tension	~ 50 mN m^{-1}	~ 0 mN m^{-1}

droplets. Due to the large surface tension, lyotropic self-assembly is not involved in the emulsification process of classical nanoemulsions [3].

The above mentioned aspects are discussed in another review dealing with the phenomenon of self-emulsification [70]. The author states that nanosized oil droplets produced by the PIT method have a lamellar liquid crystalline phase on their surfaces, therefore approaching microemulsion systems. This lamellar layer prevents coalescence and the initial surfactant concentration required to form small droplets is simultaneously reduced. These systems are generally less stable and often exhibit large particle sizes [70]. Their polydispersity leads to higher rates of Ostwald ripening when compared to nanoemulsions prepared by high-pressure homogenization [40].

In view of these aspects, it is difficult to classify the systems produced by low-energy emulsification. Some authors have based their conclusions on the principle of excluding inappropriate terminology [9, 66]. They correctly concluded that the produced systems could not possess microemulsion status due to their thermodynamic instability. However, it is not a viable conclusion that these systems can therefore automatically be regarded as nanoemulsions. The nanoemulsions produced by spontaneous emulsification methods usually comprise a higher amount of surfactant than nanoemulsions created by high shear, frequently even accompanied by a co-surfactant [42, 61, 66]. These systems therefore either represent or approach the status of microemulsion systems; in the latter case, they do not possess the same astounding thermodynamic stability. Such a hybrid system that combines the disadvantages of both microemulsions and nanoemulsions, namely high surfactant concentrations and metastability, can hardly be considered a desirable product. These hybrid formulations can apparently be produced by either high or low energy emulsification methods if sufficient amounts of surfactant and co-surfactant are present [9].

In consideration of the controversial nature of the colloidal nanosized o/w systems created by low-energy emulsification methods, the focus of this review will remain with the classical nanoemulsions produced by high-shear methods. However, the active discussion on this topic offers new prospects and raises questions of interest. Since microemulsions containing large amounts of surfactant are likely to achieve superior skin permeation of active molecules in comparison to most conventional emulsion systems and even nanoemulsions, it would be worth investigating how the hybrid nanoemulsions perform in this respect. A direct comparison of skin permeation rates achieved with conventionally produced nanoemulsions and emulsions formed by spontaneous emulsification methods would be of interest in order to determine potential differences in microstructure and properties.

However, the discussed issues might soon have to be considered in an entirely new light. Recent investigations indicate that surfactant molecules do not decrease interfacial tension in nano-sized oil droplets in water as much as expected [68]. These surprising results were obtained by vibrational sum frequency scattering experiments of mixtures of n-hexadecane oil droplets stabilized by sodium dodecyl sulfate (SDS). The adsorption of SDS onto the nanodroplets with an average radius of 83 nm was directly determined in these measurements. It was found that the interfacial density of adsorbed SDS molecules was at least one order of magnitude lower than the density at a corresponding planar interface. The achieved maximum decrease in interfacial tension for the nano-sized oil droplets was only 5 mN/m as opposed to 42 mN/m on planar interfaces [68]. These highly interesting findings indicate that surfactant molecules work differently at planar interfaces and emulsion droplets. Surfactants strongly reduce the interfacial tension between oil and water at a planar interface which previously served as a model for interfacial tension measurements. This is apparently not the case at the corresponding interface of nano-sized droplets. The data are in agreement with zeta potential measurements and might even explain the discrepancy between measured and calculated Ostwald ripening rates. These findings once again highlight the difference between kinetically stabilized emulsions, comprising nanoemulsions, and thermodynamically stable microemulsions. As opposed to the presented results for nanoemulsions, the surface tension in microemulsions is reduced by orders of magnitude [68]. It therefore remains to be investigated which mechanisms govern emulsion droplet stabilization in nanoemulsions.

II. LECITHIN AS EMULSIFYING AGENT

1. Definition, natural occurrence and properties of lecithin

Lecithin is perhaps the most widely used natural emulsifying agent and has been used in the food industry for centuries. Nowadays, it is likewise used in pharmacy, cosmetics and biotechnology as an effective dispersive and emulsifying agent [71-73]. The term lecithin is the commonly used expression for a mixture of phospholipids with adherent glycolipids and oil. As such it will be used within the scope of this review. From a chemical viewpoint, however, the name lecithin refers exclusively to sn-3-phosphatidylcholine [4, 35, 73].

Lecithins are naturally occurring amphiphiles which are major components of cell membranes of plants and animals. They are mixtures of phosphatides or phospholipids and have important functions in growth and functioning of cells [73, 74]. The most common phospholipids in lecithin mixtures are phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI), phosphatidylserine (PS), sphingomyelin, lysophospholipids and other glycerol phospholipids of complex fatty acid composition [14, 73]. By far the most abundant phospholipid is phosphatidylcholine; hence the name lecithin was commonly adopted for the substance itself as well as for mixtures of phosphatidylcholine with related phospholipids.

The polar structure of the lecithin molecules renders them useful emulsifying agents (*Figure 2*). Lipophilic side chains of esterified fatty acids are bound to the polar head group. The phosphates and nitrogen-containing moieties can be ionised, thereby representing negatively and positively charged residues, respectively. This allows for aqueous interactions, which form a mechanical barrier [14].

Although natural lecithins as classical emulsifying agents in nanoemulsions have frequently been replaced or combined with synthetic surfactants and other stabilizing additives, they are still the emulsifying agents of choice in terms of biocompatibility [6]. Natural lecithins are among the safest emulsifying agents on the market and the incidence of allergic reactions to lecithin is very rare [51, 74]. Both natural and enzyme-modified lecithins possess regulatory GRAS status [73] and can thus be safely employed in the development of lipid nanoemulsions even for sensitive routes of application [14, 43].

Lecithin mixtures can be obtained from various companies which produce a large variety of products. Such lecithin products can be derived from both animal and plant sources. Animal sources comprise milk, brain or egg yolk, of which only the latter is of practical importance [73, 75]. The more commonly used vegetable lecithins are obtained

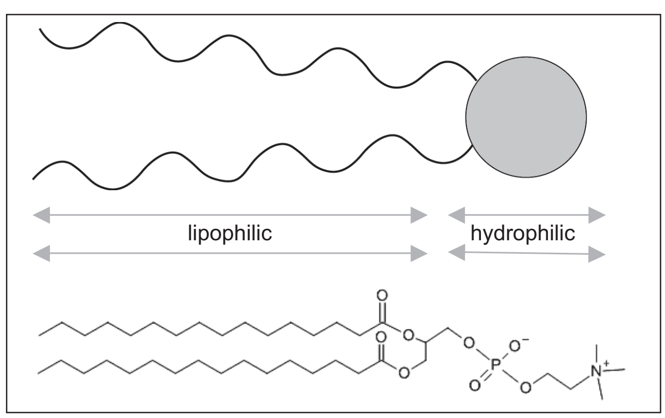

Figure 2 - Schematic structure of a phospholipid.

from oil-bearing seeds such as soybeans, sunflower kernels and rapeseed. Soybean oil has been the primary industrial source of vegetable lecithin for many years [4, 73, 76]. Pharmaceutically employed lecithin mixtures are usually derived from egg yolk or soybeans. Detailed information about lecithin processing, including isolation, modification and purification of phospholipids, can be found in the respective literature [35, 73, 77]. Apart from natural lecithin mixtures modified lecithins with specific emulsifying properties are commercially available as well. Such modifications can comprise enzymatic or chemical adaptation as well as fractionation of phospholipid molecules [35, 73].

2. Self-assembly of lecithin and phospholipid structures

A remarkable property of phospholipids is their ability to form various self-organising structures in solution, such as micelles, lyotropic liquid crystals, swollen micelles or microemulsions, emulsions, organogels or liposomes [4, 7]. Phospholipid systems comprising mixtures of phosphatidylcholine derivatives with short and long hydrocarbon chains therefore show high morphological diversity. This diversity can be caused by differences in hydration, temperature and composition of the mixtures [78]. A large body of experimental data about the structural organisation of lecithin in various media exists. The amphiphilic lecithin molecules do not undergo an abrupt transition from the solid to the fluid state; the observed intermediate states are referred to as mesophases or liquid crystals. Lamellar, hexagonal or micellar phases are examples of the various structures that can emerge through changes in temperature or hydration [7]. A detailed elucidation of the self-organisation behaviour of lecithin at o/w and w/o interfaces has been conducted by Shchipunov [4]. This article focuses on the behaviour of lecithins at the interfacial region of o/w nanoemulsions.

Phospholipids behave differently at an o/w interface according to their chemical structure [35]. PC forms lamellar structures with well-ordered mono- and bilayers and is therefore highly suitable for emulsion stabilization. In addition, liposomal structures can be formed. PE forms reversed hexagonal phases while the more hydrophilic lysophospholipids are known to form hexagonal widespread clusters. A fundamental understanding of the interfacial behaviour of different phospholipids can be used to create optimized lecithin mixtures for the production of nanoemulsions [35, 73].

3. Lecithin-based systems in drug delivery

In pharmaceutical products, natural lecithin mixtures as well as purified phospholipids and semi-synthetic or synthetic phospholipid derivatives are increasingly used as excipients. The use of natural lecithins in colloidal lipid-based vehicles is not restricted to nanoemulsion systems. Lecithins have also been employed to create microemulsions with improved skin-friendliness [69, 74]. Likewise, purified lecithins are frequently used in the development of vesicular delivery systems such as mixed micelles or liposomes [49, 79]. However, not all lecithin products are suitable for the production of any given delivery system. The success of formulation development will depend largely on the choice of components.

Phospholipid-stabilized colloidal formulations are used to efficiently solubilize and incorporate drugs. *Figure 3* shows the different structures of liposomes, nanoemulsions and solid lipid nanoparticles. Active substances can be incorporated in the core of these phospholipid aggregates or within the phospholipid layers. Apart from their function as an emulsifying agent, lecithins therefore serve to improve the solubilization of drugs, to reduce toxicity and to increase absorption [73, 80]. In lecithin-based nanoemulsions the solubility of lipophilic compounds with log P values over 2.7 is mainly dependent on the oil phase while less lipophilic compounds are primarily solubilized by phospholipids [80].

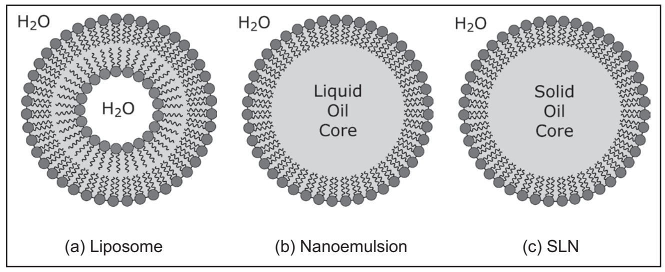

Figure 3 - The structure of nano-sized colloidal systems differs for liposomes (a), nanoemulsion droplets (b) and solid lipid nanoparticles (SLN, c) adapted from [25].

III. LECITHIN-BASED NANOEMULSIONS

1. Lecithin as emulsifier in submicron-sized emulsions

The first nanoemulsions used for parenteral nutrition and subsequently parenteral administration of lipophilic drugs were exclusively based on lecithin [6-8]. It was soon realized that these systems were suitable as vehicles for various other routes of drug delivery. The type of surfactant used was varied and a large number of natural or synthetic emulsifiers were investigated as main or co-surfactant to produce nanoemulsions with optimized properties. In general, lecithin-based nanoemulsions are highly fluid and may appear translucent or almost transparent if their droplets are small enough. They might even appear slightly bluish due to Rayleigh scattering [3]. Instability or microbial contamination can easily be detected in such translucent systems. When the droplet radius exceeds 100 nm, nanoemulsions appear hazy or white due to significant multiple scattering of light. The physical stability and shelf life of nanoemulsions is superior to that of macroscopic emulsions [3, 34].

2. Physical stability

In every emulsion system, the reservoir of excess free energy in the interfacial region results in an overall free energy that is well above the global minimum of the system. Such a colloidal system cannot be formed by spontaneous dispersion; it is thermodynamically unstable, and any apparent stability must be regarded as a purely kinetic phenomenon [81]. Following the second law of thermodynamics, the system will tend towards the lowest possible energy level. Therefore, destabilization phenomena occur over the course of time. The inherent instability of conventional macroemulsions will eventually lead to phase separation caused by processes such as aggregation, flocculation, creaming, sedimentation and coalescence [5, 35, 81].

Aggregation and flocculation are caused by collisions of particles. If dispersed single droplets adhere to each other due to attractive forces such as van der Waals interactions and repulsive forces are overcome, flocculation occurs. If such flocculated droplets merge due to film rupturing, coalescence takes place. Creaming and sedimentation, however, result from the different densities of the phases. Creaming occurs when droplets with larger size, possibly caused by coalescence, rise towards to emulsion surface. This in turn causes increased droplet collisions. If droplets become sufficiently large and free oil is formed through fusion of separate droplets, coalescence and finally cracking or breaking of the emulsion is observed [14]. Droplet coalescence is the normal and irreversible way of an emulsion to coarsen, thereby reducing the amount of Gibbs free energy by reduction of the interfacial area and leading to eventual phase separation [5, 35, 81]. In contrast, flocculation and creaming are reversible phenomena where the numbers of droplets as well as their size remain unchanged. However, the distribution of the droplets becomes inhomogeneous. This can be reversed by gentle agitation [7, 14]. The possibility of small degrees of creaming in commercial nanoemulsions is the reason for the recommendation to shake them by hand before use [14].

Another mechanism of emulsion destabilization can occur when droplet sizes are small and a certain solubility of the dispersed phase in the continuous phase is given. This phenomenon is called Ostwald ripening or molecular diffusion [3, 81]. An increase in solubility will render the small droplets thermodynamically unstable in respect to the larger ones. Diffusion of small dispersed phase droplets to larger ones occurs due to differences in solubility and interfacial LaPlace pressure. Therefore, large droplets will grow larger at the expense of small droplets and droplet sizes increase [31, 81]. Ostwald ripening occurs within the size range of 100 to 500 nm and below. It represents no threat to the stability of macroemulsion with large droplets where the rate of Ostwald ripening is very slow. Therefore, this phenomenon is a major problem only in submicron-sized emulsions when the dispersed phase has significant water solubility. The ripening process might proceed rapidly under these conditions [81].

The most accurate theory for calculating the rate of Ostwald ripening was independently established by Lifshitz and Slezov as well as by Wagner and is therefore named the Liftshitz-Slezov-Wagner (LSW) theory. It provides a useful tool for estimating the rate of Ostwald ripening in nanoemulsions, which is assumed to be diffusion-controlled. The ripening rate eventually reaches a stationary state with linear increase of the cube of the mean particle radius, r^3, with time. The rate-limiting step of droplet growth is the diffusion of the dispersed phase through the bulk phase while the passage of the solute across the interface is not hindered by any barrier [81]. According to the LSW theory, the ripening rate is directly proportional to the solubility of the dispersed phase in the continuous phase. This has been confirmed by experimental data [81]. However, certain discrepancies between calculated and measured values of Ostwald ripening rates are commonly observed. Henry *et al*. reported that the experimentally determined ripening rates for Tween-type surfactants were up to 40 times higher than the calculated rates, possibly due to micelle-mediated oil transport [48]. On the one hand, certain limitations of the LSW theory might be due to its origin, as it was established for ripening in a solid matrix [81]. On the other hand, this phenomenon could possibly be explained by recent findings concerning the behaviour of surfactants at the interface of nanoscopic oil droplets, as suggested by de Aguiar *et al*. [68].

In classical lecithin-based nanoemulsions, Ostwald ripening is reported to be of minor importance, especially in combination with highly apolar oils [5, 7]. Good physical stability is obtained by employment of lecithin as an emulsifier, which is remarkably insoluble in water, and the choice of an oil with a distinctly low water solubility. In this fashion, diffusion of the emulsified oil droplets through the aqueous medium is limited [5, 7]. However, numerous nanoemulsion formulations have been created and adapted for various applications. The choice of oil and surfactant is no longer confined to the classic excipients. Therefore, Ostwald ripening can be a limitation for the stability of reformulated lecithin-based nanoemulsions. Consequently, the optimization of formulation properties is of critical importance, especially in newly designed multicomponent systems. The following section deals with the various approaches which have been found to successfully enhance the stability of such formulations.

3. Optimization of physical stability

In order to develop nanoemulsions with prolonged physical stability, multiple mechanisms of stabilization should be employed (*Figure 4*). Apart from choosing a suitable main emulsifier, additional stabilizing agents can be incorporated. Improved stabilization of nanodroplets is in most cases achieved by means of electrochemical or steric stabilization [35], as will be detailed in the following chapter. Electrostatic repulsion can be achieved by introducing a high surface charge on droplets, which will consequently repel each other according to the DLVO-theory. Steric stabilization requires polymers or voluminous surfactants. Particle-induced stabilization is less frequently employed in lecithin-based nanoemulsions. An interesting approach in this context

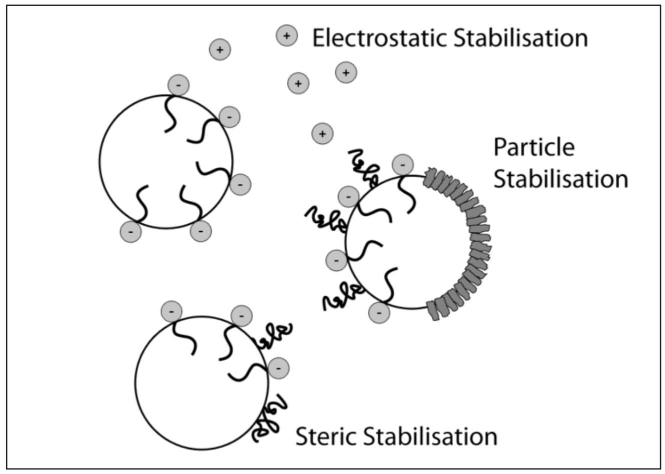

Figure 4 - Principles of emulsion stabilisation adapted from [35].

was recently conducted by Eskandar *et al*., who combined surface-active agents and silica nanoparticles as stabilizing agents [82, 83].

It is well-established that the emulsification properties of PC alone are not sufficient to form nanoemulsions with satisfying long-term stability [4, 14]. The lecithin molecules can stabilize emulsions by forming a multilamellar shell around each droplet, which acts as an effective structural-mechanical barrier. Thus, coalescence is prevented [4]. However, PC molecules do not possess a sufficiently high electrical charge which they can bring upon the droplet surface. Therefore, electrostatic stabilization between emulsion droplets stabilized with PC alone is rather poor, as uncharged nanodroplets will tend to collide and eventually coalesce [4, 14].

This can be improved by the introduction of ionogenic additives. Conveniently, natural lecithin mixtures can be employed, which contain other phospholipids able to acquire an electrical charge. These include phosphatidic acid, phosphatidylserine, phosphatidylinositol and small amounts of free fatty acids. Emulsions stabilized with mixtures of phospholipids exhibit a significant increase in resistance against flocculation since an electrostatic repulsion between the droplets favors long-term stability [4, 14]. The droplet surface charge of such nanoemulsions is usually in the negative range due to the presence of negatively charged phospholipids, especially the free fatty acids and phosphatidic acid [14, 44, 64].

Apart from charge-induced stabilization, phospholipid mixtures also lead to improved molecular packing at the interface. Nanoemulsion stability can be improved by modifying the dimensionless packing parameter S_p. The packing geometry of a molecule at the interface represents the ratio between hydrocarbon volume, optimum head group area and tail length. Lecithin has an S_p close to unity. Its molecular geometry is therefore not perfectly suited for the formation of curved surfaces [4, 56]. Surfactants with a large polar head region and single hydrocarbon chains diminish S_p. This promotes the formation of o/w emulsions. Lysolecithin, a neutral lecithin derivative with only one fatty acid residue, can be used in this fashion to stabilize o/w emulsions in combination with PC [4].

Various investigations demonstrate the importance of phospholipid composition in emulsion formation [73]. Systematic studies about the effects of different phospholipid derivatives on emulsion formation and stability were conducted by Rydhag and Wilton [84]. It was shown that the presence of PE and PI enhanced the swelling of the lamellar liquid crystalline phase of phosphatidylcholine, thus stabilizing o/w emulsions. The different hydration behaviour of differently charged phospholipid head groups causes different molecular head group space requirement and packing behaviour at the interface [7]. Therefore, commercially available lecithin mixtures of PC with its naturally occurring by-products might sufficiently serve to stabilize nanoemulsions, as already investigated [39]. By careful selection of appropriate phospholipid mixtures or derivatives the nanoemulsion composition can be kept as biocompatible as possible.

These aspects explain the widespread use of additional surface active agents for production of lecithin-based nanoemulsions. The packing parameter can be reduced by addition of more hydrophilic surfactants, thus allowing the interfacial film more flexibility to take up the curvature of nano-sized droplets [56]. Therefore, additional surfactants such as Tween 80 [18, 26] or poloxamer [85] are frequently incorporated both for steric stabilization and for modification of the dimensionless packing parameter. For the same reasons the nonionic polyethylene hydroxy stearate [86] has been investigated. It has to be noted, however, that only rather general estimations in changes of the dimensionless packing parameter can be made, as the successive structural rearrangements in lecithin mixtures are difficult to quantify [4]. Therefore, general predictions on the stability of newly developed formulations can hardly be made [85]. Numerous studies have investigated stability enhancement by using mixed surfactants; however, the mechanisms involved are not yet fully understood. Nevertheless, this concept is almost unexceptionally used in industrial emulsions [56].

Apart from different phospholipids, numerous other additives have been employed to produce either high positive or negative droplet surface charge of nanoemulsions. Charge-inducing additives proposed for positively charged systems include stearylamine [15, 87-89], chitosan [90, 91], oleylamine [92], cetyltrimethylammonium bromide [93] and recently phytosphingosine [18, 28, 39]. A more pronounced negative particle surface charge can be obtained by incorporating substances such as deoxycholic acid [15, 87]. These additives are frequently employed in order to create nanoemulsions with higher electrochemical stability. Although there is general agreement that increased zeta potential values lead to enhanced stability of emulsions, decreased stability was reported in some cases [94]. A possible explanation for these observations is the fact that the mechanical barrier functions of the lecithin film have not been taken into account [95].

Furthermore, a frequently employed strategy to produce nanoemulsions with enhanced physical stability is the addition of steric stabilizers. Both surface-active polymers such as hydroxypropylmethylcellulose (HPMC) or emulsifying agents with sterically voluminous groups such as Tween derivatives can be used in this context. Apart from their steric shielding effect, polymers or other gelling agents can be incorporated to increase the viscosity of nanoemulsions and thus prevent aggregation or creaming [96]. Moreover, surface-active cellulose ethers such as HPMC can even be employed to produce surfactant-free nanoemulsions with enhanced viscosity [97]. Since such formulations can be expected to yield increased interaction with various tissues and prolonged biological effect, HPMC might be an advantageous multifunctional co-stabilizing agent in classical lecithin-based nanoemulsions as well.

Another important parameter to characterize the surfactant film is the spontaneous mean curvature, H_0, which expresses the natural tendency of the monolayer to bend away from a flat geometry [52]. This parameter shows positive values for hydrophilic surfactants, which are frequently employed as co-surfactants in lecithin-based nanoemulsions. In theory, the addition of such hydrophilic surfactants, such as Brij 98 or Tween 80, can help to produce a more balanced HLB-value within the interfacial surfactant mixture, thus favoring interfacial film curvature [52]. This has been practically confirmed in numerous studies [18, 26, 52, 85]. The addition of a suitable co-emulsifier can enhance the flexibility of the interfacial film [87]. A high H_0 leads to the formation of elastic or flexible surfactant films, while a low H_0 favors the formation of a more rigid film [52]. Mixed surfactant films are more likely to exhibit the necessary rigid, yet flexible properties to form nano-sized droplets [56, 85]. Any changes in the microviscosity of the interfacial film can have significant impacts on emulsion stability [85].

It has to be noted that additional co-emulsifiers might influence the nature of the mixed interfacial film, which can have useful or disadvantageous effects. It was observed that the incorporation of stearylamine greatly reduced drug release of nalbuphine from lecithin-based nanoemulsions [52]. This was attributed to the tight packing of phospholipids with stearylamine, as the similar hydrophobic tails lead to ordered close packing which strengthens the interfacial layer and hinders drug release [52]. However, a sufficiently tight packing of the mixed interfacial film is necessary to obtain droplet stability [86]. Hydration forces and the viscoelasticity of the emulsifier film also influence the rate of coalescence [7].

In recent studies, approaches were made to employ mild, nontoxic surfactants such as sucrose esters [39, 98, 99]. These non-ionic surfactants are relatively innocuous and biodegradable [99]. Likewise, the use of cyclodextrins as additional stabilizing agents at the interface of o/w nanoemulsions has been investigated [39, 100]. These cyclic oligosaccharides can form inclusion complexes with fatty acid residues of the oil phase, thereby stabilizing the interfacial film [101]. Another unconventional approach to enhance the stability of lecithin-based nanoemulsions could theoretically be presented by the incorporation of proteins or peptides. Synergistic effects between lecithins and proteins have been reported in food emulsions [35]. Moreover, proteins such as whey protein are frequently used as main emulsifiers to stabilize food emulsions and have successfully been used to produce nanoemulsions [102]. However, the lack of experimental data in the pharmaceutical field shows that this idea has so far not been adapted to pharmaceutical lecithin-based nanoemulsions.

Apart from the discussed additives, an essential aspect in emulsion stability is the choice of the oil phase [43, 103]. The correct choice of the oil phase can actively contribute to enhanced physical stability by improving the interfacial film's properties. Free fatty acids present in various oils such as castor oil have been reported to act as co-surfactants [50, 64, 85], thereby providing an oil phase with superior properties. Moreover, the chemical stability of the oil phase has to be considered. Chemical degradation of important compounds leads to changes in the nature of the interfacial film, physical instability and subsequent breaking of the emulsion. In lecithin-based nanoemulsions, compounds at risk of oxidation are unsaturated fatty acid residues both in triglyceride and lecithin molecules [7]. Moreover, hydrolysis of triglycerides of the oil phase as well as of phospholipids may occur. The latter leads to the formation of free fatty acids, lysophospholipids and glycerophosphorylic compounds. These degradation products are more water-soluble than their original counterparts, which may influence their behaviour at the interface. Moreover, the formation of free fatty acids is responsible for the seemingly contradictory increase in negative zeta potential values observed in long-term stability studies of nanoemulsions or liposomes (unpublished results). Baker *et al.* reported that the release of small quantities of free fatty acids during heat sterilization and storage due to phospholipid and oil hydrolysis leads to increased negative zeta potential values, thereby conferring increased stability to these formulations [14]. However, this increase in zeta potential values can only be seen as partially beneficial since the formation of free fatty acids is accompanied by a decrease in pH value. This promotes further degradation through hydrolysis of triglycerides and phospholipids and destabilizes nanoemulsions [7, 14, 104]. Although the decrease in pH during long-term observation generally proceeds rather slowly, it is recommended to adjust the pH value right after production [7].

In order to optimise the chemical stability of the oil phase, preliminary stability studies and the use of antioxidants are recommended [5]. Phospholipids may likewise have different oxidative stability due to their variable fatty acid composition. Studies indicate that soybean lecithin might possess better oxidative stability than egg lecithin [105]. However, various other factors contribute to lipid oxidative stability in emulsions, such as oxygen availability and storage temperature.

Optimization of these conditions will have a greater impact on nanoemulsion stability than minor differences in lecithin properties.

The choice of a suitable oil phase is likewise important to minimize Ostwald ripening. Since the rate of Ostwald ripening in o/w nanoemulsions is directly related to the water solubility of the oil, not all oils are equally suitable for the production of nanoemulsions [31]. Moreover, the rate of Ostwald ripening largely depends on formulation properties. For perfectly monodisperse nanoemulsions, Ostwald ripening is negligible as it depends on solubility differences of droplets in accordance with their size. Therefore, narrow droplet size distributions are more resistant to Ostwald ripening [31] and should thus be among the primary goals of preliminary studies. Furthermore, it has been shown that the addition of hydrophobic excipients can decrease the rate of Ostwald ripening. Even the presence of small amounts of such ripening inhibitors can prevent droplet growth and enhance nanoemulsion stability. The lower the water solubility of this oil compound, the more effective stabilization of emulsions can be observed. The chemical stability of the ripening inhibitor is also of crucial importance for the stability of the formulation [81]. Squalene is a popular choice for inhibition of Ostwald ripening since squalene molecules are essentially insoluble in water and are thus unlikely to diffuse through the aqueous bulk phase [5]. Moreover, its high surface tension allows for the production of emulsions with smaller droplet sizes than most other commonly used oils when emulsified with PC [106].

To conclude, a better steric or electrochemical stabilization of nanoemulsions can be achieved by various additives. However, the composition of each formulation has to be investigated separately since chemical degradation or unexpected incompatibilities may occur. The stability of drug-loaded nanoemulsions can be impaired by incorporation of normally beneficial additives such as polymers or gelling agents [43, 104].

4. Optimization of chemical stability of drugs

The chemical stability of the incorporated drugs can be modified according to their partitioning behaviour. Drugs that are susceptible to aqueous hydrolysis can be incorporated into the oil core and therefore protected from their metabolic fate [5, 52, 107]. Moreover, incorporation of drugs into the interfacial phospholipid bilayer was found to increase the chemical stability of prednicarbate [28]. Interestingly, the partitioning of the drug seemed to be correlated to the homogenization process, as explained in *Figure 5*. Increased temperature and a higher number of cycles led to increased chemical stability of the drug even at low pressure [28]. A similar principle for enhanced solubilization of drugs by incorporation in the interfacial lecithin layer is employed in the SolEmuls technology [108]. Since the chemical stability of active compounds is a crucial aspect, further investigations on this topic would be of interest.

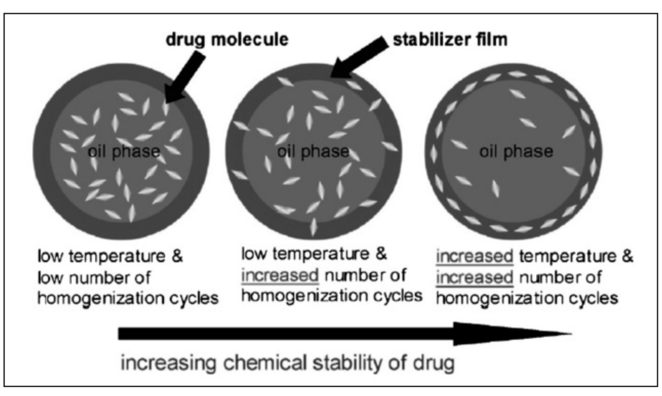

Figure 5 - Principle of chemical stabilisation of drugs in lecithin-based nanoemulsions, reprinted from reference [28] with permission from Elsevier. The possible distribution of incorporated drugs is demonstrated.

5. Effect of excess lecithin in o/w nanoemulsions

In systems containing phospholipids, various structures may be formed during production. Liposomes, lamellar or discoidal micelles, nanoparticles or network-like structures can emerge. It can therefore be assumed that such phospholipid aggregates may also be present in lecithin-stabilized nanoemulsions [80]. Early investigations have already examined the effect of excess lecithin on nanoemulsion formation. Krafft *et al*. found that an excess of lecithin decreases emulsion stability and results in the presence of vesicular lecithin aggregates [109]. It was not clarified, however, which kind of degradation processes were promoted by these vesicles. Low amounts of surfactant were therefore considered not only desirable from a biological and cost efficiency viewpoint, but also for technological reasons.

In recent years more and more investigations indicate that lecithin-based nanoemulsions can contain liposomal structures, especially when they are produced using an excess of lecithin as emulsifier. Small unilamellar vesicles as well as multilamellar structures have been reported [7, 110, 111]. Cryo-TEM images of o/w nanoemulsions often show oil droplets as homogeneously shaded circles while liposomal vesicles are seen as clear, unfilled circles (*Figure 6*) [112]. The presence of vesicles in phospholipid-stabilized o/w emulsions is well established [52, 111-113]. It was even assumed that up to 50 % of incorporated phospholipids might be present as liposomal or micellar structures [114]; however, such estimations could not be verified up to date. Other researchers have proposed to confirm the absence of liposomes in lecithin-based nanoemulsions by NMR spectroscopy [98].

Whether the presence of such structures impairs the stability and functionality of lecithin-based nanoemulsion systems is not entirely clear. On the one hand, it is well-known that the presence of phospholipids may enhance solubilization of drugs [65]. Therefore, an excess of lecithin might be advantageous. On the other hand, surplus phospholipids might promote destabilization phenomena. The rates of Ostwald ripening and coalescence in emulsions have been reported to be higher in the presence of excess surfactant and surfactant micelles due to micelle-mediated transport of oil [40, 44, 48].

In terms of biological effects, vesicles formed by surplus lecithin are regarded with concern. Early lecithin-based nanoemulsions for parenteral nutrition were reformulated due to the suspicion that excess lecithin in form of liposomes might be responsible for dyslipidemia upon long-term infusion [7]. Liposomes formed by excess phospholipids can indeed interfere with lipid metabolism and may result in hypercholesterolemia, hyperlipidemia and hepatic steatosis [8]. Stability issues due to excess lecithin, however, were not reported.

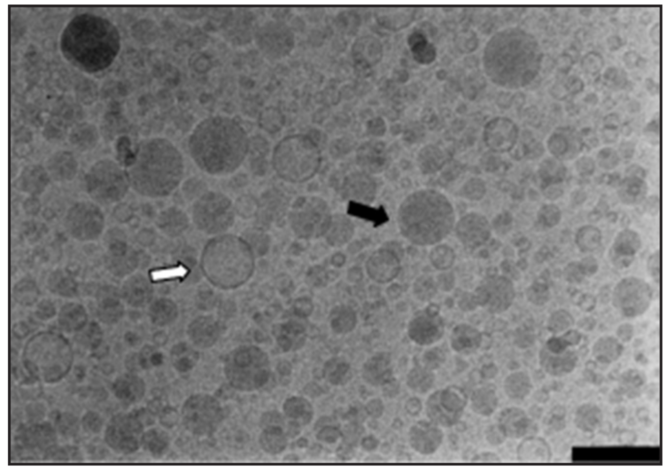

Figure 6 - Cryo-TEM image of a nanoemulsion, reprinted from reference [112] with permission from Elsevier. Emulsion droplets are seen as homogeneously shaded circles (dark arrow) while liposomal vesicles are seen as unfilled circles (white arrow). The scale bar represents 200 nm.

The formation and stability of lecithin-based nanoemulsions are still not completely understood. Likewise, commercial lecithin-stabilized emulsions show unexpected stability against steam-sterilization, which might be related to structural changes in interfacial lecithin layers [7, 113].

Overall, the role of surplus lecithin in lecithin-based nanoemulsions has not been ultimately clarified. It is uncertain whether additional lecithin-aggregates are inherent to such nanoemulsion systems or can be avoided. Successful approaches to reduce excess liposomes include both lowering of the lecithin amount and higher homogenization pressure [7]. However, a general conclusion about the influence of these structures on nanoemulsion stability has still not been established.

6. Microbiological stability

Since lecithin-based nanoemulsions were first used as parenteral formulations, microbiological contamination had to be excluded. Large volumes of parenteral nanoemulsions distributed in multidose containers have to be properly preserved [115]. Steam-sterilization by autoclaving is possible for classical lecithin-based nanoemulsions [5, 7, 14] and might even serve to further stabilize such formulations [14]. However, not all excipients and incorporated drugs might be suitable for autoclaving. In general, aseptic filtration for nano-sized emulsions is possible if their droplet size is below 200 nm and the viscosity is low; however, preservative agents should be added [116].

A considerable amount of useful experimental data on this topic has been established by Sznitowska *et al*. [113, 115, 116]. A physicochemical screening of antimicrobial agents revealed that no sufficient preservation could be achieved by any of the investigated antimicrobials. In subsequent studies, it was shown that the concentrations of free parabens in the aqueous phase were too low to achieve satisfying preservation [113, 115]. Apparently, the tested preservative agents have unsuitable diffusion behaviour. Although parabens seemed most promising, the concentration of free molecules in the aqueous phase was apparently too low, while high concentrations were found in the interfacial region. This can be ascribed mostly to the presence of lecithin rather than the oil content. More research in this field is needed to identify suitable and effective stabilizing agents. Due to the complex structure of lecithin-based nanoemulsion systems, partitioning phenomena of preservative agents may limit the contact with microorganisms in the aqueous phase [113]. Consequently, higher doses or combinations of preservatives might be required. Moreover, many preservative agents do not exhibit maximal activity at slightly alkaline pH values often required for nanoemulsion stability [115].

A number of excipients have been proposed and patented for inhibition of bacterial growth in parenteral nanoemulsions of propofol. However, only few such as EDTA have been approved by the Food and Drug Administration for marketing in the United States [14]. In recent studies, potassium sorbate was used as a mild preservative agent for topically applied lecithin-based nanoemulsions [18, 39]. No systematic efficacy tests of such formulations were conducted. However, a comparison with a batch of corresponding unpreserved nanoemulsions showed a noticeable superiority of the preserved formulations (unpublished data). This indicates that potassium sorbate at least contributes to enhanced microbiological stability. However, systematic studies are necessary to identify suitable preservatives for all routes of administration.

7. Storage conditions

Certain methods are frequently applied right after the production of lecithin-based nanoemulsions to enhance their storage stability. Such methods comprise the adjustment of pH value, sterile filtration or autoclaving and storage under an oxygen-free nitrogen atmosphere [14, 15, 117]. Usually, nanoemulsions are stored either at room temperature or slightly chilled at 4-8 °C, which is advisable to prevent chemical degradation processes.

Investigations showed that lecithin-based nanoemulsions can usually not be stored deep-frozen, since emulsion systems are often destabilized or completely phase separated upon thawing [14, 118]. Cold storage might lead to fat crystallization and subsequently to coalescence. Water crystallization induces freeze-concentration phenomena, followed by denaturation of compounds or adsorption of surfactant molecules to ice crystals [102]. In a recent publication, the effects of different storage temperatures on nanoemulsion stability have been described [102]. A storage temperature of - 24 °C led to increased particle sizes in dependence of the incorporated oil fraction and the homogenization pressure. Nanoemulsion stability against frozen storage was optimized by applying ultra-high pressure homogenization at 200-225 MPa and using formulations with low oil content; thus satisfying results in terms of storage stability were achieved.

IV. PRODUCTION OF NANOEMULSIONS

The production of nanoemulsions as reported in the literature can be divided into two fundamentally different approaches. The conventional production of nanoemulsions relies on high-energy emulsification through pressure or ultrasound. In contrast, low-energy emulsification makes use of the intrinsic physico-chemical properties of the excipients which are mixed in a specific fashion [63]. Whether or not the latter method produces nanoemulsions in the conventional sense of the word, or rather phases approaching microemulsion status, remains to be clarified. Since a large body of data can be found for both methods, all currently known methods for the formation of nano-sized formulations are presented in this context.

Preliminary studies to optimize both nanoemulsion composition and processing parameters should be performed systematically, as has been demonstrated [18, 28, 39, 97]. These preliminary studies can be accelerated by using experimental design [64, 119]. Incorporation of active molecules, however, might change the formulation parameters significantly. The interaction of drugs with nanoemulsions is complex in nature, as active molecules can be distributed to various locations [115]. Systematic studies on the destabilizing effect of drugs in consideration of their lipophilicity, ionisation and molecular weight have been conducted [27]. It is difficult to predict changes in nanoemulsion stability, as no clear correlation seems to exist between the physicochemical properties of drugs and their destabilizing potential [27]. It has also been shown that even the presence of undissolved drug may not influence the short-term stability of the systems.

The incorporation of a lipophilic drug often leads to an increase in mean oil droplet diameters, since lipophilic molecules are solubilized in the oil core. The solubility of the active molecule in the oil phase has to be established beforehand. However, it has to be considered that the surfactants used, especially lecithins, are able to enhance drug solubility. Moreover, the oil volume fraction often determines the produced particle sizes. Larger amounts of dispersed phase lead to larger particles, which might be less favorable in terms of stability. On the other hand, the solubilizing capacity for lipophilic drugs is higher as well [50]. A compromise has to be found in order to produce optimized formulations of reasonable properties and composition.

1. High-energy emulsification methods

The preparation of submicron-sized emulsions makes use of high energy input to rupture larger emulsion droplets into smaller ones. Extreme shear and high-frequency agitation can be employed to fragment the droplets, thus overcoming the LaPlace pressure which causes the resistance of droplets against deformation. However, a sufficient amount of emulsifier has to be present in the formulation to rapidly coat the new surface area [3, 34]. Drugs can be incorporated either de novo or ex tempore, meaning either before or after high-energy processing of the coarse formulation [104]. A large number of factors can influence the product, namely the composition and viscosity of the emulsion, the processing conditions as well as the temperature.

Most studies dealing with lecithin-based nanoemulsions produced by high-energy methods report particle sizes between 150 and 300 nm [18, 26, 28, 39]. Apparently, mean particle sizes below 100 nm for classical nanoemulsions are hard to achieve [98]. The lamellar structure of PC alone enables the formation of nanoemulsions with particles sizes between 100 and 300 nm [35]. Only a few authors report the successful formation of lecithin-based nanoemulsions with droplet diameters in the true nanosize region by applying higher pressure and a selective formulation composition [31, 120]. Addition of mild surface-active agents and a decrease of the oil volume fraction can also be employed to achieve this goal [98, 121].

Droplet sizes can usually be decreased by increasing the energy input due to longer homogenization time or higher pressure [35, 85]. Likewise, an increase in lecithin concentration leads to the production of smaller particles due to an increased surfactant to oil volume ratio, which in turn leads to enhanced physical stability [27, 31]. In contrast, large oil volume fractions lead to increased droplet collisions and hence coalescence during emulsification [40]. It is important to determine the optimal processing conditions for every formulation since over-processing might lead to increased or fluctuating droplet sizes [97]. Overprocessing is either caused by increased droplet collision and re-coalescence rates upon increase of homogenization cycles or insufficient emulsifier concentration in relation to the increasing interfacial area. These two phenomena are often related. If the newly created interfacial area is too large to be efficiently covered by emulsifying agents, this can trigger the coalescence process. This coalescence is often the limiting factor for droplet size reduction. A plateau in particle size reduction with time is reached, where no further decrease of droplet diameters can be achieved or droplet size even slightly increases [7, 48, 102]. An important aspect in this context is the influence of the temperature during production. Thermal energy is produced during high-pressure homogenization. Efficient cooling might limit cavitation phenomena and prevent droplet re-coalescence [82, 102]. Moreover, the viscosity of the formulation can play an important role. Increased inlet fluid viscosity could promote droplet rupturing through higher extensional stress and could weaken recoalescence phenomena [102].

1.1. High-pressure homogenization

Emulsification through high-pressure homogenization relies on forces created in suitable devices, such as shear, turbulence and cavitation [7]. The standard procedure for producing classical lecithin-based nanoemulsions comprises multiple steps [18, 26, 28, 39]. An oil phase and an aqueous phase are prepared separately under continuous magnetic stirring at about 50 °C. The oil phase often consists of soybean oil or other biocompatible oils and various lecithins, possibly with admixture of antioxidants such as α-tocopherol or other additives, including lipophilic drugs. The aqueous phase might contain additives such as hydrophilic surfactants or glycerol. All components have to be completely dissolved. Both oil and water phase should be filtered to reduce the bioburden and possible pyrogens, especially in formulations for parenteral application [7]. Subsequently, the two phases are combined and mixed with a conventional lab mixer. This pre-homogenization can be performed by employing a high-shear rotor-stator device such as an Ultra-Turrax or Silverson emulsifier. Thus, a homogenous, but coarse emulsion with droplet sizes of a few μm is produced [34]. This pre-emulsion is further stirred and re-heated to around 50 °C. Although higher temperatures of over 90 °C have been used for this production step [18], moderate heating is recommended to avoid degradation of phospholipids [50]. The emulsion is then processed with a high-pressure homogenization device such as an EmulsiFlex, a Gaulin Homogeniser or Micron Lab.

The most frequently used high-pressure (HP) homogenisers are of the piston-gap type. These devices consist of one or two piston intensifiers as well as an HP-valve. The fluid under pressure is forced through a small orifice, the HP-valve gap. The fluid is rapidly accelerated and a high pressure gradient is produced between the inlet and the outlet of the HP-valve. Consequently, phenomena such as intense shear forces, energy-bearing eddies in turbulent flow, impacts with solid surfaces as well as cavitation phenomena due to high local pressure fluctuations occur. The latter cause solvent vapour bubbles to form and collapse, which leads to disruption of droplets [7, 102]. In addition, due to shear effects and the conversion of kinetic energy into thermal energy, the fluid is subjected to short-life heating phenomena. These can be controlled by cooling devices. All of these mechanical forces serve to fragment particles to achieve nano-sized droplets.

The interest in the production of nanoemulsions increases as potent homogenization technologies are developed. A recent study reports the use of ultra-high pressure homogenization for the production of o/w nanoemulsions stabilized with whey protein [102]. Homogenization pressures up to 225 mPa were used, which corresponds to 2,000 bar pressure. Conventional high-pressure homogenization for nanoemulsion production usually employs around 500-800 bar, although higher pressures have been reported [108]. In case of the cited ultra-high pressure homogenization, the best results were obtained at 200-225 mPa pressure with or without recycling of the formulations [102]. The resulting formulations did not, however, show significantly lower particle sizes than nanoemulsions produced by ordinary high-pressure homogenization.

1.2. Microfluidisation

In submerged jet homogenisers, such as the Microfluidizer or the Nanojet, turbulent flow is the main phenomenon responsible for the rupturing of droplets, though shear and cavitation occur as well [7]. Microfluidizer devices consist of interaction chambers with microchannel architecture. The liquid sample is forced through these microchannels towards an impingement area at high pressure. Precisely controlled emulsification forces are generated, such as a laminar shear flow at the inlet and turbulent flow with cavitation through vapor bubble implosion at the outlet [102]. These mechanical forces act together to reduce the mean droplet diameter of the dispersed phase [9, 102]. The coarse pre-emulsion is prepared as described above.

A direct comparison of o/w nanoemulsions produced by high-pressure homogenization (Silverson) and microfluidization (Microfluidizer) was performed [9]. It was found that the microfluidizing device was more effective in producing stable o/w nanoemulsions at low emulsifier concentrations. However, this study did not investigate lecithin as emulsifier and the data cannot be seen as representative for the various types of different homogenization and microfluidization devices on the market. Practical aspects that have to be considered with either homogenization or microfluidization devices are the loss of sample due to adhesion in the stirrer assembly as well as the limiting sample value that can be processed. These parameters can vary for different devices [9].

1.3. High-energy ultrasonication

Another high-energy method to produce lecithin-based nanoemulsions is high-energy ultrasonication [51, 98]. Both oil and water phase are prepared separately under slight stirring and heating to around 75 °C. After mixing of the phases, coarse pre-emulsions are produced by means of mixing devices such as an Ultra-Turrax. The resulting emulsion is then ultrasonicated with appropriate devices such as a Vibra-Cell ultrasound instrument. Processing conditions have to be optimized in terms of ultrasonication time or energy input. Different observations on the influence of prolonged ultrasonication have been reported in this context [51]. Particle sizes comparable to those achieved with high-pressure-homogenization can be obtained even under mild sonication conditions [98, 121].

In summary, the vast majority of classical lecithin-based nanoemulsions is produced through high-pressure homogenization. An advantage of high-pressure homogenizers or microfluidizers is that the thermal stress can often be reduced through cooling or short processing times. Moderate processing conditions with tempatures below 45 °C and pressures between 500 and 800 bar are advisable. However, only large sample volumes of distinctly low viscosity can be processed. Despite this drawback, high-pressure homogenization has been established as a reliable standard production method for lecithin-based nanoemulsions. In contrast, the amount of data dealing with the preparation of nanoemulsions through ultrasonication is limited. Although ultrasonication appears to be a rapid and practical production method, more data about optimization of processing conditions, thermal stress and possible cooling options are required. Moreover, the smallest particle size distributions with a PDI below 0.1 have only been reported for nanoemulsions produced by high-pressure homogenization. This might represent a possible limitation for ultrasonication as a production method in this context.

2. Low-energy emulsification methods

Low-energy emulsification methods employ the physico-chemical properties of a system to form submicron-sized emulsions. These methods usually rely on a change of the spontaneous curvature of the incorporated surfactant [34]. This can be achieved in different ways as explained below. Irrespective of the questionable nature of the produced formulations, a major advantage of low-energy emulsification methods is their applicability for the formulation of thermolabile compounds [66]. However, it has to be kept in mind that no classical lecithin-based nanoemulsions can be produced with these methods.

2.1. Solvent evaporation method (solvent diffusion method)

An organic phase consisting of oil, a water-miscible solvent such as acetone and a lipophilic surfactant is mixed with an aqueous phase containing a hydrophilic surfactant and distilled water. The organic phase is injected into the aqueous phase under magnetic stirring, and upon diffusion of the organic solvent into the external aqueous phase nanodroplets are formed. The water-miscible solvent is subsequently removed by evaporation [42, 64, 122, 123]. Nanoemulsions produced by this method cannot be stabilized exclusively by lecithin, but comprise combinations of other emulsifying agents such as different types of Span- and Tween-type surfactants. In most cases, synthetic surfactants prevail. The amount of surface-active agents is higher than in classical nanoemulsions, requiring at least 10 % [64]. Particle sizes are often comparable to those achieved with high-energy homogenization [42, 64]. However, the amount of incorporated oil phase has been found to be extremely low in some investigations (1 % w/w), which renders the produced formulations rather unsuitable for incorporation of lipophilic drugs [42].

Interestingly, it was observed that the kinetics of spontaneous emulsification are more rapid when the miscibility between organic and water phase is good [42]. This observation is in support of the opinion stated by Mason *et al*. [3] that the systems created by low-energy emulsification methods are rather approaching microemulsion phase properties, where the two immiscible liquid phases have relatively high mutual solubility [3]. Moreover, detailed information about particle size distributions or long-term stability is rarely presented. It is therefore difficult to come to a definite conclusion about the exact nature of the produced formulations. Other findings indicate that so-called self-emulsifying drug delivery systems produced by low-energy emulsification might generally exhibit a large droplet size distribution and do not possess unimodal droplet population size [62, 124]. Another drawback is the potential presence of solvent or co-solvent remains in the final product [102].

Another important point worth considering is the fact that lecithin is unable to form microemulsion structures in aqueous ternary systems unless a co-surfactant or co-solvent is added [4]. This point again confirms the theory that systems formed by spontaneous emulsification, where mixtures of surfactants or solvents are required, do not possess the same microstructure as nanoemulsions formed by high shear. Low-energy emulsification in ternary lecithin/water/oil systems would only result in coarse emulsions, as first investigated by Shinoda *et al*. [59]. Therefore, the production of classical lecithin-based nanoemulsions without large amounts of synthetic co-surfactants will remain refined to high-energy emulsification methods.

2.2. The classical phase inversion temperature method

The principle of the phase inversion temperature (PIT) technique consists in varying the temperature of the system relative to the phase inversion temperature to convert bicontinuous microemulsion phases into nanoscale droplets [3, 63, 125-128]. To this end, a transition from an o/w emulsion at low temperatures to a w/o emulsion at higher temperatures is induced, and vice versa. This phenomenon is called transitional phase inversion and affects the HLB value of the system [40]. This phase inversion occurs when the relative affinity of the surfactants for the different phases is changed by the temperature. After heating and subsequent cooling, the system crosses a point of minimal surface tension and zero spontaneous curvature. This transitional region between the different macroemulsions shows the bicontinuous structure of microemulsion phases. Upon further cooling, the formation of finely dispersed kinetically stabilized droplets is promoted [34, 63].

The whole procedure is conducted under slight stirring without extreme shear [47]. The nanoemulsion formation results from a sudden break-up of this microemulsion system by either rapid cooling [126] or sudden water dilution [129] at the PIT. The latter method is also referred to as the emulsion inversion point method and is described below. It has to be noted that only nonionic polyethoxylated surfactants allow to conduct this emulsion inversion if the solubility of the surfactant is relatively balanced for both oil and water phases [63]. This again supports the notion that the resulting systems rather approach microemulsion status [3].

An overall analysis of lecithin-based nanoemulsions produced by the classical PIT method indicates that larger emulsion droplet sizes and broader particle size distributions are obtained. This polydispersity is accompanied by higher rates of Ostwald ripening [40]. Both amount and type of surfactants can be variable. Such formulations [130] are unlikely to exhibit prolonged physical stability and are stable for only a few months [126, 127]. In contrast, formulations produced by the PIT method with temperature cycling are assumed to be protected by a thick interfacial layer of surfactant, which can be reinforced by additional phospholipids [63]. These particular systems produced by temperature cycling can be seen as nanocapsules or lipid nano-capsules, as they possess a core-shell structure which is relatively rigid at room temperature. Nanoemulsion-like systems produced with the classical PIT method either require large amounts of surfactants or show inferior properties such as large particle sizes and polydispersity [63]. These observations indicate that certain colloidal systems can be produced and optimized by the PIT method. However, classical lecithin-based nanoemulsions cannot be produced by this technique. Interestingly, the phase inversion method was formerly cited as pre-emulsification method followed by subsequent high-pressure homogenization [7].

2.3. Aqueous titration method (emulsion inversion point method)

The emulsion inversion point method (EIP method) is also referred to as spontaneous emulsification method or aqueous titration method [34, 37, 42, 61, 122, 123, 131, 132]. Some authors describe it as another form of PIT method, since it relies on the same principle [40]. The only difference is that the nanoemulsion formation results from a sudden break-up of the microemulsion system by water dilution at the PIT [129], while the classical PIT method employs rapid cooling [126]. Therefore, a transition in the spontaneous curvature of the interfacial film is obtained by changing the water volume at the PIT by successively adding water. During this so-called catastrophic phase inversion minimal interfacial tension values are achieved and are said to induce the formation of fine droplets [34].

Investigations suggest that the droplet size is governed by the nature of the lamellar or bicontinuous microemulsion structure formed at the inversion point. Most studies report that a critical surfactant-to-oil ratio is needed to assure the formation of these structures and the later formed submicron-sized emulsion droplets [34]. The final emulsions are birefringent, which indicates the existence of a liquid crystalline structure within the emulsions [34]. However, long-term stability data for nanoemulsions produced in this fashion are rarely reported. Higher amounts of surfactant are needed to obtain satisfying droplet sizes and a monomodal size distribution. Surfactant amounts of up to 40 % [61, 66] and the presence of co-surfactants suggest that the resulting formulations have little in common with classical nanoemulsions. This should be emphasised for assumed o/w nanoemulsions which contain only 25 % water, but 35 % of oil phase [66]. Clearly, no distinct classification in terms of conventional nanoemulsions can be made in this case.

V. CHARACTERIZATION OF NANOEMULSIONS

The most frequently employed techniques for nanoemulsion characterization are summarized in this context. Interfacial tension measurements are omitted since recent findings [68] might soon stimulate more detailed reports about this topic. The discussed techniques can also be employed for continuous monitoring of formulation properties over extended periods of storage. In order to gain information about the formulation's stability against stress conditions, accelerated stability tests can be conducted using centrifugation, heating/cooling cycles, freeze/thawing cycles or storage at elevated temperatures [36, 61, 86].

1. Visual appearance

Lecithin-based nanoemulsions may exhibit different optical appearance in correlation to their particle size. Most of the recently described lecithin-based nanoemulsions exhibit milky white to semitransparent appearance, as their particle size is above 100 nm (*Figure 7*). Upon long-term storage, a visible separation of a nanoemulsion system into two phases has sometimes been reported: an upper concentrated emulsion and an almost clear, viscous solution (*Figure 8*). This can be ascribed to creaming phenomena, during which the less dense phase moves to the surface of the stored emulsion. However, droplet sizes remain unchanged, and the inhomogeneous distribution of the droplets is reversible upon gentle agitation [7, 56, 104].

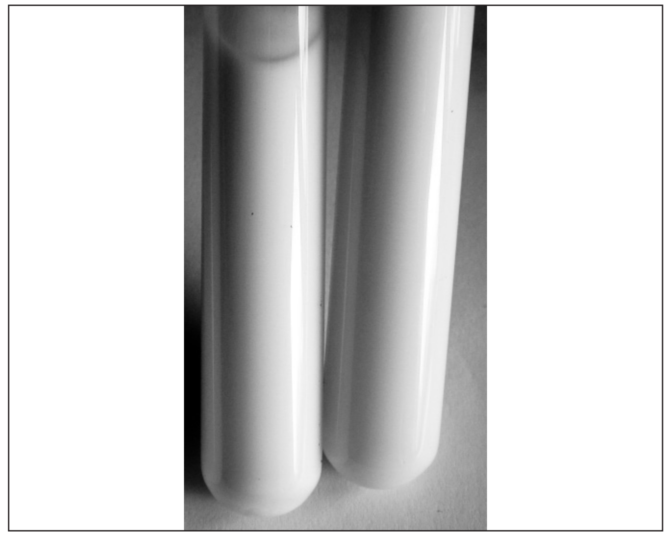

Figure 7 - Picture of a lecithin-based nanoemulsion with particle sizes around 150 nm.

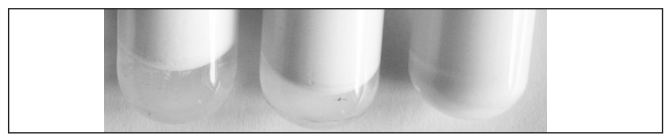

Figure 8 - Destabilisation phenomena in lecithin-based nanoemulsions (yellowish colour, appearance of an aqueous solution). However, redispersion is possible and particle sizes remain stable.

2. Mean droplet size and droplet size distribution

The droplet size distribution is one of the most important physical characteristics of nanoemulsions. It is frequently determined by dynamic light scattering (DLS) with either Zetasizer devices (Malvern) or Coulter Counter particle size analysers (Coulter Counter) [87]. It is also referred to as photon correlation spectroscopy (PCS) [112]. This laser light scattering technique is a useful tool for estimating the particle size distribution of fine particles ranging from a few nanometers to several micrometers [42, 133]. The analysis is based on the determination of fluctuations in the intensity of scattered light due to Brownian movement of the particles. The particle size is then calculated using the Stokes-Einstein relation [61, 134]. Thereby, information about the hydrodynamic mean particle diameter as well as the polydispersity index (PDI) is gained. The PDI discloses the quality of the dispersion and represents the width of the size distribution. PDI values lower than 0.1 or 0.2 indicate suitable measurements and good quality of the colloid suspension. Values close to 1 are found for samples of poor quality, which either do not represent droplet sizes in the colloidal range or exhibit a very high polydispersity [63]. As for all colloidal systems analysed by DLS, both the density and the viscosity of the nanoemulsion should be taken into account. These parameters can influence the outcome of the particle size determination [135]. The same problem has been described in context with laser diffractometry, another method of particle size determination [136]. However, it can be assumed that the dilution of the nanoemulsion samples with distilled water (1:100 or even 1:1000 v/v) as commonly performed for DLS measurements will minimize the influence of these parameters on the measurement results.

However, DLS fails to accurately describe the size composition for mixed samples, especially when the concentration of a particular size population is low [36]. Therefore, an additional particle size measurement technique is necessary for determining the amount of large emulsion droplets over 5 μm in a formulation. Single particle optical sensing is a possible method discussed for this application [5]. Moreover, a combination of DLS with sedimentation field flow fractionation can be used to characterize the size distribution of polydisperse samples correctly [36].

Likewise, DLS results provide no information about flocculation phenomena since the nanoemulsion samples are diluted prior to measurements, which leads to redispersion of potentially flocculated intact particles [98]. Therefore, another technique for analysis of aggregation phenomena should be employed. Nuclear magnetic resonance (NMR) spectroscopy has been suggested for this task [98] and can be employed to investigate the droplet size of nanoemulsions as well [87, 112]. The hydrodynamic diameter of nanoemulsion droplets can be calculated from the self-diffusion coefficient using the Stokes-Einstein equation. Interestingly, the results obtained with DLS or NMR spectroscopy can differ considerably. This might be ascribed to the complexity of nanoemulsions, which might contain oil droplets as well as liposomal vesicles [112]. The presence of the latter can also be determined by means of ^{31}P-NMR using shift reagents [98]. Moreover, fourier transform infrared spectroscopy has been used to determine microstructures formed by PC [137]. This technique can also be employed to characterize lecithin-stabilized emulsion systems or to investigate their stability [72, 138].

3. Surface characteristics: the zeta potential

The assessment of the zeta potential (ZP, ζ-potential) is a standard procedure employed to characterize lecithin-based nanoemulsions. Usually microelectrophoresis or laser Doppler electrophoresis, corresponding to the determination of droplet electrophoretic mobility in an aqueous medium, is employed using devices such as the Zetasizer (Malvern). The ZP characterizes the surface charge of emulsified oil droplets. High absolute ZP values lead to repulsive forces between particles, which might contribute to enhanced nanoemulsion stability. Large absolute surface charge values above 30 mV will lead to droplet repulsion and thus enhanced stability against coalescence [139]. However, the ZP should not be considered as guarantee for nanoemulsion stability, since other relevant factors are not expressed through this parameter [108].

The above mentioned technique requires appropriate dilution of samples. Both distilled water and electrolyte solutions or buffers have been proposed for this task. Dilution with pure distilled water might lead to fluctuating conductivity which can impair the viability of the obtained data [139]. Therefore, electrolyte solutions of distilled water and NaCl or KCl [39, 50, 85] are frequently employed to ensure constant conductivity below 50 μS/cm. However, it has to be considered that the presence of counter ions might impair the physical stability of charged nanoemulsion droplets. Measurements should therefore be conducted immediately upon dilution of samples.

4. Morphology and microstructure

Microscopy techniques such as atomic force microscopy are useful tools to confirm results obtained by DLS measurements [87, 98]. In many cases conventional transmission electron microscopy (TEM) is employed. This method is among the most important techniques for analysing microstructures, as it can visualize coexistent structures and microstructure transitions [61, 65]. However, the quality of the images can be rather poor [42] and the method is unsuitable for fluid nanoemulsion samples. Therefore, the preferred electron microscopy technique for analysis of nanoemulsions is cryo-TEM. This method avoids artifacts inherent with other electron microscopy sample preparation techniques, and it allows for a clear differentiation between emulsion droplets and other structures [5]. A probate alternative is freeze-fracture TEM, which is a highly useful, artifact-free technique for observation of diverse systems such as surfactant aggregates, microemulsions and biological systems [31]. Both methods employ rapid vitrificaton of solutions with lipid-based formations to observe microstructures. In this fashion, the presence of vesicles, micelles, liquid crystals and other structures together with nanoemulsion oil droplets can be detected [36].

5. Phase distribution of drugs

The distribution of a drug within a nanoemulsion is complex and depends on its specific properties, such as its lipophilicity, molecular weight and structure [140]. The solubility of the drug in the respective oil phase of the nanoemulsion is another crucial aspect. The drug can be distributed in four different locations, namely the oil phase, the oil/water interface, the water phase as well as the aqueous micelles or liposomal vesicles present in the water [52, 111, 112, 115]. A dynamic exchange of the drug between the different phases might further complicate analysis in this context [112]. Lipophilic molecules will be distributed within the oil core of the droplets or the phospholipid monolayer, although certain amounts might be incorporated into the phospholipid bilayer of liposomes in the aqueous phase. Hydrophilic molecules, on the other hand, will be found in the aqueous phase or in liposomes. Amphiphilic molecules will mainly be inserted into the phospholipid layer of the nanodroplets, but might also be found in liposomes or form aggregates. The distribution pattern of a drug might influence its release profile, since a sustained release can be expected for drugs which are incorporated in the oil core [140]. In order to achieve a high drug loading into the nanoemulsion core an appropriate choice of both drug and oil phase is essential. Furthermore, lecithin acts as an additional solubilizer, especially with drugs of lower lipophilicity. Different methods have been proposed for investigating the entrapment efficiency of lecithin-based nanoemulsions for various drugs, such as ultrafiltration, ultracentrifugation, gel filtration and microdialysis [36, 111, 113]. Separation methods based on ultrafiltration allow an estimation of the drug content in the oil phase, the interfacial region as well as in the external water phase of nanoemulsions [65]. Recent studies with the model drug silybin [111] suggest that ultrafiltration is the most accurate method, while both microdialysis and ultracentrifugation are much less reliable. Since ultracentrifugation eventually leads to a breakdown of the emulsion structure and possibly redistribution of the drug, its validity might be questionable in this case. A quantification of silybin phase distribution showed that more than 80 % of the drug was located in the o/w interfacial region, which can be ascribed to its poor solubility in both water and oil phase. Another method to determine the phase distribution of drugs in colloidal lipid dispersions without impairing their structure is electron paramagnetic resonance (EPR) spectroscopy [140, 141], which can be used to determine the distribution pattern of spin-labeled model compounds.

The interactions between drugs and lipids in lecithin-based nanoemulsions can have significant influence on formulation properties and should therefore be characterized for every formulation. The encapsulation of lipophilic drugs into the oil core might be reduced due to the presence of spacious hydrophilic structures of co-surfactants in the interfacial film. Moreover, drugs can interact with the surfactants and accumulate at the interface; this was observed especially for amphiphilic drugs as well as for lipophilic drugs and preserving agents such as parabens [52, 112, 115, 142]. Lipophilic drugs might intercalate between the hydrophobic tails of phospholipids, thus altering the properties of the interfacial film [85]. A direct interaction of the drug with the interfacial film is often accompanied by a change in particle size upon drug incorporation. The inclusion of drugs into the oil core of an o/w nanoemulsion usually induces a slight increase in particle sizes. If amphiphilic drug molecules are incorporated into the interfacial film, acting as additional surface active agents, particle sizes might be decreased [52, 112, 113].

Apart from the above mentioned techniques, thermoanalytical methods such as differential scanning calorimetry (DSC) can be employed to investigate the interactions between drugs and nanoemulsion phases [112]. Although caution should be applied when interpreting phase transitions in multicomponent systems, the localization of a drug in the phospholipid bilayer can be determined with some certainty [112]. In addition, the presence of certain phases such as liposomes can be determined. However, the quality of these informations might be limited due to the complex composition of commercially available lecithins [7].

6. Rheological characteristics

The viscosity of lecithin-based nanoemulsions is usually very low. It can be determined by means of various viscosimeters and rheometers suitable for highly fluid samples. The viscosity of lecithin-based nanoemulsions can be an important aspect not only in terms of product application, but also in the course of emulsification. It has been observed that, as a general tendency, smaller nanoemulsion droplet sizes can be obtained when the viscosity of the coarse emulsion increases [31]. However, this alone is not a sufficient guarantee of obtaining small droplets [42]. An increase in viscosity can lead to smaller particles since the degree of coalescence during high-pressure homogenization is reduced; the oil droplets cannot move as freely and rapidly. Therefore, an increase in viscosity of the continuous phase might decrease the droplet collision frequency [31, 102]. However, it has also been reported that oil phases with higher viscosities require higher homogenization pressures to achieve smaller particle size distributions [18, 50]. At critically high viscosity values and insufficient pressure, a strong increase in particle size can be expected. This is due to the fact that the high viscosity can also generate difficulty in lecithin movement, which prevents the surfactant molecules from rapidly covering the oil droplets during preparation. Therefore, increased coalescence phenomena occur [31].

Generally, nanoemulsions both of o/w and w/o type may exhibit a complex rheological behaviour. An overall Newtonian behavior, except at very high shear rates, has been observed [46]. However, a large range of nanoemulsion viscosities can be obtained with different ratios of oil as well as different processing conditions in terms of pressure and number of cycles [102]. The viscosity of nanoemulsions can be increased not only by addition of thickening agents such as polymers, but also by increasing the oil phase volume [43]. Semisolid preparations for topical application can conveniently be developed in this way.

VI. APPLICATION OF NANOEMULSIONS AND CURRENT RESEARCH TRENDS

1. Dermal drug delivery and cosmetics

Lipid-based drug delivery systems for increased dermal uptake of active substances are a modern alternative to possibly irritating permeation enhancers or physical methods to lower the skin barrier function [143-146]. Lecithin-based nanoemulsions fulfill certain requirements for topical formulations that cannot be entirely met by other vehicles. The use of liposomes is limited by issues such as poor drug loading and poor long-term stability. Solid lipid nanoparticles are sometimes regarded with suspicion due to safety concerns. Microemulsions have a comparatively high potential to cause skin irritation. In contrast, nanoemulsions contain non-irritating compounds and low amounts of surfactants, but can nevertheless be expected to exhibit high stability [31]. Therefore, lecithin-based nanoemulsions as colloidal carrier systems are frequently designed for dermal and cosmetic applications [39, 49]. Prolonged biological effects of various lipophilic drugs as well as increased skin permeation have been reported [28, 69, 99].

The *stratum corneum*, consisting of corneocytes embedded in a highly ordered lipid matrix, is the main barrier for dermal drug delivery [143]. Phospholipid systems offer a mild, physiological alternative to conventional formulations used to overcome this barrier. It has been shown in various studies that phospholipids in liposomes can increase skin permeation or skin accumulation of drugs due to lipid mixing of liposomal phospholipids with lipid bilayers of the skin [6, 69, 147, 148]. Phospholipids can modify these bilayer structure, thereby promoting the transcellular transport of drugs and acting as penetration enhancers [133]. Not all phospholipids, however, have the same enhancing effect on skin permeation. Some phospholipids, such as PC, have low values of gel-liquid crystalline phase transition temperature; they are consequently in a fluid state at the average skin temperature of 32 °C. The interaction of such fluid-state phospholipids with the *stratum corneum* lipids supposedly leads to a fusion and consequently enhanced permeation of incorporated drugs [149, 150]. In contrast, the rigid bilayer structure of skin lipids is apparently not affected by gel-state phospholipids [149]. Consequently, different lecithin mixtures exhibit different effects in terms of skin permeation of incorporated drugs, as shown with microemulsion systems [69]. Different phospholipids also have different effects on skin hydration and barrier function [78].

Since liposomes and nanoemulsions made from lecithin molecules have a certain structural similarity, it can be assumed that the ability of lecithin to interfere with skin lipids and its affinity to cellular membranes is not restricted to liposomal formulations. It can be derived from data obtained with liposomes that fluid-state phospholipids in nanoemulsions are likewise able to diffuse into the *stratum corneum* and enhance drug penetration [120]. Changes in skin lipid fluidity have been observed for various lecithin-based nanoemulsion systems by means of differential scanning calorimetry [26, 39]. Due to this enhanced skin lipid fluidity, lecithin might enable active molecules incorporated in the same formulation to penetrate into the skin more efficiently [149]. Overall, the amount of experimental data dealing with the effect of lecithin-based nanoemulsions on skin barrier function is rather poor. This might be due to the fact that nanoemulsions are often complex in their composition. This complicates the determination of correlations between observed effects and excipients and makes the interpretation of data difficult [61].

Lecithin-based formulations exhibit properties of both pharmaceutical drug delivery vehicles and nurturing cosmetic formulations. Poorly soluble lipophilic drugs can be incorporated in the oil core of nanoemulsions or within the interfacial layer. Lecithins may help to increase the solubility of drugs, possibly by association of the drug with the interfacial film [65]. Due to the small size and monomodal dispersion of nanoemulsion droplets, the formulation can deposit uniformly on skin. The small size of the droplets represents a large interfacial area which might favor skin permeation of active substances [25, 42, 43] and may also result in prolonged residence times in the uppermost skin layers [12]. It can be assumed that for very small particles, shunt pathways into the skin such as hair follicles or sebaceous channels can be involved as well [120]. If the affinity of the incorporated drug for the lipophilic oil core is high, slow drug release can be expected. However, if the affinity is too high, increased lag-times and reduced flux values may occur [65].

It has been reported that small lipid nanoparticles can form a film on the skin surface upon application, thus creating an occlusive effect and increased skin hydration [2]. This film formation model can serve to interpret the data obtained with film-forming nanoemulsions [2, 151]. An occlusion effect can be detrimental for penetration that is driven by a transepidermal osmotic gradient, such as for elastic vesicles [78, 124, 145, 152]. For vehicles such as lecithin-based nanoemulsions, however, skin occlusion is favorable since the enhanced skin hydration promotes the penetration of active substances. In a recent study, the formation of a thick, semisolid film composed of emulsion droplets and silica nanoparticles contributed to increased skin occlusion and consequently enhanced skin penetration of the incorporated drug all-trans retinol [151]. In addition, the emulsion film was assumed to act as a drug reservoir.

A similar effect for lecithin-based nanoemulsions containing natural cyclodextrins was recently observed. A stronger film-building capacity was observed in comparison to the control nanoemulsions, which might have contributed to the increased skin permeation of the model drug progesterone *in vitro* [39]. In another study, lecithin-based nanoemulsions incorporated in o/w creams were shown to increase skin hydration *in vivo* due to the formation of a dense film of nanoemulsion droplets [31]. Nanoemulsions have notable adhesive properties on skin surface upon administration, since the water within the formulation evaporates and a film of oil droplets is left. Due to capillary forces of the nano-sized pores, fusion and formation of a film is promoted; pressure can reinforce this effect [31]. The resulting increased skin hydration may promote the absorption of topically applied drugs or cosmetics. Another proposed mechanism of skin penetration is the diffusion of small droplets through intercorneocyte spaces [31].

Irrespective of their exact mechanism of skin penetration, lecithin-based nanoemulsions can be considered safe and innocuous formulations for dermal drug delivery, as confirmed in numerous studies [89, 153]. However, the choice of the lipid matrix and the addition of potentially skin irritating co-surfactants are highly relevant for the safety of dermatological formulations. In systematic cell culture studies the cytotoxicity of lecithin-based o/w nanoemulsions for dermal application was investigated [130]. It was found that the addition of the cationic surfactant stearylamine was toxic to all investigated cell lines while conventional lecithin-based nanoemulsions with sodium taurocholate and Tween 80 did not affect cell viability. Therefore, mild and non-irritating substances should be preferred as co-surfactants. Known skin penetration enhancers such as oleic acid can be added to increase skin penetration, but might also lead to skin disruption and skin irritation *in vivo* [87]. Likewise, non-irritating cosmetic oils should be employed, such as soybean oil, long-chain and medium-chain triglycerides or octyldecanol [65, 87].

Lecithin-based nanoemulsions are also seen as next-generation carrier systems for cosmetic and cosmeceutical purposes due to their excellent hydrating properties, rapid penetration and skin-friendliness [43, 49]. The field of modern cosmetology is concerned with the treatment of non-pathological skin and merges fluently with dermatology. Multifunctional products that focus on treatment as well as aesthetics are needed [49]. The main concerns of dermatological or cosmetic formulations can be fulfilled with the aid of nanoemulsions. Firstly, they can be used to administer active ingredients to certain skin layers while limiting the passage into the blood stream. Likewise, they can be used to improve the skin barrier function. They are well suited to achieve the goals of skin care, protection and hydration [49]. This can be especially useful for the treatment of chronic skin diseases such as atopic dermatitis or dry skin, where the applied vehicles must not further irritate or weaken the skin barrier [28].

The controlled drug targeting of certain skin layers can be optimized with nanoemulsion systems without a concomitant increase in systemic side effects. With the correct vehicle, drug flux across the skin is negligible, as is envisioned in topical therapy [154]. This was shown in an innovative approach to enhance the dermal delivery of all-trans retinol by incorporation in nanoparticle-coated nanoemulsions [83, 151]. This emulsion-based hybrid drug delivery system showed improved dermal delivery of all-trans retinol.

The fluid nature of nanoemulsion systems as well as their translucent or milky appearance gives them appealing sensorial properties and a pleasant skin feel [43, 49]. Incorporation of fragrances or cosmetic substances such as coenzyme Q10 is possible [42, 155]. Furthermore, prolonged improvements in hair condition were reported after application of a cationic nanoemulsion and nail lacquers based on aqueous polymer nanoemulsions with improved moisturising effect were developed [43, 156]. In another approach, a lecithin-based nanoemulsion as delivery system for ceramides was developed, thus overcoming the technological challenge to formulate these highly insoluble skin care substances [18].

The lack of viscosity poses a certain challenge in nanoemulsion product development, but also offers the opportunity to market nanoemulsion formulations as sprays. Furthermore, nanoemulsions can be modified with natural substances such as polymers to achieve a large variety of fluid to semi-solid or gel-like products. Hydrogel-thickened nanoemulsions stabilized by lecithin and additional Tween-type surfactants or poloxamer showed particle sizes comparable to those of fluid nanoemulsions [96]. Higher viscosity was achieved by swelling with a carbomer gel matrix after the high-pressure homogenization procedure. Carbomer-type gelling agents have been used to obtain nanoemulsions with semisolid consistence in other investigations as well [120].

In contrast to cosmetic nanoemulsions, which aim to promote the permeation of active ingredients into the skin without systemic involvement, dermal drug-loaded nanoemulsions seek to promote the deeper penetration of drugs to achieve a pharmaceutic effect. The dermal delivery of drugs such as antifungal agents, anti-inflammatory drugs or corticosteroids aims at local treatment of diseased skin. The drug penetration profile mainly depends on the physico-chemical properties of the active substance [15], but can be influenced by the chemical composition of the vehicle [2]. Drugs such as diazepam, pilocarpine, cyclosporin or azole antimycotics were reported to be more effective when applied in a nanoemulsion as opposed to conventional vehicles [15, 157]. Likewise, it was found that the corticosteroids bethamethasone valerate and bethamethasone diproprionate as well as a large number of non-steroidal anti-inflammatory drugs exhibited enhanced skin penetration and a higher anti-inflammatory effect when delivered in a nanoemulsion as opposed to a macroemulsion of the same composition and a commercial cream [24, 120].

Studies have revealed that positively charged formulations show higher interaction with various tissues [10, 158]. It is well known that all epithelia, including the *stratum corneum*, carry a negative charge upon their surface at physiological pH. This charge is due to the presence of negatively charged protein residues and selective active ion pumps. Therefore, it was assumed that *stratum corneum* cells would exhibit improved interaction with positively charged vehicles resulting in increased drug permeation and prolonged pharmacological effect. This increased degree of skin binding and biological effect was confirmed in numerous studies [6, 15, 18, 44, 154].

The first positively charged nanoemulsions were produced using stearylamine as additive [10, 15] and led to superior skin permeation of antifungal drugs through rat skin when compared to negatively charged nanoemulsions [15]. Another frequently used component for obtaining a positive surface charge is the anti-inflammatory sphingoid base phytosphingosine. This physiological base has the potential to achieve both increased drug permeation rates and improved skin care properties for lecithin-based nanoemulsions [18, 26]. In thermoanalytical studies, no increased influence of nanoemulsions containing phytosphingosine on skin lipid fluidity was observed when compared to control nanoemulsions [26, 39]. This observation supports the theory that the enhancing effect might be caused by the improved interaction between positively charged formulations and skin rather than by changes in skin lipid fluidity induced by phytosphingosine itself. In general, the effect of a positive surface charge has to be investigated separately for every novel formulation, since skin permeation rates are influenced by various factors and might vary from case to case. Therefore, the positive effect of charge-modified nanoemulsions is not entirely consistent throughout all studies [87].

In order to ensure high biocompatibility and non-irritancy of lecithin-based nanoemulsions, attempts have been made to produce stable lecithin-based nanoemulsions without the use of additional synthetic surfactants such as ethoxylated ones [31, 39]. Since it is difficult to achieve satisfying nanoemulsion properties with lecithin as sole emulsifier, different strategies have to be employed. A viable option is the addition of natural sugar surfactants such as sucrose esters [39]. Another approach is the modification of the nanoemulsion composition. A classical lecithin-based nanoemulsion with particle sizes well below 100 nm, a narrow particle size distribution and increased viscosity was developed by replacing large amounts of the aqueous phase by glycerol [31]. Enhanced skin penetration of the model dye Nile red was observed in rats after topical application of formulations containing these nanoemulsions, which was attributed to the increased skin hydration.

In vitro studies have shown that nanoemulsions can exert a significant effect on skin permeation of lipophilic drugs [26, 39, 96]. Hydrogel-thickened nanoemulsions yielded significantly enhanced skin permeation rates of camphor, menthol and methyl salicylate [96]. The mechanisms assumed to be responsible for this effect comprised increased follicular transport, high concentration gradient of the drugs and more direct diffusion of drug molecules from smaller emulsion droplets which might be more efficiently embedded in the *stratum corneum*. However, more *in vivo* studies are needed to gain data under physiological conditions and to confirm tendencies observed *in vitro* [49].

2. Parenteral drug delivery

Since classical nanoemulsions have emerged from this field, numerous studies deal with the delivery of lipophilc drugs upon intravenous injection [8, 11, 108, 112, 117, 159, 160]. A large number of marketed nanoemulsions for drug delivery are found in the field of intravenous application, comprising drugs such as diazepam (Diazepam-Lipuro), etomidat (Etomidat-Lipuro), amphotericin B (Ambisome) and propofol (Disoprivan) [6, 7, 108]. Nanoemulsions for parenteral use have additional stability and safety requirements, such as sterility achieved by filtration or autoclaving [5]. Moreover, the viscosity of such formulations should be kept low to avoid injection pain. Likewise, particle sizes well below 500 nm and narrow particle size distributions are essential for patient safety, since larger particles may cause embolism [14, 50]. A significant advantage, however, is that such formulations can be produced with equipment and expertise already established in parenteral nutrition [64, 108]. In general, o/w emulsions are considered to be more biocompatible than w/o emulsions. The latter are more viscous, remain longer at the site of injection and have higher incidences of reactogenicity [5].

Only a limited number of emulsifying agents are regarded as safe for parenteral administration apart from lecithins, such as poloxamers, Span- and Tween-type surfactants [50, 56]. Unlike its synthetic alternatives, lecithin can be completely biodegraded and metabolized and thus guarantees non-toxicity of the applied formulations [7].

Multiple benefits can be achieved by employing lecithin-based nanoemulsions for drug delivery via the parenteral route. Lipophilic drugs can be solubilized in the oil core and their potential aqueous instability is decreased, which in turn leads to increased bioavailability [5]. This was shown for lecithin-based nanoemulsions with morphine and morphine prodrugs. Both slowed release and protection against hydrolysis were achieved, which led to successful induction of anaesthesia [107]. However, such an additional protective effect is not equally pronounced for all drugs, especially if the drug has a considerable destabilizing effect on the formulation itself [104].

A slower release of incorporated drugs is another potential benefit of nanoemulsions. Since the transport of drug from the oil core through the aqueous solution can be slow for lipophilic drugs, sustained release properties are frequently reported [161, 162]. Since nanoemulsions are particulate in nature, they also have longer biological residence times and are more efficiently phagocytosed by scavenging cells than aqueous formulations. Thus they can increase drug uptake into cells [5, 64]. A retarded release and thereby prolonged activity of incorporated drugs has been observed in several studies [163]. A prolonged analgetic effect has been found for the morphin-like nalbuphine and its prodrugs both *in vitro* and *in vivo* [52]. Lecithin-based nanoemulsions slowed drug release, thereby significantly increasing the duration and potency of analgesia. Likewise, parenteral nanoemulsions as delivery vehicles for chlorambucil [164, 165] and carbamazepine [64] showed a prolonged therapeutic effect *in vivo* and *in vitro*, respectively.

The choice of the oil compound is a determinant factor for the pharmacokinetic profile of drug release from nanoemulsions. Squalene has repeatedly been shown to be a highly useful lipophilic compound for preparation of lecithin-based nanoemulsions [5]. Squalene nanoemulsions with varying concentrations of lecithin showed good stability and the slowest release of lipophilic drug rifampicin compared with numerous other oils *in vitro* [106]. Sustained *in vivo* drug release is more likely to be achieved with highly lipophilic drugs, which have a high affinity to the oil phase [5].

Nanoemulsions can be used to achieve not only sustained release, but also organ targeting [52]. Surface modification of the emulsion droplets, for example with polyethylene glycol derivatives, can also be used to achieve prolonged circulation times or even passive targeting of tumors and inflammation sites [51, 52, 98]. Enhanced cytotoxicity and pro-apoptotic activity was reported for chlorambucil in human ovarian adenocarcinoma cells [164]. Such passive targeting of tumors can also be envisioned with conventional lecithin-based nanoemulsions provided their particle size is small enough [98, 121]. Attachment of target-specific ligands or charge-inducing excipients to the droplet surfaces for site-specific targeting of drugs is perhaps a more selective approach [44, 51].

Another important benefit of parenteral nanoemulsions as opposed to parenteral solutions is related to safety issues. By incorporating drugs into nanoemulsions droplets, the direct contact of drugs with body fluids and tissues can be avoided and side-effects can be minimized, thus increasing patient compliance [50, 52, 64]. However, a limiting factor for the use of lecithin-based nanoemulsions for parenteral application is the hemolysis which might be caused by the interaction of phospholipids and erythrocytes [52, 166]. It was observed that egg PC induces moderate toxicity in erythrocytes *in vitro*, which was increased in the presence of stearylamine and decreased upon addition of Brij-type surfactants. It was assumed that the latter may slow the exchange of phospholipids due to their viscosity. Such a shielding effect was also observed with polymeric surfactants [52]. These findings suggest that optimization of lecithin-based formulations can minimize the incidence of adverse reactions.

Charge-modified nanoemulsions have been developed for parenteral application to improve their physical stability. Since the ZP of lecithin-based nanoemulsions is usually a negative one, emulsion stability after injection is impaired by physiological cations such as Na^+ or Ca^{2+}. Flocculation may occur as the negative charges on nanoemulsion droplets are neutralized by these ions [14, 35, 98]. However, the negative surface potential of lecithin-stabilized nanoemulsions is markedly pH-dependent and can be improved by adjustment of the pH to alkaline. Such formulations with higher negative ZP values should be less sensitive to the presence of electrolytes [36]. Alternatively, the development of positively charged nanoemulsions is expected to enhance the electrochemical stability upon injection. As already mentioned, positively charged formulations can be used to enhance interaction with negatively charged tissues. In the case of parenteral nanoemulsions, the additional benefit of improved stability in the circulation plays a major role [159]. Moreover, the positive charge can also be employed for the association of anionic hydrophilic substances with the oil phase, as shown for polyanionic oligonucleotides [88]. The presence of phospholipids markedly contributed to the solubilization of these drug complexes [88]. However, the frequently used cationic surfactant stearylamine is not an ideal candidate for parenteral formulations. The ongoing discussion about its potential irritancy has led to a number of studies investigating the toxicological profile of nanoemulsions with stearylamine [167, 168]. Although no acute toxicity could be detected, most recent publications focus on employing physiological compounds for inducing a positive ZP value [18, 26, 39].

An interesting lecithin-based formulation entitled Lipid Nano-Spheres (LNS) was developed by Seki *et al*. [117, 169, 170] and was investigated as parenteral drug carrier for dexamethasone palmitate. These formulations were produced from equal amounts of lecithin and soybean oil, while the control nanoemulsions showed a regular surfactant to oil ratio. The LNS system significantly prolonged the plasma half-life of dexamethasone palmitate after intravenous injection. Moreover, improved targeting into inflammation sites and increased anti-inflammatory efficacy was observed. LNS easily and selectively passed through leak capillary walls by passive diffusion, which was driven by the higher plasma concentration. The lipid nanocarriers thus showed significant advantages over conventional nanoemulsions due to their lower droplet size (20-25 versus 200-300 nm). However, the increased amount of surfactant might play an additional role in the enhanced diffusion of LNS into tissues.

Another interesting approach is the formulation of supersaturated nanoemulsions for parenteral delivery. A stable supersaturated nanoemulsion for tirilazad was developed [171]. In spite of the drug content being twice the soluble amount, good long-term stability was reported. The excess amphiphilic drug was assumed to be associated mainly with the lipid phase. Likewise, a stable supersaturated nanoemulsion formulation of docetaxel was investigated for targeted tumor delivery [172, 173].

3. Ocular drug delivery

The bioavailability of drugs upon ocular application is often limited due to insufficient water solubility of the drug and difficulties to overcome the corneal barrier [174]. Lecithin-based nanoemulsions can be employed to improve the delivery of drugs via the ocular route [22, 64] and to prolong the pharmacological effect of drugs in comparison to aqueous solutions [175, 176]. As for other routes of application, positively charged nanoemulsions have yielded improved drug bioavailability. A depot effect in rabbit cornea and conjunctiva was observed for cyclosporine delivered by positively charged ocular nanoemulsions [19]. Likewise, numerous other studies have investigated the improved permeation of positively charged nanoemulsions through the cornea, which exhibits a negative surface charge at physiological pH [17, 177]. It can be assumed that a nanoemulsion which is applied via the ocular route will separate into an aqueous phase and a drug-loaded oil phase which will form a reservoir in the conjunctival sac. Therefore, nanoemulsions can increase the local bioavailability and prolong the pharmacologic effect of lipophilic drugs when administered directly into the eye [6]. However, the potential of nanoemulsions to increase the ocular bioavailability of drugs is also dependent on the physico-chemical properties of the drug and cannot be achieved in all cases [178].

4. Oral drug delivery

In oral drug delivery, lecithin-based nanoemulsions offer the advantage of a safe, non-toxic composition since they can be made from natural edible materials [51]. The use of oils that are rich in beneficial ingredients, such as poly-unsaturated fatty acids, can give these drug delivery systems an additional nutraceutical effect. Nanoemulsions can be employed to enhance the oral bioavailability and prolong the biological effect of highly lipophilic active substances with poor oral absorption or short biological half-life [179, 180]. The solubility of lipophilic drugs can be enhanced through incorporation in o/w nanoemulsions. Furthermore, incorporated substances are shielded against the extreme conditions or efflux pumps of the gastrointestinal tract and their absorption can be increased [62, 181, 182]. Consequently, the dose necessary to obtain a therapeutic effect can be decreased and drug-related side effects can be reduced [183]. In this context, it has been shown that encapsulation of the lipophilic anti-inflammatory agent curcumin in Tween-stabilized nanoemulsions is able to protect it from its metabolic fate in the intestine, eventually increasing its activity. Both decreased droplet size and the presence of lipids are held responsible for this effect [182]. In another study, a lecithin-based nanoemulsion for the oral delivery of primaquine proved to be more efficient in terms of antimalarial activity and biodistribution in mice than a solution of the drug [183]. The oral bioavailability of cefpodoxim was compared from a lecithin-based nanoemulsion and a macroemulsion of the same composition [23]. The nanoemulsion yielded a higher absorption, which resulted in increased bioavailability comparable to that of a parenteral solution.

Another approach to improve the oral bioavailability of drugs is mucoadhesive nanoemulsions. It was shown that polymer-coated droplets led to an enhanced antidiuretic effect of desmopressin in rats [16]. Likewise, lecithin-based nanoemulsions with and without PEG-modification were successfully employed to achieve efficient co-administration of paclitaxel and curcumin to overcome multidrug resistance in tumor cells [51]. In addition, approaches for targeted drug delivery to specific anatomical sites have been reported [51, 133].

5. Other routes of application

The nasal delivery route might be a promising field of application for lecithin-based nanoemulsions. It was recently found that ergoloid mesylate incorporated in lecithin-based nanoemulsions exhibited higher nasal absorption than a solution of the drug [133]. The effect was ascribed to the higher viscosity of the nanoemulsion, a higher bioadhesive effect due to the positive ZP value and the effect of lecithin as an absorption enhancer. Another interesting approach was taken in the field of pulmonal vaccination. A cationic lecithin-based nanoemulsion was investigated as a potential carrier for DNA vaccines to the lung [184]. The results indicate that these systems are able to induce immune stimulation via the pulmonary mucosa and are suitable for pulmonary application since no cytotoxicity was observed in Calu-3 cell lines [184].

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Emulsions with particle sizes in the submicron range, especially within or at least near the actual nanoscale range of below 100 nm, are useful pharmaceutical products that can be adapted for a large number of applications. The use of natural lecithin mixtures as a main or sole emulsifier renders them highly biocompatible. Therefore, the use of such lecithin-based nanoemulsions is suitable even for sensitive routes of drug administration, such as intravenous or ocular drug delivery. Moreover, a large field of application is represented by dermal drug delivery, skin care and cosmetic products. Classical nanoemulsions can easily be produced by high-energy emulsification on a large scale. Technological progress as well as new insights into the mechanisms of nanoemulsion formation and stabilization allow for the development of formulations with excellent long-term stability. Although the role of lecithin as emulsifying agent has been established decades ago, the complex behaviour of phospholipids in nanoemulsions is still not entirely predictable. Numerous innovative approaches have been investigated for the optimization and modification of lecithin-based nanoemulsions. The versatility of these formulations renders them interesting canidates for further research in various fields of application.

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