# Manufacturing techniques and excipients used during the formulation of oil-in-water type nanosized emulsions for medical applications

**Shunmugaperumal Tamilvanan[a,b*], Sudalaimuthu Ramachandran Senthilkumar[b], Raj baskar[b], Thenrajan Raja Sekharan[b]**

[a] International Medical University (IMU) SDN BHD, No. 126, Jalan 19/155B, Bukit Jalil, 57000 Kuala Lumpur, Malaysi

[b] Sankaralingam Bhuvaneswari College of Pharmacy, Sivakasi, Tamil Nadu State, India

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## ABSTRACT

Medically, the oil-in-water nanosized emulsions are used mainly as delivery carriers for lipophilic drug molecules which show therapeutic activity when administered via parenteral, ocular and transdermal routes. To extract multifunctional activities, the nanosized emulsions containing neutral, anionic and cationic charges over dispersed oil droplets are designed with the help of variety of excipients especially emulsifiers. This type of decoration on the dispersed oil droplet's surface allows the nanosized emulsions to be useful for drug delivery and/or drug targeting to otherwise inaccessible internal organs of human body. The aim of this review is to address the various manufacturing techniques and excipients used during the formulation of the multifunctional o/w nanosized emulsions for medical applications.

**KEY WORDS:** Nanosized emulsions, manufacturing techniques, excipients, anionic, cationic, neutral

## INTRODUCTION

Nanosized or submicron emulsions can be defined as systems of at least two nearly immiscible fluids dispersed one into another in the form of droplets with diameter significantly below one micrometer. Production of nanosized emulsions is achieved for diameters ranging from 50-400 nm and a narrow droplet size distribution (1). Medically, the oil-in-water (o/w) nanosized emulsions are used mainly as delivery carriers for lipophilic drug molecules which show therapeutic activity when administered via parenteral, ocular and transdermal routes.

To extract multifunctional activities, the nanosized emulsions containing neutral, anionic and cationic charges across the surface of the dispersed oil droplets are designed with the help of variety of excipients, especially emulsifiers. Surface charge optimization allows the o/w nanosized emulsions to be useful for drug delivery and/or targeting to otherwise inaccessible internal organs of the human body (2).

In contrast to microemulsions, the o/w nanosized emulsions are thermodynamically

* Corresponding author: S. Tamilvanan, International Medical University (IMU) SDN BHD, No. 126, Jalan 19/155B, Bukit Jalil, 57000 Kuala Lumpur, Malaysia, Tel: +60-3- 2731 7484, Fax.: +60-3-8656 7229; E-mail: tamilvanan2k@yahoo.com

instable, which can significantly reduce their applicability. The thermodynamic instability behaviour of emulsions include phenomena such as flocculation, coalescence, creaming, and Ostwald ripening. The physical instability of emulsions is due to the spontaneous tendency toward a minimal interfacial area and hence a minimal surface free energy between the dispersed phase and the dispersion medium. Minimizing the interfacial area is mainly achieved by two mechanisms: first coagulation possibly followed by coalescence and second by Ostwald ripening. Coalescence is often considered as the most important destabilization mechanism leading to coursing of dispersions and can be prevented by a careful choice of stabilizers or emulsifiers. The molecular diffusion of solubilizate (Ostwald ripening), however, will occur as soon as curved interfaces are present. During Ostwald ripening, the molecular diffusion of the lipophilic components occurs from small particles to larger particles due to the difference in escaping tendency (vapor pressure) between the two. Ostwald ripening is thus directly proportional to the solubility of the lipophilic component in the dispersion medium as well as dependent on the particle size distribution. Various physical factors such as density difference between the dispersion medium and droplets, strong hydrodynamic agitation, interdroplet interaction, and the droplet interfacial visco-elasticity can affect or perturb the colloidal stability of emulsion droplets and thus their shelf life and therapeutic utility. Therefore, the stabilization of emulsion droplets is in many cases very important and desirable. There are different ways of stabilization of the o/w nanosized emulsions, all of which are related to the modification of the interface between the dispersion medium and emulsion droplets. Depending on the intended medical application of nanosized emulsions, various kinds of emulsifier molecules ranging from small surfactants or surface-active polymers to polylayered interfacial coatings produced by multicomponent emulsifier films are considered.

This review encompasses a short overview on the preparation of medically useful o/w nanosized emulsions followed by a description (examples) of selected emulsifier molecules used for emulsion stabilization. Moreover, it is emphasized that the review focuses only on nanosized emulsions (having size distribution ranging between 50 and 1000 nm with a mean droplet size of about 250 nm), which should not be confused with self-microemulsifying drug delivery systems or microemulsions that are transparent, thermodynamically stable dosage forms. Before proceeding, a brief description concerning classification of nanosized emulsions is presented.

## CLASSIFICATION OF OIL-IN-WATER NANOSIZED EMULSIONS

Based on the emulsifier combinations used in the formation of submicron emulsion droplets, the o/w nanosized emulsions can be classified into three types (Figure 1). Emulsifiers with the capacity to produce a negative charge at the o/w interface are termed anionic and those able to provide a positive charge at the o/w interface are called cationic. The literature suggests that neither triglycerides nor phospholipidic emulsifier's components of the conventional or anionic emulsions are able to significantly sustain the incorporated lipophilic drug release in simulated or real physiological environments under sink conditions. Therefore, in an attempt to prolong and/or optimize the drug release, cationic lipid or polysaccharide emulsifiers are added to the emulsions to elicit mucoadhesion with anionic ocular tissues by an electrostatic adhesion. Indeed, cationic emulsions prepared using stearylamine, oleyamine and chitosan can serve this purpose. It was initially believed and now has become clearer from many reports in the literature that an occurrence of electrostatic attraction between the cationic emulsified droplets and anionic cellular moieties of the ocular and topical skin surface tissues enhance the bioavailability of emulsions containing lipophilic drugs (3-6). There is another type of emulsion that is neutral in terms of the charge on the dispersed droplets. These are instead stabilized through steric effects exerted by the emulsifier molecule present in the emulsion formulation.

According to Capek (7), the stability of the electrostatically- and sterically-stabilized o/w nanosized emulsions can be controlled by the charge of the electrical double layer and the thickness of the droplet surface layer formed by non-ionic emulsifier, respectively. In spite of

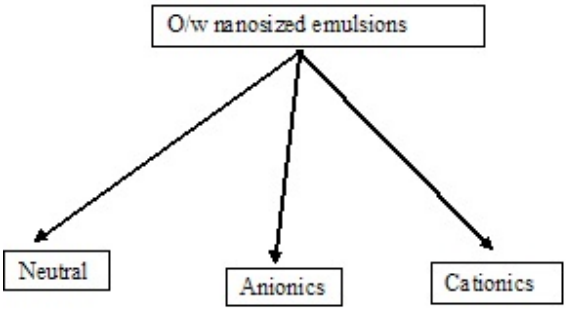


**Figure 1** Classification of oil-in-water (o/w) nanosized emulsions based on emulsifier molecules.

the similarities between electrostatically- and sterically-stabilized emulsions, there are large differences in the partitioning of molecules of ionic and non-ionic emulsifiers between the oil and water phases and the thickness of the interfacial layers at the droplet surface (7). The thin interfacial layer (the electrical double layer) at the surface of electrostatically stabilized droplets does not create any steric barrier for mass transfer. This may not necessarily be true for the thick interfacial layer formed by a non-ionic emulsifier. The sterically-stabilized oil droplets, however, can favor the transfer of materials within the intermediate agglomerates. Hence, the stability of electrosterically-stabilized emulsion ($\delta_k$) is controlled by the ratio of the thickness of the non-ionic emulsifier adsorption layer ($\delta$) to the thickness of the electrical double layer ($k^{-1}$) around the oil droplets (7).

$$[\delta / (k^{-1})] = (\delta_k) \qquad \text{Eq 1}$$

## MANUFACTURING TECHNIQUES FOR DRUG-FREE/LOADED O/W NANOSIZED EMULSIONS

To get a better idea of how to formulate the nanosized emulsion delivery systems suitable for parenteral, ocular, percutaneous, and nasal uses, the reader is referred to more detailed descriptions of methods of nanosized emulsion preparation (8,9). A hot-stage high-pressure (HP) homogenization technique or combined emulsification technique (de novo production) is frequently employed in order to prepare nanosized emulsions which exhibit the desired stability characteristics even after subjection of the emulsion to steam sterilization. Therefore, the steps involved in this technique in making placebo (drug-free) neutral, anionic and cationic emulsions can be arranged in the following order:

1. Separately weigh the oil- and water- soluble ingredients.
2. Heat both oil and water phases separately to 70° C.
3. Add the oil phase to the water phase and continue the heating up to 85° C with constant stirring to form a coarse emulsion.
4. Mix at high shear to make a fine emulsion.
5. Cool the fine emulsion formed in ice bath.
6. Homogenize the fine emulsion.
7. Cool the homogenized emulsion in ice bath.
8. Filter the emulsion through a filter of suitable porosity to remove large droplets or debris.
9. Adjust the emulsion to the desired pH.
10. Purge nitrogen/argon gas into the head space of the vials containing the emulsion.
11. Steam Sterilize the emulsion

The initial heating may be necessary for the solubilization of the respective oil and water phase components in their corresponding phases. Mixing the two phases with constant mild stirring and subsequently raising the temperature to 85° C is needed to form an initial coarse emulsion and to localize the surfactant molecules for better adsorption at the oil-water interface, respectively. The traditional droplet size-reducing steps involved during the preparation include constant mild stirring initially mixing oil and water phases, high shear/speed mixing, followed by homogenization. High pressure (HP) homogenizers are particularly suitable for continuous production of finely dispersed emulsions and are therefore of interest in the food and pharmaceutical fields (10-12). Extreme shear and high energy input have to be applied during the homogenization process to fragment the droplets of a coarse emulsion from the micro- to the nano-scale range, overcoming thus the Laplace pressure. The Laplace pressure, which causes the resistance to interface deformation and breakup, effectively increases when the droplet diameter decreases, and is therefore larger for nanosized- than for coarse emulsions (1).

## Equipment to make nanosized emulsions

High shear/speed mixing of a coarse emulsion can be performed using rotor-stator, high pressure, membrane or ultrasonic devices (11,13,14). Different equipment using HP technology are being developed, at both prototype and industrial scales, depending on the nominal pressure level. For example, jet dispersers involve two fluid jets (each from opposite bores) that collide with one another to disrupt particles. Marie et al (15) have shown that it is possible to perform oil-emulsification by direct injection of the oily phase under pressure (up to 350 MPa) into a continuous aqueous phase at low pressure. Nevertheless the oil droplets created by such a process displayed large size distributions, indicating that a more precise control of process and optimization may be required using this process.

To produce emulsions with small droplet size, microfluidization or HP homogenization is usually used. Microfluidization is a process whereby a liquid mixture is forced by high pressure through an interaction chamber, which splits the stream into two and then recombines them at ultrahigh velocities (16). The product can be recycled to reduce droplet size further. The combination of high shear, turbulence, and cavitation generated by this apparatus can produce nanosized or submicrometer emulsions with a narrow size distribution (17). For instance, Microfluidizer devices (Microfluidics™) comprise interaction chambers designed with a microchannel architecture that combines laminar extension flow at the inlet of the chamber to turbulent flow with cavitation and impact in and at the outlet of the chamber (10,14,18,19): the premix stream is divided into two fluid jets at the inlet of the chamber and the fluid velocity is accelerated due to a sudden decrease in the pipe diameter. Laminar extension flow is considered responsible for droplet disruption at the inlet of the chamber. Inside the chamber, the fluid changes its flow direction leading to enhanced particle collision and impingement on the chamber walls. The fluid jets then collide (coming from two opposite microchannels) leading to enhanced particle disruption. Another HP mixing equipment of interest is Polytron™ which also functions on a similar principle.

In HP homogenization, fluid is forced at high pressure by means of a plunger pump through a very narrow channel. Depending on the type of homogenizer, the fluid may then collide head on with another high velocity stream or hit a hard-impact ring. Droplet size is reduced by cavitation, high shear forces, and high-speed collisions with other droplets (20). Pressure, temperature, and number of passes are parameters that can be controlled and influence the efficiency and magnitude of droplet size reduction. HP-homogenizers of piston-gap type developed by manufacturers such as Avestin™, APV™ or Stansted Fluid Power™ consist of one or two piston intensifier(s) able to generate high pressure, and HP-valve equipped with ceramic needles and seat of specially engineered design. In such HP-homogenizers, the fluid under pressure is forced through a small orifice of some micrometers width, the HP-valve gap (21). The fluid accelerates over a very short distance to very high velocity and the resulting strong pressure gradient between the inlet and outlet of the HP-valve generates intense shear forces and extensional stress through the valve gap (22). Cavitation, turbulence and impact with solid surfaces takes place at the outlet of the valve gap (23,24). Due to shear effects and conversion of kinetic energy into heat, the fluid travelling through the HP-valve is accompanied by short-life heating phenomena that can be controlled by efficient cooling devices (25,26). All these mechanical forces are expected to disrupt particles down to the submicron range. This type of equipment can deliver pressure up to 150-200 MPa (high pressure homogenization, HPH) and even more for the latest developments, i.e. up to 300-400 MPa for ultra-high pressure homogenization (UHPH). On the basis of a numerical simulation, Floury et al (24) attempted to understand the flow pattern of oil-in-water emulsions (20%, w/w, sunflower oil) stabilized by Tween 20 (1%, w/w) in UHP-homogenizer. Their results suggested that: (i) extensional flow taking place before and in the HP-valve gap probably led to droplet deformation and breakup; (ii) turbulence, recirculation and cavitation phenomena taking place at the outlet and downstream of the HP-valve gap could contribute to additional breakup but also to re-coalescence of oil droplets.

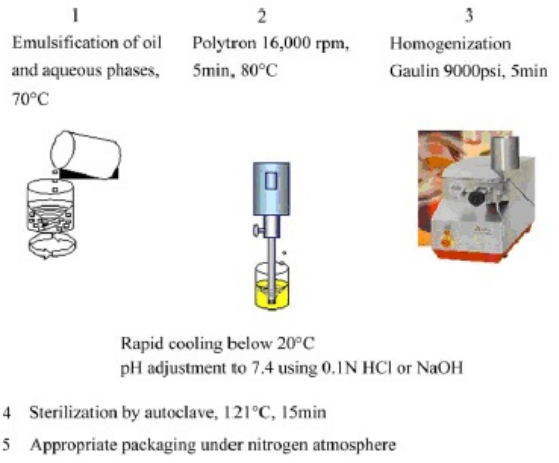


**Figure 2** Preparation of o/w nanosized emulsion (de novo method) [by permission from Elsevier (27)].

## Approaches to incorporate drug into oil phase or at the oil-water interface

There are four different approaches (27) to incorporate lipophilic drugs or heat labile molecules into the oil phase or at the o/w interface of the nanosized emulsions:

(A) extemporaneous drug addition,
(B) de novo emulsion preparation,
(C) an interfacial incorporation approach, which includes the recently developed SolEmul® technology, and
(D) incorporation of antibodies, DNA protein, oligonucleotide or heat labile molecules

### (A) extemporaneous drug addition

Cohen et al (28), when looking for a new galenic presentation form for amphotericin B with better ocular tolerance over the commercially available Fungizone® eye drops, incorporated the drug directly into the preformed 20% emulsion, Intralipid®. However, after addition of the solid drug particles or drug solution, several physical changes such as phase separation, precipitation or creaming may occur thus limiting such practices in o/w nanosized emulsion preparations. Therefore, ocular active lipophilic agents are not normally incorporated into the emulsions by this extemporaneous addition method.

### (B) de novo emulsion preparation

In principle, the lipophilic drug molecules (thermostable) should however be incorporated by a de novo process as described earlier. Thus, the drug is initially solubilized or dispersed together with an emulsifier in suitable single-oil or oil mixture by means of heating. The water phase containing the osmotic agent with or without an additional emulsifier is also heated and mixed with the oil phase by means of high-speed mixers. Further homogenization takes place to obtain the needed small droplet size range of the emulsion. A terminal sterilization by filtration, or steam, then follows. The emulsion thus formed contains most of the drug molecules within its oil phase or its oil-water interface. This is a generally accepted and standard method to prepare lipophilic drug-loaded nanosized emulsions for parenteral, ocular, percutaneous, and nasal uses, as illustrated in Figure 2. This process is normally carried out under aseptic conditions and nitrogen or argon atmosphere to prevent both contamination and potential oxidation of sensitive excipients.

### (C) Interfacial incorporation approach

Since many drugs of commercial interest generally have a solubility that is too low in FDA approved oils, Lance et al (29) proposed a method to incorporate such drugs into the interfacial o/w layer of the emulsion droplets. This can be achieved by initially dissolving the drug along with the phospholipid (emulsifier) in an organic solvent, instead of in the oil.
Following the solvent evaporation, the obtained phospholipids/drug co-mixture is used in the de novo production of the emulsions (30). However, this approach suffers from possible drug nanocrystal formation and from the use of organic solvent during the emulsion preparation process. To overcome such drawbacks, a novel SolEmul® technology was developed in which an additional high speed homogenization step is included to mix the drug with emulsion. The drug particles are micronized to the nanosize range prior to incorporation into the emulsion. By this technique, adequate amounts of lipophilic drugs can be substantially incorporated into the

lipophilic core or intercalated between the selected emulsifier molecular films at the o/w interface of the emulsions. The drugs reported to have been incorporated by this novel approach are amphotericin B, carbamazepine and itraconazole (31-34). However, it should be emphasized that all the lipophilic drug molecules that have been incorporated into the emulsions by SolEmul® technology are meant only for parenteral use (31-34) and so far no ocular, nasal and topical active agents have been incorporated by this approach although there appears to be no regulatory reason to exclude this technical improvement when designing emulsion formulations for these applications.

**(D) incorporation of antibodies, DNA protein, oligonucleotide or heat labile molecules**

Both extemporaneous drug addition (method A) into the preformed emulsion and de novo emulsion preparation (method B) are useful for the incorporation of heat labile molecules into the o/w nanosized emulsions. For example, cyclosporin A was successfully incorporated without drug degradation into the emulsion by following the de novo method (35). The extemporaneous addition of the solid drug or drug previously solubilized in another solvent or oil to the o/w nanosized emulsions is not a favoured approach as it might compromise the integrity of the emulsion. However, since therapeutic DNA and single stranded oligonucleotides (oligos) or siRNA are water soluble due to their polyanionic character, the aqueous solution of these compounds can be added directly to the o/w cationic nanosized emulsion in order to interact electrostatically with the cationic emulsion droplets and thus associate/link superficially at the oil-water interface of the emulsion (36-38). When administered via parenteral and ocular routes, the release of the DNA and oligos from the associated emulsion droplet surfaces should therefore initially be dependent on the affinity between the physiological anions of the biological fluid and cationic surface of the emulsion droplets. The mono- and di-valent anions in the biological fluid available in parenteral route is plasma and in ocular topical route is tear fluid, aqueous humor and vitreous. Moreover, these biofluids contain multitude of macromolecules and nucleases. There is a possibility that endogenous negatively charged biofluid's components could dissociate the DNA and oligos from cationic emulsion. It is noteworthy to conduct during the preformulation development stages an in vitro release study for therapeutic DNA and oligos-containing cationic nanoemulsion in these biological fluids and this type of study could be considered as an indicator for the strength of the interaction occurring between DNA or oligo and the emulsion (39). Interestingly, the stability of oligos (a 17-bases oligonucleotide, partially phosphorothioated) was validated using a gel-electrophoresis method. After incorporating the oligos into the cationic nanosized emulsion as well as during in vitro experiments of oligo-containing emulsion in vitreous fluid at different time periods, the emulsions were phase separated by Triton X-100 and then the degradation of oligos was monitored using gel-electrophoresis (39). No appearance of new bands was observed in comparison to the standard aqueous oligos solution. This result indicated that the oligos did not undergo degradation under the conditions applied to prepare a sterile emulsion.

In order to enable the nanosized emulsion to target inaccessible tissues, homing devices/ligands such as antibodies and cell recognition proteins are usually linked onto the particle surface. Various methods have been employed to couple ligands to the surface of the nanosized emulsions with reactive groups. These can be divided into covalent and noncovalent couplings. Noncovalent binding by simple physical association of targeting ligands to the nanocarrier surface has the advantage of eliminating the use of rigorous, destructive reaction agents and conditions. Common covalent coupling methods involve formation of a disulfide bond, cross-linking between two primary amines, reaction between a carboxylic acid group and a primary amine, reaction between a maleimide and a thiol, reaction between a hydrazide and an aldehyde, and reaction between a primary amine and free aldehyde (40). For antibody- conjugated anionic emulsions, the reaction of the carboxyl derivative of the coemulsifier molecule with free amine groups of the antibody and disulfide bond formation between coemulsifier derivative and reduced antibody were the two reported conjugation techniques (41-43). However, by the formation of a thio-ether bond between the free maleimide reactive group already localized at the o/w interface of

the emulsion oil droplets and a reduced monoclonal antibody, the antibody-tethered cationic emulsion was developed for active targeting to tumor cells (44,45).

## EMULSIFIERS TO STABILIZE THE O/W NANOSIZED EMULSIONS FOR MEDICAL APPLICATION

In general, the o/w nanosized emulsion should be formulated with compatible vehicles and additives. The components of internal and external phases of nanosized emulsion should be chosen to confer enhanced solubility and stability to the incorporated lipophilic drug. In addition, the selected excipients should also preferably be chosen to favorably influence the biofate or therapeutic index of the incorporated drug following administration via parenteral, ocular, percutaneous, and nasal routes. This section is a comprehensive presentation of the general considerations concerning excipient selection and their optimum concentrations.

Prior to the formulation design of the emulsions, data is needed concerning the drug solubility in the oil vehicle. Additionally, prerequisite information is needed about compatibility of the oil vehicle with other formulation additives and the established ocular/skin tissues-oil vehicle matching before the dosage form can be prepared. Table 1 lists the common emulsion excipients and the oils suitable for dissolving or dispersing lipophilic drugs of ocular/parenteral interest. Since oils are triglycerides, care must be taken to minimize oxidation. Therefore, antioxidants such as α-tocopherol (0.001-0.002% w/w) may be included in a typical emulsion formulation for medical applications. The final oil-phase concentration in emulsions meant for ocular use is now widely accepted to be at or below 5% w/w taking into account that the emulsion must be kept in a low-viscosity range of between 2 and 3 centipoises, which also is the optimal viscosity for ocular preparations (46). However, for all other medical uses, the amount of oil may be varied but generally is within 5-20% w/w. Sometimes, a mixture of oils may be employed to facilitate drug solubilization in the oil phase. Jumaa and Müller (47,48) reported the effect of mixing castor oil with medium chain triglycerides (MCT) on the viscosity of castor oil. The oil combination at the ratio of 1:1 (w/w) led to a decrease in the viscosity of castor oil and simultaneously to a decrease in the interfacial tension of the oil phase. This phenomenon was related to the free fatty acids contained in castor oil, which can act as coemulsifiers, thereby resulting in lower interfacial tension and, simultaneously, in a more stable formulation in comparison with the other oil phases. In addition to the digestible oils from the family of triglycerides, including soybean oil, sesame seed oil, cottonseed oil, and safflower oil, which are routinely used for making medical emulsions, alternative biocompatible ingredients such as α-tocopherol and/or other tocols were also investigated for drug delivery purposes via o/w emulsions (49,50). But the emulsions formed from tocols are often considered as microemulsion systems with few exceptions (49,50).

**Table 1** Common Excipients used for formulation of o/w nanosized emulsions

| Oils | Emulsifiers | Cationic lipids and polysaccharide | Miscellaneous |
|---|---|---|---|
| Sesame oil | Cholesterol | Stearylamine | ?- Tocopherol |
| Castor oil | Phospholipids (Lipoid) | Oleylamine | Glycerin |
| Soyabean oil | Polysorbate 80 and 20 (Tween 80 and 20) | Chitosan | Xylitol |
| Paraffin oil | Transcutol P | | Sorbitol |
| Paraffin light | Cremophor RH | | Thiomersal |
| Lanolin | Poloxamer 407 | | EDTA |
| Vaseline | Poloxamer 188 | | Methyl paraben |
| Corn oil | Miranol C 2 M and MHT | | Propyl paraben |
| Glycerin monostearate | Tyloxapol TPGS | | |
| Medium-chain monoglycerides | | | |
| Medium-chain triglycerides | | | |
| Squalene | | | |

Unlike spontaneously forming thermodynamically stable microemulsion systems that require a high surfactant concentration (20% and higher) and an alkanol component, the kinetically stable nanosized emulsions can be prepared by using relatively lower surfactant concentrations. For example, a 20% o/w nanosized emulsion may only require a surfactant concentration of 1-5%. The kinetic stability of the nanosized emulsions can be achieved by creating a barrier at the oil-water

interface, protecting the emulsion from breakage (7). These barriers may be of electrostatic or steric nature and prevent emulsion droplets from direct contact. The most common way to stabilize emulsions is by surfactant adsorbed at the interface between the droplets and dispersion medium. Surfactant adsorption layers do not only reduce the interfacial tension but can also provide an electrical charge to the emulsion droplets (ionic surfactants) or create the strong steric barrier via bulky molecular groups directed toward the dispersion medium (non-ionic surfactants).

Traditionally, lecithins or phospholipids are the emulsifiers of choice to produce o/w nanosized emulsions. However, additional emulsifiers preferably dissolved in the aqueous phase are usually included in the emulsion composition. A typical example of the aqueous soluble emulsifiers are nonionic surfactants (e.g., Tween 20) which are preferred because they are usually less irritant than their ionic counterparts. The nonionic block copolymer of polyoxyethylene-polyoxypropylene, Pluronic F68 (Poloxamer 188), is included to stabilize the emulsion through strong steric repulsion. However, surfactants such as Miranol MHT (lauroamphodiacetate and sodium tridecethsulfate) and Miranol $C_2$ M (cocoamphodiacetate) were also used in earlier ophthalmic emulsions (51). It should be added that commercially available cyclosporin A-loaded anionic emulsion (Restasis®) contains only polysorbate 80 and carbomer 1342 at alkaline pH to stabilize the anionic emulsion. To prepare a cationic emulsion, cationic lipids (stearyl and oleylamine) or polysaccharides (chitosan) are added to the formulation. Strikingly, a stable emulsion based on chitosan-lecithin combination was also reported (52). Conversely, a cationic emulsion based on an association of poloxamer 188 and chitosan without the incorporation of lecithin was prepared and also demonstrated adequate stability (47,53). Recently, a report from our group also indicated the stability of oil droplets through the cation conferring chitosan along with poloxamer 188 as a mixed emulsifier (54). Since the free fatty acid generating phospholipid emulsifier molecule is omitted from the nanosized emulsion system, the stable nanosized emulsion produced from chitosan-poloxamer emulsifier combination has the potential to attenuate a microclimate acidic pH in the vicinity of oil phase, oil-water interface and water phase of the emulsion (54). These non-phospholipid-based emulsions should therefore be able to incorporate the acid-labile molecules like therapeutic peptides and proteins, and to delineate the scope of applying lyophilization process for the development of a solid or dry emulsion.

Oil in water emulsion compositions based on a tocopherol (or a tocopherol derivative) as the disperse phase has been described in a patent granted to Dumex (55). Interestingly, the emulsifying agent used to make tocol-based emulsions is restricted to vitamin E TPGS (D-alpha-tocopheryl polyethylene glycol 1000 succinate) presumably because adequate toxicological data is not yet available for other derivatives. According to a patent by Nakajima et al (56), functional emulsions for use in food, drugs and cosmetics were reported. These emulsions were stabilized with various span products such as span 80, span 40, etc.

### Advantages of charge stabilized nanosized emulsions

At present, emulsions stabilized by positively charged, cationic surfactants are most often used as colloidal drug carriers (37). Kim et al (57) used an emulsion of squalene in water stabilized by the cationic surfactant 1,2-dioleoyl-sn-glycero-3-trimethylammonium-propane (DOTAP), which facilitated gene transfer in biological fluid even in the presence of 90% serum in the dispersion medium. The emulsion droplets play the role of mucosal gene carriers and can form stable complexes with DNAs. Here the DNA was incorporated in the emulsion by the de novo method. Compared with liposomal carriers, cationic emulsions demonstrated a 200 fold increase in transfectional efficacy in both lungs and tissues (57,58). The nature of oil as the disperse phase, is another important factor that can affect the applicability of such emulsions for transfection. Three different oils were used for the disperse phase: soybean oil, linseed oil, and squalene (58). The transfection activities of the nanosized emulsion carriers in the presence of serum followed the order squalene > soybean oil > linseed oil, and the squalene emulsions were also most stable. From this data, the authors concluded that stability of a carrier system is a necessary requirement to form

stable complexes with DNA, and this stability determines the in vivo transfection.

The literature indicates that the interaction between cationic liposomes and polyanionic macromolecules like DNA is dependent on their ratio, and at the ratio of maximum transfection there occurs a prominent aggregation phenomenon leading to destabilization of formulation or desorption of DNA from the formulation (59). Furthermore, Simberg et al (60) suggest that an understanding of the interplay between lipoplex composition, its interaction with serum, hemodynamics and target tissue properties (susceptibility to transfection) could explain the biodistribution and efficient in vivo transfection following intravenous administration of cationic lipid–DNA complexes (lipoplexes) in a murine model. However, it is interesting to see what could happen when the cationic nanoemulsion is applied to in vitro cell culture models in the presence of serum. The serum stability of emulsion/DNA complex was reported (61). Further studies are, however, necessary to be carried out to understand clearly the origin of the serum stability of this emulsion. In addition the transfection efficiency of this emulsion was not affected by time up to 2 h post-emulsion/DNA complex formation. This suggests that the o/w cationic nanosized emulsion complexed with DNA is stable to allow routine laboratory manipulation.

The o/w nanosized emulsions stabilized by both cationic and anionic lipids were investigated in order to compare the degree of binding and uptake by specific cells that over-expressed tumor receptors (62). Immunoemulsions were prepared by conjugating an antibody to the surfactant molecule via a hydrophobic linker and then the antibody-conjugated surfactant was used to make the emulsion by the de novo method. The anionic stabilized emulsions showed decreased stability leading to phase separation after 20 days of storage. The reduced stability of anionic immunoemulsion could be attributed to the rapid decrease of the zeta-potential caused by the positively charged conjugated antibody and consequently, due to a lower electrostatic repulsion between the colloidal droplets (62). On the other hand, immunoemulsions stabilized by both anionic and cationic emulsifiers exhibited a multifold increase in cell binding in contrast to the emulsions without antibodies.

Positively charged o/w nanosized emulsions were also found to be effective vehicles to improve the skin permeability of incorporated lipophilic molecules in dermatological applications (63). Because epithelial cells of the skin carry a negative surface charge they show a high selectivity and permeability to positively charged solutes. Thus, positively charged nanosized emulsions are promising systems for enhancing the skin permeability for drugs included in the colloidal droplets. The authors also showed that ceramides could be successfully delivered transdermaly by means of nanosized emulsions stabilized by a positively charged interfacial layer of the naturally occurring molecule, phytosphingosine. Other applications of nanosized emulsions as carriers, stabilized by ionic surfactants, in the pharmaceutical and cosmetic fields have been reviewed by Solans et al (64) and Tamilvanan (65).

Anionic phospholipids are also commonly utilized for the stabilization of drug-carrying nanosized emulsion droplets both individually and in binary mixtures (66). Soybean lecithin and modified phospholipid, n-hexanoyl lysolecithin (6-PC), alone and as 1:1 mixtures were used as stabilizers of medium-chain triglyceride (MCT) droplets in water (66). Although individual uncharged phospholipids provide emulsion droplets with a moderate negative charge for stabilization, mixed phospholipids produce much more stable emulsions and a large negative zeta-potential. A possible explanation for this phenomenon is related to the increased incorporation of polar compounds from the soya lecithin into the mixed interfacial film when 6-PC is present. This interfacial film acts as a stabilizer by forming a high energy barrier that repels adjacent droplets and leads to the formation of stabilized emulsified droplets. The stability of the emulsion did not noticeably change, even in the presence of the model destabilizing drug, indomethacin, demonstrating the high potential for such mixed emulsifiers for the formulation of colloidal drug delivery systems (66). Lysolecithin has one fatty acid ester chain removed from the glycerol backbone, in addition, lysolecithin is toxic (destroys RBC cell membranes). Furthermore, although the role of

phospholipids is essential for the stability of the emulsions, possible cataractogenic effects due to the phosphatidyl choline and, basically, to a derivative of the same, lysophosphatidyl, have been described by different authors (67,68).

A new class of surface-active dialkyl maleates can be utilized for emulsion polymerization (69). Here, the emulsion droplets of monomeric maleates are self-stabilized and simultaneously serve as liquid "reactive storage carriers". Three types of head group in the dialkyl maleates were studied- non-ionic, cationic, and zwitterionic with different lengths of hydrophobic alkyl chain. Cationic and zwitterionic dialkyl maleates with the longest alkyl chains - $C_{16}H_{33}$ and - $C_{17}H_{35}$ provided the best stability for o/w nanosized emulsions. When compared with the data obtained for the well-known non-ionic surfactant $NPEO_{10}$ (nonylphenol-poly (ethylene oxide)) and the cationic CTAB (cetyltrimethyl ammonium bromide), an excellent stabilizing capacity especially for the cationic maleates can be stated. Whereas non-ionic dialkyl maleates show almost the same emulsifying ability and stability as $NPEO_{10}$, the cationic derivatives of these novel surfactants are more effective in stabilization than the traditional CTAB.

Sometimes anionic surfactants are especially added to emulsion droplets for the stabilization of "reactive storage carriers" subjected to further chemical transformation. Sodium dodecyl sulfate (SDS) was utilized to stabilize miniemulsion droplets which in the subsequent step were polymerized and formed PBCA (poly(n-butylcyanoacrylate)) nanoparticles, suitable for targeting drug delivery to specific cells (70). It is worth mentioning that SDS is predominantly used to achieve required miniemulsion stability (71). In some cases, however, cationic surfactants are also used in miniemulsion formulations, which were reported first in the late seventies of the 20th century. In general, however, stability of miniemulsions does not depend on the sign of the surfactant charge and is mainly determined by the surfactant coverage of the reactive carriers (miniemulsion droplets). The same factor is also crucial for the size of miniemulsion droplets after steady-state miniemulsions are obtained (71).

**Advantages of neutral-charged (sterically-stabilized) nanosized emulsions**

In many cases, however, greater emulsion stability can be achieved without imparting a significant surface charge to the emulsion droplets, by means of steric stabilization (7). Non-ionic surfactants possessing bulky hydrophilic groups like polyoxyethylene (PEO) protruding into the dispersion media decrease coalescence arising from droplet collisions. Another contribution to the steric stabilization of emulsions by nonionic surfactants is provided by the close packing of PEO chains at the droplet surface. The compact packing of PEO chains at the droplet surface creates stearic stabilization because little or no interpenetration of PEO chains on different droplet surfaces occurs due to entropic repulsion (72). Large head groups carrying simultaneously charges of opposite signs, such as in zwitterionic surfactants, can cause similar effects. In polar dispersion media of low to medium ionic strength, these groups are, as a rule, strongly solvated (hydrated in the most common case of $H_2O$) (73). Voluminous and on an average almost non-charged hydration shells, surrounding the emulsion droplet possess a significant steric rigidity and can also effectively stabilize emulsions. There are, however, only a few examples in the literature that use zwitterionic surfactants as effective emulsion stabilizers. For example, lecithin was used for the stabilization of perfluorooctyl bromide (PFOB) in water emulsions, to be used as oxygen-carrying system in a bio-artificial liver device (74). The Sauter mean diameter of 0.2 μm PFOB emulsion droplet in water was obtained by high-pressure homogenization. The emulsion was stable for several months even at a volume fraction of 20%. Non-ionic surfactants are more often used for emulsion stabilization than zwitterionic phospholipids because they are synthetically manufactured, can be well defined analytically and have significantly less batch to batch variation than naturally occurring (egg yolk, soybean) lecithins).

The non-ionic surfactant Span-83, was used for stabilizing water droplets in oil to form a reactive storage carrier for the synthesis of calcium carbonate nanoparticles by means of a two membrane system (75). Firstly, an aqueous emulsion was prepared in kerosene stabilized by

0.02596 wt % Span and containing $CO_3^{-2}$ ions in the droplets of the dispersed phase. The oil phase contained also a 0.02792 M solution of bis(2-ethylhexyl) hydrogen phosphate (2DEHPA), a well-known molecular carrier for the transportation of metal ions across emulsion liquid membranes (ELM). In the second stage, a $CaCl_2$ aqueous solution filled dialysis tube was placed into the oil-in-water emulsion and due to the reaction between $CO_3^{-2}$ and $Ca^{2+}$ ions in the aqueous droplets, $CaCO_3$ nanoparticles were obtained. Similarly, ZnS nanoparticles were prepared in inverse water-oil-emulsion (76). The stabilization of emulsions was provided by the addition of 5 wt% of Span 80 or Span 20, respectively, to the oil phase (cyclohexane). The dispersed phase contained a mixture of zinc acetate and thioacetamide, which react upon heating to form ZnS. The authors demonstrated that for the preparation of ZnS nanoparticles the use of Span 20 was more favourable because of the smaller emulsion droplet size and therefore lower and more homogeneous size of the final particles. Another advantage was the higher stability of Span 20 against hydrolysis as compared to Span 80.

In general, fulfilling both stabilization mechanisms (smaller droplet size and lesser susceptibility of surfactant toward chemical degradation) simultaneously leads not only to the highest emulsion stability but also to lesser sensitivity to changes in the external conditions such as pH, ionic strength, and temperature. Therefore the use of mixtures of different classes of surfactants for emulsion stabilization is frequently the most effective solution in many practical cases.

## Advantages of nanosized emulsions stabilized by mixed or multicomponent emulsifier molecules

Sometimes mixtures of natural zwitterionic surfactants used for emulsion stabilization contain small amounts of polar compounds which can be incorporated into the adsorption layer and lead to a modest droplet charge which additionally stabilizes the emulsion (65). Surface layer with charged natural admixtures reported by Trotta et al (66) is only a particular case of a very large class of emulsion-stabilizing systems based on a tailored application of ionic–zwitterionic surfactant mixtures. Mixtures of dipalmitoylphosphatidylcholine (DPPC) and homologues and dimyristoylphosphatidyl-ethanolamine (DMPE) phospholipids were utilized by Ishii and Nii (77) for stabilizing model drug-carrying oil-in-water nanosized emulsions. In contrast to the data, the main stability factor was found to be the optimal average hydrophilic-lipophilic balance (HLB) value of the stabilizers mixture, defined similarly for non-ionic surfactants (66). For example, emulsions prepared with mixtures of dimyristoylphosphatidylcholine (DMPC, zwitterionic) and DMPE behaved similarly to emulsions prepared by DMPC alone. This fact was explained by the equivalence of HLB values for both surfactants used, regardless of their ionic nature. However, the ionic character of a surfactant like DMPE (and therefore the charge of respective emulsion droplets) can be affected by the pH of the dispersion medium.

The o/w nanosized emulsion stabilized by mixed ionic/non-ionic surfactants revealed very high physical stability and was found to be most appropriate for dermatological applications as a ceramide-carrying colloidal system (63). Greater emulsion stability was achieved by the combination of the non-ionic steric stabilizer Tween 80 and the phospholipid co-stabilizers phytosphingosine and phosphatidyl-ethanolamine.

## 'Stealth' property of nanosized emulsions: in vitro demonstrations

Anionic emulsion formulations capture apolipoproteins along with other plasma proteins within minutes after an infusion in human blood, facilitating their fast elimination. In contrast, cationic emulsions reveal a much longer retention time in the plasma, Moreover, cationic colloidal carriers can promote the penetration of therapeutic agents into cell surfaces possibly via an endocytotic mechanism (53). To improve the drug targeting efficacy of colloidal carriers of anionic emulsions and to further prolong the circulating effect of the cationic emulsions, a mixed stabilizers film at the oil/water droplet interface composed of non-ionic Poloxamer 188 and ionic lipoid E80 and stearylamine/oleylamine was created combining the effects of electrostatic and steric barriers at the oil/water interface (78). Competition between steric repulsion by poloxamer and electrostatic attraction by ionic

components led to the sensitive adsorption of small molecular weight proteins like apolipoproteins, albumins by the surface of colloidal carriers whereas all emulsion droplets were effectively shielded from the adsorption of larger proteins like immunoglobulins, fibrinogen, etc. enhancing the shelf-life of emulsion formulations in the blood (Figure 3). The apoA-I along with apoA-IV have been suggested to modulate the distribution of apoE between the different lipoprotein particles in the blood and thereby affect their clearance (78). In addition, the attachment of apoE would greatly alter the in vivo distribution of fat emulsions since this protein is a ligand for the apoE-specific receptors on the liver parenchymal cells. The higher the preferential adsorption of apoA-I onto the cationic emulsion droplets, the more intensified the displacement/redistribution of apoE would, therefore, be expected to occur on these types of cationic emulsion formulations in the blood (78). Indeed, the ratio of apoA-I to apoA-IV was very close to 1 for Lipofundin® MCT 10% whereas it was about 0.26 for deoxycholic acid-based anionic emulsion and above 5 for oleic acid-based anionic emulsion and both cationic emulsions.

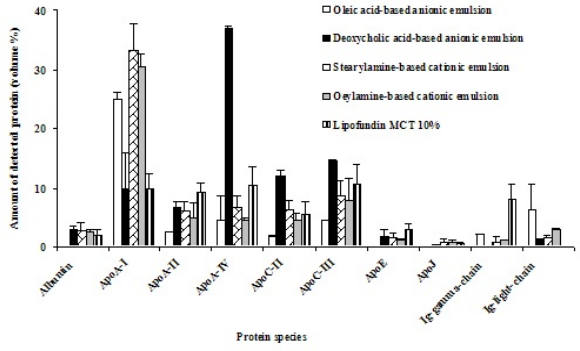


**Figure 3** Amount of major proteins on the 2-D gels of plasma proteins adsorbed on emulsions with negative or positive surface charge in comparison with Lipofundin MCT 10% [by permission from Elsevier (61)].

## Advantages of stabilizers in nanosized emulsions

In some practical cases where emulsions are applied under real, sometimes quite harsh conditions, such as high temperature or shear stresses, the stabilization of colloidal carriers by conventional surfactants (ionic as well as non-ionic) can appear to be insufficient to keep the initially acceptable emulsion properties intact. For ionic surfactants as stabilizers, the stabilization mechanism based on the electrical double layer fails at high amounts of electrolyte. The situation for non-ionic surfactants as stabilizers is only slightly better because their molecules are typically not strongly adsorbed (79,80). Therefore, the most effective way to stabilize emulsions by creation of a protective adsorption layer is the use of amphiphilic macromolecular compounds or polymeric surfactants (79). In contrast to the commonly used monomolecular surfactants, polymeric surfactants do not only adsorb much stronger but also retain this ability under high electrolyte concentration or/and high temperature (79,80). This is made possible due to a special molecular design of surface active polymers which have in their structure anchor groups responsible for strong adsorption at the interface and stabilizing groups protruding from the interface into the dispersion medium and forming a bulky layer with thicknesses of several nanometers.

Most often used stabilizers for the preparation of emulsions, in the fields of agrochemicals, pharmaceuticals and personal care products, are either block or graft copolymers. In block copolymers, the hydrophobic blocks reside at the surface or even partly penetrate in the oil droplet, making trains or short loops whereas the hydrophilic blocks protrude in the dispersion medium as loops or tails providing steric stabilization (80). As examples, PEO-PPO-PEO triblock copolymer (commercially available as "Pluronics") or PPO-PEO-PPO can be mentioned. Triblock copolymers are, however, not the most efficient stabilizers because the PPO chain is not hydrophobic enough to attach strongly at the o/w interface (81). The surface activity of these polymeric surfactants is rather the result of a rejective anchoring or negative enthalpic energy change of the PPO group because of its low solubility in water and most oils. Alternative and more efficient graft copolymers consist of a polymeric backbone attached to the interface and several chains dangling into the continuous phase and forming at the interface a "brush" structure.

A typical example of commercial graft was described (82). Here, mixtures of Polyoxyethylene-660-12-hydroxystearate (Solutol HS15) with the anionic lipid composition Lipoid S75 were employed to enhance the long term as well as accelerated (by freezing and centrifugation) stability of oil-in-water nanosized emulsions. Emulsion stabilized by phospholipids displayed a stable behaviour after autoclaving and centrifugation but de-emulsified after freezing. In contrast, emulsions prepared only with Solutol HS15 demonstrated a significant change in particle size after autoclaving. The best results were obtained using a stabilizer mixture revealing a combination of electrostatic stabilization mechanism typical for the anionic phospholipids and the steric stabilization mechanism originating from non-ionic polymeric surfactant. The combination of stabilization mechanisms improved the emulsion's stability, compared to the emulsion's stability prepared using only the individual surfactants.

The commercially available cationic block-copolymer Eudragit E100 was utilized as both emulsion stabilizer and solidifying agent upon further drying (83). Due to the specific properties of Eudragit E100, no surfactant or organic solvent additives were employed in order to fulfil common ecological, toxicological, and manufacturing safety requirements during the preparation of a redispersible dry emulsion.

The naturally occurring polymers cyclodextrins are commonly used in pharmaceutical aqueous formulations for inclusion of less soluble or instable drugs. Their use, however, is complicated by poor interaction with biological membranes. To overcome this difficulty, the cyclodextrins were chemically modified, including grafting of fatty acid chains to the hydroxyl groups in order to provide the optimum surface activity to the polymer (84). Upon addition of Miglyol to an aqueous phase, nanocapsules with an oily interior were formed spontaneously. These nanocapsules had an average size of approximately 300 nm and revealed an excellent long-term stability for at least 5 months. Interestingly, the most appropriate properties for nanocapsules formation were observed for cyclodextrin derivatives with linear $C_6$ chains. Compounds possessing longer aliphatic chains caused the formation of large polydisperse aggregates and were therefore unsuitable for encapsulation. The preparation of nanocapsules with modified cyclodextrins without the addition of surfactants is a promising tool for the development of novel drug delivery systems. A variety of current approaches to increase emulsion stability with help of emulsifier molecules have been summarized in a recent review by Grigoriev and Miller (85).

### Miscellaneous additives

Additives other than antioxidants such as preservatives (e.g., benzalkonium chloride, chlorocresol, parabens) are included in emulsions to prevent microbial spoilage of multidose medical emulsions. α-Tocopherol is a good example of an antioxidant used to obtain a desirable stabilized emulsion under prolonged storage conditions. The presence of components of natural origin such as lecithin or oils with high calorific potential renders the emulsion a good medium to promote microbial growth when it is packaged in multidose containers. Pharmaceutical products when distributed into multidose containers, especially for parenteral and ocular administrations, should be properly preserved against microbial contamination and proliferation during storage under normal conditions and proper use. Incorporation of preservatives in single-dose vials is also a common procedure if filtration is used as a sterilization method (65). Sznitowska et al (86) studied the physicochemical compatibility between the lecithin - stabilized emulsion and 12 antimicrobial agents over two years of storage at room temperature. Preliminary physicochemical screening results indicated that the addition of chlorocresol, phenol, benzyl alcohol, thiomersal, chlorhexidine gluconate, and bronopol should be avoided due to the occurrence of an unfavorable pH change followed by coalescence of lecithin-stabilized droplets of the emulsion.

Furthermore, the efficacy of antimicrobial preservation was assessed using the challenge test according to the method described by the European Pharmacopoeia.

Despite good physicochemical compatibility, neither the parabens nor benzalkonium

chloride showed satisfactory antibacterial efficacy in the emulsion against the tested microorganisms and consequently were not suitable for preservation. Therefore, higher concentrations of antimicrobial agents or their combinations may be required for efficient preservation of the lecithin-stabilized emulsion probably because of unfavorable phase partitioning of the added antimicrobials within the different internal structures of the emulsion (86). This finding clearly indicates that the possible electrostatic attraction between the negatively charged lipid moieties of the mixed emulsifying film formed around the anionic emulsified oil droplets (69) and the quaternary cationic ammonium groups of the preservative is not the plausible cause for the reduced activity of the benzalkonium chloride. Thus, the possible intercalation of this surfactant in either the cationic or anionic interfacial mixed emulsifying film is likely to occur, preventing benzalkonium chloride from eliciting its adequate preservative action (86).

For the development of cationic emulsions in ophthalmology, the use of quaternary ammonium compound (QAC) for their cationic property rather than their preservative effect is being considered. Therefore, Liang et al (87) suggested the use of lipophilic cetalkonium chloride (CKC), one of the longest alkyl-chain components, as a cationic agent in ophthalmic emulsions. With a highly lipophilic QAC, the distribution between the oil and aqueous phases of the emulsion is modified because of the affinity toward the oil phase, further favoring the cationic agent role over the preservative role. Using in vivo confocal microscopy (IVCM) for in vivo tissues images and impression cytology (IC) for ex vivo epithelium inflammatory marker expression in correlation with standard immunohistology for deep infiltration and apoptosis, the same author assessed the toxicological effects of benzalkonium chloride/CKC emulsion/solution formulations on the ocular surface of rabbits following multiple topical applications (87). These in vivo and ex vivo experimental approaches demonstrated that ocular surface toxicity was reduced by using an emulsion instead of a traditional solution and that a CKC emulsion was safe for ocular administration.
Overall, it is preferable to formulate nanosized emulsions devoid of preservative agents and fill it in sterile single-dose packaging units to prevent potential contamination. It should be pointed out that the two available ocular emulsion products (Refresh Endura® and Restasis®, Allergan, Irvine, CA) on the market are preservative free and packed in single-use vials. Currently there is no commercial parenteral emulsion which contains preservatives and research concerning the problem of preservation of nanosized emulsion is very limited (88-91).

## CURRENT AND NEAR FUTURE DIRECTION

### Colloidal particles stabilized emulsions

In addition to surfactants, polymers and biomolecules, colloidal particles have long been recognised to stabilise droplets, e.g. as in Pickering emulsions (92). Examples of various colloidal particle types used to stabilize emulsions include $BaSO_4$, crystalline ferric oxide, carbon black, bentonite, kaolinite clay, latex, and silica particles. The effectiveness of emulsion stabilisation by particles depends on the particle size, shape, concentration, and wettability (expressed as the contact angle at the three phase boundary and is equivalent to the hydrophile-lipophile balance (HLB) of a surfactant), as well as the level of particle aggregation as controlled by particle-particle interaction (93). It is proposed that the conventional model for emulsion stabilisation by solid particles assumes the formation of a "densely packed" layer at the oil-water interface, which prevents droplet coalescence by a steric barrier mechanism. If charged, particles may give rise to electrostatic repulsion, which further enhances emulsion stability.

Many commercial products based on emulsions (for cosmetic and food uses) include both surfactants and particles, hence the characterisation and mechanisms of stabilisation for emulsions with mixed interfacial layers are of great importance (94). These droplet/surfactant/particle systems are also of interest in designing low-surfactant emulsion systems with enhanced stability (95). Tambe and Sharma (96) reported the improvement in the stability of emulsions stabilised with calcium carbonate particles following the addition of stearic acid. This synergistic effect was attributed to the

adsorption of surfactant on solid particles resulting in the change in particle wettability. A strong emulsification synergy in stabilising o/w emulsions between hydrophilic colloidal silica (Ludox) and non-ionic polyoxyethylene surfactants added from the water phase has been demonstrated by Midmore (95). The synergy was attributed to the POE micellar adsorption or POE chain bridging between silica particles which resulted in better interfacial adsorption. Synergistic stabilisation of oil-in-water emulsions by a mixture of non-ionic surfactants and hydrophilic silica nanoparticles has recently been studied by Binks et al (94) highlighting the importance of preparation protocol. Attachment energy of a silica particle at the oil-water interface was correlated to emulsion stability. Velev et al (97) reported that the adsorption of lysine onto negatively charged latex particles enabled interfacial adsorption due to the reduction of hydration forces and electrostatic repulsion between droplets and particles. The influence of droplet-particle electrostatic interactions on emulsion stability was studied by Lan et al (98) and Binks and Whitby (99). Addition of cationic surfactant CTAB to negatively charged hydrophilic silica dispersions as the aqueous phase of emulsions, resulted in enhanced stability at low CTAB concentrations, i.e. the region where CTAB is preferably adsorbed onto silica surface and promoted interfacial adsorption due to neutralisation of surface charge and optimisation of particle hydrophobicity. This synergistic emulsion stabilization comes from three sources: CTAB (i) hydrophobizes silica nanoparticles by partial charge neutralization, (ii) promotes nanoparticle aggregation, and (iii) reduces interfacial tension. However, when the CTAB concentration is such so that both droplets and silica surfaces are positively charged, emulsion stability is significantly reduced.

Simovic and Prestidge (100) showed that nanoparticle layers significantly influence the release kinetics of a model lipophilic drug (di-butyl-phthalate (DBP)) from polydimethylsiloxane o/w emulsions; either sustained or enhanced release can be achieved depending on the nanoparticle layer structure and drug loading level. Nanoparticle layers can be engineered to facilitate a range of release behaviours and offer great potential in the delivery of poorly soluble drugs. Particle stabilised emulsions have been extensively studied in terms of stabilisation mechanisms (99,101), synergy with common emulsifiers (98, 102), and interfacial properties (103,104). However, few reports have focused on their carrier properties as dermal delivery vehicles, e.g. penetration and targeting skin layers. Recently, the influence of nanoparticle coating of submicron (nanosized) oil-in-water emulsion droplets on the in vitro release and dermal delivery characteristics, with particular emphasis on potential controlled release and targeted skin delivery of all-trans-retinol was reported by Eskandar et al (105). Medium-chain triglyceride o/w emulsions have been stabilised with mixed interfacial layers composed of lecithin or oleylamine and hydrophilic silica nanoparticles using a simple cold high pressure homogenization technique. These emulsion based hybrid drug delivery systems showed improved topical delivery of all-trans-retinol; nanoparticle layers significantly improved the performance of o/w emulsions as encapsulation and delivery systems for all-trans-retinol. Therefore, emulsion-based hybrid drug delivery systems should have a potential for drug delivery.

## CONCLUSION

The preparation by de novo method or SolEmul® technology allows the production of emulsion particles in nanosized range with acceptable stability without the separation of oil and water phases. Based on the conferration of surface/interfacial charges by the incorporation of selective excipients onto the emulsion systems, the o/w nanosized emulsions can conveniently be classified into neutral, anionic and cationic. Whereas the ionic emulsifier can impart repulsive forces between similarly charged electrical double layers to emulsified oil droplets, the non-ionic emulsifier can provide the interactive particles with the steric stabilization. Furthermore, addition of particular excipients to the emulsion system prevents the opsonization of emulsion particles and thus increases substantially the circulation time of emulsion particles inside the bloodstream for reaching the intended target site in vivo. Accumulating knowledge thus suggests that constant progress in better understanding the principles and processes governing the various issues related to o/w nanosized emulsions has brought major

improvements in the efficacy of parenteral or nonparenteral drug delivery systems.

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## LIST OF ABREVIATIONS

| | |
|---|---|
| CTAB | cetyltrimethyl ammonium bromide |
| CKC | cetalkonium chloride |
| 2DEHPA | bis(2-ethylhexyl) hydrogen phosphate |
| DBP- | di-butyl-phthalate |
| DMPC | dimyristoylphosphatidylcholine |
| DMPE | dimyristoylphosphatidylethanolamine |
| DOTAP | 1,2-dioleoyl-sn-glycero-3-trimethylammonium-propane |
| DPPC | dipalmitoylphosphatidylcholine |
| EDTA | ethylenediamine tetraacetic acid |
| ELM | emulsion liquid membranes |
| HLB | hydrophilic-lipophilic balance |
| HP | high-pressure |
| HPH | high pressure homogenization |
| IC | impression cytology |
| IVCM | in vivo confocal microscopy |
| MCT | medium-chain triglyceride |
| NPEO10 | nonylphenol-poly (ethylene oxide) |
| O/w- | oil-in-water |
| PBCA | poly(n-butylcyanoacrylate) |
| 6-PC- | n-hexanoyl lysolecithin |
| PEO | polyoxyethylene |
| PFOB | perfluorooctyl bromide |
| QAC | quaternary ammonium compound |
| SDS | Sodium dodecyl sulfate |
| Solutol HS15 | Polyoxyethylene-660-12-hydroxystearate |
| TPGS | D-alpha-tocopheryl polyethylene glycol 1000 succinate |
| UHPH | ultra-high pressure homogenization. |

## REFERENCES

1. Mason TG, Wilking JN, Meleson K, Chang CB, Graves SM. Nanoemulsions: formation, structure, and physical properties. J Physics: Condensed Matter, 18:635-666, 2006.

2. Tamilvanan S. Formulation of multifunctional oil-in-water nanosized emulsions for active and passive targeting of drugs to otherwise inaccessible internal organs of the human body. Int J Pharm, 381:62-76, 2009.

3. Vandamme TF. Microemulsions as ocular drug delivery systems: recent developments and future challenges. Prog Retin Eye Res, 21:15-34, 2002.

4. Tamilvanan S, Gursoy RN, Benita S. Emulsion-based delivery systems for enhanced drug absorption. Pharm Tech, 131:156-161, 2002.

5. Lallemand F, Felt-Baeyens O, Basseghir K, Behar-Cohen F, Gurny R. Cyclosporine A delivery to the eye: a pharmaceutical challenge. Eur J Pharm Biopharm, 56:307-318, 2003.

6. Piemi MP, Korner D, Benita S, Marty JP. Positively and negatively charged submicron emulsions for enhanced topical delivery of antifungal drugs. J Control Rel, 58:177-187, 1999.

7. Capek I. Degradation of kinetically-stable o/w emulsions. Adv. Colloid Interf. Sci, 107:125-155, 2004.

8. Klang SH, Benita S. Design and evaluation of submicron emulsions as colloidal drug carriers for intravenous administration, in Benita S (ed), Submicron Emulsion in Drug Targeting & Delivery. *Harwood Academic*, The Netherlands, pp 119-152, 1998.

9. Benita S, Levy MY. Submicron emulsions as colloidal drug carriers for intravenous administration: Comprehensive physicochemical characterization. J Pharm Sci, 82:1069-1079, 1993.

10. Keck CM, Müller RH. Drug nanocrystals of poorly soluble drugs produced by high pressure homogenisation. Eur J Pharm Biopharm, 62:3-16, 2006.

11. Sanguansri P, Augustin MA. Nanoscale materials development- a food industry perspective. Trends Food Sci Tech, 17:547-556, 2006.

12. Mehnert W, Mäder K. Solid lipid nanoparticles. Production, characterization and applications. Adv Drug Deliv Rev, 47:165-196, 2001.

13. Schubert H, Ax K, Behrend O. Product engineering of dispersed systems. Trends Food Sci Tech, 14:9-16, 2003.

14. Schultz S, Wagner G, Urban K, Ulrich J. High-pressure homogenization as a process for emulsion formation. Chem Eng Tech, 27:361-368, 2004.

15. Marie P, Perrier-Cornet JM, Gervais P. Influence of major parameters in emulsification mechanisms using a high-pressure jet. J Food Eng, 53:43-51, 2002.

16. Washington C, Davis SS. The production of parenteral feeding emulsions by microfluidizer. Int J Pharm, 44:169-176, 1988.

17. Pinnamaneni S, Das NG, Das SK. Comparison of oil-in-water emulsions manufactured by microfluidization and homogenization. Pharmazie, 58:554-558, 2003.

18. Perrier-Cornet JM, Marie P, Gervais P. Comparison of emulsification efficiency of protein-stabilized oil-in-water emulsions using jet, high pressure and colloid mill homogenization. J Food Eng, 66:211-217, 2005.

19. Stang M, Schuchmann H, Schubert H. Emulsification in high-pressure homogenizers. Eng Life Sci, 1:151-157, 2001.

20. Patravale VB, Date AA, Kulkarni RM. Nanosuspensions: a promising drug delivery strategy. J Pharm Pharmacol, 56:827-840, 2004.

21. Floury J, Legrand J, Desrumaux A. Analysis of a new type of high pressure homogeniser. Part B. Study of droplet break-up and recoalescence phenomena. Chem Eng Sci, 59:1285-1294, 2004.

22. Stevenson MJ Chen XD. Visualization of the flow patterns in a high pressure homogenizing valve using a CFD package. J Food Eng, 33:151-165, 1997.

23. Diels AMJ, Callewaert L, Wuytack EY, Masschalck B, Michiels CW. Inactivation of Escherichia coli by high-pressure homogenisation is influenced by fluid

viscosity but not by water activity and product composition. Int J Food Microb, 101:281-291, 2005.

24. Floury J, Bellettre J, Legrand J, Desrumaux A. Analysis of a new type of high pressure homogeniser. A study of the flow pattern. Chem Eng Sci, 59:843-853, 2004.

25. Grácia-Juliá A, René M, Cortés-Muň oz M, Picart L, López-Pedemonte T, Chevalier D. Effect of dynamic high pressure on whey protein aggregation: a comparison with the effect of continuous short-time thermal treatments. Food Hydrocolloids, 22:1014-1032, 2008.

26. Picart L, Thiebaud M, René M, Guiraud JP, Cheftel JC, Dumay E. Effects of high pressure homogenisation of raw bovine milk on alkaline phosphatase and microbial inactivation. A comparison with continuous short-time thermal treatments. J Dairy Res, 73:454-463, 2006.

27. Tamilvanan S, Benita S. The potential of lipid emulsion for ocular delivery of lipophilic drugs. Eur J Pharm Biopharm, 58:357-368, 2004.

28. Cohen T, Sauvageon-Martre H, Brossard D, D'Hermies F, Bardin C, Chast F, Chaumeil JC. Amphotericin B eye drops as a lipidic emulsion. Int J Pharm, 137:249-254, 1996.

29. Lance MR, Washington C, Davis SS. Structure and toxicity of amphotericin B/triglyceride emulsion formulations. J Antimicrob Chemother, 36:119-128, 1995.

30. S.S. Davis, C. Washington, Drug emulsion, European Patent 0,296, 845 A1 (1988).

31. Buttle S, Schmidt RH, Müller RH. Production of amphotericin B emulsions based on SolEmuls technology, Fourth World Meeting on Pharmaceutics, Biopharmaceutics and Pharmaceutical Technology, Florence, April 8-11, 2002, pp. 1535-1536.

32. Müller RH, Schmidt S. SolEmuls technology for i.v. emulsions of poorly soluble drugs: amphotericin B, Fourth World Meeting on Pharamceutics, Biopharmaceutics and Pharmaceutical Technology, Florence, April 8-11, 2002, pp. 1451-1452.

33. Akkar A, Müller RH. Formulation of intravenous carbamazepine emulsions by SolEmuls® technology. Eur J Pharm Biopharm, 55:305-312, 2003.

34. Akkar A, Müller RH. Intravenous itraconazole emulsions produced by SolEmuls technology. Eur J Pharm Biopharm, 56:29-36, 2003.

35. Tamilvanan S, Khoury K, Gilhar D, Benita S. Ocular delivery of cyclosporin A. I. Design and characterization of cyclosporin A-loaded positively-charged submicron emulsion. STP Pharma Sci, 11:421-426, 2001.

36. Hagigit T, Abdulrazik M, Orucov F, Valamanesh F, Hagedorn M, Lambert G, Behar-Cohen F, Benita S. Topical and intravitreous administration of cationic nanoemulsions to deliver antisense oligonucleotides directed towards VEGF KDR receptors to the eye. J Control Rel, In Press, 2010.

37. Tamilvanan S. Oil-in-water lipid emulsions: implications for parenteral and ocular delivering systems. Prog Lipid Res, 43:489-533, 2004.

38. Teixeira H, Dubernet C, Puisieux F, Benita S, Couvreur P. Submicron cationic emulsions as a new delivery system for oligonucleotides. Pharm Res, 16:30-36, 1999.

39. Hagigit T, Nassar T, Behar-Cohen F, Lambert G, Benita S. The influence of cationic lipid type on in vitro release kinetics of antisense oligonucleotide from cationic nanoemulsions. Eur J Pharm Biopharm, 70:248-259, 2008.

40. Nobs L, Buchegger F, Gurny R, Allemann E. Current methods for attaching targeting ligands to liposomes and nanoparticles. J Pharm Sci, 93:1980-1992, 2004.

41. Song YK, Liu D, Maruyama KZ, Takizawa T. Antibody mediated lung targeting of long-circulating emulsions. PDA J Pharm Sci Technol, 50:372-377, 1996.

42. Lundberg BB, Griffiths G, Hansen HJ. Conjugation of an anti-B-cell lymphoma monoclonal antibody, LL2, to long-circulating drug-carrier lipid emulsions. J Pharm Pharmacol, 51:1099-1105, 1999.

43. Lundberg BB, Griffiths G, Hansen HJ. Cellular association and cytotoxicity of anti-CD74-targeted lipid drug-carriers in B lymphoma cells. J Control Rel, 94:155-161, 2004.

44. Goldstein D, Nassar T, Lambert G, Kadouche J, Benita S. The design and evaluation of a novel targeted drug delivery system using cationic emulsion-antibody conjugates. J Control Rel, 108:418-432, 2005.

45. Goldstein D, Gofrit O, Nyska A, Benita S. Anti-HER2 cationic immunoemulsion as a potential targeted drug delivery system for the treatment of prostate cancer. Cancer Res, 67:269-275, 2007.

46. Lee VHL, Robinson JR. Review: Topical ocular drug delivery: Recent developments and future challenges. J Ocul Pharmacol, 2:67-108, 1986.

47. Jumaa M, Müller BW. Physicochemical properties of chitosan-lipid emulsions and their stability during the autoclaving process. Int J Pharm, 183:175-184, 1999.

48. Jumaa M, Müller BW. The effect of oil components and homogenization condition on the physicochemical properties and stability of parenteral fat emulsions. Int J Pharm, 163:81-89, 1998.

49. Constantinides PP, Tustian A, Kessler DR. Tocol emulsions for drug solubilization and parenteral delivery. Adv Drug Deliv Rev, 56:1243-1255, 2004.

50. Constantinides PP, Han J, Davis SS. Advances in the use of tocols as drug delivery vehicles. Pharm Res, 23:243-255, 2006.

51. Muchtar S, Benita S. Emulsions as drug carriers for ophthalmic use. Coll Surf A Physicochem Eng Aspects, 91:181-190, 1994.

52. Ogawa S, Decker EA, McClements DJ. Production and characterization of o/w emulsions containing cationic droplets stabilized by lecithin-chitosan membranes. J Agric Food Chem, 51:2606-2812, 2002.

53. Calvo P, Remuñá - López C, Vila - Jato JL, Alonso MJ. Development of positively charged colloidal drug carriers: Chitosan-coated polyester nanocapsules and submicro-emulsions. Coll Polym Sci, 275:46-53, 1997.

54. Tamilvanan S, Ajith Kumar B, Senthilkumar SR, Baskar R, Raja Sekharan T. Stability assessment of injectable castor oil-based nanosized emulsion containing cationic droplets stabilized by poloxamer-chitosan emulsifier films. AAPS

PharmSciTech. 2010 published online May 22, 2010. DOI: 10.1208/s12249-010-9455-3.

55. Sonne MR. Tocopherol compositions for delivery of biologically active agents, USPTO Patent Full-Text and Image Database No. 6,193,985, Dumex, Copenhagen, 2001; pp. 1-21.

56. Nakajima M, Nabetani H, Ichikawa S, Xu QY. Functional emulsions. US Patent 6538019, 2003.

57. Kim TW, Chung H, Kwon IC, Sung HC, Shin BC, Jeong SY. Airway gene transfer using cationic emulsion as a mucosal gene carrier. J Gene Med, 7:749-758, 2005.

58. Kim YJ, Kim TW, Chung H, Kwon IC, Sung HC, Jeong SY. The effects of serum on the stability and the transfection activity of the cationic lipid emulsion with various oils. Int J Pharm, 252:241-252, 2003.

59. Liu Y, Mounkes LC, Liggitt HD, Brown CS, Solodin I, Heath TD, Debs RJ. Factors influencing the efficiency of cationic liposome-mediated intravenous gene delivery. Nat Biotechnol, 15:167-173, 1997.

60. Simberg D, Weisman S, Talmon Y, Faerman A, Shosshani T, Barenholz Y. The role of organ vascularization and lipoplex-serum initial contact in intravenous murine lipofection. J Biol Chem, 278:39858-39865, 2003.

61. Yi SW, Yune Y, Kim TW, Chung H, Choi YW, Kwon IC, Lee EB, Jeong SY. A cationic lipid emulsion/DNA complex as a physically stable and serum-resistant gene delivery system. Pharm Res, 17:314-320, 2000.

62. Goldstein D, Sader O, Benita S. Influence of oil droplet surface charge on the performance of antibody-emulsion conjugates. Biomed Pharmacother, 61:97-103, 2007.

63. Yilmaz H, Borchert HH. Design of a phytosphingosine-containing, positively charged nanoemulsion as a colloidal carrier system for dermal application of ceramides. Eur J Pharm Biopharm, 60:91-98, 2005.

64. Solans C, Izquierdo P, Nolla J, Azemar N, Garcia-Celma MJ. Nanoemulsions. Curr Opin Colloid Interface Sci, 10:102-110, 2005.

65. Tamilvanan S. Oil-in-Water Nanosized Emulsions: Medical Applications, in Gad SC (ed), Pharmaceutical Manufacturing Handbook. Chap. 7.4., John Wiley & Sons Publishers, NJ, USA, pp 1329-1368, 2008.

66. Trotta M, Pattarino F, Ignoni T. Stability of drug-carrier emulsions containing phosphatidylcholine mixtures. Eur J Pharm Biopharm, 53:203-208, 2002.

67. Kador PF, Kinoshita JH. Phospholipid effects on the rat lens transport systems. Exp Eye Res, 26:657-665, 1978.

68. Cotlier E, Baskin M, Kresca L. Effects of lysophosphatidyl choline and phospholipase A on the lens. Invest Ophthalmol, 14:697-701, 1975.

69. Abele S, Sjöberg M, Hamaide T, Zicmanis A, Guyot A. Reactive surfactants in heterophase polymerization. 10. Characterization of the surface activity of new polymerizable surfactants derived from maleic anhydride. Langmuir, 13:176-181, 1997.

70. Weiss CK, Lorenz MR, Landfester K, Mailänder V. Cellular uptake behavior of unfunctionalized and functionalized PBCA particles prepared in a miniemulsion. Macromol Biosci, 7:883-896, 2007.

71. Landfester K. Synthesis of colloidal particles in miniemulsions. Annu Rev Mater Res, 36:231-279, 2006.

72. Dale PJ, Kijlstra J, Vincent B. The temperature stability of single and mixed emulsions stabilized by nonionic surfactants. Colloids Surf A Physicochem Eng Asp, 291:85-92, 2006.

73. Yaseen M, Lu JR, Webster JRP, Penfold J. The structure of zwitterionic phosphocholine surfactant monolayers. Langmuir, 22:5825-5832, 2006.

74. Moolman FS, Rolfes H, van der Merwe SW, Focke WW. Optimization of perfluorocarbon emulsion properties for enhancing oxygen mass transfer in a bio-artificial liver support system. Biochem Eng J, 19:237-250, 2004.

75. Hu Z, Deng Y, Sun Q. Synthesis of precipitated calcium carbonate nanoparticles using a two-membrane system. Colloid J, 66:745-750, 2004.

76. Naskar MK, Patra A, Chatterjee M. Understanding the role of surfactants on the preparation of ZnS nanocrystals. J Colloid Interface Sci, 297:271-275, 2006.

77. Ishii F, Nii T. Properties of various phospholipid mixtures as emulsifiers or dispersing agents in nanoparticle drug carrier preparation. Colloids Surf B Biointerfaces, 41:257-262, 2005.

78. Tamilvanan S, Schmidt S, Müller RH, Benita S. In vitro adsorption of plasma proteins onto the surface (charges) modified-submicron emulsions for intravenous administration. Eur J Pharm Biopharm, 59:1-7, 2005.

79. Tadros ThF, Vandamme A, Levecke B, Booten K, Stevens CV. Stabilization of emulsions using polymeric surfactants based on inulin. Adv Colloid Interface Sci, 108-109:207-226, 2004.

80. Tadros ThF. Principles of emulsion stabilization with special reference to polymeric surfactants. J Cosmet Sci, 57:153-169, 2006.

81. Benichou A, Aserin A, Garti N. Double emulsions stabilized with hybrids of natural polymers for entrapment and slow release of active matters. Adv Colloid Interface Sci, 108-109:29-41, 2004.

82. Jumaa M, Müller BW. Parenteral emulsions stabilized with a mixture of phospholipids and PEG-660-12-hydroxy-stearate: evaluation of accelerated and long-term stability. Eur J Pharm Biopharm, 54:207-212, 2002.

83. Cui F, Wang Y, Wang J, Feng L, Ning K. Preparation of redispersible dry emulsion using Eudragit E100 as both solid carrier and unique emulsifier. Colloids Surf A Physicochem Eng Asp, 307:137-141, 2007.

84. Memisoglu E, Bochot A, Sen M, Sharon D, Duchêne D, Hincal AA. Amphiphilic β-cyclodextrins modified on the primary face: synthesis, characterization and evaluation of their potential as novel excipients in the preparation of nanocapsules. J Pharm Sci, 91:1214-1224, 2002.

85. Grigoriev DO, Miller R. Mono- and multilayer covered drops as carriers. Current Opin Colloid Interf Sci, 14:48-59, 2009.

86. Sznitowska M, Janicki S, Dabrowska EA, Gajewska M. Physicochemical screening of antimicrobial agents as potential preservatives for submicron emulsions. Eur J Pharm Sci, 15:489-495, 2002.

87. Liang H, Brignole-Baudouin F, Rabinovich-Guilatt L, Mao Z, Riancho L, Faure MO, Warnet JM, Lambert G, Baudouin C. Reduction of quaternary ammonium-induced ocular surface toxicity by emulsions: an in vivo study in rabbits. Molecular Vision, 14:204-216, 2008.

88. Jumaa M, Furkert FH, Müller BW. A new lipid emulsion formulation with high antimicrobial efficacy using chitosan. Eur J Pharm Biopharm, 53:115-123, 2002.

89. Pongcharoenkiat N, Narsimhan G, Lyons RT, Hem SL. The effect of surface charge and partition coefficient on the chemical stability of solutes in o/w emulsions. J Pharm Sci, 91:559-570, 2002.

90. Han J, Washington C. Partition of antimicrobial additives in an intravenous emulsion and their effect on emulsion physical stability. Int J Pharm, 288:263-271, 2005.

91. Swietlikowska DW, Sznitowska M. Partitioning of parabens between phases of submicron emulsions stabilized with egg lecithin. Int J Pharm, 312:174-178, 2006.

92. Pickering SU. Emulsions. J Chem Soc Trans, 91:2001-2021, 1907.

93. Aveyard R, Binks BP, Clint JH. Emulsions stabilized solely by colloidal particles. Adv Colloid Interface Sci, 100-102:503-546, 2003.

94. Binks BP, Desforges A, Duff DG. Synergistic stabilization of emulsions by a mixture of surface-active nanoparticles and surfactant. Langmuir, 23:1098-1106, 2007.

95. Midmore BR. Synergy between silica and polyoxyethylene surfactants in the formation of o/w emulsions. Colloids Surf., A, 145:133-143, 1998.

96. Tambe DE, Sharma MM. Factors controlling the stability of colloid-stabilized emulsions: I. an experimental investigation. J Colloid Interface Sci, 157:244-253, 1993.

97. Velev OD, Furusawa K, Nagayama K. Assembly of latex particles by using emulsion droplets as templates. 1. Microstructured hollow spheres. Langmuir, 12:2374-2384, 1996.

98. Lan Q, Yang F, Zhang S, Liu S, Xu J, Sun D. Synergistic effect of silica nanoparticle and cetyltrimethyl ammonium bromide on the stabilization of O/W emulsions. Colloids Surf A, 302:126-135, 2007.

99. Binks BP, Whitby CP. Nanoparticle silica-stabilised oil-in-water emulsions: improving emulsion stability. Colloids Surf A, 253:105-115, 2005.

100. Simovic S, Prestidge CA. Nanoparticle layers controlling drug release from emulsions. Eur J Pharm Biopharm, 67:39-47, 2007.

101. Binks BP, Lumsdon SO. Influence of particle wettability on the type and stability of surfactant-free emulsions. Langmuir, 16:8622-8631, 2000.

102. Binks BP, Rodrigues JA, Frith WJ. Synergistic interaction in emulsions stabilized by a mixture of silica nanoparticles and cationic surfactant. Langmuir, 23:3626-3636, 2007.

103. Simovic S, Prestidge CA. Adsorption of hydrophobic silica nanoparticles at the PDMS droplet-water interface. Langmuir, 19:8364-8370, 2003.

104. Simovic S, Prestidge CA. Colloidosomes from controlled interaction of submicrometer triglyseride droplets and hydrophilic silica nanoparticles. Langmuir, 24:7132-7137, 2008.

105. Eskandar NG, Simovic S, Clive A. Prestidge. Nanoparticle coated submicron emulsions: sustained in-vitro release and improved dermal delivery of all-trans-retinol. Pharm Res, 26, 1764-1775, 2009.