International Journal of Pharmaceutics 387 (2010) 120–128

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Mixed micelle formation with phosphatidylcholines: The influence of surfactants with different molecule structures

Christopher Rupp, Hartwig Steckel, Bernd W. Müller*

Department of Pharmaceutics and Biopharmaceutics, Christian Albrecht University Kiel, Gutenbergstraße 76, 24118 Kiel, Germany

ARTICLE INFO

Article history:
Received 7 October 2009
Received in revised form 2 December 2009
Accepted 4 December 2009
Available online 18 December 2009

Keywords:
Phosphatidylcholine
Sucrose esters
Packing parameter
Mixed micelles

ABSTRACT

The number of mixed micellar (MM) drug products being introduced into the commercial pharmaceutical market is very limited although there is need for alternative dosage forms for poorly soluble active drug substances. While known systems are composed of phosphatidylcholine and bile salts, it was the aim of this study to investigate if alternative surfactants are able to form isotropically clear solutions over a broad range of concentrations and at higher ratios of phosphatidylcholine (PC). It was a particular challenge of this work to find a MM system with a unimodal particle size distribution since it is known that surfactants often form vesicles with phospholipids instead of MM. The theoretical approach behind this work was the transfer of the packing parameter concept, which describes the molecular association of one amphiphilic species, to the organisation behaviour of two different amphiphilic species (water-insoluble phospholipid + surfactant leading to MM). Therefore the influence of the surfactant molecular geometry on the ability to form MM with phospholipids was investigated. A homologous series of two different surfactant classes, namely polyglycerol esters and sucrose esters, with a large hydrophilic head region leading to a smaller packing parameter were analysed regarding their ability to form clear MM solutions with PC. For comparison, surfactants with no strictly defined partition between a polar head and a non-polar tail (e.g. Poloxamer 188) were tested. Decaglycerol laurate and especially sucrose laurate (SL) were superior compared to all other tested surfactants with respect to their ability to form clear solutions with hydrogenated PC (hPC) at a higher ratio and over a broad range of concentrations while unsaturated PC showed an inferior performance to form MM. The favourite MM system composed of SL with 0.5 weight fractions of hPC formed about 20 nm sized MM in a concentration range of 1.0–80 mg/mL and showing a unimodal particle size distribution with a PDI value <0.1. The results of the study have shown that the transferred packing parameter concept is applicable to the tested surfactants to describe their ability forming mixed micelles with PC.

1. Introduction

The raising number of new lipophilic and poorly soluble drug candidates demand for the development of application vehicles with higher solvent power (Lukyanov and Torchilin, 2004). Various approaches to solubilise drugs, such as inclusion complexes with cyclodextrins, microemulsions or liposome formulations (Müller and Albers, 1992; Lawrence and Rees, 2000; Barenholz, 2001) have been described in the literature so far. However, all these systems are not generally applicable to every active drug substance, e.g. cyclodextrins need special guest molecule structures for complexation, or exhibit other disadvantages, e.g. microemulsion systems are characterized by high surfactant concentrations which mostly are not well tolerable. The stability of liposomes encapsulating lipophilic drugs within the bilayer may be affected due to drug/PC interactions (Sharma and Sharma, 1997; Crosasso et al., 2000; Krishnadas et al., 2003). With respect to parenteral application, one can deduce from liposome research that phospholipids represent the only class of excipients offering unique benefits for a surface active ingredient as they are non-toxic, parenterally well tolerated, and exhibit a high biocompatibility. However, phospholipids are forming bilayer structures and typically not micelles which could be used to solubilise a water-insoluble drug. Under specific conditions, phospholipids and phospholipid derivatives do form mixed micelles instead of vesicles when being combined with suitable hydrophilic surfactants (Helenius and Simons, 1975; Hammad and Müller, 1998a; Mu et al., 2005). Mixed micelles offer a high potential drug delivery system with many advantages as they contain a hydrophobic core which can be used to encapsulate pharmaceuticals with poor water solubility. Furthermore, due to their small size (usually smaller than 60 nm) MM show an enhanced vascular permeability and accumulate in pathological areas with leaky

* Corresponding author. Tel.: +49 431 8801333; fax: +49 431 8801352.
E-mail address: bwmueller@pharmazie.uni-kiel.de (B.W. Müller).

0378-5173/$ – see front matter © 2009 Published by Elsevier B.V.
doi:10.1016/j.ijpharm.2009.12.018

vasculature, as in the case of tumours. To avoid a rapid clearance a particle size <200 nm is required for the drug carriers as opsonization is reduced for such small particles (Mayer et al., 1989; Marjan and Allen, 1996). Mixed micelles can be produced by combining natural phospholipids with specific surfactants. In a classical MM system, the phospholipid serves as a water insoluble but swellable amphiphilic component (Shankland, 1970) next to a water soluble surfactant. There are different theoretical models for the formation and shape of mixed micelles described in the literature. The best characterised MM system is the bile salt/lecithin mixed micellar solution which has been extensively investigated (Shankland, 1970; Rosoff and Serajuddin, 1980; Hammad and Müller, 1998a). A widely accepted molecular model for mixed micelles composed of bile salts and phosphatidylcholine is the mixed disk model proposed by Mazer et al. (1980) which is analogous to that suggested by Small et al. (1969). Mazer's model describes bile salt molecules to be not only positioned around the perimeter of the PC bilayer but also being incorporated into the bilayer interior. Even though bile salt/lecithin solutions are the most common mixed micellar systems still under research (Sznitowska, 2008), several problems occurred with this system. Slight changes in the bile salt/phospholipid ratio e.g. result in significant changes in MM size and structure (Shankland, 1970; Lichtenberg et al., 1983; Krishnadas et al., 2003). A bile salt molecule holding a steroidal backbone does not represent the typical structure of a surfactant with a strict partition between a polar head and a non-polar tail. So Small specified those molecules (like the bile salts) with a slight distinction in polarity as class IIIB lipids or amphiphilics. The organisation behaviour of typical amphiphilic molecules with a strictly defined separation from the polar part on the one side and the non-polar part on the other side of the molecule is demonstrated by the packing parameter concept (PPC) according to Israelachvili et al. (1977). The packing parameter $p = V/A_o \times l_c$ allows a rough prediction how the aggregate geometry of an amphiphilic species could look like. V and l_c are the total volume and the extended length of the non-polar chain(s), respectively, and A_o is the optimal head group area of the molecule. Regarding this concept, amphiphilic molecules with p-values between 0.5 and 1.0 geometrically appear like a truncated cone, and an accumulation will result in a lamellar organisation with a bilayer formation such as vesicles or liposomes (Israelachvili et al., 1977; Corkery, 2002). Micelles can be expected if $p \leq 0.5$ due to the fact that the respective molecules are defined by a conical shape whereby an assembly of such molecules above the CMC will force the hydrophobic ends into the inner core and the polar head regions will build the outer shell (driven by an entropic advantage; Tanford, 1978). The smaller the packing parameter, the more spherical the micelle geometry can be expected. Therefore, phosphatidylcholines (PC) with a p-value ≥ 0.5 (Segota and Tezak, 2006) are not able to form micelles solely. However, it was shown that when lamellae and micelle forming molecules are mixed together they form various types of structures like vesicles or micelles depending on their proportions (Ollivon et al., 2000). A theoretical approach behind the presented work is the transfer of the packing parameter concept which describes the organisation of one amphiphilic species, to the organisational behaviour of the combination of two different amphiphilic species, a water-insoluble phospholipid and a hydrophilic surfactant. With this transferred model which is schematically shown in Fig. 1, the combination of a surfactant and a phospholipid resulting in a mixed micelle can be explained. The influence of the surfactant molecular structure on the ability to form mixed micelles with phospholipids was investigated in order to find a homogeneous binary system composed of phosphatidylcholine next to a suitable surfactant which permits the existence of only one generated structure, indicating a unimodal particle distribution at higher ratios of PC to surfactant and over a broad range of total surfactant concentrations. Requirements for the tested surfactants were a small packing parameter according to Israelachvili, that means a large hydrophilic head group which is explicitly separated from the hydrophobic ending. Beside these molecular conditions the surfactant further should have a HLB-value $\gg 10$ (HLB = hydrophilic lipophilic balance) to assure an adequate water solubility. As a close match to this, sucrose esters as disaccharides offer an optimal large hydrophilic head region which forces the hydrophobic acyl chains into a v-formation which will result in spherical micelles. Moreover polyglycerol esters as a second class of surfactants were investigated for their ability to form clear solutions with phosphatidylcholine. A homologous series with different fatty acids ranging from C_{12}–C_{18}, of both different surfactant classes was tested. Further, there are more similarities between those two surfactant classes as both exhibit a head region with noticeably potent hydrophilic hydroxyl-units (compared to a polysorbate's ether groups) and the non-polar tail does not bear any hydrophilic element at all. For comparative purpose, different other types of surfactants were also included into the study,

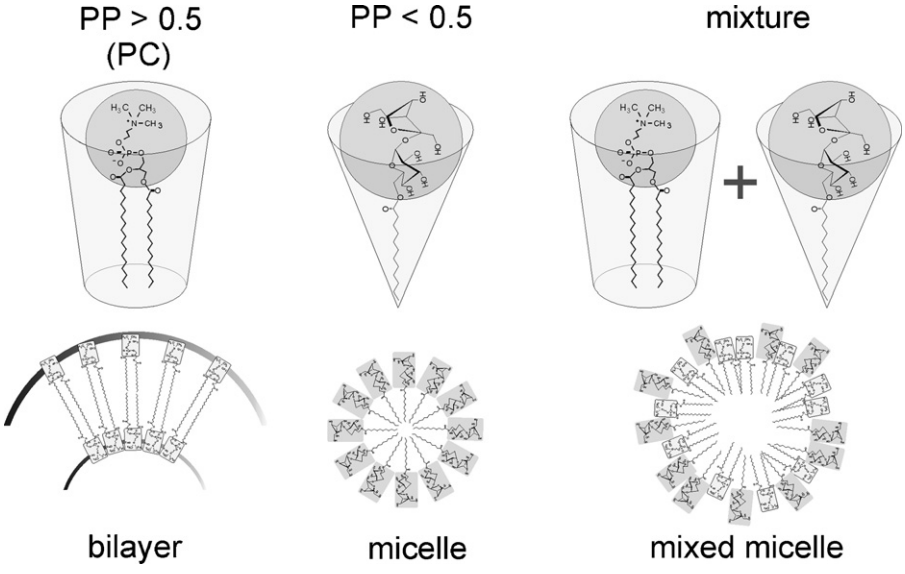

Fig. 1. Hypothetic mixed micelle formation of a non-conical amphiphil with a p-value > 0.5 (e.g. PC) and a surfactant with a p-value < 0.5 (e.g. a sucrose ester).

although those surfactants showed different molecular geometries as they do not feature such a strict and explicit separation from a polar to a non-polar part of the molecule. The parenterally tolerated Poloxamer 188 as a block copolymer and Solutol HS 15, with a hydrophilic hydroxyl group as interruption factor in the lipophilic tail, were chosen. The reason for combining poloxamer with hPC was to have a counter-example compared to those surfactants that are characterised by a strictly defined separation from a polar part to a non-polar part and thus, can be classified by the PPC. Poloxamer as a copolymer composed of POE and POP blocks shows a defined partition between a polar and a non-polar moiety within its chemical structure. Further the high HLB-value (24) is defined by those POE and POP moieties. However, the HLB-value demonstrates only the molecular weight of the hydrophilic chemical groups related to the entire molecular weight of the poloxamer macromolecule. Hence, by knowing the HLB value, it is not evident in which arrangement the hydrophilic moieties are located. But to be classified by the PPC and according to our proposal for the application of the (transferred) PPC to form MM (Fig. 1), the whole surfactant needs to appear in a conical shape to accept small *p*-values. Furthermore polysorbate 20 (Tween 20), polasorbate 80 (Tween 80) and the sodium salt of cholic acid (CA), offering a steroidal backbone, were investigated. Different types of phosphatidylcholines were included into the study, e.g. hydrogenated PC, unsaturated PC (uPC), and dipalmitoylphosphatidylcholine (DPPC).

2. Materials and methods

2.1. Materials

Hydrogenated phosphatidylcholine, hPC (Phospholipon® 100H) which is composed of at least 98% stearic and palmitic acid with a purity of 99%, and unsaturated phosphatidylcholine, uPC (Phospholipon® 90G), with a purity of also 99% were from Phospholipid GmbH (Cologne, Germany). 1,2-Dipalmitoyl-L-phosphatidylcholine (DPPC) was purchased from Lipoid GmbH (Ludwigshafen, Germany). Sucrose esters as SL (sucrose laurate, D-1216), SM (sucrose myristate, M-1695), and SP (sucrose palmitate, D-1616) which all present a mixture of mono- and diesters, and polyglycerol esters (PGE) as L-7D (decaglycerol laurate), M-10D (decaglycerol myristate) and SW-10D (decaglycerol stearate) were from Mitsubishi Chemical Corporation (Tokyo, Japan). The surfactants Poloxamer 188 and Solutol HS 15 (polyethylene glycol 660 ester of 12-hydroxy stearic acid) were a gift from BASF (Ludwigshafen, Germany). The surfactants Tween 80 (polysorbate 80), Tween 20 (polysorbate 20) and cholic acid (sodium salt, CA) as well as the lipophilic fluorescent dye DPH (1,6-diphenyl-hexatriene) were purchased from Sigma–Aldrich (Munich, Germany). NaH_2PO_4 and Na_2HPO_4 were obtained from Merck (Darmstadt, Germany). The used water was of double-distilled quality.

2.2. Methods

2.2.1. Preparation of MM (preparation of the samples)

All micellar and mixed micellar solutions or dispersions were prepared by a direct dispersion method which was shown to be equivalent to the film-forming or the evaporation method (Lichtenberg et al., 1979). The water-insoluble phospholipid component and the water soluble surfactant were dispersed together in phosphate buffer 0.067 M at pH 7.4 (Hammad and Müller, 1998a,b) using a thermostated magnetic stirrer (Sznitowska, 2008). The buffer solution was added after filtration (0.22 μm). Starting at a higher temperature of 60 °C in order to obtain an optimal hydration of the PC above its thermotropic transition temperature (Lichtenberg et al., 1983), the samples got equilibrated at 37 or 25 °C for at least 24 h. Considering the fact that most of the applied surfactants represent mixtures of mono- or diesters (sucrose esters and polyglycerol esters) or at least are not purified (Polysorbate, Solutol HS 15 and Poloxamer 188) the weight fraction was used instead of the molar fraction to describe the different ratios of PC to surfactant in the prepared solutions. The total surfactant concentration (PC + surfactant) of the stock solutions was kept constant in a range from 1.0 mg/mL to 80 mg/mL (mainly 10 or 50 mg/mL) at a weight fraction of the particular PC ranging from 0.0 to 0.7 (WF_{PC}0.0–0.7) which represents a content of 0–70 wt% of the particular PC.

2.2.2. Transmission measurements

In order to determine the maximum amount of PC which can be solubilised by the respective surfactant, light transmission measurements of the prepared MM systems or dispersions were carried out at 660 nm using a UV spectrophotometer (Kontron Instruments, Munich, Germany). Before starting a measurement, the samples were equilibrated for 24 h at room temperature (RT). Each sample was examined without being filtrated to avoid sample manipulation by eliminating agglomerates or larger vesicles. A light transmission rate of 100% and otherwise no reduction in the light transmission, respectively, indicates a complete solubilisation of the PC and the formation of a clear mixed micellar solution. At the point of an abrupt decrease in transmission, the system is oversaturated with PC and no further embedding of PC molecules by surfactant molecules into MM or maybe mixed vesicles occurs. All measurements were done in triplicate. The values reported are the average of at least two independent samples.

2.2.3. Size determination

Dynamic light scattering (DLS) was used to measure the size as hydrodynamic diameter and the size distribution of occurring particles as micelles, mixed micelles or vesicle–micelle mixtures. DLS determinations were made at a constant scattering angle in all cases (Wei et al., 2009; Kaushik et al., 2007; Zhou et al., 2008) using a photon correlation spectrometer (Zetasizer Nano ZS, Malvern Instruments Ltd., UK-Malvern). All measurements were carried out in triplicate at 25 °C after 5 min of equilibration and all values reported are the average of at least three independent samples. To avoid any loss of particles like larger vesicles, the produced samples generally were analysed without a dilution and filtration step to get information on every species that emerged after sample preparation. In some cases samples with a higher total surfactant concentration of 50 mg/mL were filtrated (0.45 μm). As basis of evaluation of the DLS results, the found volume weighted diameter of the particles and the polydispersity index (PDI) of each investigated sample were utilised. Using DLS technique, the random movement of colloids is monitored according to fluctuations in the intensity of the scattered light. From this, a decaying exponential correlation function is derived, and it contains the translational diffusion coefficient *D*. According to the Stokes–Einstein relation, *D* is inversely proportional to the dynamic viscosity η and the radius of an idealized sphere *r* with *k* as the Boltzmann constant and *T* as the absolute temperature. Thus, if the temperature is held constant, the diffusion coefficient for a sphere with the given radius *r* will be solely dependent on the dynamic viscosity of the sample (Fillafer et al., 2007).

$$D = \frac{kT}{6\pi\eta r}$$

To compensate the influence of a higher viscosity at higher total surfactant concentrations, the viscosities of the different dilutions were measured with the SV-10 Vibro viscometer (Malvern Instruments Ltd., Worcestershire, UK) because it is known that a change in viscosity results in a failure on determined particle size (Fillafer et al., 2007).

2.2.4. Surface tension measurements (CMC determination)

The total surfactant concentration of the stock solution was kept constant at 5.0 or 10.0 mg/mL for every micellar or mixed micellar solution. Based on these solutions a geometrical dilution series was prepared and every dilution was stored at RT over 24 h for equilibration before starting a measurement. The solutions of the amphiphilic substances with concentrations above the CMC reached equilibrium within 6 h whereas those with concentrations below the CMC required a stabilisation time up to 24 h. The surface tension measurements were made for each dilution and the stock solution. The equilibrium surface tension of each sample was measured at 22 °C with a K12 tensiometer using the Wilhelmy plate technique (Krüss GmbH, Hamburg, Germany). The CMC was determined from the plot of the surface tension against the logarithm of the concentration. Measurements were done in triplicate and all data reported are the average of at least two independent samples.

2.2.5. Determination of fluorescence intensity (CMC determination)

A DPH stock dispersion of 2×10^{-7} M in phosphate buffer 0.067 M at pH 7.4 (Hammad, 1998; Hammad and Müller, 1998a,b) was prepared and different surfactant solutions of various concentrations were diluted with the DPH buffer solution. The samples were equilibrated overnight in a dark chamber at 25 °C (Chattopadhyay and London, 1984). A fluorescence plate reader (Polarstar optima, BMG LABTECH GmbH, Offenburg, Germany) was used to measure the DPH fluorescence. The wavelengths of excitation and emission were 355 and 428 nm, respectively. The temperature was controlled at 25 °C. The CMC was determined by plotting the fluorescence intensity versus total surfactant concentrations of the micelle system or MM system. The CMC was estimated from the point at which the slope of the intensity showed a sharp increase which is due to micelle or mixed micelle formation and the solubilisation of increasing amounts of the lipophilic fluorescent dye DPH.

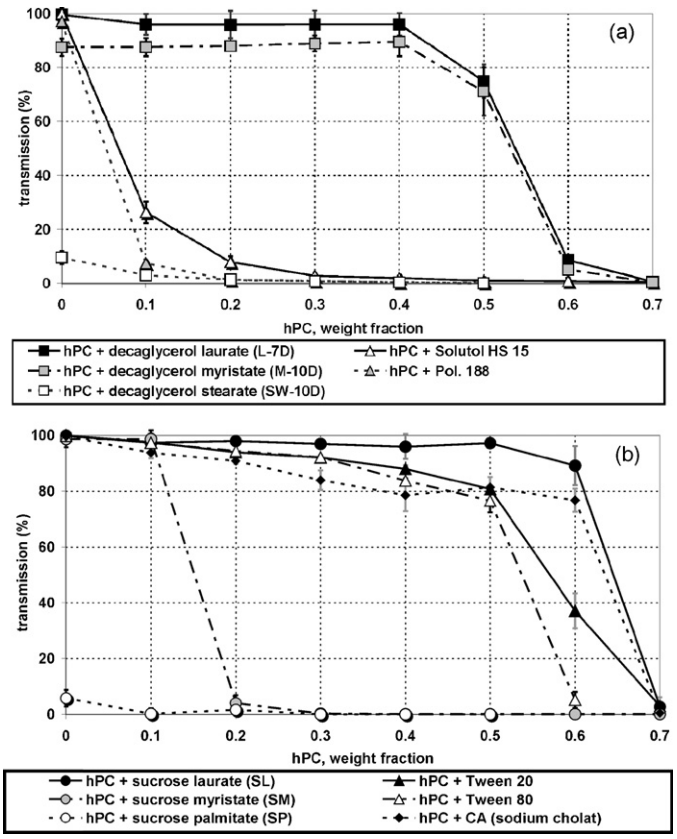

Fig. 2. (a) Solubilisation of hPC by polyglycerol esters and other surfactants at a constant total surfactant concentration of 10 mg/mL. (b) Solubilisation of hPC by sucrose esters and other surfactants at a constant total surfactant concentration of 10 mg/mL.

3. Results and discussion

3.1. Solubilisation of PC by surfactants – light transmission data

The formation of mixed micelles composed of a water-insoluble phospholipid and a water soluble surfactant results in an isotropically clear solution (Lichtenberg et al., 1979). The maximum amount of hydrogenated phosphatidylcholine (hPC) that could be incorporated into an isotropically clear solution by different surfactants is shown in Fig. 2a and b. In all cases, the end point of hPC solubilisation is characterised by a sharp decrease of the light transmission. The surfactants Poloxamer 188 and Solutol HS 15 were found to be unsuitable to form a clear solution with hPC at all. They were incapable to solubilise even the weight fraction of 0.1 hPC ($WF_{hPC}0.1$) without a sharp decrease in transmission (Fig. 2a).

Copolymers like Poloxamer 188 are forming polymeric micelles which represent a separate class of micelles (Torchilin, 2002). The hydrophobic core of those micelles consists of PPO blocks and these are surrounded by an outer shell of the hydrated PEO above the CMC. But there are no monomers which exhibit a spatial separation from a polar head region to an absolutely non-polar tail. Hence, the block copolymer does not feature the structural requirements for a conical shape which is required for a MM formation with hPC as it is postulated by the transferred PPC (Fig. 1).

Solutol HS 15 is exhibiting a hydrophilic element (hydroxyl group at the C_{12} of the stearic acid) within the lipophilic chain which could act as an interruption factor with regard to the association with hPC into MM. The hydrophilic moiety (hydroxyl-unit) within the lipophilic tail impedes an explicit separation from a polar head to an absolutely non-polar tail. A co-association with hPC in order to form MM is not possible because the molecule does not fit the transferred PPC.

The observed inability of Poloxamer 188 to form clear solutions with hPC is in agreement with the results of Hammad (1998) who found that even at a of weight fraction 0.05 of unsaturated PC were incapable to form MM with Poloxamer 188. Decaglycerol stearate as the most lipophilic polyglycerol ester (PGE) was not able to form clear solutions with hPC at all whereas the other two tested PGEs as L-7D and M-10D were suitable to form MM at weight fractions of solubilised hPC of up to 0.4 (Fig. 2a). With an increasing length of the fatty acid, the lipophilic volume of the PGEs increases and due to that the ability to form clear solutions with hPC decreases. Similar results could be obtained for the homologous series of the tested sucrose esters (Fig. 2b). SL as the most hydrophilic sucrose ester, featuring the smallest lipophilic volume, incorporated a maximum weight fraction of 0.6 hPC, whereas the more lipophilic sucrose esters, SM and SP, were found to solubilise only 0.1 WF_{hPC}. Polysorbate 20, polysorbate 80 and cholic acid sodium salt (CA) were able to solubilise hPC at 0.5 and 0.6 WF_{hPC} (in the case of CA).

The more lipophilic SEs as well as PGEs showed an inferior ability to arrange with hPC to an isotropically clear solution. Contingently, those surfactants featuring a larger lipophilic volume according to the PPC, exhibit a negative packing entropy of their voluminous tails in the aggregates (MM) (Nagarajan, 2002). Accordingly, the shorter chained SL (or L-7D) revealed a preferential chain packing with hPC to an (MM) isotropically clear solution. Hence, the lipophilic chain length might be a limiting factor for a MM formation with hPC. A requirement for a MM system to serve as a drug carrier system is the formation of an isotropically clear solution over a broad range of

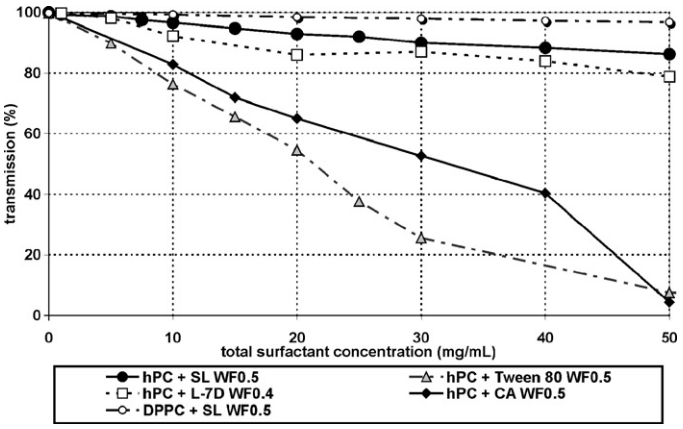

Fig. 3. Solubilisation of hPC and DPPC with different surfactants at increasing total surfactant concentrations.

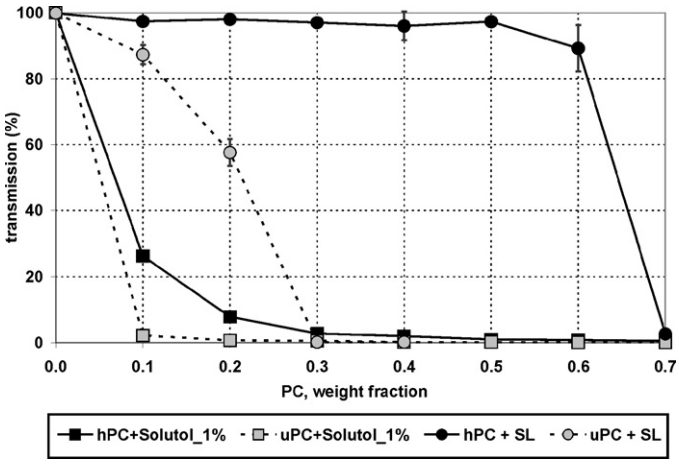

Fig. 4. Solubilisation of hPC and uPC by SL and Solutol HS 15.

total surfactant concentrations. The ability of the investigated systems to form clear solutions is depending on the total surfactant concentration (Fig. 3). While solutions of polysorbate 80 or CA in combination with hPC at a WF_{hPC}0.5 appeared turbid at higher total surfactant concentrations, L-7D and SL were found to form clear solutions with hPC even at higher total surfactant concentrations of >10 mg/mL. At higher total surfactant concentrations polysorbate or CA were unable to hold hPC solubilised if the weight fraction of hPC was at 0.5. With an increase in total surfactant concentration more hPC precipitated resulting in a continuous decrease in transmission (Fig. 3).

Polysorbates (Tween 20, 80) are exhibiting a polar head region which is geometrically separated from a hydrophobic tail and by that they should be classified by the transferred PPC. In Comparison to sucrose esters PEG demonstrates the hydrophilic head regions of a polysorbate molecule. The hydrophilicity caused by a PEG-chain (polysorbate) is less remarkable compared to the hydrophilicity caused by many hydroxyl-units (sucrose in a sucrose ester). Contingently, the closeness of the hydrophilic elements within the hydrophilic head region could be decisive for a hydrophilic surfactant to form MM with hPC. Accordingly one can deduce that the hydrophilicity should only be located at the polar head region and more potent hydrophilic elements should be present (–OH (sucrose) instead of ether (PEG)). Thus, polysorbate 20 or 80 (Tween 20 or 80), offering a large polar head but no less hydrophilic, were not able to form clear solutions with hPC.

Hammad (1998) also found polysorbate 80 to be unqualified to form a mixed micellar solution with lecithin at higher total surfactant concentrations even at smaller ratios of lecithin/polysorbate 80. It is known that CA, as a bile salt, forms MM with lecithin also at higher total surfactant concentrations (Lichtenberg et al., 1979; Cohen and Carey, 1991; Hammad and Müller, 1998a). The same has also been found in this work (data not shown), but if hydrogenated phosphatidylcholine is present instead of unsaturated PC, the ability of CA to form clear solutions with PC at higher total surfactant concentrations disappeared (Fig. 3). Previous investigations revealed that the ability of bile salts and phosphatidylcholine to preferably form mixed micelles instead of vesicles depends on the number of unsaturated fatty acids in the lecithin (Cohen and Carey, 1991; Booker et al., 1992). Lecithins containing more highly unsaturated acyl chains were found to form more favourably mixed micelles instead of building bilayer vesicles when being mixed with bile salts (Cohen and Carey, 1991). Interestingly, the ability of SL to form isotropically clear solutions with phosphatidylcholine could be further enhanced by a switch from hPC to DPPC. Those systems with 0.5 WF_{DPPC} resulted in a system offering almost no change in light transmission at highest total surfactant concentrations of 50 mg/mL. This phenomenon can also be explained with the transferred packing parameter concept: DPPC holds a different *p*-value compared to hPC due to a smaller lipophilic volume induced by the absence of the more hydrophobic stearic acid enabling a tighter arrangement with SL in associated particles.

In order to reveal the influence of the double bond within the unsaturated fatty acid (oleic acid) of uPC, selective surfactants were combined with both hPC or uPC at different PC ratios maintaining a constant total surfactant concentration of 10 mg/mL (Fig. 4). In case uPC was next to SL, SL did not form clear solutions with uPC but precipitated instead even at a lower ratios of uPC. An explanation for that can also be given by the packing parameter concept since uPC (in comparison to hPC) holds a larger lipophilic volume due to the double bond, leading to a bend in the hydrophobic chain resulting in an increased *p*-value. This inadequate larger *p*-value of uPC hindered a MM formation with SL. In addition, the bend within the oleic acid of uPC complicated an arrangement with SL so that MM formation is sterically hindered. Solutol HS 15 was not able to form a clear solution with neither hPC nor uPC. The presence of unsaturated PC even implicated a more intense decrease in transmission (Fig. 4). Also in this case the hydroxyl group in the lipophilic chain forms a structural barrier for the association with PC to form MM. The result that hPC has a better ability to form MM as uPC is contrary compared to previous experiments on the MM formation of bile salt/lecithin mixtures (Cohen and Carey, 1991). However a bile salt represents an untypical surfactant and the organisation with lecithin into MM is based on the mixed disk model which cannot be applied to MM with sucrose or polyglycerol esters as surfactants.

3.2. DLS analysis and size determination

In an aqueous mixture of two different amphiphilic species, not only the ratio of surfactant to phospholipids (Almog et al., 1986) but also the total surfactant concentration can have an influence on the formation of mixed micelles, vesicles or else (Lichtenberg et al., 2000). The common lecithin/bile salt mixed micellar systems show a variation in size of the particles as the proportion of lecithin to bile salt varies (Shankland, 1970). A favourite MM system, however, should show no change in size, shape or particle distribution over a broad range of total surfactant concentrations. The dependence of the total surfactant concentration on the size measured as hydrodynamic diameter and the polydispersity index (PDI) for three different MM systems is shown in the Fig. 5a–c.

At different total surfactant concentrations particle size fluctuations can be observed and PDI values near 1.0 at total surfactant concentrations higher than 15 mg/mL if polysorbate 80 was mixed

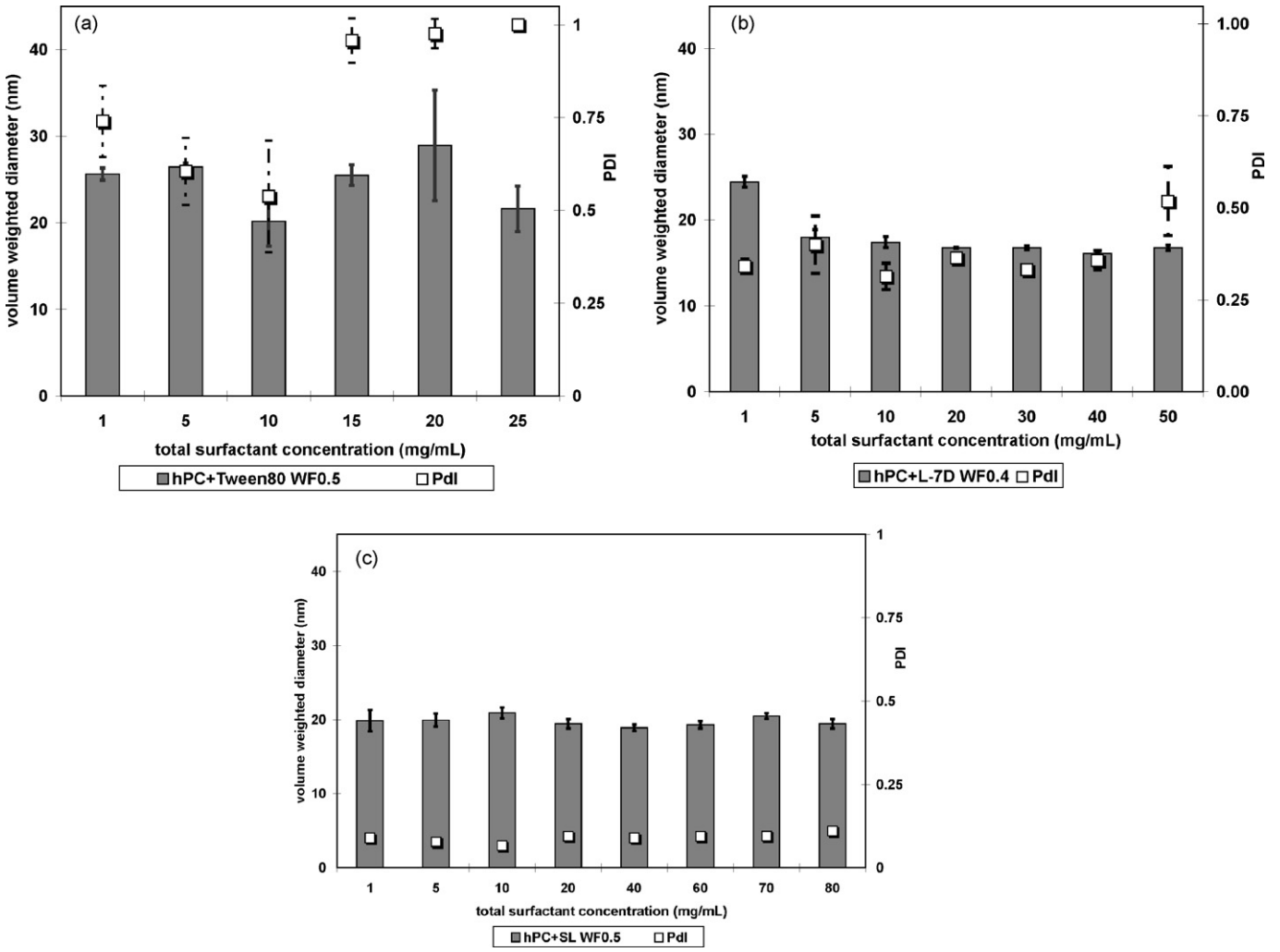

Fig. 5. (a) Influence of total surfactant concentration on size and PDI of the MM system hPC + Tween 80 at a WF_{hPC}0.5. (b) Influence of total surfactant concentration on size and PDI of the MM system hPC + L-7D at a WF_{hPC}0.4. (c) Influence of total surfactant concentration on size and PDI of the MM system hPC + SL at a WF_{hPC}0.5.

with hPC at WF_{hPC}0.5 (Fig. 5a). These high PDI values represent a broad particle size distribution excluding a unimodal particle size distribution. It has already been reported that polysorbate 80 forms mixed micelles as well as vesicles when mixed with hydrogenated PC (Lim and Lawrence, 2004a,b). Due to the fact that the system hPC and polysorbate 80 at WF_{hPC}0.5 got turbid at higher total surfactant concentrations, those bad PDI values may be caused by precipitated hPC.

These size measurement results further re-emphasise the finding that polysorbate 80 is not able to form clear solutions with hPC because there is no geometrical arrangement possible for hPC and polysorbate 80 to form MM as it was postulated by the transferred PPC. Possibly the hydrophilicity caused by a PEG-chain (polysorbate polar head region) is less remarkable to force a sufficient conical shape as is needed for a MM formation with hPC (Fig. 1).

Mean particle size and polydispersity of the system hPC and L-7D at WF_{hPC} 0.4 with increasing total surfactant concentration is shown in Fig. 5b. No change in size at total surfactant concentrations between 5 and 50 mg/mL occurred. Only at lower concentrations (about 10 mg/mL) a slight increase in size was observed which may be explained by dilution effects (close to CMC). The PDI value was below 0.4 for most samples. This high PDI value represents a broader particle size distribution indicating that besides MM other aggregates were formed in the solution. At the highest concentration of 50 mg/mL the highest PDI value of about 0.5 was measured. Increasing total surfactant concentrations (over 50 mg/mL) led to a change in the sample composition. The system then was oversaturated with hPC resulting in an agglomeration of hPC which also can be shown by the decrease in light transmission (Fig. 3).

Fig. 5c shows the mean particle size and PDI at increasing total surfactant concentrations of the favourite MM system of hPC and SL at a WF_{hPC}0.5. The mean particle size at all concentrations (1–80 mg/mL) was about 20 nm without fluctuations. The PDI values over this broad range of total surfactant concentration were in most cases $\leq$0.1.

Micelles of decaglycerol laurate (at WF_{hPC}0.0, Fig. 6a) with a size of approximately 8.5 nm were found to be slightly smaller than micelles of decaglycerol myristate (at WF_{hPC}0.0, Fig. 6b) with a diameter of approx. 10.5 nm which can be explained by the longer fatty acid chain of M-10D (C_{14}) compared to the lauric acid in L-7D. The presence of hPC led to MM formation with an expected larger mean diameter. At a total surfactant concentration of 1% (10 mg/mL) the MM sizes of hPC and L-7D at WF_{hPC} 0.2–0.6 were approximately in the range of 19–24 nm regardless of the content of hPC (Fig. 6a). If M-10D is used as surfactant the particle size increased to 24–29 nm at WF_{hPC} of 0.1–0.6 (Fig. 6b). Fig. 6a and b generally reveals that mixed micelles composed of decaglycerol laurate (L-7D) and hPC were slightly smaller than MM composed of decaglycerol myristate (M-10D) and hPC which is probably due

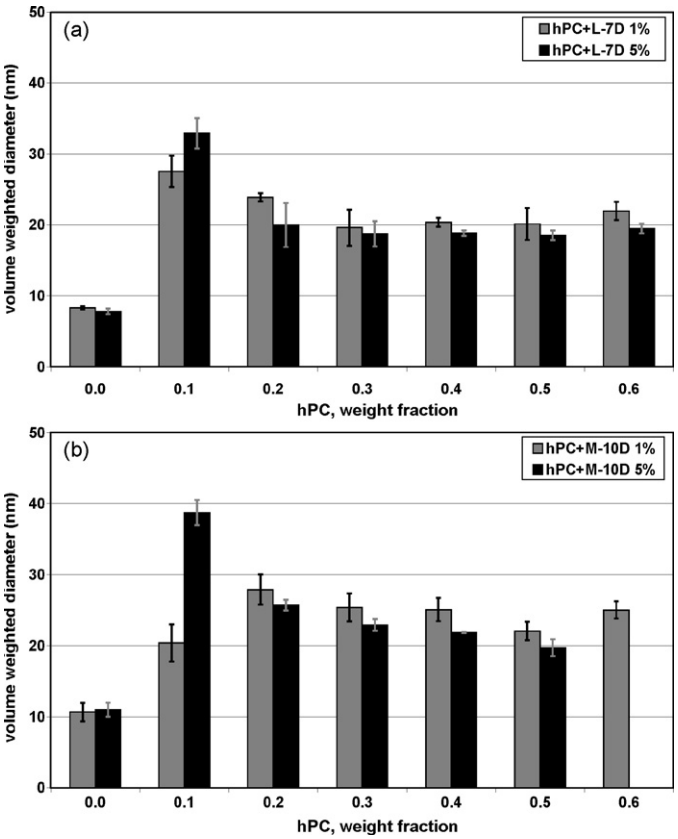

Fig. 6. Influence of total surfactant concentration on size of MM systems with polyglycerol esters and increasing weight fractions of hPC. (a) L-7D and (b) M-10D.

to the different length of the fatty acids. By increasing the total surfactant concentration to 5%, no significant changes in the mean particle sizes occurred if the WF_{hPC} was 0.2–0.6 in both systems. Surprisingly, in both systems the particle sizes increased at higher total surfactant concentrations of 5% if the weight fraction of hPC declined to WF_{hPC} 0.1, although the values were corrected for the influence of the increased viscosity. The reason for that is attributed to a change in vesicle shape or the formation of larger micelles. SL micelles were found to have an average size of about 7–8 nm at a WF_{hPC}0.0 (Fig. 7). Co-mixing of hPC resulted in an increase in size. Systems containing hPC at WF_{hPC}0.1 or WF_{hPC}0.2 were found to be 9–13 nm large at 1% or 5% total surfactant concentration, explainable by an integration of hPC into the SL micelles forming mixed

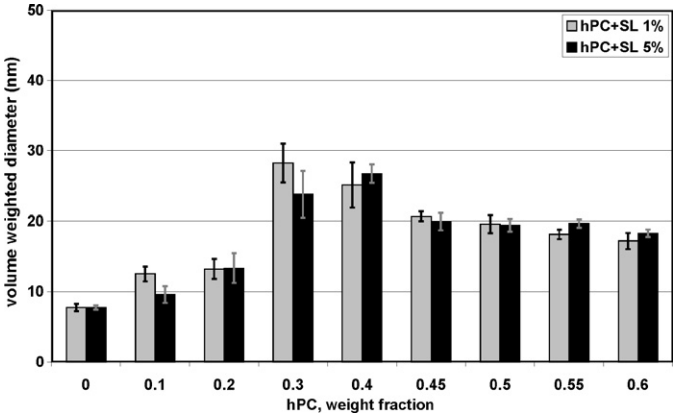

Fig. 7. Influence of total surfactant concentration on size of MM systems with sucrose laurate and increasing weight fractions of hPC.

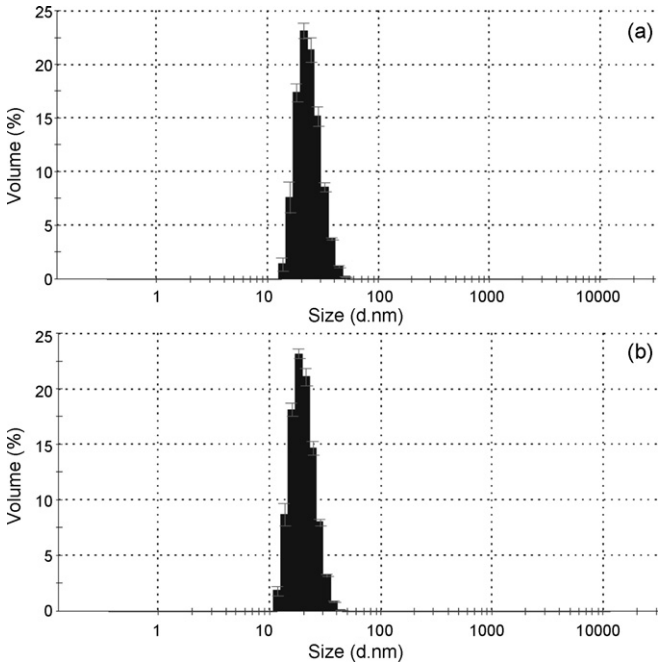

Fig. 8. Volume weighted diameter of the favourite MM system hPC + SL WF_{hPC}0.5 at a total surfactant concentration of 1% (a) and 5% (b).

micelles. At 0.3 weight fraction, the system turned into a bimodal particle distribution with one species at 13 nm (not shown in Fig. 7) and another at approximately 28 nm at 1% total surfactant concentration. The system formed at this weight fraction larger aggregates which were not further investigated. At higher weight fractions of hPC SL was able to form mixed micellar sized particles with hPC which showed no change in size at different total surfactant concentrations. The combination of SL with hPC at WF_{hPC} of 0.45–0.6 resulted in 18.5–21 nm sized particles with no change in diameter at a total surfactant concentration of 10 or 50 mg/mL. The favourite MM system composed of SL and 0.5 weight fractions of hPC (WF_{hPC}0.5) displays a unimodal particle distribution with particle sizes of approximately 20 nm, indicating mixed micelles, and an excellent PDI value of 0.1 (Figs. 5c and 8a, b). Fig. 8 illustrates the volume weighted diameter of this favourite MM system at different total surfactant concentrations of 1% (Fig. 8a) and 5% (Fig. 8b). The results were calculated considering the determined dynamic viscosities (0.95 mPa s at 1% and 1.25 mPa s at 5%) and accordingly no change in size could by observed for different total surfactant concentrations (Fig. 8).

3.3. CMC – determination

The CMC values for SL were approximately 0.23 mg/mL obtained by surface tension measurements and approximately 0.21 mg/mL obtained by fluorescent measurements, respectively. The CMC value for sucrose monolaurate being reported in literature is about 0.14 mg/mL (Becerra et al., 2008) which is in relatively good agreement with the actually determined ones. SL is a sucrose ester with a mixture of mono- and dilauric acid esters and due to the more hydrophobic dilauric acid component a decrease of the CMC can be expected (Traube, 1891; Aranow and Witten, 1958). The addition of either hPC or DPPC as phosphatidylcholine component at a weight fraction of 0.5 resulted in a slight decrease in surface tension and CMC (Fig. 9a). A drop in CMC to approximately 0.18 mg/mL for the MM system with hPC and SL at a WF_{hPC}0.5 and approximately 0.14 mg/mL could be observed if DPPC was present instead of hPC. The reduction of the CMC due to the addition of phos-

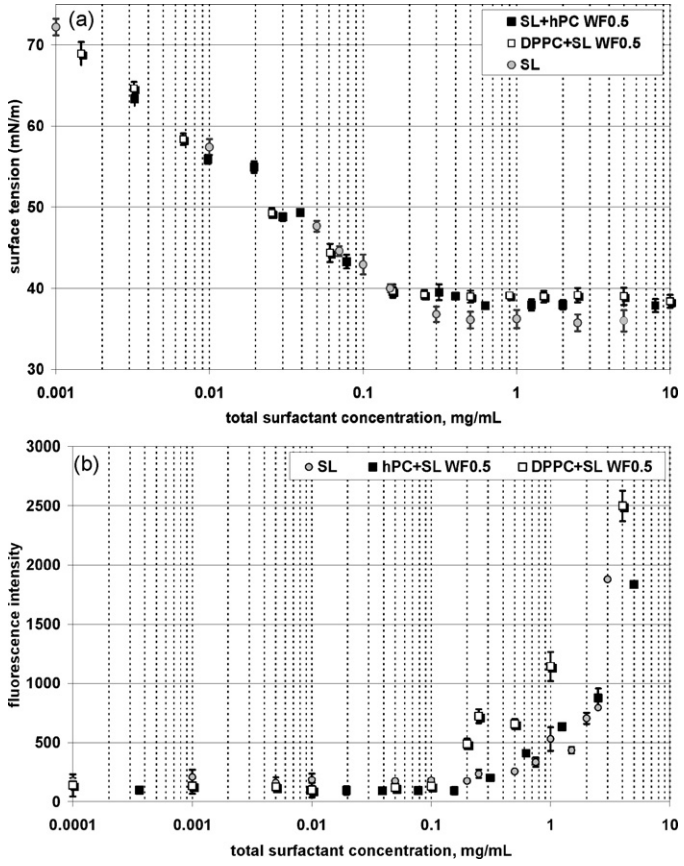

Fig. 9. Determination of the CMC for SL and SL/hPC or SL/DPPC MM systems containing a WF_{hPC} of 0.5 by (a) surface tension measurements and (b) fluorescence measurement technique.

phatidylcholine for both hPC and DPPC could be confirmed by the fluorescent measurements (Fig. 9b). It is known that the addition of phospholipids to a surfactant leading to a MM system can result in a reduction of the CMC value. Hammad found that the classical mixed micellar system of sodium cholate and lecithin showed a lower CMC value compared to sodium cholate itself (Hammad, 1998).

4. Conclusions

In general, the modified packing parameter concept from Israelachvili could successfully be transferred to the application of MM systems: Hydrophilic surfactants that feature a strictly defined partition within their molecular geometry on the one side and exhibit a small value of the modified packing parameter (<0.5) due to a large equilibrium polar head (featuring potent hydrophilic elements) and a shorter absolutely hydrophobic tail (which leads to a smaller lipophilic volume) on the other side are promising candidates to form MM systems with saturated PCs. The following can be concluded with respect to the application of the transferred PPC to the formation of MM with hydrogenated PC:

- The surfactants' HLB has to be at a higher value to assure adequate water solubility.
- The surfactant has to appear (geometrically) in a conical shape. An explicit structural breakup within the molecular geometry between a large polar head and an absolutely non-polar tail is necessary.
- The surfactants' hydrophilicity is only located at the polar head region and it is defined by potent hydrophilic elements (–OH units (sucrose esters) better than ether (PEG); polysorbate 20 or 80 were not able to form MM with hPC).
- A smaller lipophilic volume (V_c) was found to be more important than the loss of lipophilic chain length (l_c) by the use of shorter chained surfactants (e.g. SL) with regard to form MM with hPC.
- The non-polar tail should not bear any hydrophilic element (Solutol HS 15, exhibiting a hydroxyl group within the stearic acid was unable to form clear solutions with hPC).

Although the *p*-value of the surfactant should be small, the smaller *p*-value should not be caused by a longer hydrophobic tail (l_o) but by a large equilibrium polar head region. The more lipophilic SEs as well as PGEs revealed an inferior ability to arrange with hPC to an isotropically clear solution in comparison to the short chained sucrose laurate or polyglycerol laurate. As a result, the lipophilic chain length might be a limiting factor for a MM formation with hPC as it hindered a tighter packing in the inner mixed micellar core (next to hPC). As a result , mixed micellar systems were successfully produced with polyglycerol esters and sucrose esters (next to hPC). Short chain fatty acids as hydrophobic part of the amphiphilic (surfactant) molecules were shown to produce stable MM systems over a broad range of concentrations. Sucrose laurate forms isotropically clear solutions with hPC (or DPPC) exhibiting a unimodal particle distribution with particle sizes of approximately 20 nm, indicating mixed micelles, and an excellent PDI value of 0.1, even at higher ratios of hPC (WF_{hPC}0.5 or 0.6) and over a broad range of total surfactant concentrations. In comparison to the commonly used lecithin/bile salt MM where unsaturated PC (lecithin) is required for a MM formation, the introduced novel MM systems are formed with saturated PC types which are more stable against oxidative degradation. The direct dispersion method is a simple production method for the introduced mixed micellar systems owing the benefit of avoiding organic solvents.

References

Almog, S., Kushnir, T., Lichtenberg, D., 1986. Kinetic and structural aspects of reconstitution of phosphatidylcholine vesicles by dilution of phosphatidylcholine–sodium cholate mixed micelles. Biochemistry 25, 2597–2605.
Aranow, R.H., Witten, 1958. Theoretical derivation of Traube's rule. J. Chem. Phys. 28, 405–409.
Barenholz, Y., 2001. Liposome application: problems and prospects. Curr. Opin. Colloid Interface Sci. 6, 66–77.
Becerra, N., Toro, C., Zanocco, A.L., Lemp, E., Günther, G., 2008. Characterization of micelles formed by sucrose 6-O-monoesters. Colloids Surf. A 327, 134–139.
Booker, M.L., Ahrendt, S.A., Lillemoe, K.D., Pitt, H.A., 1992. Distribution of phosphatidylcholine molecular species between mixed micelles and phospholipid-cholesterol vesicles in human gallbladder bile: dependence on acyl chain length and unsaturation. J. Lipid Res., 1485–1492.
Chattopadhyay, A., London, E., 1984. Fluorimetric determination of critical micelle concentration avoiding interference from detergent charge. Anal. Biochem. 139, 408–412.
Cohen, D.E., Carey, M.C., 1991. Acyl chain unsaturation modulates distribution of lecithin molecular species between mixed micelles and vesicles in model bile. Implications for particle structure and metastable cholesterol solubilities. J. Lipid Res. 32, 1291–1302.
Corkery, R.W., 2002. The anti-parallel, extended or splayed-chain conformation of amphiphilic lipids. Colloids Surf. B 26, 3–20.
Crosasso, P., Ceruti, M., Brusa, P., Arpicco, S., Dosio, F., Cattel, L., 2000. Preparation, characterization and properties of sterically stabilized paclitaxel-containing liposomes. J. Control. Release 63, 19–30.
Fillafer, C., Wirth, M., Gabor, F., 2007. Stabilizer-induced viscosity alteration biases nanoparticle sizing via dynamic light scattering. Langmuir 23, 8699–8702.
Hammad, M.A., 1998. Solubility and Stability of Drugs in Mixed Micelles. Ph.D. thesis.
Hammad, M.A., Müller, B.W., 1998a. Increasing drug solubility by means of bile salt-phosphatidylcholine-based mixed micelles. Eur. J. Pharm. Biopharm. 46, 361–367.
Hammad, M.A., Müller, B.W., 1998b. Solubility and stability of tetrazepam in mixed micelles. Eur. J. Pharm. Sci. 7, 49–55.
Helenius, A., Simons, K., 1975. Solubilization of membranes by detergents. Biochim. Biophys. Acta, Rev. Biomembr. 415, 29–79.
Israelachvili, J.N., Mitchell, D.J., Ninham, B.W., 1977. Theory of self-assembly of lipid bilayers and vesicles. Biochim. Biophys. Acta, Biomembr. 470, 185–201.
Kaushik, P., Vaidya, S., Ahmad, T., Ganguli, A.K., 2007. Optimizing the hydrodynamic radii and polydispersity of reverse micelles in the Triton

X-100/water/cyclohexane system using dynamic light scattering and other studies. Colloids Surf. A 293, 1–3, 162–164.
Krishnadas, A., Rubinstein, I., Oenyueksel, H., 2003. Sterically stabilized phospholipid mixed micelles: in vitro evaluation as a novel carrier for water-insoluble drugs. Pharm. Res. 20, 297–302.
Lawrence, M.J., Rees, G.D., 2000. Microemulsion-based media as novel drug delivery systems. Adv. Drug Deliv. Rev. 45, 89–121.
Lichtenberg, D., Opatowski, E., Kozlov, M.M., 2000. Phase boundaries in mixtures of membrane-forming amphiphiles and micelle-forming amphiphiles. Biochim. Biophys. Acta, Biomembr. 1508, 1–19.
Lichtenberg, D., Robson, R.J., Dennis, E.A., 1983. Solubilization of phospholipids by detergents structural and kinetic aspects. Biochim. Biophys. Acta, Rev. Biomembr. 737, 285–304.
Lichtenberg, D., Zilbermann, Y., Greenzaid, F., Zamir, S., 1979. Structural and kinetic studies on the solubilization of lecithin by sodium deoxycholate. Biochemistry 18, 3517–3525.
Lim, W.H., Lawrence, M.J., 2004a. Influence of surfactant and lipid chain length on the solubilisation of phosphatidylcholine vesicles by micelles comprised of polyoxyethylene sorbitan monoesters. Colloids Surf. A 250, 449–457.
Lim, W.H., Lawrence, M.J., 2004b. Aggregation behaviour of mixtures of phosphatidylcholine and polyoxyethylene sorbitan monoesters in aqueous solution. Phys. Chem. Chem. Phys. 6, 1380–1387.
Lukyanov, A.N., Torchilin, V.P., 2004. Micelles from lipid derivatives of water-soluble polymers as delivery systems for poorly soluble drugs. Adv. Drug Deliv. Rev. 56, 1273–1289.
Marjan, J.M.J., Allen, T.M., 1996. Long circulating liposomes: past, present and future. Biotechnol. Adv. 14, 151–175.
Mayer, L.D., Tai, L.C.L., Bally, M.B., 1989. Influence of vesicle size, lipid composition, and drug-to-lipid ratio on the biological activity of liposomal doxorubicin in mice. Cancer Res. 49, 5922–5930.
Mazer, N.A., Benedek, G.B., Carey, M.C., 1980. Quasielastic light-scattering studies of aqueos biliary lipid systems. Mixed micelle formation in bile salt-lecithin solutions. Biochemistry 19, 601–615.
Mu, L., Elbayoumi, T.A., Torchilin, V.P., 2005. Mixed micelles made of poly(ethylene glycol)-phosphatidylethanolamine conjugate and d-[alpha]-tocopheryl polyethylene glycol 1000 succinate as pharmaceutical nanocarriers for camptothecin. Int. J. Pharm. 306, 142–149.
Müller, B.W., Albers, E., 1992. Complexation of dihydropyridine derivatives with cyclodextrins and 2-hydroxypropyl-[beta]-cyclodextrin in solution. Int. J. Pharm. 79, 273–288.
Nagarajan, R., 2002. Molecular packing parameter and surfactant self-assembly: the neglected role of the surfactants tail. Langmuir 18, 31–38.
Ollivon, M., Lesieur, S., Grabielle-Madelmont, C., Paternostre, M., 2000. Vesicle reconstitution from lipid-detergent mixed micelles. Biochim. Biophys. Acta, Biomembr. 1508, 34–50.
Rosoff, M., Serajuddin, A.T.M., 1980. Solubilization of diazepam in bile salts and in sodium cholate–lecithin–water phases. Int. J. Pharm. 6, 137–146.
Segota, S., Tezak, D., 2006. Spontaneous formation of vesicles. Adv. Colloid Interface Sci. 121, 51–75.
Shankland, W., 1970. The equilibrium and structure of lecithin–cholate mixed micelles. Chem. Phys. Lipids 4, 109–130.
Sharma, A., Sharma, U.S., 1997. Liposomes in drug delivery: progress and limitations. Int. J. Pharm. 154, 123–140.
Small, D.M., Penkett, S.A., Chapman, D., 1969. Studies on simple and mixed bile salt micelles by nuclear magnetic resonance spectroscopy. Biochim. Biophys. Acta, Lipids Lipid Metab. 176, 178–189.
Sznitowska, M., 2008. Paclitaxel solubility in aqueous dispersions and mixed micellar solutions of lecithin. Chem. Pharm. Bull. 56, 70–74.
Tanford, C., 1978. The hydrophobic effect and the organization of living matter. Science 200, 1012–1018.
Torchilin, V.P., 2002. PEG-based micelles as carriers of contrast agents for different imaging modalities. Adv. Drug Deliv. Rev. 54, 235–252.
Traube, J., 1891. Annalne Chemie 265, 27–55.
Wei, Z., Hao, J., Yuan, S., Li, Y., Juan, W., Sha, X., Fang, X., 2009. Paclitaxel-loaded Pluronic P123/F127 mixed polymeric micelles: formulation, optimization and in vitro characterization. Int. J. Pharm. 376, 176–185.
Zhou, Z., Chaibundit, C., D'Emanuele, A., Lennon, K., Attwood, D., Booth, C., 2008. Solubilisation of drugs in worm-like micelles of block copolymers of ethylene oxide and 1,2-butylene oxide in aqueous solution. Int. J. Pharm. 354, 82–87.