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Solubilization of poorly water-soluble drugs by mixed micelles based on hydrogenated phosphatidylcholine

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ABSTRACT

A remarkable part of newly developed active pharmaceutical ingredients is rejected in early phase development and will never find a way to a patient because of poor water solubility which is often paired with poor bioavailability. Considering such arising solubility problems the development of application vehicles like mixed micelles (MM) is a challenging research topic in pharmaceutical technology. While known classical MM systems are composed of phosphatidylcholine and bile salts, it was the aim of this study to investigate if alternatively developed MM systems were superior in solubilization of different hydrophobic drugs. The novel MM were also comprised of phosphatidylcholine and (contrarily to bile salts) different other suitable surfactants forming binary MM. As model water-insoluble drug substances two benzodiazepines, diazepam and tetrazepam, and the steroid estradiol were chosen. In this study the solubilization capacities of newly developed MM were compared to those of classical lecithin/bile salt MM systems and different other surfactant containing systems. The MM system with sucrose laurate and hydrogenated PC (hPC) at a weight fraction of 0.5 was found to be superior in drug solubilization of all investigated drugs compared to the classical lecithin/bile salt mixed micelles. Further, a polysorbate 80 solution, also at 5%, was inferior with regard to solubilize the investigated hydrophobic drugs. The MM sizes of the favorite developed MM system, before and after drug incorporation, were analysed by dynamic light scattering (DLS) to evaluate the influence of the drug incorporation. Here, the particle sizes, before and after drug incorporation, remained constant, indicating a stable formation of the solubilizate. Further the critical micelle concentration (CMC) of MM before and after drug incorporation was analysed by three different determination techniques. Constant CMC-values could be obtained regardless if diazepam was encapsulated within the MM or unloaded MM were analysed.

1. Introduction

Nowadays about 40% of newly developed active pharmaceutical ingredients are rejected in early phase development and will never find a way to a patient because of their poor water solubility leading to bioavailability problems (Lukyanov and Torchilin, 2004). Furthermore, up to 70% of drug molecules coming from synthesis have solubility problems (Keck et al., 2008). Considering such arising solubility problems the development of application vehicles with a high solvent power is a challenging research topic in pharmaceutical technology. Different efforts to improve the solubility of drugs using a capable vehicle to enclose hydrophobic drugs, such as inclusion complexes with cyclodextrins, microemulsions, dendrimers or liposome formulations (Müller and Albers, 1992; Lawrence and Rees, 2000; Barenholz, 2001) have been established so far. However, all these systems exhibit disadvantages, e.g. cyclodextrins need special guest molecule structures for complexation. Microemulsion systems are characterised by high surfactant concentrations which mostly are not well tolerable and those systems are often only stable at an explicit composition of surfactants, cosurfactants, oil and water. As a promising parenterally well tolerated vehicle, liposomes are formed by bilayer lipid membranes enclosing an aqueous core which exhibits cargo space for exclusively hydrophilic actives (Svenson, 2009). Multilamellar liposomes further enable also hydrophobic actives to be arranged in-between as well, but by that the size of such vehicles will increase in order to allow higher drugs amounts to be encapsulated. Once a lipophilic drug has been attached in-between the bilayers, it could possibly act as an interfering factor within the bilayer formation and may decrease the stability (Sharma and Sharma, 1997; Crosasso et al., 2000; Krishnadas et al., 2003). Unfortunately, most liposomes are energetically metastable and eventually will re-organise to planar bilayers (Svenson, 2009). 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

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as they are non-toxic, parenterally tolerated, and exhibit a high bio-compatibility. But phospholipids are forming bilayer structures when dispersed into an aqueous surrounding (Carey and Small, 1970) and, thus, are not able to form a hydrophobic inner core. In classical mixed micelles, water-insoluble phospholipid molecules are located next to a surfactant (bile acid) holding a hydrophobic core which can be used to encapsulate poorly soluble drugs (Hammad and Müller, 1998a,b). MM present a convenient drug delivery system as they are thermodynamically stable (in comparison to liposomes), nano-sized vehicles with sizes of usually 5–60 nm. Thus, MM show an enhanced vascular permeability and accumulate in pathological areas with leaky vasculature, as in the case of tumours. To avoid a rapid clearance, a particle size <200 nm is required for the drug carriers to inhibit extravasation from normal vasculature (Mayer et al., 1989; Marjan and Allen, 1996). Further, MM enclose the hydrophobic drug already in a molecularly dispersed state which could lead to an enhanced bioavailability (because a dissolution process will be omitted in comparison to, e.g. nano-crystals). Thus, different studies made in the past (Muranishi et al., 1979; Amedee-Manesme et al., 1991) and also in latest time (Mrestani et al., 2010) confirm about an increased oral bioavailability due to the application of mixed micellar systems. Although it is possible for phospholipids to form mixed micelles, only one mixed micellar system has found its way to the pharmaceutical drug market. In Konakion® MM or Cernevit® (and in earlier times Valium® MM) unsaturated phosphatidylcholine (lecithin) is mixed with glycocholic acid (sodium salt) representing the classical MM system composed of lecithin/bile salt. A drawback of the classical MM is that a change in the lecithin/bile salt ratio or in the total concentration leads to a change in the MM size and shape (Shankland, 1970; Lichtenberg et al., 1983; Krishnadas et al., 2003). Polymeric micelles (Chiappetta and Sosnik, 2007) and mixed micelles composed of derivatised PEGylated phospholipids (Mu et al., 2005) are in the focus of investigation in many research groups and a lot of promising findings are achieved on those systems. However, there is no formulation entry on the pharmaceutical drug market successfully accomplished yet.

The combination of a water-soluble surfactant and a water-insoluble phospholipid can result in a formation of (single-) surfactant micelles, mixed micelles or mixed dispersions. A micellar or mixed micellar formation will generate an isotropically clear solution (Lichtenberg et al., 1979). Here, micelles are made up of one surfactant species showing sizes usually smaller than 10 nm. Mixed micelles are made up of at least two different species; in our study a water-soluble surfactant and a water-insoluble phospholipid. This definition is in agreement with the one given by Carey and Small (1970). Due to the presence of the water-insoluble phospholipids MM are known to exhibit a diameter that can be greater than those for typical micelles (Ashok et al., 2004; Lukyanov and Torchilin, 2004; Dabholkar et al., 2006; Rupp et al., 2010). If a binary system comprises a higher content of a water-insoluble PL and less amounts of a water-soluble surfactant which are not able to solubilze sufficient amounts of the insoluble PL, the system becomes oversaturated with PL. In that case water-insoluble PL-aggregates will be dispersed into a PL-saturated surfactant solution and a dispersion will be generated. If no additional high energy input takes place – like due to hear shear forces or a high pressure homogenisation – no nm-sized dispersed particles can be expected.

In this study the solubilization capacities of different surfactant containing systems (micelles, mixed micelles, dispersions) for three water-insoluble drug substances were achieved. As model water-insoluble drug substances two benzodiazepines, diazepam and tetrazepam, and the steroid estradiol were chosen. In earlier studies it was found that sucrose laurate (SL) is able to form isotropically clear solutions with hydrogenated phosphatidylcholine (hPC) offering unimodal distributed (PDI < 0.1) mixed micelles with sizes of about 20 nm (data not shown) over a broad range of total concentrations and at higher ratios of hPC (Rupp et al., 2010). The aim of this study was to find a strictly unimodal distributed nano-sized vehicle system containing higher ratios of well tolerated hydrogenated (and thus more stable) phosphatidylcholine on the one hand, and on the other hand this vehicle should possess more solubilization power for poorly soluble drugs compared to the classical mixed micelles or/and other surfactant systems. Especially, the earlier developed MM systems (Rupp et al., 2010), composed of hPC or DPPC and SL (50%, w/w) which were found to form isotropically clear solutions with a unimodal particle distribution over broad ranges of total surfactant concentrations, should be tested regarding their solubilization capacities for different poorly soluble drugs. For this purpose, different surfactants were combined with altering weight fractions (WF_{hPC}) of hPC or DPPC (WF_{hPC} ranging from 0.0 to 0.6) leading to micelles (WF_{hPC}0.0), mixed micellar systems or dispersions.

For comparison, different classical mixed micellar systems of lecithin/bile salt and also polysorbate 80 and hydroxylpropyl-β-cyclodextrine (HP-β-CD) solutions were prepared. Each system was loaded with the above mentioned poorly soluble drugs and the solubilization capacities were compared. The size of the most beneficial mixed micellar systems, before and after drug incorporation, were analysed by DLS to evaluate the influence of the drug incorporation. Furthermore, the CMC before and after drug incorporation was analysed for a favourite MM system.

2. 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 (SE) as SL (sucrose laurate, D-1216) and SM (sucrose myristate, M-1695) and polyglycerol esters (PGE) as L-7D (decaglycerol laurate) and M-10D (decaglycerol myristate) were delivered by Mitsubishi Chemical Corporation (Tokyo, Japan). The sucrose esters and polyglycerol esters represent to about 95% a mixture of monoesters (approx. 80%) and diesters (approx. 15%) as it is certified by the manufacturer (Mitsubishi Chemical Corporation or Mitsubishi-Kagaku Foods Corporation, Tokyo, Japan). The remaining 5% are of free sucrose, sulphated ash or moisture (Mitsubishi Chemical Corporation or Mitsubishi-Kagaku Foods Corporation, Tokyo, Japan). The surfactant Tween 80® (Polysorbate 80) as well as the lipophilic fluorescent dye DPH (1,6-diphenyl-hexatriene) were purchased from Sigma–Aldrich (Munich, Germany). The hydroxylpropyl-β-cyclodextrine (Cavasol W 7 HP®, HP-β-CD) was obtained from ISP (International Specialty Products Inc., Cologne, Germany). The poorly soluble drug diazepam was purchased from Synopharm (Hamburg, Germany) and tetrazepam from Sanofi-Aventis (Munich, Germany). β-17-Estradiol as well as the bile salts glycocholic acid sodium salt (GCA) and cholic acid sodium salt (CA) 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.

3. Methods

3.1. 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 and 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) using a thermostatted 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 were equilibrated at 37 °C 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 80) the weight fraction was used instead of the molar fraction to describe the different ratios of PC to surfactant in the prepared solutions. A purity of 95% (content of potential micelle forming substances) on the PGE and SE was assumed for the weight calculation. The total surfactant concentration (PC + surfactant) of the stock solutions was kept constant in a range from 1.0 mg/mL to 50 mg/mL at a weight fraction of the particular PC ranging from 0.0 to 0.6 (WF_{PC}0.0–0.6) which represents a content of 0–60 wt% of the particular PC.

3.2. Solubilization capacity determination

Excess amounts of the respective hydrophobic drug were added to 10 mL of the different micellar or mixed micellar solutions or dispersions (preparation see Section 3.1) in vials which were then shaken in a thermostatted water bath (SW-20C; Julabo Labortechnik, Seelbach, Germany) at 25 °C or 37 °C for at least 24 h. After another 24 h storage at RT the samples reached equilibrium. Excess amounts of the respective poorly soluble drug were separated by 12 min centrifugation at 12,000 rpm in a centrifuge (Biofuge A; Heraeus Instrument GmbH, Hannover, Germany).

Of the supernatant solutions, 0.5 mL were properly diluted with a methanol/water mixture (70:30, v/v; in the case of diazepam and estradiol) or with a mixture of a 0.01 M aqueous solution of KH_2PO_4 (adjusted to pH 4.2 with H_3PO_4) and acetonitrile (40:60, v/v) if tetrazepam was the drug and then subjected to HPLC analysis. Each run was repeated at least twice. All data reported are the average of at least three independent samples. For the calibration curve, different concentrations (at least five) in a range from 1 μg/mL to 10 μg/mL were prepared by dilution from a stock solution of the respective poorly soluble drug in adequate solvent mixture and the dilution was made with same solvent mixture. The concentration absorption relationship obeyed the Beers–Lambert law (r^2 not less than 0.999).

3.3. HPLC analysis

3.3.1. Diazepam

A mobile phase consisting of a mixture of double-distilled water and methanol (30:70, v/v) at a flow rate of 1 mL/min and wave length of 254 nm was utilised for the HPLC (Waters Corp. Milford, MA, USA) analysis of diazepam. The instrument which was used consisted of an RP-18 column (150 mm × 4.6 mm; 5 μm) (Merck; Darmstadt, Germany), a high precision pump (Waters 600E Multisolvent Delivery System), an autosampler (Waters Inline Degasser AF and a Waters 717 plus autosampler) and a Waters UV detector (Waters 996 Photodioden Array Detektor), and the software used for analysis was Waters Empower 1154.

3.3.2. Tetrazepam

The mobile phase consisted of a mixture of a 0.01 M aqueous solution of KH_2PO_4 (adjusted to pH 4.2 with H_3PO_4) and acetonitrile (40:60, v/v), the flow rate was 1.4 mL/min, and the detection was at a wavelength of 254 nm. The instrument as well as the column were equal to those described for diazepam.

3.3.3. Estradiol

The mobile phase consisted of a mixture of double-distilled water and methanol (30:70, v/v) at a flow rate of 1.4 mL/min and a wavelength of 280 nm was selected for the HPLC analysis of estradiol. The instrument as well as the column were equal to those described above.

3.4. Size determination

Dynamic light scattering (DLS) was used to measure the size as volume weighted 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 using a photon correlation spectrometer which allows size measurements of particles with sizes between 0.6 nm and 6 μm (Zetasizer NanoZS, Malvern Instruments Ltd., UK). The Zetasizer NanoZS uses a laser at the light wavelength of 633 nm. 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. As basis of evaluation of the DLS results, the found volume weighted hydrodynamic diameter of the particles and the polydispersity index (PDI) of each investigated sample were utilised. 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 shift of the measured particle size (Fillafer et al., 2007). The DLS technique was also used to analyse the size of different dilutions made of empty or drug loaded MM solutions to evaluate the sizes of the respective MM system above and beyond the CMC.

3.5. Surface tension measurements (CMC determination)

The total surfactant concentration of the stock solution was kept constant at 5.0 mg/mL or 10.0 mg/mL for every 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.

3.6. 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 was prepared and different mixed micellar 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 nm 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 MM system. The CMC was estimated

from the point at which the slope of the intensity showed a sharp increase which is due to a mixed micelle formation and the solubilization of increasing amounts of the lipophilic fluorescent dye DPH.

4. Results and discussion

4.1. Solubilization by micelles and HP-β-CD

The sucrose esters were found to be superior in diazepam solubilization compared to all other investigated solubilizing systems (Fig. 1a) which is visualised by the highest slopes in the linear graphs of each solubilizing system. All surfactant containing systems were analysed in a concentration range from 0.5% to 5% (5–50 mg/mL), which was above the respective CMC of each surfactant. SM solubilized more diazepam than SL which can be explained by the more hydrophobic acyl chain of the myristic acid ester. For the same reason, the myristic acid ester of decaglycerol (M-10D) exhibited a higher solubilization capacity for diazepam compared to the less hydrophobic laurylic acid ester (L-7D). The solubilization capacities of diazepam decrease as follows: SM > SL > polysorbate 80 > M-10D > L-7D > HP-β-CD. The β-cyclodextrin derivate (HP-β-CD) was found to be a poor solubilization aid for both tested benzodiazepines (diazepam, Fig. 1a and tetrazepam, Fig. 1b). In contrast, the solubilization capacity of HP-β-CD for the steroid estradiol was superior which is in agreement with earlier reports in the literature (Brewster et al., 1988). HP-β-CD solubilized more estradiol than any micellar solution. In a different study it was shown that SM is not able to form isotropically clear MM solutions with higher ratios of hPC (data not shown). For this reason the solubilization capacities of SM and also for M-10D were not shown for tetrazepam and estradiol. Accordingly, only the solubilization capacities of SL and L-7D, and not SM or M-10D, are discussed because in previous studies it was found that only these two surfactans were able to form MM with hPC and exhibit a reasonable solubilization capacity. For both tested benzodiazepines the solubilization capacities decrease in the order SL > polysorbate 80 > L-7D > HP-β-CD whereby the solubilization capacities for diazepam were found to be invariably higher compared to tetrazepam. A different solubilization profile was achieved for estradiol. While the sucrose ester SL also solubilized higher amounts of estradiol compared to the polyglycerol ester L-7D, polysorbate 80 showed an enhanced solubilization effect in comparison to SL.

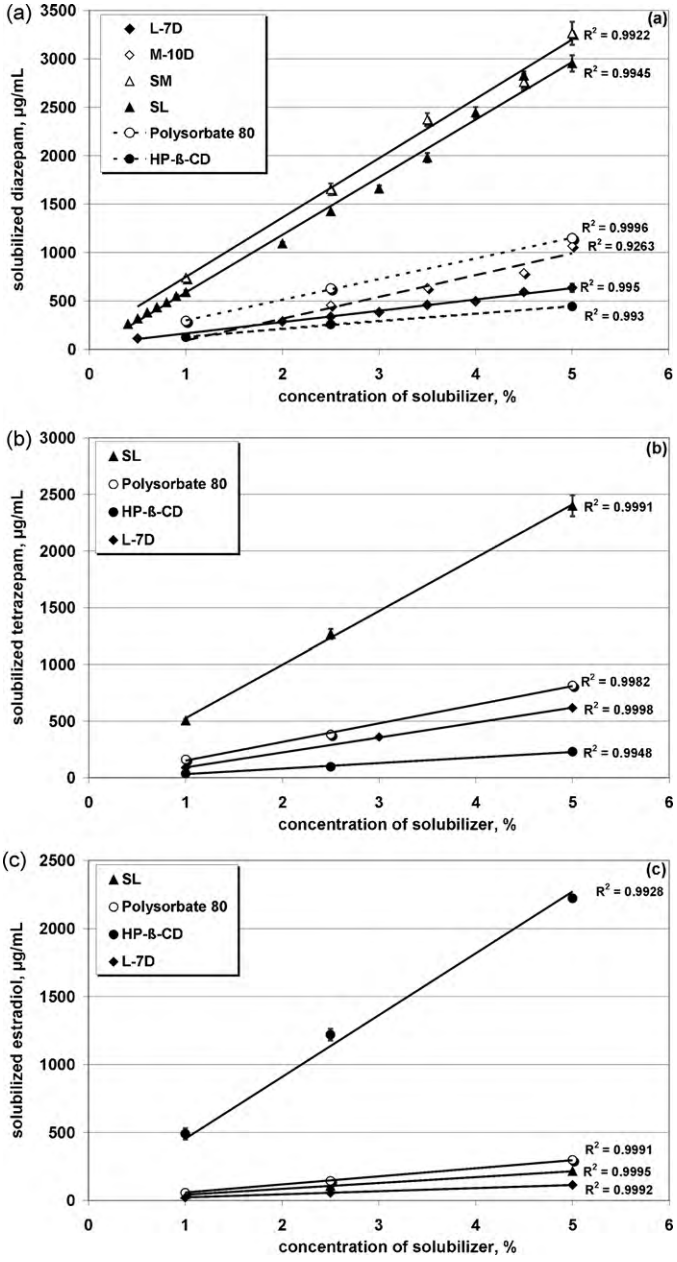

Fig. 1. (a) Diazepam, (b) tetrazepam and (c) estradiol. Micellar and HP-β-CD solubilization of different poorly soluble drugs at increasing concentrations of the respective solubilizer ($n = 6$).

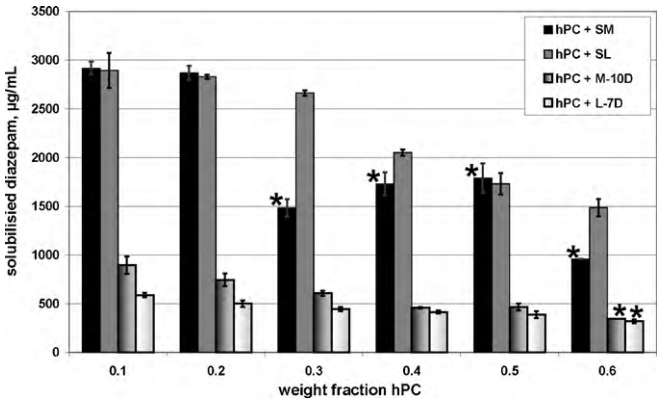

Fig. 2. Solubilization capacity for diazepam in different surfactant containing systems (mixed micelles or dispersions, *); ($n \geq 6$).

4.2. Diazepam solubilization by mixtures of a SE or PGE and hPC

In previous studies, it was shown that SM only forms MM with hPC up to a weight fraction of 0.2 (WF_{hPC}) of hPC. No isotropically clear solutions, indicating MM, could be observed if the WF_{hPC} was higher 0.2. The same was found for the PGEs L-7D and M-10D in a combination with a weight fraction of 0.6 of hPC (Rupp et al., 2010). Thus, the marked bars represent dispersions instead of mixed micellar solutions, Fig. 2. The solubilization capacity of diazepam for all surfactant containing solutions (MM) was equal or higher when M-10D instead of L-7D was next to hPC. The same was found for SEs next to hPC. SM, being more hydrophobic, solubilized more diazepam than SL when being next to hPC in a MM solution (Fig. 2). Dispersions of SM and hPC (e.g. at a WF_{hPC} 0.3) indicated a decline in solubilization capacity for diazepam (compared to the MM solutions).

Generally, the combination of SEs with hPC was superior regarding the solubilization capacity for diazepam compared to the combination of PGE with hPC, independent of the applied WF_{hPC}. Hence, the solubilization capacity for diazepam was 4–6 times higher (at the respective hPC content) if the MM were composed

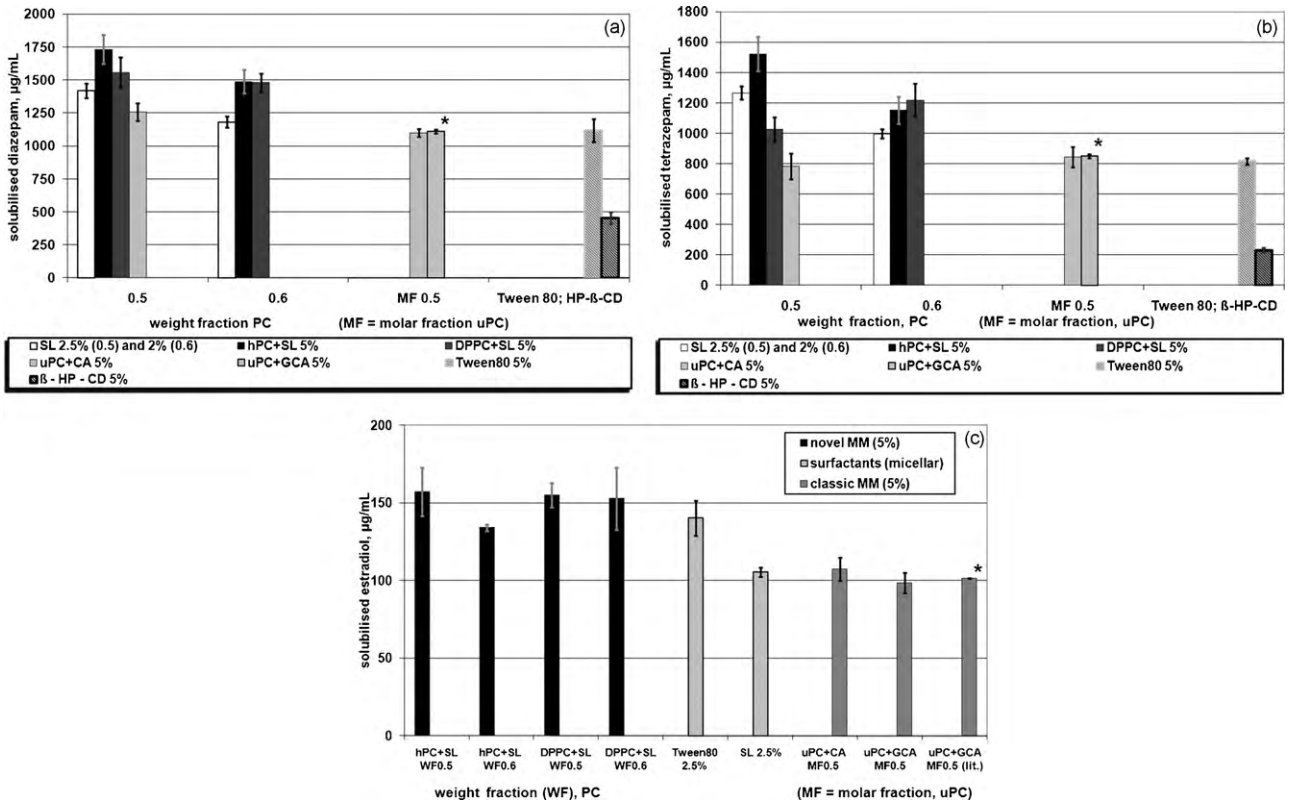

Fig. 3. (a) Solubilization capacity for diazepam in novel MM systems in comparison to classical lecithin/bile salt MM (c = 5%, 50 mg/mL) and other solubilizing systems (*, literature value, Hammad, 1998). (b) Solubilization capacity for tetrazepam in novel MM systems in comparison to classical lecithin/bile salt MM (c = 5%, 50 mg/mL) and other solubilizing systems (*, literature value, Hammad and Müller, 1998b). (c) Solubilization capacity for estradiol in novel MM systems in comparison to classical lecithin/bile salt MM (c = 5%, 50 mg/mL) and other solubilizing systems (*, literature value, Hammad, 1998).

of SL and hPC instead of a PGE (L-7D or M-10D) and hPC, regardless the WF_{hPC}. Increasing amounts of hPC resulted in a continuous decrease in diazepam solubilization when SL was mixed with hPC. However, even at higher ratios of hPC ($WF_{hPC}0.5$) a substantial solubilization capacity for diazepam could be observed (Fig. 2) holding an isotropically clear MM solution.

4.3. Solubilization of water-insoluble drugs by different solubilizing systems

In the following, solubilization capacities of different poorly soluble drugs obtained with MM systems composed of hPC or DPPC with SL were compared to those of classical (lecithin/bile salt) MM systems and different other solubilizing systems (Fig. 3a–c). All shown solubilizing systems were analysed at a constant total surfactant concentration of 50 mg/mL (5%) with the exception of pure micellar SL solutions (2% and 2.5%, white bars in Fig. 3a–c). For comparative purpose, the micellar solubilization capacity of SL (solely) was determined at the same SL concentration as in the corresponding MM systems (hPC/DPPC + SL at $WF_{PC}0.5$ or 0.6). The classical MM were prepared at a molar fraction of lecithin at 0.5 and partially at a lecithin weight fraction of 0.5 (Fig. 3a and b). The solubilization capacities, achieved by classical MM, for diazepam (Fig. 3a) or tetrazepam (Fig. 3b) and estradiol (Fig. 3c) were found to be equal to that described in literature (Hammad, 1998; Hammad and Müller, 1998b). The developed MM systems with hPC or DPPC and SL ($WF_{PC}0.5$ or 0.6) were found to be superior in drug solubilization of all investigated drugs compared to the classical lecithin/bile salt mixed micelles (Fig. 3a–c) regardless whether the classical MM were self prepared or compared to the literature value (Hammad, 1998). A benefit of an enhanced solubilization capacity with the novel developed MM was not only obtained compared to classical MM but also compared to the corresponding SL solutions at concentrations of 2.0% and 2.5% (as they were applied in the presented novel MM). There is a significant increase in solubilization capacity due to the presence of hPC (or DPPC) in combination with SL in MM ($WF_{PC}0.5/0.6$) compared to solutions with SL solely at corresponding concentrations (Fig. 3a–c). As a favourite MM system, hPC + SL $WF_{hPC}0.5$ (at c = 5%) solubilized a 3.5-fold amount of diazepam compared to HP-β-CD and about 1.5-fold more than polysorbate 80 or the classical lecithin/bile salt MM, whereby all solutions held the same amount of solubilizing agent at 5%. The solubility of diazepam in water has been reported to be 49 µg/mL (Ashok et al., 2004). The favourite developed MM system with SL and 0.5 weight fractions of hPC showed an increase in aqueous diazepam solubility of up to ~3500% at a total surfactant concentration of 50 mg/mL. It has also been reported in literature that the increase in solubility for diazepam by sterically stabilized DSPE-PEG 5000/PC (80:20) MM was ~600% at 12 mg/mL (Ashok et al., 2004). Accordingly, the introduced new MM system exhibits an enhanced solubilization capacity for diazepam, even compared to sterically stabilized DSPE-PEG 5000/PC MM. The same MM system containing 0.5 weight fraction hPC next to SL was found to be the most beneficial MM system with regard to solubilizing tetrazepam. Significantly higher amounts of tetrazepam were solubilized if 0.5 weight fractions hPC were attended instead of 0.6 ($WF_{hPC}0.6$). Further, a change from hPC to DPPC led to a decrease in solubilization capacity of tetrazepam if the PC content was 0.5 ($WF_{hPC\ or\ DPPC}$). This could be explained by the more lipophilic tail of hPC compared to DPPC, where no stearic acid is present. Thus, the core of MM composed of 0.5 weigth fractions of hPC next to SL would be more lipophilic compared to the system of DPPC + SL WF0.5, offering a tighter bonding of tetrazepam. In contrast, it was found that a MM system of 0.6 weight fractions hPC next to SL did not solubilize

higher amounts of tetrazepam compared to the MM system containing 0.6 weight fractions DPPC (instead of hPC) next to SL. Those two MM systems (DPPC + SL WF_{PC}0.5/0.6) exhibited approximately the same solubilization capacities for tetrazepam. The MM system hPC + SL WF0.5, solubilized the 6.5-fold amount of tetrazepam compared to HP-β-CD and about 1.8-fold more than polysorbate 80 or the classical lecithin/bile salt MM, whereby all solutions held the same amount of solubilizing agent at 5%. Therefore, the developed MM system with 0.5 weight fractions hPC next to SL showed the greatest benefit for the solubilization of tetrazepam. In comparison to the classic lecithin/bile salt mixed micelles, the new MM system was found to solubilize approx. 75% more tetrazepam at the same total surfactant concentration of 50 mg/mL, regardless whether cholic acid sodium salt or glycocholic acid sodium salt were used next to 0.5 weight or molar fractions of lecithin (Fig. 3b, grey bars). The introduced MM systems with SL and 0.5 or 0.6 weight fractions of hPC or DPPC further revealed also a higher solubilization capacity for the steroid estradiol compared to classical MM, even though there was not such a distinctive benefit found as for the benzodiazepines. Whereby, the classical lecithin/bile salt mixed micelles again could be replicated leading to the same solubilization capacities for estradiol (Fig. 3c) as they were published before (Hammad, 1998). SL solely, at the same concentration as it was applied in the MM (2% SL/WF_{PC}0.6 and 2.5% SL/WF_{PC}0.5),

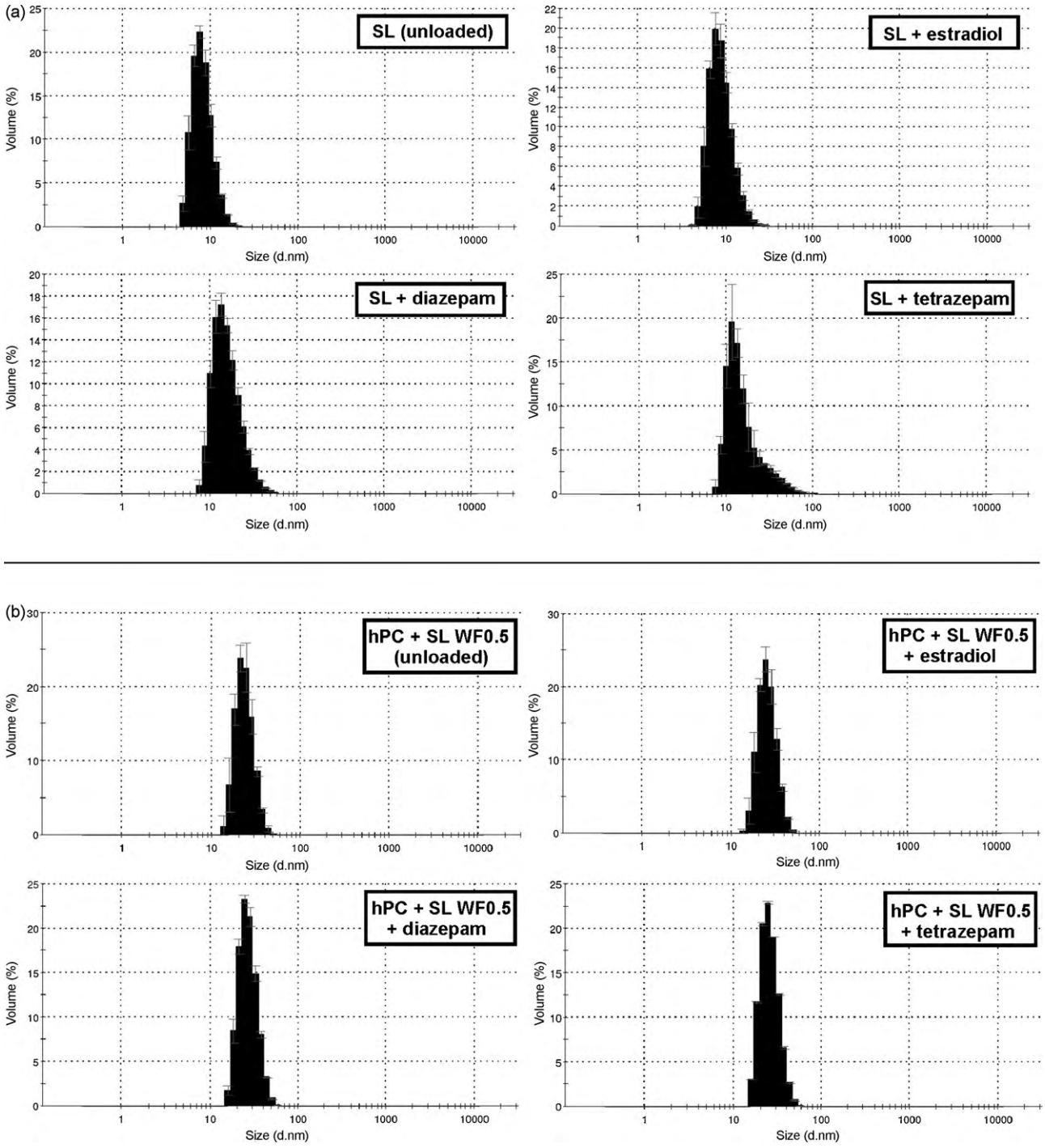

Fig. 4. (a) Sizes of empty and drug loaded SL micelles as volume weighted diameter ($n = 3$); SL concentration = 1% (10 mg/mL). (b) Sizes of empty and drug loaded hPC + SL WF_{hPC}0.5 mixed micelles as volume weighted diameter ($n = 3$); MM concentration = 1% (10 mg/mL).

solubilized significantly less estradiol compared to the respective MM which emphasizes the benefit of MM. However, polysorbate 80 at 25 mg/mL was found to solubilize approximately the same amount of estradiol as the novel developed MM at a concentration of 50 mg/mL, and the cyclodextrine HP-β-CD exceeded all applied solubilizing systems by far regarding the solubilization capacity for the steroid estradiol (Fig. 1c).

4.4. Influence of drug incorporation on micellar and mixed micellar sizes

SL micelles were found to have a volume weigthed hydrodynamic diameter of 8 nm in an unloaded state at a concentration of 1% (10 mg/mL) (Fig. 4a). The incorporation of the lipophilic steroid estradiol led to a slight increase in the volume weighted diameter to 9 nm and thus did not influence the size of SL micelles. The addition of benzodiazepines resulted in a change in the observed size and most notably in a broadening of the size distribution of diazepam or tetrazepam loaded SL micelles compared to empty SL micelles (Fig. 4a). After equilibrium, the mean hydrodynamic diameter of diazepam loaded SL micelles increased from 8 nm (empty state) to about 17 ± 2 nm, and an equilibrium tetrazepam loading resulted in a micellar size of 18 ± 2 nm (Fig. 4a). One can deduce from those findings that the benzodiazepines were not only incorporated within the micellar core but possibly were also integrated into the micellar shell or were adsorbed on the outer shell leading to transformed micelles. Such an interaction between micelle forming amphiphilic molecules and the lipophilic drug, which ought to be incorporated within the hydrophobic core, could reduce the stability of those drug loaded micelles. This could lead to a decreased stability of the solubilizate as the drug molecules might interact with the water, e.g. in terms of a hydrolysis. Another reason for this remarkable size-shift might be given by a change in the morphology of the micelles due to the drug incorporation. Maybe a transformation from presumably more spherical micelles to rodlike micelles occurred after an incorporation of the hydrophobic drug molecules into the micellar system.

Selected MM systems were also analysed by the DLS technique with the objective to determine MM sizes before and after an equilibrium drug loading. The findings were different for the investigated favourite MM systems compared to those achieved for micellar SL systems: the favourite system composed of SL with 0.5 weight fractions of hPC formed MM with sizes of about 21 ± 3 nm (as mean volume weighted diameter by DLS) in an unloaded state. Almost no change in MM size or shape occurred after drug loading, neither for diazepam or tetrazepam nor for estradiol (Fig. 4b). Hence, after incorporation of diazepam or tetrazepam MM sizes of 25 ± 2 nm were measured indicating just a slight increase while estradiol inclusion led to equally sized MM of about 22 ± 2 nm (Fig. 4b). Accordingly, the mixed micellar sizes – before and after drug incorporation – remained constant, indicating a stable formation of drug incorporated into the mixed micelles.

4.5. CMC of unloaded and drug loaded mixed micellar systems

CMC-values for the MM system hPC + SL $WF_{hPC}0.5$ in an unloaded state and after an equilibrium diazepam loading were determined by three different measurement techniques. The CMC-values for the mixed micelles obtained by surface tension measurements were approx. 0.18 mg/mL before and approx. 0.20 mg/mL after diazepam loading (Fig. 5). The stable CMC-values before and after diazepam inclusion could be confirmed by fluorescent measurements (Fig. 6a and b) whereas the same CMC-values were observed. Thus, the introduced mixed micellar was found to be unaffected by the incorporation of diazepam. As a third determination technique DLS size measurements were performed at

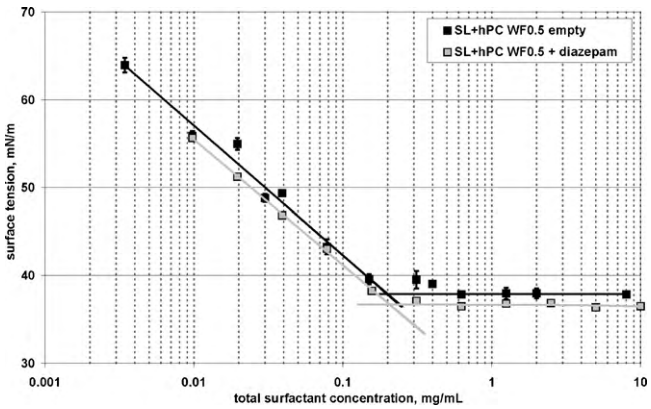

Fig. 5. Determination of the CMC for empty and drug loaded hPC + SL $WF_{hPC}0.5$ mixed micelles by surface tension measurements.

a dilution series of the unloaded (Fig. 7a) and diazepam loaded (Fig. 7b) MM system at concentrations above and below the CMC as it was achieved by surface tension and fluorescent measurements. At concentrations above the CMC unchanged sizes for the MM were detected which then vanished by undergoing the CMC. This can be explained by the hydrophobic effect: while the water-insoluble hPC was attached by SL molecules in soluble mixed micelles at concentrations above the CMC, hPC molecules precipitated (driven by the hydrophobic effect) after falling below the mixed micellar CMC. Due to the disappearance of mixed micelles an agglomeration of hPC was forced by the water and thus, no mixed micelles could be observed any more but formation of larger particles was observed (Fig. 7a and b) which most probably is caused by precipitated hPC. This finding would also explain the high PDI-values (not shown)

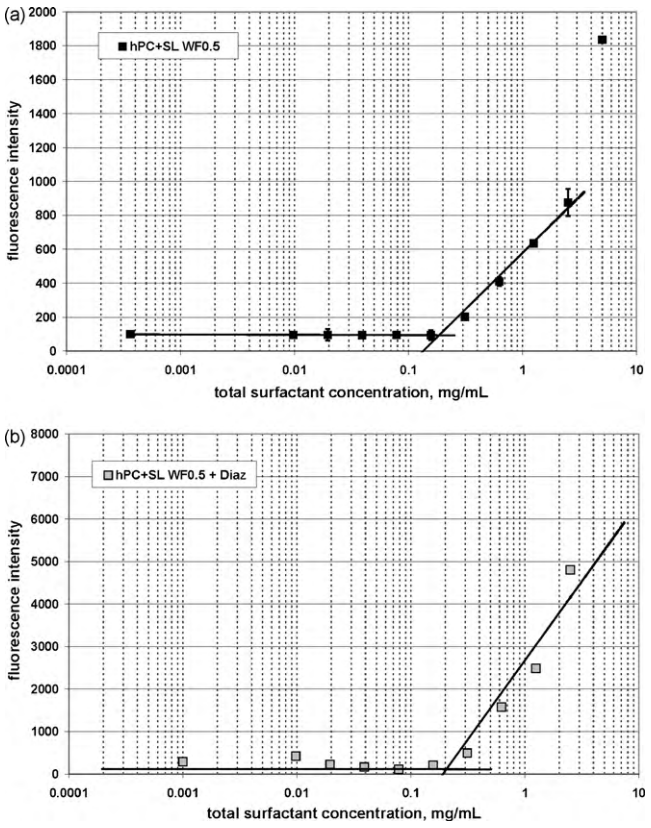

Fig. 6. Determination of the CMC by fluorescence measurement technique for (a) empty hPC + SL WF0.5 mixed micelles and (b) diazepam loaded hPC + SL $WF_{hPC}0.5$ mixed micelles.

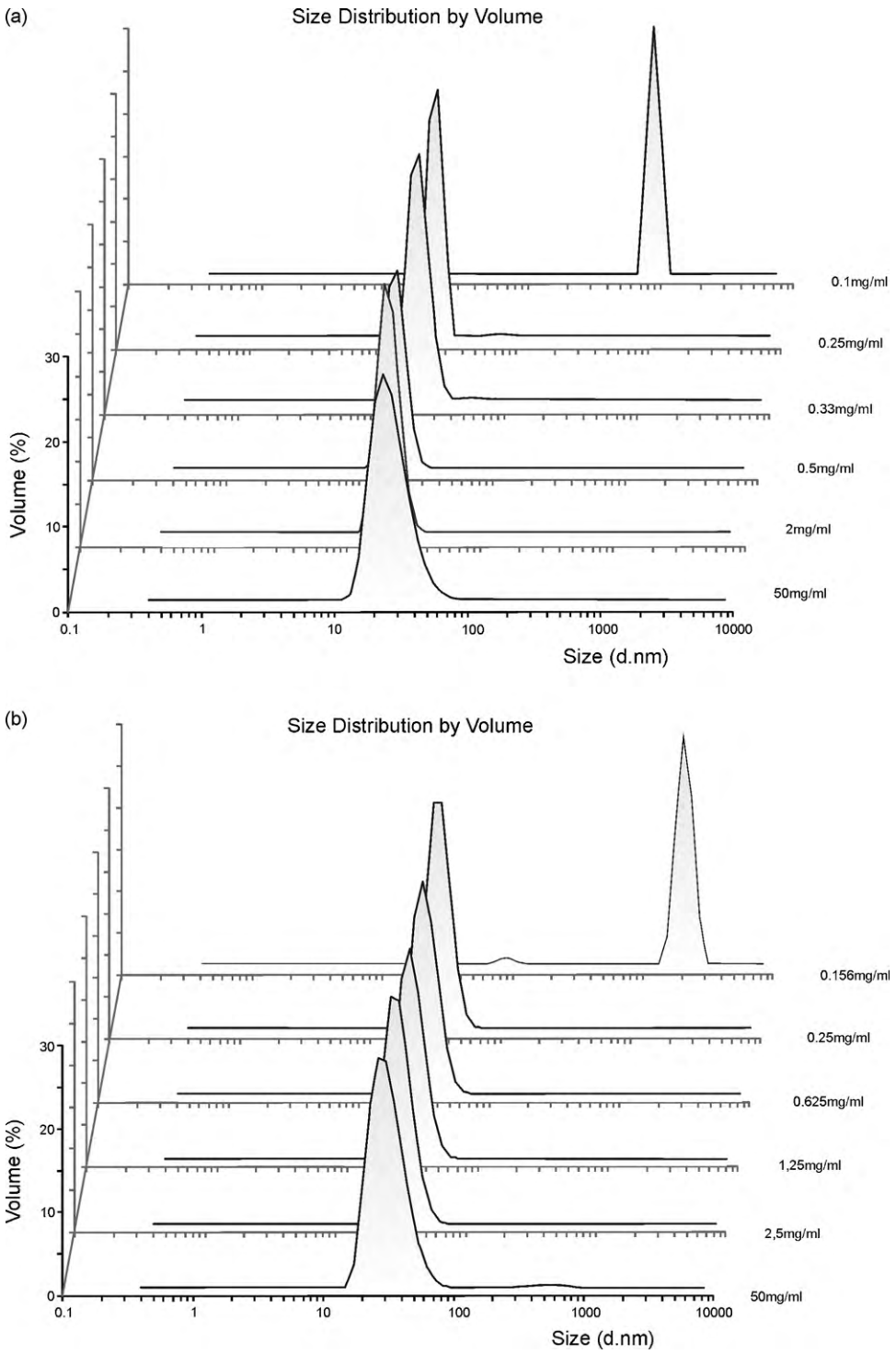

Fig. 7. Determination of the CMC by DLS technique for (a) empty hPC + SL WF_{hPC}0.5 mixed micelles and (b) diazepam loaded hPC + SL WF_{hPC}0.5 mixed micelles. Stability of MM against dilution.

measured for the sample with increased dilution. Accordingly, it was found that the three different measurement techniques all led to equal results and confirmed that all solubilization experiments were done well above the CMC.

5. Conclusions

The introduced developed MM systems containing SL and hPC or DPPC were found to be promising solubilization vehicles for all tested poorly soluble drugs. Sucrose laurate (SL) as the most hydrophilic sucrose ester solubilized higher amounts of hydrophobic drugs than any other tested sucrose- or polyglycerol ester in combination with higher ratios of hPC in MM. Thus, SL was the most suitable tested surfactant for the formation of MM next to hPC. Furthermore, the MM system composed of SL and higher ratios of hPC (WF_{hPC}0.5) was found to be superior compared to classical lecithin/bile salt MM with regard to enhance the solubilization capacity for the tested hydrophobic drugs. Moreover, the novel MM solubilized more diazepam or tetrazepam than solutions of

polysorbate 80 or a cyclodextrine (HP-β-CD) at the same concentration of 5%. The finding of constant CMC-values for the unloaded as well as diazepam loaded mixed micelles supports a stable solubilizate formulation. The solubilization of the tested hydrophobic drugs did not lead to notable changes in the observed volume weighted hydrodynamic diameter of the MM system. Whereas the micellar sizes of SL solely significantly changed after incorporation of diazepam or tetrazepam assuming a reduced stability of the micellar solutions. The developed MM systems are composed of saturated PC types instead of unsaturated PC (lecithin) as it is required for a MM formation with bile salts which makes the new MM become more stable against oxidative degradation. The applied hPC is parenterally well tolerated but further studies have to clarify if the mixture with SL also results in a high bio-compatibility and a low haemolytic activity.

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