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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