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Lipoamino acid-based micelles as promising delivery vehicles for monomeric amphotericin B

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ABSTRACT

Lipoamino acid-based micelles have been developed as delivery vehicles for the hydrophobic drug amphotericin B (AmB). The micellar solubilisation of AmB by a gemini lipoamino acid (LAA) derived from cysteine and its equimolar mixtures with the bile salts sodium cholate (NaC) and sodium deoxycholate (NaDC), as well as the aggregation sate of the drug in the micellar systems, was studied under biomimetic conditions (phosphate buffered-saline, pH 7.4) using UV–vis spectroscopy. Pure surfactant systems and equimolar mixtures were characterized by tensiometry and important parameters were determined, such as critical micelle concentration (CMC), surface tension at the CMC (γ_{CMC}), maximum surface excess concentration (Γ_{max}), and minimum area occupied per molecule at the water/air interface (A_{min}). Rheological behaviour from viscosity measurements at different shear rates was also addressed. Solubilisation capacity was quantified in terms of molar solubilisation ratio (χ), micelle–water partition coefficient (K_M) and Gibbs energy of solubilisation (ΔG_s°). Formulations of AmB in micellar media were compared in terms of drug loading, encapsulation efficiency, aggregation state of AmB and *in vitro* antifungal activity against *Candida albicans*. The LAA-containing micellar systems solubilise AmB in its monomeric and less toxic form and exhibit *in vitro* antifungal activity comparable to that of the commercial formulation Fungizone.

1. Introduction

Invasive fungal infections are a major cause of concern in immunocompromised individuals, such as AIDS patients and those undergoing immunosuppressive chemotherapy, including cancer patients and organ transplant recipients. Amphotericin B (AmB), a hydrophobic polyene antibiotic from *Streptomyces* sp., remains the drug of choice in the therapy of systemic fungal infections (Allen, 2010; Laniado-Laborín and Cabrales-Vargas, 2009). AmB is known to bind membrane sterols forming complexes that associate to form transmembrane pores (channels), inducing permeability to K^+ and leakage of small molecules. Its selectivity for fungi results from its higher affinity for ergosterol when compared to the cholesterol-containing membranes of mammalian cells (Janout et al., 2015; Laniado-Laborín and Cabrales-Vargas, 2009; Neumann et al., 2013).

Due to its poor water solubility, AmB is formulated as a colloidal suspension for intravenous administration using a bile salt, sodium deoxycholate, as the solubilising agent to increase the drug bioavailability. Bile salts, which are the major components of bile, are biological surfactants involved in the metabolism and excretion of cholesterol in mammals, playing an important role in the solubilisation and transport of lipid-soluble endogenous molecules from the gut, such as cholesterol, bilirubin and fatty acids (Moghimipour et al., 2015; Monte et al., 2009; Yeap et al., 2013). Bile salts are characterized by a hydrophobic steroid backbone with hydrophilic hydroxyl groups that vary in number, position and orientation, distributed on the same side of the almost planar molecular structure, with methyl groups located on the opposite side. As a consequence of their facial amphiphilic nature, bile salts exhibit a complex self-assembly behaviour with distinct aggregation properties that differ from those of conventional surfactants, namely a stepwise aggregation process over a broad concentration

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range (Calabresi et al., 2007; Madenci and Egelhaaf, 2010; Moghimipour et al., 2015; Natalini et al., 2014).

Despite its efficacy, deoxycholate-solubilised AmB (Fungizone) is associated with severe toxic side effects, namely nephrotoxicity (Barratt and Bretagne, 2007; Kun Han et al., 2007; Laniado-Laborín and Cabrales-Vargas, 2009; Wasko et al., 2012), which prompted the development of more efficient and safer delivery agents, including polymeric micelles (Charvalos et al., 2006; Jain and Kumar, 2010; Shao et al., 2010; Vandermeulen et al., 2006; Wang et al., 2009), liposomes (Allen and Cullis, 2013; Barratt and Bretagne, 2007, 2007; Torchilin, 2005), nanoparticles (Burgess et al., 2013; Kun Han et al., 2007; Patel and Patravale, 2011; Tan et al., 2014; Zia et al., 2015) and self-emulsifying delivery systems (Bhattacharyya and Bajpai, 2012; Narang et al., 2007). The pharmacokinetics, toxicity and activity of AmB were found to be strongly influenced by the type of formulation (Allen and Cullis, 2013; Hamil, 2013; Serrano et al., 2013; Torrado et al., 2008). Lipid-based formulations of AmB with improved safety profiles have reached the market, however their high cost and the higher doses required to achieve therapeutic results equivalent to Fungizone limit their widespread use (Allen and Cullis, 2013; Barratt and Bretagne, 2007; Hamil, 2013). The fact that these formulations will be coming off patent in the near future offers the opportunity for generics manufacturing but also a challenge for the development of novel AmB formulations aiming at non-parenteral administration (Serrano et al., 2013; Torrado et al., 2008).

The amphiphilic nature of the drug is responsible for its self-assembly behaviour in aqueous media, leading to water-soluble dimmers or oligomers that can further associate to form water-insoluble poly-aggregates (Hamil, 2013; Serrano et al., 2013; Wasko et al., 2012). Toxicity of AmB towards mammalian cells has been attributed to its water-soluble aggregated forms, in contrast to the drug monomers and water-insoluble aggregates (Charvalos et al., 2006; Espada et al., 2008; Wasko et al., 2012). Therefore, the non-selective toxicity of AmB can be reduced by controlling its aggregation state. Lipid-bile salt mixed micellar systems have been shown to decrease both the state and the extent of aggregation of AmB thus reducing drug cytotoxicity without affecting its antifungal activity (Barratt and Bretagne, 2007; Janout et al., 2015; Wasko et al., 2012).

Dimeric or gemini surfactants, whose molecules are formed by two hydrophobic alkyl chains and two polar head groups linked by a spacer at the level of the head groups, have unique properties when compared to their single-chain counterparts, namely lower critical micelle concentration (CMC), better surface activity and higher solubilising power towards hydrophobic drugs (Ménard et al., 2012; Menger and Keiper, 2000; Zana, 2002). In turn, the strong self-aggregation properties of the gemini surfactants are known to influence the aggregation behaviour of bio(macro) molecules and polymers in solution (Han and Wang, 2011).

The physicochemical properties and the aggregation behaviour of gemini surfactants in solution, including the type of supramolecular aggregate formed, can be easily tuned by modifications on their molecular structure, such as hydrophobic chain length, nature (length, hydrophobicity, flexibility) of the spacer, type of polar head groups (ether, carboxylate, sulphate, sulphonate, phosphate, quaternary ammonium, amino acid, carbohydrate), type of bond linking the spacer to the hydrocarbon chains and the polar headgroups, and symmetry of the molecule (Faustino et al., 2009a, 2014a; Menger and Keiper, 2000; Zana, 2002). In particular, aqueous solutions of gemini surfactants characterized by a short spacer chain can show high viscosity, peculiar rheological behaviour, and/or viscoelastic properties associated with the ability of these amphiphiles to form wormlike micelles at relatively low concentration (Bhadani et al., 2014; Zana, 2002).

The aggregation properties and self-assembling behaviour of gemini surfactants in aqueous media have been extensively studied, in particular the bis(quaternary ammonium) surfactants or bis(Quats) of the type *m–s–m*, where *m* and *s* designate the number of carbon atoms in the hydrophobic chains and in the spacer, respectively (Menger and Keiper, 2000; Zana, 2002). These compounds showed lower CMC, higher efficiency in surface tension reduction and enhanced antibacterial activity when compared to the conventional quaternary ammonium surfactant. However, the bis(Quats) are stable to chemical and enzymatic degradation which rised environmental concerns regarding its toxicity to the aquatic environment (Morán et al., 2004; Pérez et al., 2014; Pinazo et al., 2011).

We have recently synthesized an anionic gemini lipoamino acid from cysteine (LAA, Fig. 1) and characterized its supramolecular behaviour in aqueous media and in the presence of biological (macro) molecules of pharmaceutical relevance (Faustino et al., 2009b, 2010, 2011, 2012, 2014b). Lipoamino acids are amino acid-based surfactants obtained from the condensation of natural amino acids with fatty acids or their derivatives that meet the requirements of both biological and ecological compatibility (Bordes and Holmberg, 2015; Faustino et al., 2014a; Morán et al., 2004; Pérez et al., 2014; Pinazo et al., 2011). Furthermore, lipoamino acids can be produced by biotechnological procedures, such as fermentation or enzymatic catalysis, in alternative to chemical synthesis, thus becoming very attractive as green surfactants for biomedical and biotechnological applications (Faustino et al., 2014a; Foley et al., 2012; Morán et al., 2004; Pérez et al., 2014; Pinazo et al., 2011).

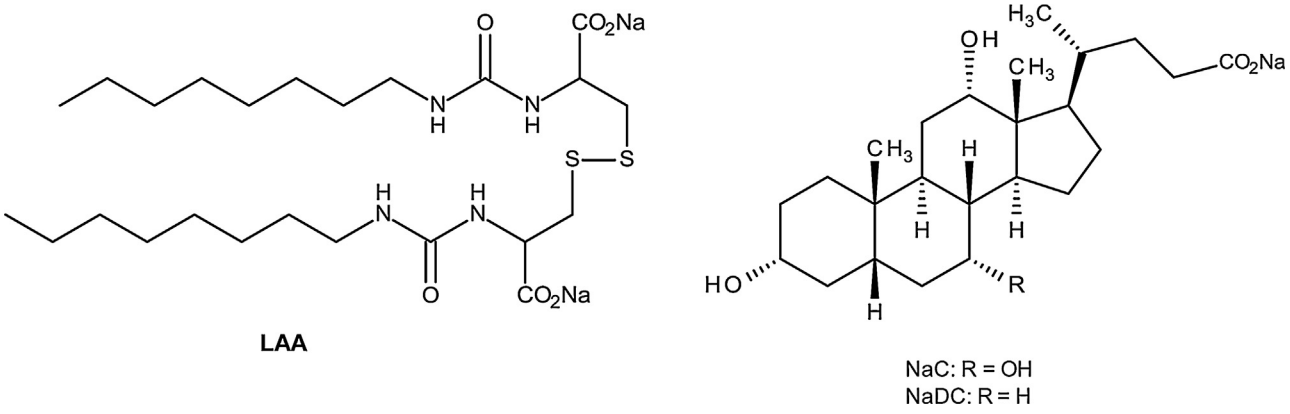

Fig. 1. Chemical structures of gemini lipoamino acid (LAA) and bile salts sodium cholate (NaC) and sodium deoxycholate (NaDC).