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# Solubilization power of an amino acid-based gemini surfactant towards the hydrophobic drug amphotericin B

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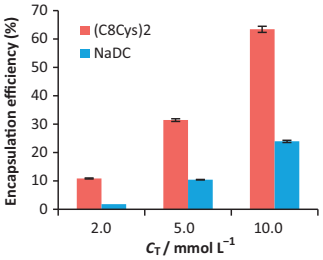
## HIGHLIGHTS

- $(C_8Cys)_2$, a gemini surfactant derived from cysteine, solubilizes amphotericin B.
- Solubilization power was quantified in terms of drug loading and efficiency.
- $(C_8Cys)_2$ is a better solubilizing agent deoxycholate in Fungizone®.
- Deoxycholate forms a shear-thinning gel at concentrations higher than 0.010 mol $L^{-1}$.

## GRAPHICAL ABSTRACT

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## ABSTRACT

Amphotericin B (AmB) is a hydrophobic polyene antibiotic used in the therapy of systemic fungal infections. Due to its poor water solubility, it is formulated as a colloidal suspension for intravenous administration using deoxycholate as the solubilizing agent (Fungizone). However, severe toxicity of the formulation has prompted the development of more efficient and safer delivery agents. Gemini amino acid-based surfactants constitute a promising alternative to the biological bile salt surfactant(s), as both biocompatible and biodegradable drug carriers. The supramolecular behaviour of an anionic gemini surfactant derived from cysteine, $(C_8Cys)_2$, was characterized by tensiometry and its solubilization capability towards the hydrophobic drug AmB was evaluated under biomimetic conditions (phosphate buffered-saline, pH 7.4). Important aggregation parameters, such as critical micelle concentration (CMC), surface tension at the CMC ($\gamma_{cmc}$), maximum surface excess concentration ($\Gamma_{max}$) and minimum area occupied per molecule at the water/air interface ($A_{min}$) were determined. Solubilization power was quantified in terms of molar solubilization ratio ($\chi$), micelle-water partition coefficient ($K_p$) and Gibbs energy of solubilization ($\Delta G_s^0$). The aggregation state of AmB in the micellar media was assayed by UV–Vis spectroscopy and *in vitro* antifungal activity was evaluated. Results were compared with the ones obtained for sodium deoxycholate (NaDC) and discussed in terms of drug loading, efficacy and efficiency. Complementary rheological measurements were performed as NaDC solutions formed a shear-thinning gel at concentrations of 0.01 mol $L^{-1}$ and higher, in the experimental conditions employed.



## 1. Introduction

Amphotericin B (AmB), a hydrophobic polyene antibiotic from *Streptomyces* sp., remains the drug of choice for the treatment of most systemic fungal infections [1–3]. Its antifungal activity results from its high affinity for sterols, primarily ergosterols of fungal and yeast cell membranes, forming membrane channels and inducing $K^+$ leakage [1–3]. The name of the drug derives from its amphoteric nature due to the presence of a carboxyl group on the main ring and a primary amino group on the mycosamine ring (Fig. 1).

AmB has poor water solubility but is able to self-associate in solution as a result of its amphiphilic structure. In aqueous

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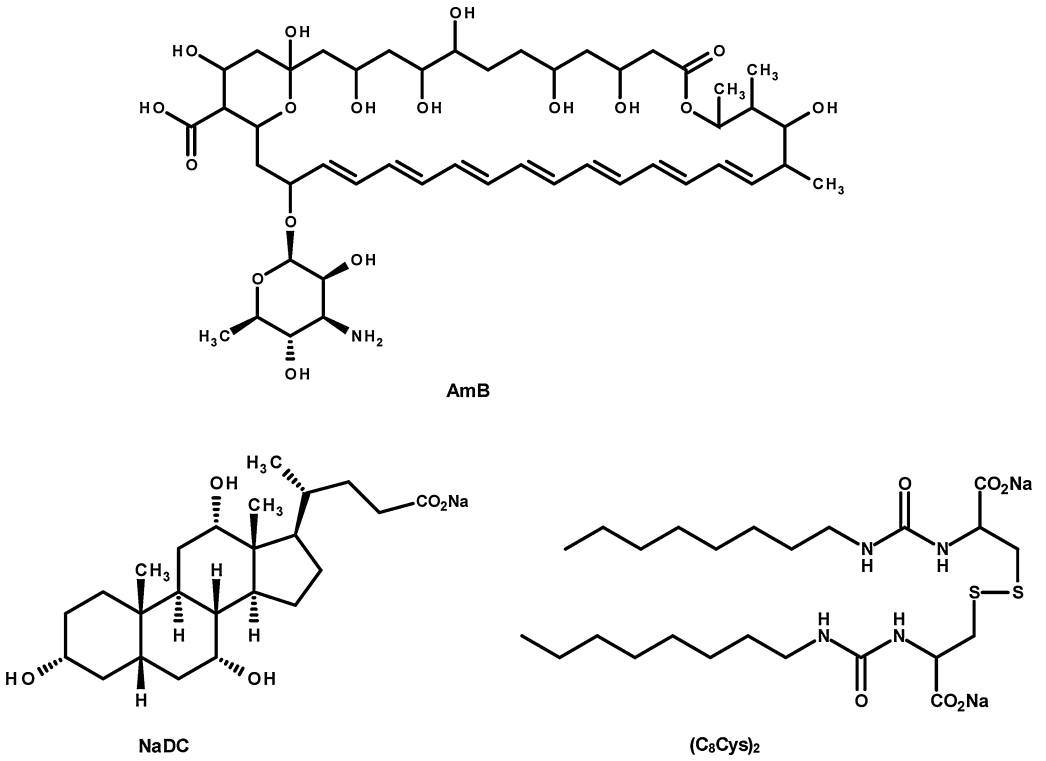


**Fig. 1.** Chemical structures of antifungal polyene antibiotic amphotericin B (AmB), sodium deoxycholate (NaDC) and anionic gemini surfactant $(C_8Cys)_2$ derived from cysteine.

suspensions the drug is known to exist as a mixture of monomers and soluble oligomers as well as insoluble aggregates [4–8]. The concentration of each species in solution depends on total drug concentration and on the method of preparation, namely the concentration of organic solvent in the suspensions [7]. The nature and proportion of the different forms of AmB in solution are correlated with the toxicity of the drug to cholesterol-containing membranes of mammalian host cells [4–8]. These toxic effects are associated with the water-soluble oligomers but not with monomeric AmB and insoluble aggregates, thus selectivity and affinity of AmB for membrane ergosterol or cholesterol is dependent on the aggregation state of the drug [8].

Bile salts, such as sodium deoxycholate (NaDC, Fig. 1), are biological surfactants involved in the solubilization and delivery of liposoluble biomolecules from the gut [9–11], that have been used as delivery vehicles for hydrophobic drugs, thus increasing drug bioavailability while simultaneously decreasing toxic or undesired side-effects [12–14]. Despite its efficacy, deoxycholate-solubilized AmB (Fungizone), in clinical use for several decades, is associated with severe nephrotoxicity [1], which prompted the development of more efficient and less cytotoxic formulations [15–20]. Lipid-associated formulations of AmB with improved safety profiles have been commercialized, however their high cost and the higher doses required to achieve the same therapeutic results of Fungizone limited their widespread use [15,16].

Gemini amino acid-based surfactants are biocompatible and biodegradable surfactants obtained from the condensation of natural amino acids with fatty acids or their derivatives [21–23]. This type of surfactant molecule is formed by two polar amino acid head groups and two hydrophobic alkyl chains connected by a spacer at or near the amino acid residues. Due to their dimeric structure, geminis usually show lower critical micelle concentration (CMC), better surface activity and higher solubilizing power when compared to conventional (single-chain) surfactants [24–26], thus constituting promising drug and gene delivery agents [27–30]. This class of surfactants has attracted considerable attention as they are prone to production by biotechnological processes (either fermentation or enzymatic catalysis) in alternative (or in combination with) chemical synthesis [31–33]. Protein hydrolysates from bioindustrial waste can also be used as raw materials for production of amino acid-based surfactants, thus allowing the conversion of secondary products into high added-value compounds [34,35].

Our group has recently synthesized a anionic amino acid-based gemini surfactant derived from cysteine, $(C_8Cys)_2$ (Fig. 1) and studied its supramolecular behaviour in aqueous media both in the absence and in the presence of biological (macro)molecules [36–40]. In the present work $(C_8Cys)_2$ is characterized and evaluated as a potential delivery agent for AmB and compared with NaDC used in the commercial formulation Fungizone. The supramolecular behaviour of the gemini surfactant under biomimetic conditions (phosphate-buffered saline, pH 7.4) was characterized by tensiometry and its solubilizing power towards AmB was assayed by UV–visible spectroscopy. The effect of encapsulation on the efficacy of AmB and on the aggregation state of the drug was also addressed as AmB toxicity is associated with its aggregation state in solution [5–8]. Results are discussed in terms of drug efficacy, drug loading, encapsulation efficiency and toxicity related to the aggregation state, and compared to the ones obtained for NaDC. Complementary rheological measurements were performed as NaDC formed a gel at the higher concentrations studied.

## 2. Materials and methods

### 2.1. Materials and sample preparation

The gemini surfactant $(C_8Cys)_2$ was synthesized according to established literature procedures [36–40] and fully characterized as described elsewhere [36,37]. Amphotericin B from *Streptomyces* sp. and sodium deoxycholate (99% purity) were supplied by Sigma and used as received. Fungizone, consisting of 45% AmB and 35% NaDC (the balance being sodium phosphate and sodium chloride), was