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Physico-biochemical studies on cationic gemini surfactants: Role of spacer

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ABSTRACT

Two series (ester/amide and polymethylene spacers) of cationic gemini surfactants have been synthesized and their critical micelle concentrations (CMC) and surface properties are determined by conductometry and tensiometry. Ester based geminis are having lower CMCs than polymethylene spacer based ones. Interaction of geminis with plasmid DNA (pEGFP-N1) has been studied using agarose gel electrophoreses to optimize lipoplex (surfactant + DNA) composition. DNA binding shows a dependence on nature of the spacer. MTT (3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide) based cell viability assays were obtained up to optimized lipoplex molar ratio on human lung cancerous cell (A549). The cytotoxicity of lipoplxes on A549 are found [DNA] dependent. Cytotoxicity of gemini surfactants (without DNA), on both A549 and human lung normal cells (L132), has also been checked. The MTT results manifest selective toxicity towards A549 at relatively lower concentration than L132. The amide gemini (cleavable) competes well with polymethylene spacer based geminis. However, former has better biodegradability. The nature of the spacer plays a key role not only in self-assembling properties but also in DNA binding/cytotoxicity.

1. Introduction

Gemini or dimeric surfactants, consisting of two monomeric surfactant molecules (single alkyl tail) with the head groups covalently linked through a flexible or rigid spacer, are potentially introduced in surfactant research [1–4]. In the last two decades, they have been architecturally/structurally exposed in various emerging areas as promising surfactants over to their monomeric counterparts. Among them, cationic gemini surfactants have been widely exploited because of relatively straight forward synthetic route [1], better physiochemical properties [5], greater ability to bind negatively charged moiety (e.g., DNA or bovine serum albumin) and relatively low cytotoxicity towards animal cell lines [1,3,6,7] compared to anionic and nonionic gemini surfactants. Recently, many studies have been carried out for developing a variety of cationic gemini surfactants as a next generation surfactant for biomedical and industrial applications such as potential gene delivery agent [7,8], drug entrapment and release [9], antimicrobial activity [10], templates for nanoparticle synthesis [11], as corrosion inhibitors [12] and as erythrocyte protectors against hypotonic hemolysis [13]. However, cationic gemini surfactants still have certain environmental concerns and toxicity issues [14].

Nowadays, the focus of researcher shifted to change the nature of the head group such as pyridinium [15–17], imidazolium [17–19], dimethyl and diethyl quaternary ammonium [20], pyrrolidinium [21], piperidinium [22] and amino acids [23–25] or architecture of alkyl tail part such as cholesterol [26–29], pyrenyl [30], esterquat [31] symmetrical and unsymmetrical alkyl tails [4]. However, spacer aspect of gemini surfactants mostly limited to change in its length [1,4,19,32]. Only, a few reports are available related to the effect of nature of the spacer on the properties of cationic gemini surfactants and their applications [27,33–35]. Further, very little work has been available regarding biocompatible spacers [36,37]. Looking at the limited studies and environmental issues researchers are attracted to synthesize a new class of cleavable spacer based cationic gemini surfactants.

The presence of biocompatibility, in the gemini surfactants (mainly spacer), can influence the several parameters and facilitate the pH induced mobility in endocytosis process. They can be easily (enzymatically) be hydrolyzed to non-toxic molecules and can safely removed from the living organisms [38]. Though, traditional quaternary gemini surfactants have been extensively studied [1,2,4,39] and also utilized for some biomedical applications [3,6,9,13,40], no report has been found related to DNA binding and cytotoxicity of biocompatible spacer based cationic gemini surfactant. Therefore, it is of genuine interest to check the influence of biocompatible spacer based cationic gemini surfactants with respect to biological applications.

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Recently, large difference in micellization (mainly CMC), surface properties, DNA binding and cytotoxicity have been observed with isosorbide spacer based cationic gemini surfactants over conventional ones [41]. To continue our research work in this direction, we have synthesized and characterized six new biocompatible spacer based cationic gemini surfactants with dodecyl, tetradecyl and hexadecyl alkyl chain (***m*** = 12, 14 or 16) and compared their solution behavior with polymethylene spacer based gemini surfactants. The surface and self-assembling properties of these surfactants have been obtained by conductivity and surface tension measurements. Binding of plasmid DNA (pEGFP-N1) with all the gemini surfactant has been evaluated by agarose gel electrophoresis experiment to optimize lipoplex (gemini + DNA) molar composition. The optimized lipoplex ratio has been further used to perform MTT assay based cytotoxicity study of cationic gemini surfactants with different contents (0.2 and 1.0 μg) of plasmid DNA on human lung cancer cell (A549). In the absence of DNA, cell viability assays have also been performed with gemini surfactants on A549 as well as human lung normal cell (L132).

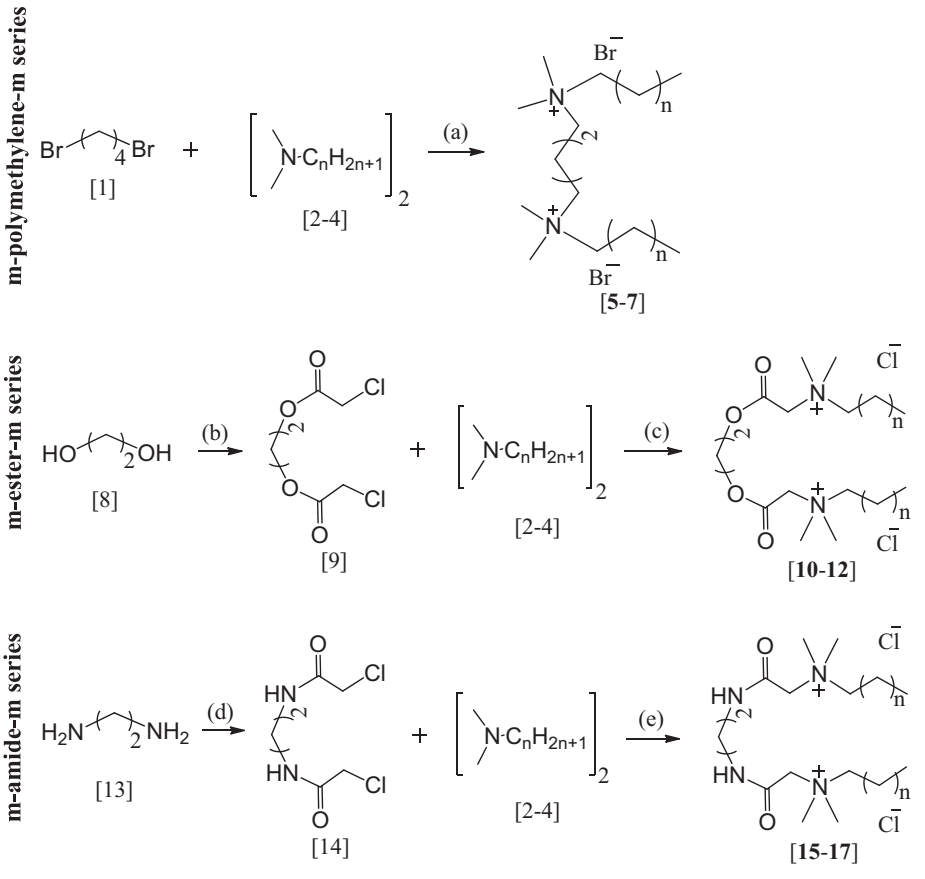
2. Experimental section

2.1. Materials

1,4-Dibromobutane (99%, Sigma Aldrich), ethylene glycol (99%, Sigma Aldrich), ethylenediamine (99%, S.D. Fine Chemicals), chloroacetylchloride (CAC, 98%, S.D. Fine Chemicals, used after simple distillation), *N*,*N*-hexadecyldimethyl-1-amine (95%, Sigma Aldrich), *N*,*N*-tetradecyldimethyl-1-amine (95%, Sigma Aldrich) and *N*,*N*-dodecyldimethyl-1-amine (95%, TCI Chemicals) are used as received. Chemicals for gel electrophoretic and cytotoxicity study were purchased from Hi-media (USA) and used as received. Plasmid DNA (pEGFP-N1), human lung adenocarcinoma cell line (A549) and normal cell line (L132) are purchased from the National Centre for Cell Science, Pune, India. De-ionized double distilled water (1–2 $\mu S \cdot cm^{-1}$) was used throughout.

2.2. Synthesis

Synthesis and characterization of spacers **9** and **14** have been carried out as reported earlier (see S1 in Supporting information) [42]. Two series (polymethylene and biocompatible spacer) of gemini surfactants are synthesized by a modification in the methodology adopted earlier (see Scheme 1) [43]. 1,4-Dibromobutane (**1**, 4.32 g, 0.02 mol), 1,2-bischloroacetoxyethane (**9**, 4.30 g, 0.02 mol) and 1,2-bischloroacetylinediamine (**14**, 4.26 g, 0.02 mol) are refluxed with various alkylamines [*N*,*N*-dimethyldodecyl-1-amine (**2**, 8.75 g, 0.041 mol), *N*,*N*-dimethyltetradecyl-1-amine (**3**, 9.90 g, 0.041 mol) and *N*,*N*-dimethylhexadecyl-1-amine (**4**, 11.05 g, 0.041 mol)] in an appropriate ratio of dry ethylacetate:DCM, dry ethanol:ethylacetate or dry ethanol for 24 to 48 h under N_2 atmosphere with guard tubing



n = 12; [2] Gemini **5**, n = 10; Gemini **6**, n= 12; Gemini **7**, n = 14
n = 14; [3] Gemini **10**, n = 10; Gemini **11**, n= 12;Gemini **12**, n = 14
n = 16; [4] Gemini **15**; n = 10; Gemini **16**, n= 12; Gemini **17**, n = 14

Scheme 1. Synthesis route of biocompatible and polymethylene spacers based gemini surfactants. Reaction conditions: (a) dry ethanol:ethylacetate (5:5), 48 h, reflux, 60–80%; (b) DCM, reflux 8 h, 50 °C, 83%; (c) dry ethylacetate:DCM (8:2), 12 h, reflux, 40–60%; (d) triethylamine, $CHCl_3$, addition at 0–5 °C, 4–5 h, R.T., 41%; (e) dry ethanol, 24 h, reflux, 60–85%.