Colloids and Surfaces B: Biointerfaces 146 (2016) 523–531

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

journal homepage: www.elsevier.com/locate/colsurfb

Vesicles from pH-regulated reversible gemini amino-acid surfactants as nanocapsules for delivery

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ARTICLE INFO

Article history:
Received 9 March 2016
Received in revised form 23 June 2016
Accepted 27 June 2016
Available online 28 June 2016

Keywords:
Gemini amino-acid surfactant
pH-regulated
Vesicle
Mechanism
Nanocapsule

ABSTRACT

Reversible transition from micelles to vesicles by regulating pH were realized by gemini amino-acid surfactants *N*,*N*'-dialkyl-*N*,*N*'-diacetate ethylenediamine. Measurement results of ζ-potential at different pH and DLS at varying solvents revealed that the protonation between H^+ and double —N—CH_2COO^- groups (generating —NH^+—CH_2COO^-), expressed as pK_{a1} and pK_{a2}, is the key driving force to control the aggregation behaviors of gemini surfactant molecule. Effect of pH on the bilayer structure was studied in detail by using steady-state fluorescence spectroscopy of hydrophobic pyrene and Coumarin 153 (C153) respectively and fluorescence resonance energy transfer (FRET) from C153 to Rhodamine 6G (R6G). Various pH-regulated and pH-reversible self-assemblies were obtained in one surfactant system. Vitamin D_3 was encapsulated in vesicle bilayers to form nano-VD_3-capsules as VD_3 supplement agent for health care products. By using the electrostatic attraction between Ca^{2+} and double $-COO^-$ groups, nano-VD_3-capsules with Ca^{2+} coated outermost layers were prepared as a formulation for VD_3 and calcium co-supplement agent. DLS and TEM were performed to check stability and morphology of the nano-capsules. It is concluded that the pH-regulated gemini amino-acid surfactants can be used to construct colloidal systems for delivering hydrophobic drugs or nutritions without lipids at human physiological pH level.

1. Introduction

Surfactant based self-assemblies, particularly in the form of micelles and vesicles, have gained widespread application in biosciences [1], functional soft materials [2,3], drug [4] and gene deliveries [5], etc. The precise control over the self-assembling process, including assembling time, condition of vesiculation, vesicle's size and stability [6], is crucial for enabling these applications.

Various methods have been introduced to control the transition between micelles and vesicles [7–9]. Usually, the micelles are formed when the concentrate of the surfactant is above the critical micelle concentrate ("cmc"). The aggregation behaviors are controlled by electrostatic, van der Waals, hydrophobic, or steric interactions as well as their delicate balance [10]. According to different surfactants and conditions, micelles can be finely transformed to vesicles to achieve the most appropriate properties of the aggregates (such as the size, charge, stability, and surface activity). Usually, vesicles are preferably formed by the surfactants with double hydrophobic chains because of the steric interactions among hydrophobic groups. Furthermore, vesicles can also be obtained by mixing cationic and anionic surfactants due to the electrostatic attraction. It is significant to find the guidance to design and control the self-assembled structures of mixed surfactants so as to gain the desired characteristics in applications. Catanionic mixed surfactants have been considered as a promising system to construct complex self-assembled nanostructures [11–15]. To enable controllable or regulated self-assembly systems with special characteristics, new functional surfactants or mixtures of different types of surfactants are constantly being developed and formulated. Zhang et al. [16] recently developed CO_2-switchable viscoelastic wormlike micellar fluids by utilizing commercially available low-cost anionic sodium dodecyl sulfate (SDS) and small-molecule tertiary diamine *N*,*N*,*N*',*N*'-tetramethyl-1,3-propanediamine (TMPDA) with a stoichiometric ratio of 2:1. Due to the CO_2-stimuli-responsive TMPDA, the macroscopic rheological properties and self-assembled microstructures can be reversibly tuned by cyclically bubbling and removing CO_2. Wang et al. [17] established photo-controlled reversible supramolecular assemblies by synthesizing light-sensitive surfactant 1-[10-(4-phenylazophenoxy)decyl]-pyridinium bromide (AzoC10) and compounding with α-cyclodextrin (α-CD). The host-guest assembly and disassembly between azobenzene and α-CD by external photostimuli can be used as a smart strategy to build up molecular shuttles, motors, and machines [18,19]. Recently,

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http://dx.doi.org/10.1016/j.colsurfb.2016.06.054
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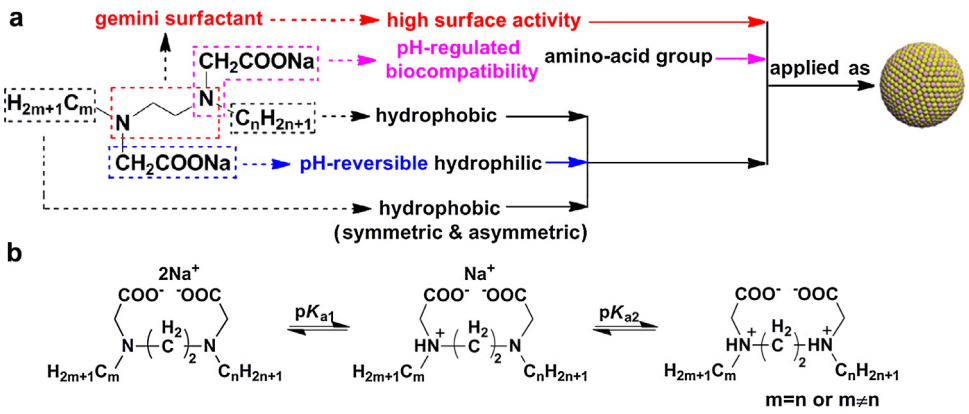

Scheme 1. Design schematic of the gemini pH-sensitive surfactant. a) design principle of *N*,*N*'-dialkyl-*N*,*N*'-diacetate ethylenediamine; b) surfactant type transformation adjusted by pH.

Table 1
pK_a values of *N*,*N*'-dialkyl-*N*,*N*'-diacetate ethylenediamine.

Gemini surfactants	pK_{a1}	pK_{a2}
Ace(8)-2-Ace(8)	7.59	7.02
Ace(10)-2-Ace(10)	7.83	6.59
Ace(12)-2-Ace(12)	8.41	6.55
Ace(8)-2-Ace(10)	8.44	4.73
Ace(10)-2-Ace(12)	8.40	4.44
Ace(10)-2-Ace(14)	8.31	4.34
Ace(8)-2-Ace(12)	8.51	4.33
Ace(12)-2-Ace(14)	8.10	4.67
Ace(10)-2-Ace(16)	8.33	4.58

Lin [20] synthesized a pH-responsive single chain surfactant *n*-decylphosphoric acid (DPA), which can be switched between two states, sodium decylphosphoric or disodium decylphosphoric, by adjusting pH value. Direct transformation of a "1-2" surfactant pair to a "1-1" pair was realized in the catanionic surfactant mixture of DPA and cetyltrimethylammonium bromide (CTAB) and consequently, pH-regulated self-assembled structures, such as spherical micelle, wormlike micelle, vesicle, and lamellar structure, were fabricated.

It is praiseworthy for a single low-molecular-weight surfactant to have complex or subtle self-assembly behaviors in aqueous solution [21–23]. As surfactants are widely used in commercial and industrial fields, it is of great significance to endow surfactants with environmental friendly and biocompatible characteristics. Based on the above considerations, a series of pH-regulated amino-acid surfactants *N*,N'-dialkyl-*N*,*N*'-diacetate ethylenediamine was designed and synthesized (Scheme 1a). A gemini structure was chosen as the molecule skeleton in order to obtain unique self-assembly properties superior to the conventional single-chain surfactants [24]. The low irritating amino-acid type was selected in order to obtain better biocompatibility and environmental safety. A —CH_2COOH group was attached to a nitrogen atom belonging to the spacer —N—CH_2CH_2—N— to endow the surfactant with pH-switchable surface activity. More importantly, double pH-stimuli —N—CH_2COO^- groups could regulate the gemini surfactant as bivalent, monovalent anionic or zwitterionic by adjusting pH value (Scheme 1b). One attractive approach is to mix different types of surfactants (such as anionic and zwitterionic surfactants, etc.) in the aqueous solution and thus utilize electrostatic effects to regulate self-assemblies at specified pH values. In our previous work, a series of fundamental studies have been made to investigate the surface and colloidal properties of the symmetric Ace(n)-2-Ace(n) and asymmetric Ace(m)-2-Ace(n) solution under varying pH conditions according to pK_a values [25,26] (listed in Table 1). It was concluded that *N*,*N*'-dialkyl-*N*,*N*'-diacetate ethylenediamine had high surface activity at alkaline pH, strong ability to form vesicles at isoelectric point pH range, and pH-recyclability at acidic pH. The vesicles formed in asymmetric Ace(m)-2-Ace(n) solution were observed to undergo a clear size change from large to small and finally swell slightly in a wider pH range from alkaline to weak acid. Both pH-reversibility and pH-regulation of the self-assembly process can be accomplished by this single gemini surfactant.

Many kinds of lipophilic active components (bioactive lipids, nutraceuticals and drugs) need to be incorporated into aqueous formulations to become suitable for commercial consumption as foods, health care products, and pharmaceuticals [27]. Lipophilic active compounds are usually dissolved in fat- or lipid-soluble medium and emulsified with H_2O to achieve a considerable drug loading capacity [28,29]. Therefore, suppliers prefer to prepare oil-in-water emulsions, microemulsions, or nanoemulsions as delivery system for practical applications, which is in conflict with current health recommendations [30]. From a consumer's perspective, it is generally not desirable to supplement the target nutrition while intaking oils or lipids.

In this study, we used detailed physical measurements to investigate the formation and regulation of pH-reversible vesicles and aimed to create an aqueous colloidal system without any lipidic co-solvents to encapsulate Vitamin D in vesicle bilayers formed by single gemini amino-acid surfactant. Vitamin D (VD) plays a critical role in controlling calcium transport, bone metabolism, and renal calcium reabsorption [31], but cannot be synthesized endogenously [32]. This substance typically comes in two different molecular forms: VD_2 (ergocalciferol) and VD_3 (cholecalciferol). In humans, VD_2 potency is less than VD_3, which can be synthesized in human skin after exposure to sunlight [33]. Here we shall present results of encapsulating VD_3 in vesicle bilayers formed by single gemini amino-acid surfactant. Considering that the main physiological function of VD_3 is to promote the adsorption of intestinal mucosa to calcium and that the double carboxylic acid can chelate with bivalent Ca^{2+}, we will show schemes to construct Ca^{2+}-coated-nano-VD_3-capsules with the Ca^{2+} surrounding the outermost carboxylic acid ions. The resultant capsules can be taken as a combined calcium and VD_3 cosupplementation agent [34,35].

2. Experimental methods

2.1. Materials

Gemini surfactants Ace(n)-2-Ace(n) and Ace(m)-2-Ace(n) were previously synthesized in our laboratory [25,26]. Pyrene (purity 98%), SDS and cetyl trimethyl ammonium bromide (1631, purity