Colloids and Surfaces A: Physicochem. Eng. Aspects 327 (2008) 95–102

Contents lists available at ScienceDirect

Colloids and Surfaces A: Physicochemical and Engineering Aspects

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

Interactions in a nonionic surfactant and chitosan mixtures

I. Pepić*, J. Filipović-Grčić, I. Jalšenjak

Department of Pharmaceutics, Faculty of Pharmacy and Biochemistry, University of Zagreb, A. Kovačića 1, PO Box 156, 10 000 Zagreb, Croatia

ARTICLE INFO

Article history:
Received 20 July 2007
Received in revised form 4 June 2008
Accepted 5 June 2008
Available online 13 June 2008

Keywords:
Triblock copolymers
Chitosan
Nonionic surfactants
Polyelectrolytes
Surface tension

ABSTRACT

Interactions in mixtures of nonionic surfactant, Lutrol F127, and cationic polyelectrolyte, chitosan from water solution and acetate buffer (pH 6.0 or 6.5 and ionic strength 0.1 and 0.5) have been investigated at two different temperatures (25 and 34 °C). Critical aggregation concentration (cac), critical micellization concentration (cmc) and the minimum area of Lutrol F127 molecule at the air/solution interface (a), were determined using the surface tension measurements. The addition of chitosan to Lutrol F127 solutions shifted the cac and cmc to higher values; the effects were more pronounced for the cmc and in acetate buffer. The Lutrol F127 adsorption at the air/solution interface strongly depended on the equilibria in the bulk-phase, i.e. on the extent of the protonation of chitosan amino groups. The results were discussed in terms of electrostatic, hydrophobic and intra- and inter-polymer interactions.

1. Introduction

PEO/PPO/PEO triblock copolymers are nonionic surface-active agents with diverse applications (dispersing, antifoaming, emulsifying and solubilizing agents) [1]. In novel drug and gene delivery systems, PEO/PPO/PEO triblock copolymers are often considered as “functional excipients” playing an important and essential role in formulation [2,3]. They are usually referred to by their trade names (Pluronics, Lutrol, Monolan, Synperonics and Supronic) or generic name (poloxamers) [4]. Their aqueous solubility, aggregation behaviour, and applications are strongly dependent on compositions of the hydrophilic poly(ethylene oxide) (PEO) block and hydrophobic poly(propylene oxide) (PPO) block, as well as on the molecular weight of copolymers. Among others commercially available triblock copolymers, Lutrol F127, denoted as F127 (poloxamer 407) has been chosen for this investigation because of its aggregation behaviour. At low concentrations, F127 exists in solutions as individual coils (unimers) and thermodynamically stable micelles are formed with increasing copolymer concentration.

The micelle formation involving triblock copolymers appears to be more complex than conventional low molecular weight surfactans [1]. The shape of F127 micelles is spherical. It is generally accepted that the core of the micelles consists of dehydrated PPO groups. An outer shell of hydrated PEO groups surrounds the hydrophobic core [5,6]. Wanka et al. [5] have studied phase diagrams of pluronic copolymer and found that F127 has a relatively simple behaviour. In the temperature region up to about 37 °C, the copolymer solution is present as an isotropic phase up to approximately 20% of copolymer, and beyond that concentration, the cubic-phase is present. The solution with copolymer micelles are expected at more than 10-fold lower concentration of F127. The copolymer forms micelles when present above the critical micelle concentration (cmc). Micelle formation is also critically dependent upon temperature, and the temperature at which micelles are formed is critical micelle temperature (cmt). In F127 solutions, there is the strong decrease in the cmc with increasing temperature, which can be more than four orders of magnitude with a temperature increase from 15 to 40 °C.

Besides concentration and/or temperature, an important factor of copolymers aggregation to be considered is their sensitivity to the presence of additives, since they constitute usual drug delivery formulations (e.g. electrolytes, low molecular weight surfactant or drugs). Pandit et al. [7] showed that addition of salting-out electrolytes (Na_3PO_4, Na_2SO_4 and NaCl) in F127 solutions decreases cmt, whereas salting-in electrolyte (NaSCN) has the opposite effect. The slight changes in the cmc of F127 in the presence of naproxen, indomethacin [8] and pilocarpine HCl [9] were noticed, whereas propofol induced micelle formation in aqueous F127 solutions at lower cmc [10]. The interaction between F127 and nonionic surfactants (hexaethylene glycol mono-*n*-dodecyl ether) resulted in spontaneous formation of mixed micelles indicating that nonionic surfactants interacted strongly with both the unassociated and

---

* Corresponding author. Tel.: +385 1 46 12 608; fax: +385 1 46 12 691.
E-mail addresses: ipepic@pharma.hr (I. Pepić), jelena.filipovic-grcic@fbf.htnet.hr (J. Filipović-Grčić), ijalsenjak@gmail.com (I. Jalšenjak).

0927-7757/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.colsurfa.2008.06.009

associated forms of F127 [11]. Although several researches have explored fundamental behaviour of F127 copolymers, as well as the effect of some additives (electrolytes, drugs and surfactants), little is known about F127 micellization in the presence of polyelectrolyte additives.

The physicochemical aspects of F127/polyelectrolyte mixtures could have fundamental and practical importance (e.g. drug delivery systems). Since chitosan is a positively charged linear polysaccharide having numerous pharmaceutical applications (e.g. pharmaceutical forms and drug delivery systems) [12], it was chosen as an example of a polyelectrolyte. Chitosan is a random copolymer, containing β $(1 \rightarrow 4)$ D-glucosamine and *N*-acetyl-D-glucosamine units and it is water-soluble in dilute acid aqueous solutions. The physicochemical properties of chitosan solutions can be controlled by the manipulations of solution conditions (temperature, pH, ionic strength, concentration, and solvent). Chitosan carries the free amino and hydroxyl groups along its backbone. The pK_a value of free amino group is around 6.5. Because of that chitosan exhibits pH dependent solubility; at $pH > 6.5$ chitosan solutions exhibit phase separation, while for $pH < 6.5$ it is soluble and carries a positive charge due to the presence of protonated amino groups [13]. The different degree of deacetylations and/or different solution conditions have influence on charge density along the chains and, therefore, on the flexibility of chitosan chains. Conformation of the chitosan polymer is a function of its chain flexibility and solution conditions [14]. Chain flexibility facilitated inter- and/or intra-molecular interaction between chitosan chains [15,16]. Within investigated pH range (i.e. pH 1–5), chitosan molecules of the same deacetylation degree have different inter- and/or intra-molecular electrostatic repulsive forces [15]. Chitosans with different degree of deacetylations, in different solution conditions may be in a rod shape, a random coil, or a compacted sphere [14]. These properties, together with the hydrophobicity of the backbone, may play an important role on its interactions with amphiphilic molecules [17]. Additionally, Amiji [18] has found the self-aggregation behaviour of chitosan in aqueous solution caused by intermolecular hydrophobic interactions, with strong influence of the ionic strength of the medium.

To the best of our knowledge, there is no report related to the aggregation behaviour and surface activity of systems containing F127 and chitosan polyelectrolyte. The aim of this work is to provide more information on the interactions between the nonionic surfactants, F127, and cationic polyelectrolyte, chitosan. The chosen experimental conditions are of considerable importance for the stability during the storage and during the possible application (e.g. ocular surface) of the mixed systems.

2. Materials and methods

2.1. Materials

The triblock copolymer, Lutrol® F127 (BASF) (generically poloxamer 407) (molecular weight 12 600) was used as received. On the basis of molecular weights, Lutrol® F127 can be represented by the formula $(EO)_{100}(PO)_{65}(EO)_{100}$. Low-viscous chitosan (molecular weight 150 000, deacetylation degree 84.5%, Fluka) was used as received. According to the supplier, chitosan contained 0.35% insoluble matter and 0.60% ash as impurities. Analytical grade acetic acid and sodium acetate (Sigma) were used for the preparations of acetate buffer solutions pH 6.0 (100 mM acetic acid: sodium acetate, ionic strength 0.1), and pH 6.5 (500 mM acetic acid: sodium acetate, ionic strength 0.5). Double distilled water (specific conductivity <1 $\mu S\,cm^{-1}$) was used for the preparation of all solutions. All others chemicals were reagent grade.

2.2. Systems preparation

Stock solutions of the copolymer (10%, w/v) were prepared by dissolving F127 copolymers in double distilled water or acetate buffer for 48 h at 4 °C. Stock solution of chitosan (1%, w/v) was prepared in aqueous acetic acid (0.5%, v/v) by stirring for 24 h at room temperature. The solution was filtered through medium porosity filter in order to exclude the trace of undissolved chitosan. The mixed F127/chitosan systems were prepared from F127 and chitosan stock solutions with a constant chitosan concentration (0.005 or 0.01 or 0.015%, w/v) and variable F127 concentrations (1×10^{-5} to 5%, w/v) by diluting with water or acetate buffer (pH 6.0 or 6.5).

Additionally, the solutions of chitosan only or F127 only were prepared by dilution of stock solutions with water or acetate buffer (pH 6.0 or 6.5) in the concentration range 1×10^{-5} to 5×10^{-2}% (w/v). All systems were kept in water circulating bath at 25 ± 0.02 °C or 34 ± 0.02 °C to equilibrate for at least 24 h before surface tension measurements. The pH measurements were made for all solutions prepared with Seven Multi pH meter (Mettler Toledo). All subsequent experiments were carried out in triplicate with freshly prepared solutions.

2.3. Time effect on surface tension

The solutions were gently stirred after being placed in the cell to enhance diffusion. In order to reach equilibrium value, conditioning time was allowed between the placement of the solution in the measurement cell and the actual reading [19,20]. Equilibrium of 30 min was found to be sufficient for F127, chitosan, and mixed F127/chitosan systems, so this period was utilized for subsequent measurements.

2.4. Surface tension measurement

The surface tension measurements were performed with K-100C tensiometer (Krüss) applying the density corrections needed. Surface tension values (σ), as a function of concentration (c), were determined employing the Du Noüy ring method. The surface tension of the neat water and acetate buffer solutions pH 6.0 and 6.5 were always higher than 71.49 $mN\,m^{-1}$ at 25 °C and 70.28 $mN\,m^{-1}$ at 34 °C in each set of measurements. The measurements were made at two different temperatures using Heating Immersion Circulator MB (Julabo) attached to water bath with temperature stability within 0.02 °C. The readings were taken in triplicate and were reproducible, with the standard deviation being less than 4% of the mean value.

3. Result and discussion

3.1. Surface properties of chitosan solutions

The surface tension of chitosan in water and acetate buffer solutions (pH 6.0 and 6.5) were determined for the concentration range 6.66×10^{-10} to 3.34×10^{-6} $mol\,dm^{-3}$ at two temperatures (25 and 34 °C). The surface tension data for chitosan in water are given in the insert of Figs. 1 and 2 at 25 °C (pH 5.06) and 34 °C (pH 5.21), respectively. The constant values of surface tension (~71.45 $mN\,m^{-1}$ at 25 °C and ~70.26 $mN\,m^{-1}$ at 34 °C) close to the surface tension of pure water (71.99 $mN\,m^{-1}$ at 25 °C and 70.52 $mN\,m^{-1}$ at 34 °C) [21] were observed. The constant values of surface tension indicate that chitosan molecules are excluded from the air/solution interface, indicating absence of surface activity. At a given pH, the amino groups of chitosan molecules are protonated, which gave the chitosan hydrophilic character in bulk solutions. Schultz et al. [22] described analogous result explaining that chitosan molecule