Synergistic Effects in Mixed Micellization between Natural and Synthetic Block Copolymers

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ABSTRACT

For applications of block copolymer mixtures as potential new nano-vehicles for delivery, understanding the components mixing characteristics are of special interest. In our previous study [1], we showed that interactions between a biological charged diblock-copolymer like protein, beta-casein, and a synthetic uncharged synthetic triblock copolymer, Lutrol F-127, lead to the formation of mixed micelles. Here we report on synergistic effects found in some mixing ratios, and the mixed micellization characteristics, obtained by Isothermal Titration Calorimetry (ITC), Cryogenic-Transmission Electron Microscopy (cryo-TEM), Small-Angle X-Ray Scattering (SAXS) and Dynamic Light Scattering (DLS). At low Lutrol mole fractions we have observed a complicated micellization process characterized by two critical mixed micelles concentrations (CmMC). Positive and negative synergistic effects of Lutrol were revealed.

Keywords: beta-casein, mixed micellization, synergism, block copolymers, CMC

1 INTRODUCTION

In the last decades much research was dedicated to studying polymers and surfactants interactions in solution. In the present work, we continue to investigate the mixed micellisation of two amphiphilic macromolecules - Lutrol F-127 and the milk protein beta-casein. Using ITC we characterized the mixed micellization process at different temperatures and at neutral pH, and analyzed the effect of protein and Lutrol concentration on the interaction. Cryo-TEM, SAXS, and DLS were applied to characterize the dimensions and morphology of the mixed aggregates.

Pluronic compounds self-assemble in aqueous solution above the CMC, or when the temperature exceeds the critical micellization temperature (CMT). The micelles' structure is well described by the "core-corona" model, in which a spherical core composed of the hydrophobic PPO chain is surrounded by a corona of hydrated PEO units. The of a large, hydrophobic C-terminal domain and a polar milk protein beta-casein (24kD, 209 amino acids) is also an amphiphilic block copolymer at neutral pH [2], consisting negatively charge N-terminal domain. In the last few years we studied the self-assembly behavior of beta-casein at different temperatures, pH and ionic strength conditions [3, 4, 5]. At neutral pH, micelles of oblate shape form at 16 °C and above, and the micelles shape and dimensions remain nearly constant in the temperature range of 24-35 °C [3].

Thermodynamic characteristics of the Lutrol/beta-casein mixed micellization, including the cooperativity, were studied by ITC and DSC. Using ITC, SAXS and cryo-TEM we have proved the two very different classes of macromolecules strongly interact to form mixed core-corona micelles. All the experiments indicate formation of well-defined micelles [1].

Here we focus on the synergism facets in mixtures of the two macromolecular amphiphiles - Lutrol and beta-casein. The application of the block copolymers mixtures as potential new nano-vehicles for drug delivery [6] or as effective stabilizers for pharmaceutics and cosmetic emulsions and suspensions may be of a significant value.

2 EXPERIMENTAL SECTION

2.1 Materials and solutions preparation

Bovine beta-casein (>99%; Sigma-Aldrich) was dissolved in pH 7.0 phosphate buffer containing 5.65 mM Na_2HPO_4, 3.05 mM NaH_2PO_4, and 0.05 mM NaCl. The buffer has ionic strength (IS) of 0.05.

Lutrol F-127 ($EO_{101}PO_{56}EO_{101}$) was a gift from BASF Chemical Company. It has an average molecular weight of 11880 (MWmin = 9840; MWmax = 14600). Micellar Lutrol solutions were prepared using the same buffer. They were added drop-wise into beta-casein solutions with stirring up to the required mole ratio. Solutions prepared at Lutrol-to protein ratios of 0, 0.125, 0.25, 0.35, 0.5, 0.75, and 1.0 were gently stirred at room temperature for 24 hours before use.

2.2 Methods

ITC. The interactions between the two block copolymers were characterized by ITC using a VP-ITC calorimeter (MicroCal), at 29°C. The reaction cell (V = 1.43 mL) was filled with degassed buffer, or individual block copolymer solution of various concentrations. A micellar solution of each of the block copolymers, or mixtures of the two were injected into the reaction cell in 28 steps of 10 mL each, and the heat flow was measured. During the titration, the stirring speed was 310 rpm. The duration of each injection was 20 s, and the equilibration time between consecutive injections was 3 min. Calorimetric data analysis was done with the Origin 5.0 software (MicroCal). The recorded information was organized in enthalpy plots showing the change in enthalpy vs. injectant concentration. The CMC was determined from the peak value of the differential enthalpy vs. injectant concentration plot.

Cryo-TEM. Micellar solutions of beta-casein, Lutrol, and mixtures of the two polymers were examined by cryo-TEM. Specimens were prepared in the controlled environment vitrification system at 29 °C. A small drop of each solution was placed on a perforated carbon film supported on a TEM copper grid, held by tweezers. The grid was blotted by a piece of filter paper to form 100–250 nm thick films within the micropores in the carbon-coated lacey polymer layer supported on the grid. The specimen was then plunged into a reservoir of liquid ethane cooled by liquid nitrogen, to ensure vitrification and prevent ice crystals formation. The vitrified specimen was mounted onto an Oxford CT-3500 cryogenic sample holder cooled with liquid nitrogen to below -170 °C. All samples were studied under low-dose conditions in an FEI T12 G^2 TEM, operating at 120 kV. Images were recorded on a Gatan US1000 2k x 2k high resolution cooled CCD camera and processed with the Digital Micrograph software. The ramp-shaped optical density gradients in the background were digitally corrected.

SAXS. We used a small-angle diffractometer (Molecular Metrology SAXS system) with Cu Kα. The solutions were sealed in thin-walled glass capillaries of about 1 mm in diameter and 0.01 mm wall thickness, and measured under vacuum. Capillaries were placed in multi-capillary holder connected to JULABO circulators, designed for temperature-controlled applications.

The scattering intensity I*(h)* was recorded at the scattering vector *h* in the interval $0.07 < h < 2.7$ nm^{-1}. The scattering vector is defined by $h=4\pi sin(\theta)/\lambda$, where 2θ is the scattering angle and λ the radiation wavelength.

The scattering intensity was then normalized with respect to time, solid-angle, primary beam intensity, capillary diameter, transmission, and the Thompson factor [7]. Scattering of the solvents, empty capillary and electronic noise were subtracted.

Samples showed a peak near $h \sim 0.1$ nm^{-1}, indicating strong repulsive interactions between the micelles. To simplify SAXS interpretation, we fitted the part of $h>0.2$ nm^{-1} to an oblate ellipsoid model, that in earlier SAXS and cryo-TEM studies we found applicable to beta-casein micelles at similar concentration and pH conditions [7].

Modeling was done using the formula for ellipsoid as described by Feigin and Svergun [8]

$$F_{ellip}^2(hr,\nu) = \int_0^1 F(hr)^2[hr(1+x(\nu^2-1))^{0.5}]^2 dx \quad (1)$$

where *h* is the scattering vector, F_{ellip} is the form factor of an oblate ellipsoid with *r, r, νr* half axis's and F(*hr*), the form factor for a sphere of radius *r* given by:

$$F(hr) = 3\frac{\sin hr - hR\cos hr}{(hr)^3} \quad (2)$$

I(h) the scattering intensity, equals:

$$I(h) = N\rho^2V^2F^2(hr,\nu) = BV^2F_{ellip}^2(hr,\nu) \quad (3)$$

with $B = N\rho^2$, where *N* is the number of micelles, ρ the electron density difference between micelles and solution, and *V* the average volume of one micelle.

DLS. Size values were determined using a Malvern Nano ZetaSizer model ZEN 3600 (Malvern Instruments Ltd, UK) at 30 °C.

3 RESULTS AND DISCUSSION

Mixed micellization of beta-casein and Lutrol has not been studied so far in detail. We showed in a previous work [1] that although both block copolymers display very different structures, they interact to form mixed micelles. In the present work accent was done on synergistic effects on CMC and size of aggregates in the mixed micellization.

Experimental CmMC values were calculated from ITC experiments at 30, 25 and 15 °C. Theoretical CmMC values were calculated by using the phase separation model for ideal mixing case.

$$\frac{1}{CmMC} = \frac{\alpha_{Lutrol}}{CMC_{Lutrol}} + \frac{1-\alpha_{Lutrol}}{CMC_{beta-casein}} \quad (4)$$

where α is the mole fraction of one of the components.

Theoretical and experimental dependences of the CmMC values on Lutrol mole fraction in the mixture, at different temperatures, are showed in the Figs. 1, 2.

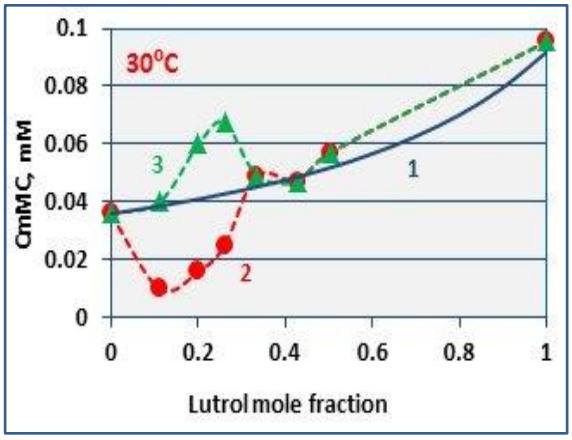

Figure 1: Dependency of the CmMC on Lutrol mole fraction: at 30 °C: 1 – theoretical curve; 2, 3 – experimental $CmMC_1$ and $CmMC_2$ values.

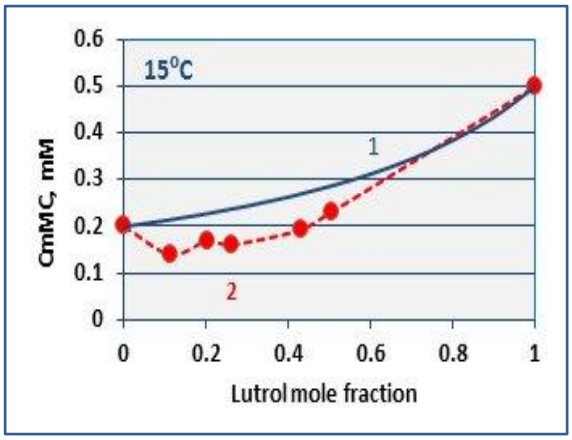

Figure 2: Dependency of the CmMC on Lutrol mole fraction: at 15 °C: 1 theoretical curve; 2 – experimental CmMC

Figure 1 shows a complicated micellization process at 30°C and low Lutrol mole fractions, characterized by two CmMC values. $CmMC_1$ is found to be lower than the theoretical one, and $CmMC_2$ is higher. At Lutrol mole fractions exceeding 0.4 the block copolymer mixture is characterized by only one CmMC value, almost identical to the theoretical one.

It was expected that temperatures lower than the Lutrol critical micellization temperature (CMT=29 °C) could result in failure of the mixed micellization. However, experiments done at 25 °C (not shown) and 15 °C (Fig. 2) show that Lutrol molecules are participating in mixed micelle formation. Moreover, some positive synergistic effect of Lutrol was revealed in these cases. Binding (interactions) between the block copolymers at temperatures below Lutrol CMT is confirmed upon titration of beta-casein into Lutrol (Fig. 3). At all the investigated temperatures (15, 25 and 30 °C) characteristic sigmoidal ITC curves were obtained, strongly indicating interactions between the two components and mixed micellization.

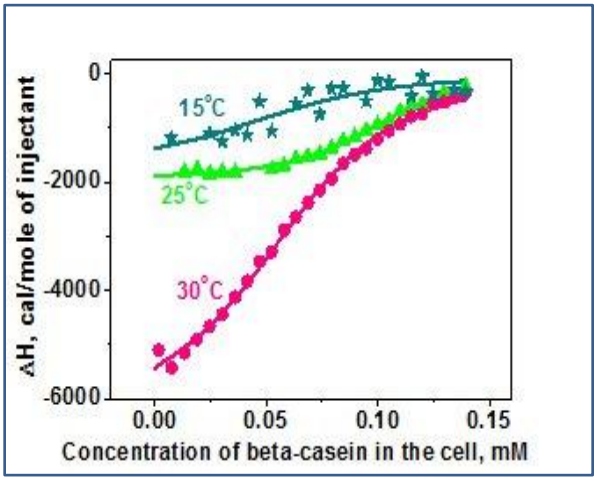

Figure 3: Binding of beta-casein to Lutrol at the different temperatures: difference ITC curves of titration of micellar beta-casein solution into Lutrol solution and into the buffer.

Interestingly, a different, more complex behavior is found in the reversed experiments, e.g., when Lutrol is titrated into beta-casein solutions, as shown in Fig. 4. This can be partially explained by the creation of several mixed micelles populations, as supported by the finding of two CmMC values, as described in Figure 1, and in the discussion above.

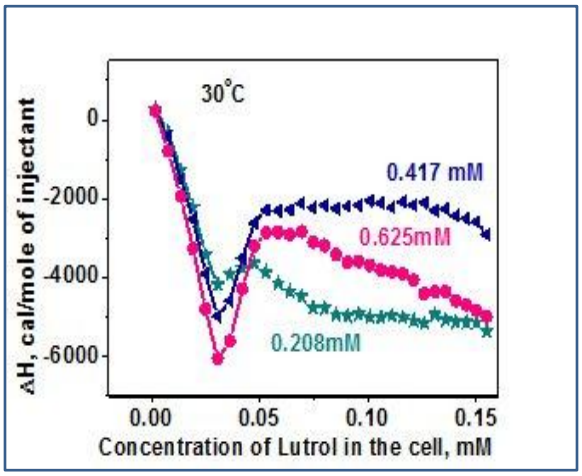

Figure 4: Binding of Lutrol to beta-casein: difference ITC curves of titrating micellar Lutrol into beta-casein at different concentrations above its CMC and into the buffer.

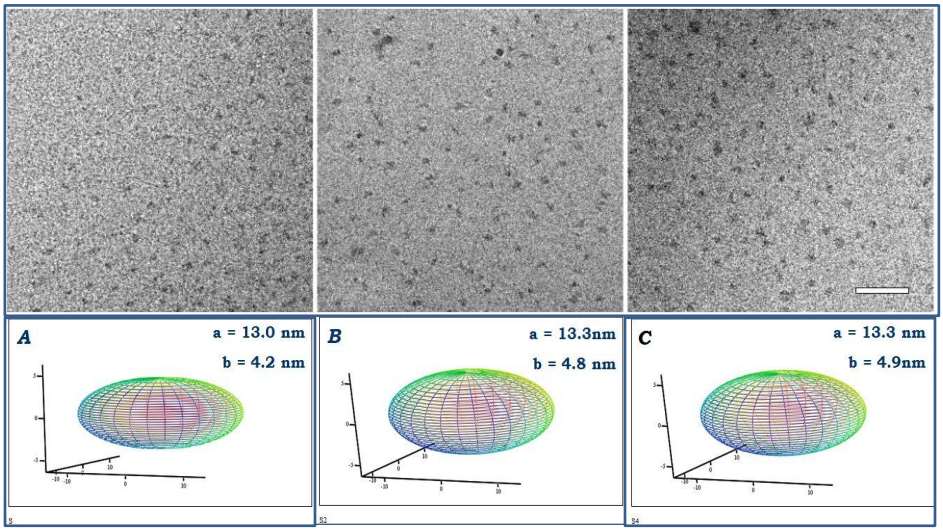

Figure 5: Cryo-TEM (bar = 100 nm) images and SAXS models for (A) beta-casein, (B) 0.25:1 and (C) 1:1 mixed Lutrol/protein micelles at 30°C. Beta-casein concentration in all panels is 2wt% (0.833 mM).

Cryo-TEM indicates some changes in the oblate micellar shape and size (Fig. 5). upon mixed micellization. Similar results are obtained by SAXS. Quantitative analysis of the SAXS data shows mainly an increase of the small oblate micelle diameter compared with the radial cross section. DLS results (Fig. 6) agrees well with the microscopy and SAXS data.

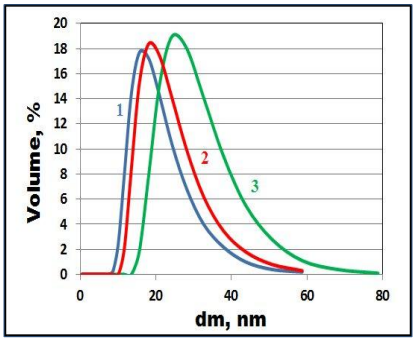

Figure 6: DLS-results for 1 -beta-casein micelles; 2 - 0.25:1, and 3 - 1:1 mixed Lutrol/protein micelles, at 30°C

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