



α_s -Casein–PE6400 mixtures: Surface properties, miscibility and self-assembly



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ABSTRACT

Surface properties, miscibility and self-assembly of mixtures of a food-grade α_s -casein and the triblock copolymer PE6400 (PEO₁₃–PPO₃₀–PEO₁₃) were examined. The properties at the surface were determined by surface pressure measurements for a 1:1 molar mixture. Comparison of the calculated isotherms show attractive interactions at surface pressures above 9 mN/m. The miscibility gaps of solutions containing 0.004–0.2 mmol/l α_s -casein and 0.02–0.1 mol/l polymer were examined. It was found that a one-phase region exists at distinct mixing ratios and temperatures. Comparison of the cloud points of mixtures of α_s -casein and PE6400 with pure α_s -casein showed that the presence of the triblock copolymer enhanced the solubility of the protein. The ζ -potential of the α_s -casein solution decreased by addition of PE6400 to zero. Our results thus suggest that α_s -casein and PE6400 are miscible. The results of the cloud point and ζ -potential measurements were explained by formation of a mixed aggregate where the PPO chains are anchored inside the hydrophobic part of the α_s -casein while the PEO chains cover the charged hydrophilic part of the α_s -casein thereby leading to an increase of the cloud point and a decrease in ζ -potential. This is in agreement with the attractive interactions between α_s -casein and PE6400 as observed via surface pressure measurements at the surface.

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1. Introduction

Investigations focus increasingly on determining the properties of naturally occurring compounds which are surface active and self-assemble into micellar structures suitable for the delivery of hydrophobic functional ingredients. These structures are of interest to various industries, including the food, pharmaceutical and personal care industries.

Examples of naturally occurring surface active compounds are the caseins which are the main protein source in milk. Caseins consist of several proteins with four principal ones being α_{s1} -, α_{s2} -, β - and κ -casein in bovine milk [1,2]. The structural units of proteins are amino acids having different hydrophobicities. When amino acids with similar hydrophobicities are sequentially arranged in the chain, distinct hydrophilic or hydrophobic blocks are formed. The

location of such blocks depends on the conformation of the chain. Caseins are highly surface active due to a lack of a secondary structure. Hydrophobic and hydrophilic residues can therefore readily assume suitable configurations at interfaces [1]. The distribution of hydrophilic and hydrophobic dominated parts in the different caseins was identified by determining the regions of the casein sequence which adsorb at hydrophobic surfaces by self-consistent field theory [3]. Results of these calculations led to conclude that β -casein is a diblock polymer where the C-terminal region has hydrophobic and the N-terminal hydrophilic properties, while α_{s1} -casein is a triblock polymer with two hydrophobic ends and a hydrophilic center [3,4].

Aside from such naturally occurring block copolymers, a huge number of synthesized block copolymers are commercially available. The PEO_x–PPO_y–PEO_x surfactants (tradename Pluronic PEs (BASF) or Synperonic PE Series (Croda)) have, for example, structural characteristics that are similar to the α_{s1} -casein. They consist of a hydrophobic polypropylene oxide (PPO) block in the center being enclosed by two hydrophilic polyethylene oxide (PEO)

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blocks. The aggregation properties of $\text{PEO}_x\text{-PPO}_y\text{-PEO}_x$ surfactants are extensively examined and shown to be highly temperature sensitive [5]. At low temperatures and concentrations, the surfactant is present as a monomer, while an increase in temperature and concentration leads to micelle formation, the size of which is influenced by temperature. A further increase in temperature and concentration leads to the formation of liquid crystalline phases [5].

Self-assembled structures of caseinates and β -caseins [6] or $\text{PEO}_x\text{-PPO}_y\text{-PEO}_x$ [7] were shown to be useful carrier systems for physiologically active components such as nutraceuticals or pharmaceuticals. Self-assembly in mixtures of triblock copolymers and various surfactants were examined [8–11]. Properties of mixtures of caseins and classical surfactants were investigated at interfaces [12]. Moreover, their ability to stabilize foams [13,14] and emulsions [15,16] were thoroughly studied. To the best of our knowledge, only one study investigated the self-assembly of triblock copolymers and caseins [17], namely β -casein, a natural diblock copolymer, mixed with $\text{PEO}_{101}\text{-PPO}_{56}\text{-PEO}_{101}$ (Lutrol).

In this study, we examined the surface properties, miscibility and self-assembly of α_s -casein and $\text{PEO}_{13}\text{-PPO}_{30}\text{-PEO}_{13}$. This polymer was chosen because it is easy to handle as it is liquid and water-soluble. Furthermore $\text{PEO}_{13}\text{-PPO}_{30}\text{-PEO}_{13}$ seems to be a good starting point to examine the influence of the PEO resp. PPO content in the molecule systematically in further studies. The triblock copolymer-like structure of the α_s -casein and the results of Portnaya et al. [17] led us to hypothesize that α_s -casein forms mixed aggregates with other block copolymers due to structural similarities. There are however two important differences in comparison to the study of Portnaya et al. [17]. First, we examined α_s -casein, a triblock copolymer rather than using β -casein, a diblock copolymer and second, the $\text{PEO}_x\text{-PPO}_y\text{-PEO}_x$ triblock copolymer used in our study is of much lower molecular weight and less hydrophilic than the one used by Portnaya et al. [17]. Hence, we will discuss our result in the context of the results obtained by Portnaya et al. [17] in terms of the different molecular architectures.

2. Materials and methods

2.1. Preparation of surfactant solutions

Solutions were prepared in 20 mmol/l imidazole/HCl buffer (Carl Roth GmbH, Karlsruhe, Deutschland) at pH 6.6. $\text{PEO}_{13}\text{-PPO}_{30}\text{-PEO}_{13}$ (Tradename PE6400, BASF, Ludwigshafen, Germany) with a molecular weight of 2900 g/mol. It was used as received. The α_s -casein rich casein with molecular weight of 23,000 g/mol, a protein content of 0.689 ± 0.013 g protein/g powder and a composition of 0.539 ± 0.013 g/g α_s -casein, 0.318 ± 0.011 g/g β -casein and 0.15 ± 0.017 g/g κ -casein was fractionated and protein stock solutions were prepared as described previously [18,19]. It should be mentioned, that α_s -casein is composed of α_{s1} - and α_{s2} -casein, but an analysis of the α_{s1} - and α_{s2} -casein content is not possible with the method used in this study. Henceforth the α_s -casein rich casein fraction is referred to α_s -casein. Below, both PE6400 and α_s -caseins are referred to as surfactants. Double-distilled water was used throughout the entire study unless stated otherwise. All glassware was soaked in diluted Deconex 11 universal solution (Borer Chemie AG, Zuchwil, Switzerland) overnight and rinsed extensively with double-distilled water prior to experiments.

2.2. Surface pressure

Monolayers were prepared and compressed by a Langmuir–Blodgett trough (KSV Minitrough, KSV NIMA, Espoo, Finland) made of Teflon with two moveable barriers and a surface

of 243.02 mm². The surface pressure π was measured by a Wilhelmy plate. Aliquots of 30 μl of the surfactant solutions having a concentration of $c = 0.45$ mmol/l were spread at the surface. After an equilibration time of 30 min, the monolayer was compressed by moving the barriers at a speed of 20 mm/min while the surface pressure was recorded as a function of the surface area A . Values of the calculated elasticity E were smoothed using a moving average filter with a span of 10 (Matlab, Ver. 6.0, Mathworks, Natick, USA).

The Langmuir–Blodgett trough was cleaned with water, ethanol (Carl Roth GmbH, Karlsruhe, Germany) and chloroform (Sigma–Aldrich Chemie GmbH, Steinheim, Germany). The success of the cleaning process was determined by measuring the surface pressure of water as a reference. Water with a conductivity of 0.055 $\mu\text{S}/\text{cm}$ (Purelab Classic, Elga) was used for the experiment.

2.3. Surface tension

The surface tension of the samples was measured using the du Noüy ring method (STA-1 Sinterface Technologies, Berlin, Germany). Prior to each measurement, the surface tension of water was measured to ensure that the ring and the vessel were surfactant-free. Surface tension was recorded as a function of time. Corrections after Harkins and Jordan were used [20]. The surface tension of water was determined as $71.41 \pm 0.05 \times 10^{-3}$ N/m at 22 °C, which is in agreement with values reported in the literature [21].

In order to calculate the critical micelle concentration (CMC), results of measurements of the equilibrium surface tension $\bar{\gamma}_{\text{eq}}$ below the $\bar{\gamma}_{\text{eq}}$ plateau were fitted with a second order polynomial and the CMC was determined at the concentration c at which the polynomial fit intersected the plateau. The surface concentration Γ was calculated using the Gibbs isotherm for ionic surfactants [22],

$$\Gamma = -\frac{1}{2RT} \left(\frac{d\bar{\gamma}_{\text{eq}}}{d \ln c} \right)_{p,T}, \quad (1)$$

where R is the ideal gas constant, T is the absolute temperature and c is the concentration.

The area A occupied by every molecule at the surface is given as [22]

$$A = \frac{1}{\Gamma \cdot N_A}, \quad (2)$$

where N_A is the Avogadro constant. The maximal surface concentration Γ_{max} and the minimum surface area A_{min} respectively are found at the CMC. All calculations were carried out with Matlab (Ver. 6.0, Mathworks, Natick, USA).

2.4. Turbidity

The absorbance was measured in a UV spectrophotometer (8435, Hewlett Packard Development Company LP, Palo Alto, USA) at 400 nm as a function of temperature to determine the turbidity of the solutions. To adjust the temperature, the measurement cells of the photometer were thermostatted with a water bath (Julabo EH, Julabo, Seelbach, Germany). Sample temperature was adjusted and recorded with a thermometer (GMH 3710 High Precision Digitalthermometer, Greisinger Electronics, Regenstauf, Germany) with a PT100 probe (GTF 401, 1/10 DIN, Greisinger Electronics, Regenstauf, Germany). Disposable polystyrene cuvettes (Brand GmbH + CO KG, Wertheim, Germany) were used. Samples were allowed to equilibrate for 30 min at each temperature prior to carrying out the measurements.

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