Fluid Phase Equilibria 360 (2013) 435–438

Contents lists available at ScienceDirect

Fluid Phase Equilibria

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

Short communication

Behavior of Triton X-114 cloud point in the presence of inorganic electrolytes

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ARTICLE INFO

Article history:
Received 28 March 2013
Received in revised form
20 September 2013
Accepted 23 September 2013
Available online 2 October 2013

Keywords:
Cloud point
Nonionic surfactant
Binodal curve
Inorganic electrolytes

ABSTRACT

Nonionic surfactants display different behaviors in aqueous solution, depending on their concentration and the presence of small amounts of salts and/or ionic surfactants. Here, cloud point values of the aqueous two-phase micellar system formed by the nonionic surfactant Triton X-114 were determined in water and in McIlvaine buffer (pH 6.5), either in the presence or absence of various salts at different concentrations. The presence of salts lowered the cloud point values according to the sequence: 0.25 M $MgSO_4{\cdot}7H_2O > 0.5$ M $NaCl > 0.05$ M $MgSO_4{\cdot}7H_2O \approx 0.5$ M $CaCl_2{\cdot}2H_2O \approx 0.1$ M $NaCl > 0.1$ M $CaCl_2{\cdot}2H_2O$. These results demonstrate that the presence of electrolytes influences the cloud point of Triton X-114 system and that knowledge of this parameter is paramount before starting partitioning studies.

1. Introduction

Aqueous two-phase micellar systems (ATPMS) are formed by surfactant agents that are amphiphilic molecules composed of a hydrophilic part and a hydrophobic one. These molecules can exhibit different behaviors in aqueous solution depending on their concentration. At concentrations above the critical micelle concentration (CMC), they form aggregates known as micelles, where the hydrophobic tails flock to the interior while the hydrophilic heads remain on the outer surface so as to minimize and maximize their contact with water, respectively [1–3]. Homogeneous micellar isotropic solutions of some surfactants, when subject to certain conditions such as increased temperature and salt addition, can spontaneously separate into two immiscible aqueous phases [1,4,5].

For polyethylene oxide-derived surfactants, such as Triton X-114 (Fig. 1), a temperature increase promotes phase separation due to thermal motion of water molecules that affects interaction/solvation of micelles. Therefore, with increasing temperature micelles start to interact with each other resulting in a micellar network [5]. The temperature at which the phase separation takes place is known as the “cloud point”, whose value depends on the surfactant structure and concentration, as well as on the presence of additives [6]. It is well known that the addition of certain salts may lower the cloud point value, as a result of stronger electrostatic interactions between salts and water molecules in comparison to the hydrogen bonds between the surfactant polar heads and water molecules. In other words, water molecules are mainly addressed to solvation of salt ions and leave space for van der Waals interactions among the surfactant molecules in micelles, which thus tend to form an entangled gel-like net. Such a phenomenon, known as “salting out” [7,8], is responsible for a decrease in the cloud point.

The addition of salts, even at micromolar concentrations, can significantly influence the partitioning of a biomolecule in ATPMS. Although salts distribute almost evenly between the two phases of an aqueous two-phase micellar system, there is little but significant difference in the partition coefficient of salt ions, which may generate an electrostatic potential difference between the phases able to influence the partitioning of charged molecules [9]. The presence of inorganic salts can result in a modification of both intra and intermicellar interactions as a result of electrostatic screening [4]. As these systems have been used to extract several biomolecules

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http://dx.doi.org/10.1016/j.fluid.2013.09.053

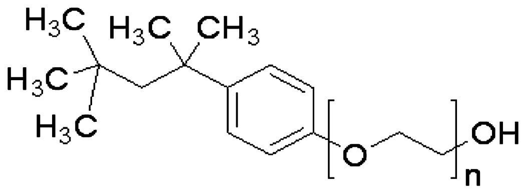

Fig. 1. Chemical structures of polyethylene glycol tert-octylphenyl ether (Triton X-114).

such as antibiotics [10,11], green fluorescent protein [12] and other proteins [13–15], the knowledge about the effect of salt addition on the binodal curve of an ATPMS is of paramount importance before studying the biomolecule partitioning. Furthermore, as various industrial sectors like the pharmaceutical and cosmetic ones employ nonionic surfactants in their formulations, knowledge of the cloud point in the presence of salts is important for formulations stability, since it can phase separate with an increase in temperature [16].

In this work, we measured the cloud points and coexistence curves of the ATPMS of the nonionic surfactant Triton X-114, either in the presence or absence of different salts. This preliminary study is part of a broader investigation aiming at the recovery of biomolecules by ATPMS formed by Triton X-114.

2. Materials and methods

2.1. Materials

Polyethylene glycol *tert*-octylphenyl ether (Triton X-114) was purchased from Sigma-Aldrich (St. Louis, MO, USA). All the other reagents were of analytical grade and used as received. All solutions were prepared in McIlvaine buffer obtained by mixing variable volumes of stock solutions of 0.2 M disodium phosphate and 0.1 M citric acid so as to ensure the desired pH (6.5). Water purified through a Millipore Milli-Q ion-exchange system (Bedford, MA, USA) was employed. The glassware used in all the experiments was washed at first with a 50:50 ethanol–1 M sodium hydroxide solution, then with a 1 M nitric acid solution, rinsed copiously with ultrapure water, and finally dried in an oven.

2.2. Experimental procedure

Experiments were carried out with Triton X-114 at different concentrations (0.5%, 1.0%, 3.0%, 5.0%, 7.0%, 9.0% and 15.0%, w/w) either in water or in Mcllavine buffer pH 6.5. The binodal curve at pH 6.5 was also determined in the presence of different salts, specifically NaCl (0.05, 0.1 and 0.5 M), $CaCl_2·2H_2O$ (0.1 and 0.5 M) and $MgSO_4·7H_2O$ (0.5 and 0.25 M). The Triton X-114 concentration varied from 0.5% to 15% (w/w) in the presence of NaCl and from 1% to 5% (w/w) for the other two salts. Cloud point measurements were performed by the method described by Albertsson [17] and modified by Blankschtein et al. [5], which consists in visually identifying the temperature at which solutions with known concentrations of a given surfactant become visually cloudy. The components of the micellar system were added to 10 mL-glass tubes so as to obtain a total mass of 3.0 g. The solutions were well mixed in an end-to-end mixer (Barnstead/Thermolyne, Dubuque, IA, USA) at 8 rpm for 1 h at room temperature. The solutions were transferred to a refrigerated bath at 10 °C (temperature at which the solutions exhibited a single and clear phase), and then the temperature was slowly raised (by 0.2 °C). The temperature at which the solution first became cloudy, indicating the onset of phase separation, was taken as the cloud point. All experiments were performed in triplicate.

3. Results and discussion

Fig. 2 illustrates the cloud point behavior of Triton X-114 solutions either in water or in McIlvaine buffer at pH 6.5 as well as in the presence of different salts.

It can be seen that the cloud point values in buffer were lower than in pure water, likely due to changes induced by the buffer salts in the intra and intermicellar interactions and, then, in the solution

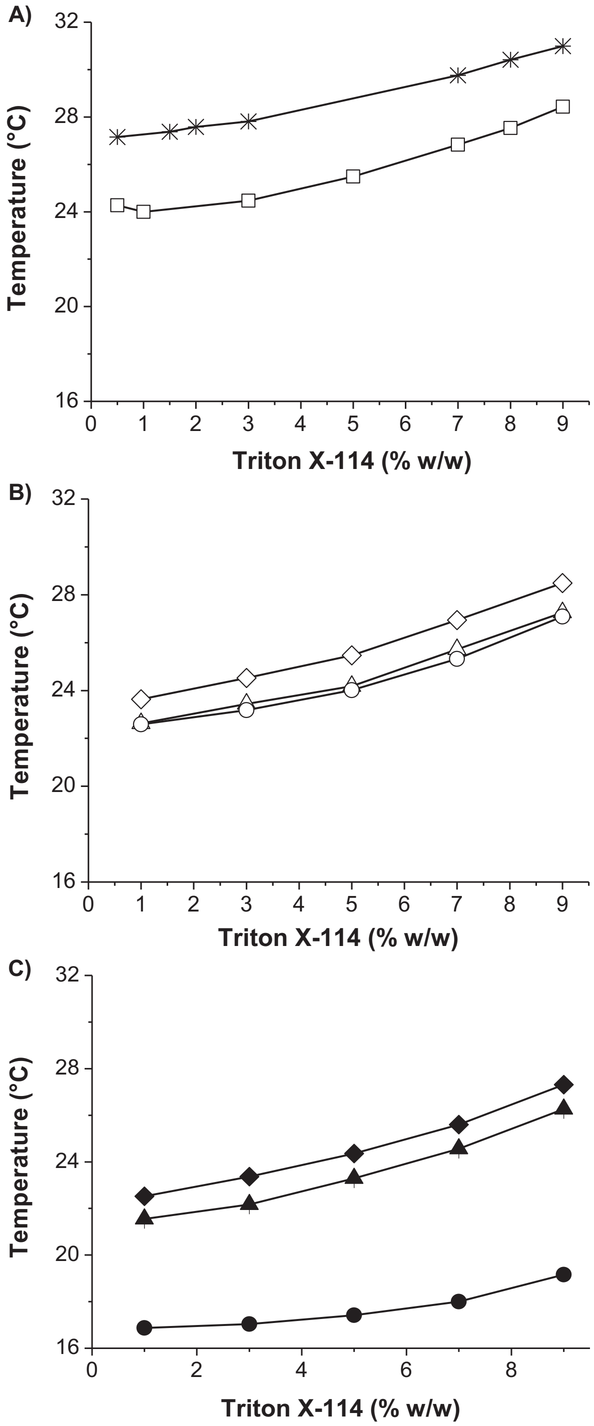

Fig. 2. Coexistence curves of the Triton X-114 system (a) in pure water (×) and in McIlvaine buffer at pH 6.5 (□); (b and c) in McIlvaine buffer at pH 6.5 in the presence of 0.1 M NaCl (△), 0.5 M NaCl (▲), 0.1 M $CaCl_2·2H_2O$ (◊), 0.5 M $CaCl_2·2H_2O$ (♦), 0.05 M $MgSO_4·7H_2O$ (○), 0.25 M $MgSO_4·7H_2O$ (●). Error bars correspond to 95% confidence level.