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Short communication

Cloud point of nonionic surfactant Triton X-45 in aqueous solution

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Abstract

Triton X-45, a nonionic surfactant with a low hydrophile–lypophile balance value and dispersible in aqueous solution at room temperature, has a Krafft point above the room temperature. The cloud point of Triton X-45 in an aqueous solution is different from the conventional aqueous nonionic surfactant micelle solution. It was further confirmed by a determination of the effect of additives on the phase behavior of aqueous solutions containing Triton X-45. The experimental fact eliminates the prevalent concept that the cloud point of Triton X-45 is below room temperature, which is helpful to exploit a biocompatible medium for a microbial growth and then for whole cell microbial transformation in a nonaqueous medium.

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1. Introduction

Krafft point of a surfactant is the temperature above which the solubility of the surfactant increases dramatically in an aqueous solution, and is interpreted as the melting point of a hydrated solid surfactant. The concept of Krafft point has been applied extensively to ionic surfactants, but has rarely been observed for nonionic surfactants [1]. At the Krafft point, the solubility of the surfactant is equal to its critical micelle concentration (CMC). Above the Krafft point, the total solubility of the surfactant increases dramatically because of the formation of micelles. However, only surfactant monomers are presented and the solubility is drastically limited at a temperature below Krafft point. Thus, a solution containing surfactant is cloudy below its Krafft point, but becomes clear at temperatures above its Krafft point.

There is a lower consolute temperature in an aqueous nonionic surfactant micelle solution at the surfactant concentration above its critical micelle concentration. The aqueous nonionic surfactant micelle solution at above a certain temperature or in the presence of a certain additives, phase separation occurs to form a surfactant dilute phase and a surfactant-rich phase or

0927-7765/\$ - see front matter © 2007 Elsevier B.V. All rights reserved. doi:10.1016/j.colsurfb.2007.07.013 coacervate phase. Such a system is called a cloud point system and the phase separation temperature is called a cloud point. The dilute phase is an aqueous solution containing surfactant monomers or micelles. The coacervate phase concentrates most of the surfactant that dissolves or swells some water [2].

Cloud point system has been utilized as a novel separation method known as cloud point extraction [3,4]. Its merits are easy to manipulate, reliable to scale up, simple and effective to operate. Especially, it provides a mild environment so that cells or proteins will not be damaged and can be exploited as a new nonaqueous medium for biocatalysis and biotransformation [5,6]. Triton X-45, a nonionic surfactant with a low hydrophile–lypophile balance (HLB) value, is dispersible in aqueous solutions and stable as a cloudy phase at a room temperature. It is believed to be a nonionic surfactant with a low cloud point [3,7] and has been applied in bioseparation process [8,9].

In the present study, we presented an experimental fact that Triton X-45 had Krafft point above room temperature and with a relative higher cloud point in aqueous solutions. The relatively higher Krafft point made the aqueous solutions containing Triton X-45 was cloudy under the room temperature condition. The cloudy phenomenon of an aqueous solution containing a nonionic surfactant at a temperature above its cloud point was different characters comparing to that of below its Krafft

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Fig. 1. Basic structure of Triton X series.

point, which had been confirmed by their biocompatibility for microbial growth and then in which for whole cell microbial transformation.

2. Experimental

2.1. Materials

Nonionic surfactant, Triton X series, with a basic molecular structure as shown in Fig. 1, were obtained from Fluke without further purification. The basic parameters that referenced from product information were listed in Table 1. Deionized water was used in determination of cloud point.

2.2. Determination of cloudy temperature

An aqueous nonionic surfactant micelle solution was heated or cooled with a temperature step of 0.5 °C in every time and then stabilization of 2 min for thermoequilibrium. Cloud point was determined visually by noting the temperature at which turbidity is observed or disappeared. The average of the temperatures of appearance and disappearance of turbidity was taken as the cloud point. But for an aqueous solution containing dispersible nonionic surfactant, such as dispersible Triton X-45 in water under room temperature, only the temperature of cloudy disappearance was taken as Krafft point.

2.3. Microbial growth in aqueous solution containing nonionic surfactant

Baker's yeast was grown aerobically at $30 \,^{\circ}$ C in two steps, i.e., activation culture in a nutriment medium for 1 day and then growing culture in a culture medium for 2 days. The nutriment medium consisted of 2 g of glucose, 2 g of peptone and 1 g of yeast extract in every100 ml of tap water. The culture medium consisted of the nutriment medium containing a mixture surfactant with a volume ratio of 10:1. The mixture surfactant is a mixture of Triton X-114 to Triton X-45 with different volume ratio. In the activation culture, 20 ml of the nutriment medium inoculated with the microorganism was filled in a 250-ml flask and shaken at 200 rpm under $30 \,^{\circ}$ C for 1 day. After the activation of culture, 2 ml of the activated culture broths was withdrawn

Table 1 Basic parameters of Triton X series

| Trade name | Average ethylene unit | Cloud point ^a (°C) | CMC (ppm) | HLB |
|--------------|--------------------------|----------------------------------|-----------|------|
| Triton X-45 | 4.5 | Dispersible | 57 | 9.8 |
| Triton X-114 | 7.5 | 25 | 100 | 12.3 |
| Triton X-100 | 9.5 | 66 | 130 | 13.4 |

^a Aqueous solution containing 1% (v/v) nonionic surfactant.

and added to each of the 250 ml flasks, which contains 22 ml of the culture media, and shaken at 200 rpm under $30 \,^{\circ}$ C. After 2 days' culture, the biomass was determined by wet cell weight (WCW) after centrifugation at 2500 rpm for 5 min.

3. Results and discussion

3.1. Cloud point of Triton X-45 in aqueous solution

The phase behavior of 10 ml water containing 3% (v/v) of Triton X-45 was changed with temperature as shown in Fig. 2. At low temperature, such as 20 °C, Triton X-45 was dispersible in the aqueous solution, and an opaque, stabile and white dispersible phase formed. It is usually misunderstood as cloud point phenomenon and the below room temperature is taken as its cloud point [3,7]. An analogue of a water-ionic surfactant mixture at a temperature below the Krafft point, the mixture Triton X-45-water system separated into two phases, i.e., an opaque hydrated solid phase was formed, after keeping the mixture at 20 °C for more than 8 h. It indicated that the temperature of 20 °C was below its Krafft point. However, with the temperature continuing to increase to $35 \,^{\circ}$ C, the dispersible phase turned into a clear, blue and transparent phase. The transparency can be detected from the background as shown in Fig. 2. However, the blue color that results from a light scatter of micelle particle cannot be detected in Fig. 2. With the temperature increase to 38 °C, the transparent aqueous surfactant solution separated into two clear phases. The same as cloud point system, the upper phase (about 2 ml) was an aqueous solution containing a little of the surfactant and the lower phase concentrated most of the surfactant with blue color. The two phases can be detected from their different refraction as shown in Fig. 2. A further increase of the temperature to 45 °C, the volume of surfactant-rich phase decreased to 0.5 ml. Water containing Triton X-45 was clear in the temperature range of about 35-38 °C. This indicates the



Fig. 2. Phase change of water containing 3% (v/v) Triton X-45 with different temperature.



Fig. 3. Cloud point of water containing different concentration of dispersible Triton X-45.

presence of a cloud point at $38 \,^{\circ}$ C, above which the solution became cloudy, and a Krafft point at $35 \,^{\circ}$ C, below which also the solution became cloudy. The presence of cloud point and Krafft point of an aqueous nonionic surfactant solution has also been reported in literature [1]. The cloudiness of surfactant dispersible in water at a temperature below Krafft point may be one of the reasons that the cloud point of Triton X-45 is believed to be below the room temperature [3,7].

Cloud points of water containing different concentration of dispersible Triton X-45 were showed in Fig. 3. The cloud point increased with the increase of surfactant concentration. The same trend has also been reported for Triton X series surfactant solutions, such as aqueous Triton X-114 and Triton X-100 solutions [10]. However, it must be pointed out that the cloud point of Triton X-45 aqueous solution is at a temperature between the cloud point of Triton X-114 and that of Triton X-100. It is conflicted with a rule that the cloud point of the same series of nonionic surfactants decreases with the decrease of hydrophile–lypophile balance (HLB) value as shown in Table 1, which indicates that there is a difference between the cloud point phenomenon of water containing dispersible Triton X-45 and that of an aqueous nonionic surfactant micelle solution.

3.2. Effect of additives on cloud point of Triton X-45

An effect of addition of the same series of nonionic surfactant, such as Triton X-100 at total surfactant concentration 5% (v/v) or Triton X-114 at total surfactant concentration 10% (v/v) at various volume ratios, on cloud points of Triton X-45 was showed in Fig. 4. The cloud point of Triton X-45 in presence of Triton X-114 or Triton X-100 exhibited two distinct regions: at the Triton X-45 volume ratio below about 40%, the mixture surfactant formed an aqueous surfactant micelle solution and has a relatively lower cloud point, whereas at the Triton X-45 volume ratio above about 40%, the mixture surfactant was dispersible in water and had a relatively higher cloud point.

In the aqueous surfactant micelle solution region, the cloud point decreased dramatically with the increase of the volume



Fig. 4. Effect of the mixture of the same series of nonionic surfactants with Triton X-45 on its cloud point. *Symbols*: diamond represents the Krafft point of aqueous solution containing dispersible nonionic surfactants; circle represents the cloud point of nonionic surfactant micelle aqueous solution; triangle represents the cloud point of aqueous solution containing dispersible nonionic surfactants. The blank one is a mixture of Triton X-114 and Triton X-45 with a total surfactant concentration 10% (v/v); the filled one is a mixture of Triton X-100 and Triton X-45 with a total surfactant concentration 5% (v/v).

ratio of Triton X-45, and the magnitudes of the cloud point were affected by the kinds of the additive surfactants, i.e., the cloud point of the mixture of Triton X-100 and Triton X-45 was higher than that of the corresponding mixture of Triton X-114 and Triton X-45. Our experimental results consisted with literature result that the cloud point of mixture Triton X-45 and Triton X-114 can be adjusted anywhere between 0 and 22 °C at Triton X-45 fraction below 15% (v/v) [8,9]. Studies on the relationship between a mixture surfactant and its cloud point have shown that the cloud point of an aqueous mixture nonionic surfactant micelle solution is at a temperature between the cloud points of those two kinds of surfactants and the magnitude is proportional to the mole ratio of those two kinds of surfactant. From the above experimental fact, it can be deduced that the cloud point of Triton X-45 should be below the room temperature. This may be one of the reasons that the cloud point of Triton X-45 is believed to be below room temperature [3,7]. In the region of water containing dispersible mixture surfactant, there were a Krafft point and a relatively higher cloud point value, which consisted with the cloud point of water containing dispersible Triton X-45 as shown in Fig. 2. The cloud point and Krafft point also decreased with the increase of the volume ratio of Triton X-45. However, the effect of the addition of different surfactants were insensitive, i.e., the addition of Triton X-100 or Triton X-114 had nearly the same value in our experimental errors as shown in Fig. 4. The change trend of cloud points was also different from the relationship about the cloud point of an aqueous surfactant micelle solution containing two kinds of mixture surfactants.

The different cloud point between aqueous nonionic surfactant micelle solution and aqueous solution containing dispersible nonionic surfactant was further testified by addition of glycerol



Fig. 5. Effects of glycerol concentration on cloud point of aqueous glycerol solution containing 5% (v/v) dispersible Triton X-45.

as shown in Fig. 5. Polar organic solvent, such as low molecular weight of polyethylene glycol and glycerol, are widely used to increase the solubility of poorly water-soluble compound in an aqueous solution. Thus, the solubility of hydrophobic part of surfactant, and therefore the surfactant as a whole, will be increased in those solutions. Thus an addition of glycerol should lead to an increase of cloud point of an aqueous surfactant micelle solution. This was confirmed by many experiment facts in literatures [11,12]. However, the cloud point of aqueous glycerol solution containing dispersible Triton X-45 decreased with the increase of glycerol fraction in our experimental concentration range. It further indicated that the cloud point of the aqueous solution containing dispersible Triton X-45 was different from that of aqueous surfactant micelle solution.

3.3. Biocompatibility of aqueous media containing nonionic surfactant

Cloud point system has been exploited as a novel two-phase partitioning system for biotransformation [13]. The biocompatibility of a nonionic surfactant in an aqueous solution is a precondition for whole cell microbial transformation involving cofactor regeneration and/or multiple enzymes catalysis process. Although the mechanism about the cloud point of an aqueous solution containing dispersible Triton X-45 remains unclear, distinguishing the cloudiness of an aqueous Triton X-45 system between above its cloud point and below its Krafft point, at least, makes sense for development of the nonionic surfactant in an aqueous solution as a novel nonaqueous medium for whole cell microbial transformation. As shown in Fig. 6, at the volume ratios of Triton X-45 below 40%, the cloud point of the aqueous surfactant micelle solution was lower than the microorganism growing culture temperature and a relatively higher biomass was achieved. However, with the volume ratios of Triton X-45 increase to the ranges of the nutriment medium containing dispersible surfactant, the biomass decreased rapidly in our experimental errors. The inhibition of volume ratio of Triton X-



Fig. 6. Cell growth in aqueous solution containing different volume ratio of mixture surfactant. The total volume surfactant fraction is aqueous solution to mixture surfactant 10:1. The filled bar is the biomass in cloudy surfactant system of nonionic surfactant micelle solution at a culture temperature above its cloud point. The open bar is the biomass in cloudy surfactant system of aqueous solution containing dispersible surfactant at a culture temperature below its Krafft point.

114 to Triton X-45 in the aqueous solution on the baker's yeast growth was fitted into the range of aqueous solution containing dispersible mixture surfactant as shown in Fig. 4. It indicated that the nutriment medium containing dispersible mixture surfactant was non-compatible for the microorganism growing culture. The biocompatibility of a nonionic surfactant to the microorganism is related to its phase structure in an aqueous solution. The mixture nonionic surfactants in an aqueous solution formed a cloud point system under the culture temperature, which was biocompatible. It has been developed as a novel nonaqueous medium for the microbial transformation of acetophenone to produce chiral 1-phenylethanol by the viable whole cell, in which the toxic substrate/product to the microorganism are in situ extracted to the coacervate phase [14]. The biocompatibility of Mycobacterium sp. in a cloud point system has also been reported in our previous works [5,15].

4. Conclusions

Triton X-45, with Krafft point above the room temperature and dispersible in aqueous solution, shows a different cloud point from that of an aqueous surfactant micelle solution. Although the mechanism behind the cloud point needs further study, the experimental fact is helpful to exploit a biocompatible medium for a microbial growth and then for the whole cell microbial transformation.

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