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Transferring of red *Monascus* pigments from nonionic surfactant to hydrophobic ionic liquid by novel microemulsion extraction

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ABSTRACT

Nonionic surfactant micelle aqueous solution as well as cloud point system is a potential separation/reaction medium. Downstream processing for recovery of product and regeneration of surfactant is indispensable. Adjustment of pH in the dilute phase of cloud point extraction is a common strategy for separation of ionizable organic compound from nonionic surfactant. However, this strategy is no longer suitable for recovery of ionizable organic compound with strong hydrophobicity due to the high solubilization of the ionic state of hydrophobic organic compound in micelles. Setting extractive fermentation of intracellular *Monascus* pigments in nonionic surfactant micelle aqueous solution to produce high color hue of red *Monascus* pigments as an example, transferring of hydrophobic *Monascus* pigments from nonionic surfactant to ionic liquid by novel hydrophobic ionic liquid–nonionic surfactant–water Winsor I microemulsion extraction and then back-extraction of the ionizable *Monascus* pigments from the ionic liquid by ionic liquid–water two-phase extraction were carried out. Nearly 80% yield was achieved in the complete process for recovery of *Monascus* pigments. It provides a novel strategy for stripping of hydrophobic ionizable organic compound from nonionic surfactant micelle aqueous solution.

1. Introduction

Surfactant forms micelles in an aqueous solution. Surfactant micelles have the capability to solubilize various species with a very broad polar spectrum, which makes surfactant micelle aqueous solution study extensively as reaction and separation media, such as micellar catalysis for solving the agent incompatibility in preparative organic chemistry [1–3]. Especially, nonionic surfactant micelle aqueous solution separates into a dilute phase and coacervate (surfactant-rich) phase above a certain temperature. This two-phase system is known as cloud point system and the phase separation temperature is called cloud point. Application of cloud point system for extraction of metal ions, organic compounds, and biomaterials is a well known technique in analysis field [4]. Due to the excellent biocompatibility of nonionic surfactant, nonionic surfactant micelle aqueous solution is also applied as a non-aqueous medium for enzymatic reaction, such as elimination of lignin inhibitor in the enzymatic saccharification of cellulose [5]. Our group has developed cloud point system as a novel medium of extractive fermentation for enhancement of hydrophobic compound solubility, elimination of product inhibition, and prevention of product from further degradation [6,7].

Submerged culture of *Monascus anka* produces at least six molecular structures of pigments, which are classified into three groups based on their colors, i.e. yellow pigments (monascin and ankaflavin), orange pigments (monascorubrin and rubropunctation), and red pigments (monascorubramine and rubropuntamine) [8]. The major components of *Monascus* pigments are strongly depended on the nutrients in culture medium as well as culture condition (such as pH). The hydrophobic *Monascus* pigments are majorly accumulated intracellularly in conventional submerged aqueous culture. Recently, nonionic surfactant micelle aqueous solution is further developed for extractive fermentation of intracellular *Monascus* pigments [9], in which secretion of intracellular product and regulation of secondary microbial metabolites are fulfilled at the same time [10,11]. Especially, extractive fermentation in the nonionic surfactant Triton X-100 micelle aqueous solution with high MSG (monosodium glutamate) concentration produces the high color hue of red *Monascus* pigment derivates with MSG (monosodium glutamate) residue (Fig. 1). The *Monascus* pigments are ionizable products with relatively strong hydrophobicity (log*P* is approximately 2 for its nonionic form while 0.3 for its ionic form [12]). Organic product in its ionic state has very limited

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solubilization in the nonionic surfactant micelles or the coacervate phase of cloud point system. Thus, most of ionizable products can be back-extracted from nonionic surfactant micelle aqueous solution by cloud point extraction via the adjustment of pH in dilute phase [13–17]. However, hydrophobic ionizable product in its ionic state prefers to partition into the coacervate phase of cloud point system [18–20], which makes adjustment of pH no longer suitable for back-extraction of the hydrophobic ionizable product from nonionic surfactant.

Thanks to the discovery of novel physical and chemical properties of ionic liquids (ILs), such as hydrophobic IL [Bmim]PF_6 (1-butyl-3-methylimidazolium hexafluorophosphate) exhibiting character of organic solvent. The organic solvent character of hydrophobic ILs has already utilized as a medium for hydrophobic IL–water two-phase extraction [21]. Meanwhile, hydrophobic IL [Bmim]PF_6, which replaces the organic solvent in organic solvent–nonionic surfactant Triton X-100–water ternary system, forms a novel [Bmim]PF_6-nonionic surfactant Triton X-100–water ternary Winsor I microemulsion, where oil in water microemulsion phase (W_m) coexists with excess IL phase [22]. The corresponding conventional Winsor I microemulsion (organic solvent–nonionic surfactant–water ternary system) extraction has been studied extensively for stripping of organic product from nonionic surfactant micelle aqueous solution. However, there is conflict between the extractive capacity of excess oil phase and the forming of W_m phase. In generally, relatively strong polar organic solvents have high extractive capacity while the nonionic surfactant is also partially extracted into the excess oil phase [23]. Similar to the conventional Winsor I microemulsion extraction, hydrophobic ILs provide the chance for transferring organic product from nonionic surfactant micelle aqueous solution to excess IL phase by the novel Winsor I microemulsion extraction. Furthermore, the peculiar characters of hydrophobic ILs also lead to the novel Winsor I microemulsion extraction have some potential advantages, such as relatively high phase transfer temperature (81 °C) [24] for operation at room temperature, very limited solubility of [Bmim]PF_6 in water at room temperature for removal of the contaminated nonionic surfactant in the excess IL phase by washing with water [25], and extraction of organic compounds with broad polar spectrum [21].

In the present work, recovery of *Monascus* pigments (Fig. 1) from extracellular broth after extractive fermentation in nonionic surfactant micelle aqueous solution was set up as a model of stripping hydrophobic ionizable organic product from nonionic surfactant micelle aqueous solution. First, the impossibility of recovery of *Monascus* pigments from nonionic surfactants by adjustment of pH in cloud point extraction was checked experimentally. Then, the effect of pH on transferring of *Monascus* pigments from nonionic surfactant micelle aqueous solution to excess [Bmim]PF_6 phase by Triton X-100–[Bmim]PF_6–water Winsor I microemulsion extraction was examined. Finally, adjustment of pH for back-extraction of *Monascus* pigments from IL by [Bmim]PF_6–water two-phase extraction was demonstrated.

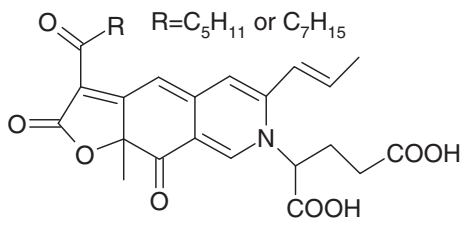

Fig. 1. Red *Monascus* pigment derivates with MSG residue.

2. Materials and methods

2.1. Extractive fermentation of Monascus pigments

Extractive fermentation of red *Monascus* pigment derivates with MSG residue (Fig. 1) in Triton X-100 micelle aqueous solution was conducted following in a similar procedure to our previous work [10]. *M. anka* (China Center of Industrial Culture Collection, CICC 5013) was maintained on potato dextrose agar (PDA) medium (potato 200 g, glucose 20 g and agar 15–20 g, per liter of tap water) at 4 °C. Inoculum culture was conducted at 30 °C and 200 rpm for 30 h in a 250 ml Erlenmeyer flask with 50 ml of working volume. The medium composition of inoculum culture was glucose 20 g, yeast extract 3 g, peptone 10 g, KH_2PO_4 4 g, KCl 0.5 g, and $FeSO_4{\cdot}7H_2O$ 0.01 g (per liter of tap water). One milliliter of inoculum culture was added into 250 ml Erlenmeyer flask containing 50 ml of work volume. The fermentation culture medium consisted of glucose 50 g, KH_2PO_4 5 g, $CaCl_2$ 0.1 g, $FeSO_4{\cdot}7H_2O$ 0.01 g, MSG 30 g, and Triton X-100 50 g, per liter of tap water. The initial pH was adjusted to 4.5 with 10% (V/V) hydrochloric acid. The flask was shaken at 30 °C and 200 rpm for 7 days. After submerged culture, fermentation broth was centrifuged to removal of mycelia and the clear supernatant was used for the following downstream processing.

2.2. Stability of Monascus pigments

After extractive fermentation, one milliliter of broth was put into 10 ml tube, which was adjusted with 20% (V/V) hydrochloric acid or 2 M NaOH to different pH ranging from 2 to 12. The tubes were incubated in 30 °C, shaken at 200 rpm for overnight. The broths with different pH as well as 1 ml of original fermentation broth were diluted with 70% aqueous ethanol solution (V/V, pH = 2) to volume of 2 ml. *Monascus* pigments (Fig. 1) had a broad absorbance with two peaks at approximately 430 nm and 510 nm (referring in Fig. 2A). The *Monascus* pigments concentration was determined at 510 nm by visible spectrum analysis via proper dilution with 70% aqueous ethanol solution (V/V, pH = 2). The pH stability of *Monascus* pigments was represented by the fraction of residual pigments at a certain pH to the original extracellular fermentation broth.

2.3. Cloud point extraction

The pH of clear supernatant of fermentation broth was approximately 7, which was adjusted with 20% (V/V) hydrochloric acid or 2 M NaOH to different pH ranging from 2 to 10. The broth with a certain pH (1 ml) was put into 10 ml tube and incubated into 65 °C (above the cloud point of the extracellular fermentation broth containing nonionic surfactant) water bath for at least 3 h. The broth was separated into two phases, where the lower one was coacervate phase and the upper one was dilute phase. The dilute phase was transferred into another tube. In order to compare the partitioning of *Monascus* pigments between the coacervate phase and the dilute phase of cloud point system based on the same volume, 70% aqueous ethanol solution (V/V, pH = 2) was added into the coacervate phase and the dilute phase to volume of 2 ml, respectively. The *Monascus* pigments concentration was determined at 510 nm by visible spectrum analysis. Meanwhile, *Monascus* pigments concentration in the fermentation broth was also determined at 510 nm. Thus, the fraction of *Monascus* pigments in the coacervate phase or dilute phase was determined.

2.4. Winsor I microemulsion extraction

The pH of fermentation broth was adjusted with 20% (V/V) hydrochloric acid or 2 M NaOH to different pH ranging from 2 to