Mixed Reverse Micellar Systems (MRMS) for Extraction of Erythromycin: Effect of Surfactant Concentration on Forward Extraction Step

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Abstract—Mixed reverse micellar systems (MRMS) of sodium bis(2-ethylhexyl)sulfosuccinate (AOT) and zwitterionic surfactants 3-(*N*,*N*-Dimethyloctadecylammonio) propanesulfonate (SB3-18) in iso-octane were used for the forward extraction of erythromycin from the aqueous phase to organic phase for the first time. The effect of surfactant concentration on the percentage of erythromycin transferred was investigated in detail. It was found that sufficient AOT (20 g/L) concentration was required for the solubilising of erythromycin in MRMS. Erythromycin was successfully solubilised in MRMS with higher percentage of erythromycin transfer which was 98.13% at 60.0 g/L of AOT concentration.

Keywords— Mixed reverse micellar, extraction, zwitterionic surfactants, Erythromycin

1. INTRODUCTION

Erythromycin is classified as a macrolide antibiotic which is normally produced by Saccharopolyspora through fermentation process [1]. Erythromycin is extensively used due to its benefit of low side effect and good curative effect, and it is also utilized as the raw material for second and third generation of semi-synthetic derivatives of macrolide [2]. Erythromycin is generally purified from fermentation broth via three conventional steps: filtration (removal of biomass), extraction (recovery) and subsequent crystallization (purification). In the industrial, butyl acetate solvent extraction is one of the prominently used techniques. However, there are certain drawbacks associated with the use of solvent extraction which including high consumption of chemicals as solvent and less cost effective [3]. Therefore, it is very significant to explore n alternative method in order to establish a successful erythromycin recovery process.

Reverse micellar extraction is another attractive liquid-liquid extraction method for biological products, as many biochemicals including amino acids, proteins, enzymes, and nucleic acids can be solubilized within and recovered from such solutions without loss of native function/activity [4]. Reverse micelles system consists of several components including surfactant, co-surfactant, oil, and water. They are aggregates of surfactant molecules with an inner core of water molecules dispersed in a continuous organic solvent medium [5]. Reverse micelle technique is based on two steps: i) a target protein is selectively solubilized into the organic phase

(forward extraction), and ii) the protein is subsequently stripped into the aqueous phase (backward extraction) by the addition of fresh aqueous buffer, also called stripping solution. Factors affecting the performance of reverse micelle system are rather complicated, including the nature and concentration of the target protein, pH, the concentration and species of ions, type and concentration of surfactant, and the composition of reverse micelles [6].

Today, reverse micelle has increasingly receiving attention from researchers in proteins extraction, owing to its impressive potential for continuous operation and scaling up [7]. Previously, a published literature reported that the addition of second surfactant into ionic surfactant could modify the interface and produce considerable change in the elastic rigidity of the interface [8]. Mixed reverse micelle formed by ionic and zwitterion surfactants is a new discovery in colloid and surface area which aims to form more flexible system in the physicochemical properties compared with single-surfactant reverse micelles. According to a previous research, mixed reverse micelles possess better synergistic performance [9]. In the present paper, we attempted the use of mixed reverse micelle from ionic AOT and SB3-18 zwitterion surfactant to extract erythromycin from the aqueous phase, with a purpose to evaluate the effectiveness of combining two types of surfactants in antibiotic extraction.

2. METHODOLOGY

A. Chemicals

In this research, erythromycin powder was obtained from Sigma Aldrich and iso-octane was used as solvent. Sodium bis(2-ethylhexyl)sulfosuccinate (AOT) as the anionic surfactant and 3-(*N*,*N*-Dimethyloctadecylammonio) propanesulfonate (SB3-18) were purchased from Sigma Aldrich. The structure of erythromycin, AOT and SB3-18 are presented in Figure 1.

B. Methodology

The aqueous phase that consisted of potassium chloride, KCL was obtained from E. Merk. For forward extraction, 5g/L erythromycin was dissolved in the aqueous solution at certain amount of KCL. Forward extraction was carried out by slowly injecting 10 mL of erythromycin solution into 10 mL AOT/SB3-18/iso-octane solution by stirring the solution until a clear phase was obtained (30 min). All experiments were carried out at room temperature. Flowchart of the experiment is shown in Figure 2.

3. RESULTS AND DISCUSSIONS

A. Effect of AOT Concentration

In the current study, erythromycin solubilisation in aqueous phase using MRMS AOT/SB3-18 was attempted. To study the effect of surfactant concentration, AOT was introduced at various concentrations (0.0 – 200.0 g/L) by maintaining the pH, salt concentration, erythromycin concentration, and molar ratio as shown in the Figure 3. When MRMS system of AOT/SB3-18 was used, clear phases were formed and solubilisation of erythromycin was generated in higher percentage. At 20.0 g/L of AOT concentration, erythromycin started to solubilize with the percentage transferred was 60.90 %. Therefore, we can hypothesize that the critical micelle concentration (CMC) of mixed AOT-SB3-18 surfactant was between 0.0-20.0 g/L of AOT which was lower than CMC of a single AOT (40g/L) [10]. Thus, another test for accurate value of CMC is required.

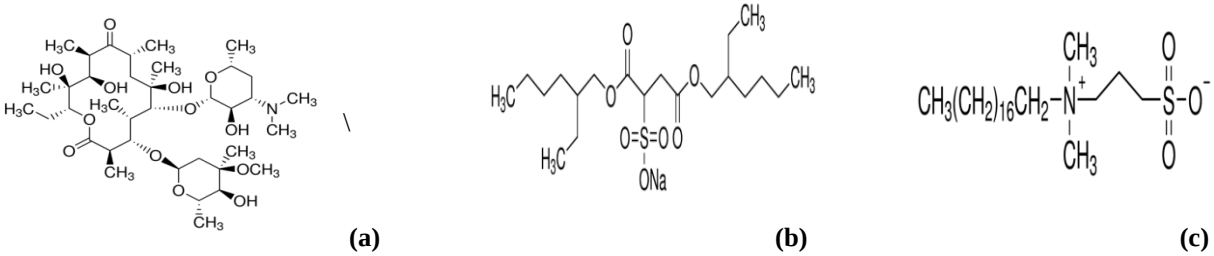

Figure 1: Structure of (a) erythromycin, (b) AOT, (c) SB3-18

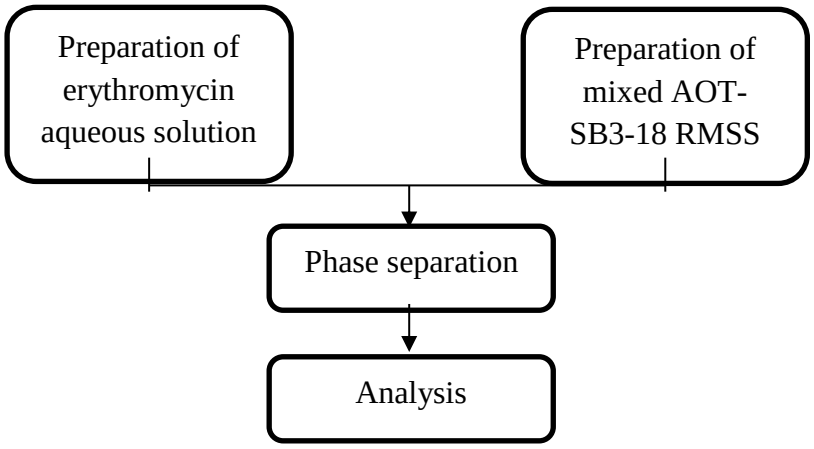

Figure 2: Flowchart of the MRMS experiment

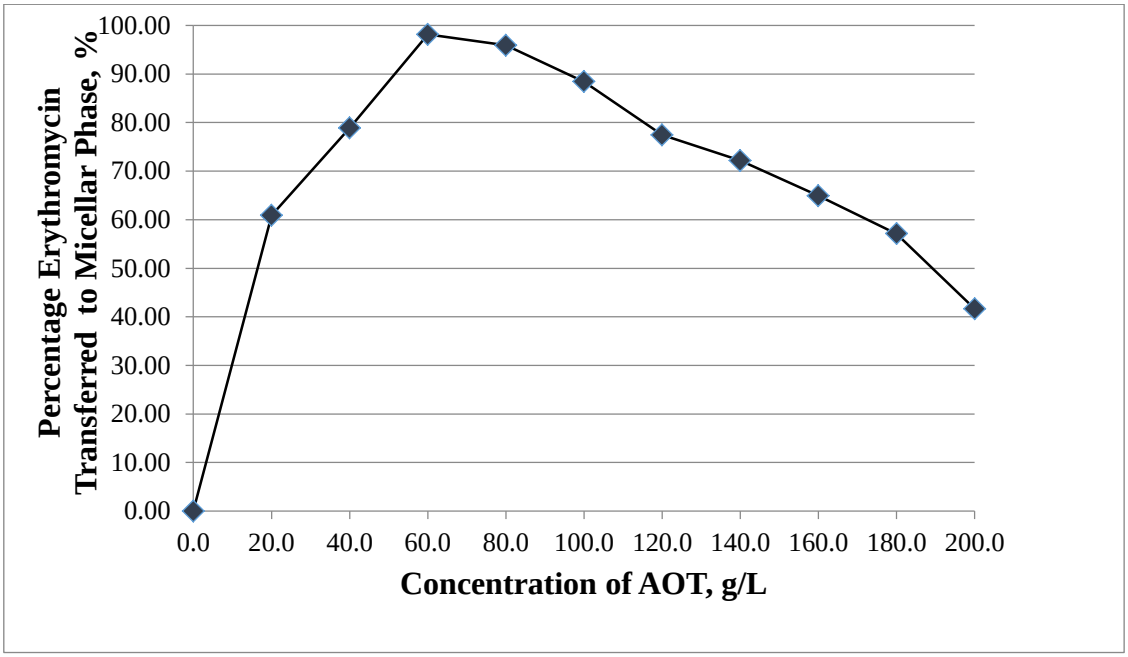

Figure 3: Final concentration of Erythromycin, E_{of} lies in the organic phase at different surfactant concentrations. Experimental conditions: Initial concentration of Erythromycin in aqueous phase, E_{ai}, 5g/L; amount of KCl, 10 g/L; molar ratio AOT:SB3-18, 10:1; pH, 8.5±0.1; stirring speed, 350 rpm; stirring time, 30 minutes; room temperature.

The critical micelle concentration (CMC) is defined as the concentration of a surfactant at which micelles start to aggregate and form micelles in the solution [11]. When the different types of surfactants are mixed together, frequently, it would display synergism or cooperative interactions in their effects on the properties of the system [12]. This synergism can be attributed to non-ideal mixing effects in the aggregates, which results in CMC of mixed micelle solution that, are substantially lower than a pure component alone [13]. As stated by Nagarajan [14], the mixture of ionic and non-ionic surfactants led in the decrease of electrostatic interactions and formed larger size micelles than the two pure component micelles. It was noted that reverse micelles extraction was controlled by electrostatic, steric and hydrophobic interactions between the bio-molecules and micelles [15]. The reduction of interactions in the repulsive head group happening at the surface resulted in larger size micelles than in either of the pure component micelles, hence the CMC value of the mixture can be lesser than that of either of the pure components.

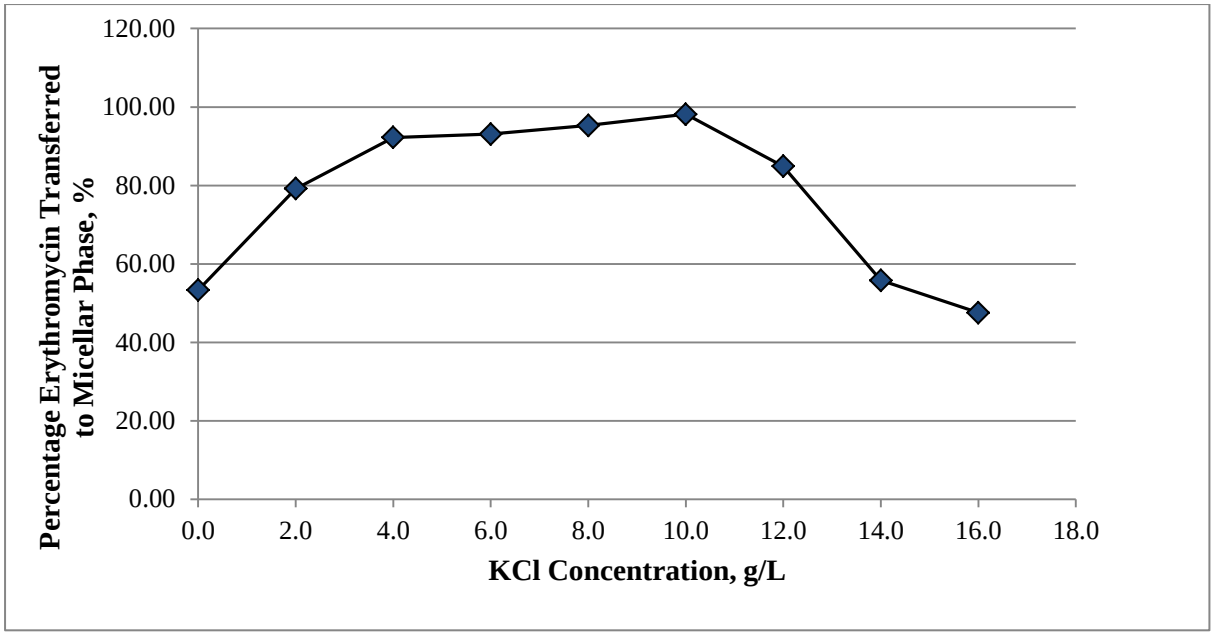

Figure 4: Final concentration of Erythromycin, E_{of} lies in the organic phase at different KCL concentrations. Experimental conditions: Initial concentration of Erythromycin in aqueous phase, E_{ai}, 5g/L; 60 g/L erythromycin concentration, molar ratio AOT:SB3-18, 10:1; pH, 8.5±0.1; stirring speed, 350 rpm; stirring time, 30 minutes; room temperature.

The resulted aqueous solution after the solubilisation of erythromycin was observed to be clear phases and a transparent interface when the concentration of AOT was varied from 20-140 g/L, thereby indicative of the successful solubilisation of erythromycin in the reverse micelle phase. However, increasing the AOT concentration from 80.0 - 200.0 g/L has significantly dropped, the percentage of erythromycin solubilized erythromycin from 95.87 % to 41.65 % and slight precipitation was observed at the interface of the solution. As clarified by Harikrishna *et al.* [15], the reasons for lower extraction yield at high surfactant concentrations in the forward extraction were because of the de-assembling of reverse micelles due to intermicellar collision and hindrance to the diffusion of solute by surfactant aggregates. Meanwhile, Juang *et al.* [16] appointed that, the bio-molecule solubilisation will increase as the surfactant concentration is increased because of the increment in the size of the micelle. However, an increase in the size of the reverse micelles would lead to the decrease of steric hindrance of the reverse micelles and the increase of the transfer of large protein molecules which are known to be the predominant contaminants.

The solubilisation percentage obtained was in the range of 41.65- 98.13% in the MRMS system. High solubility of erythromycin in mixed micelle solution shows that MRMS technique has a high potential for antibiotic recovery at low surfactant concentration. Zwitterion surfactant was expected to reduce the inhibitory power of AOT on enzyme by diluting the surface charge density at the interfacial region [17]. Previous researchers reported, the use of classical ionic surfactants may lead to protein unfolding, due to strong electrostatic interactions between the polar head groups and the protein charges [18]. Therefore, to weaken the interaction, zwitterion surfactant molecule was introduced to facilitate the solubilisation of erythromycin in the organic phase.

B. Effect of Salt Concentration

A present of salt is necessary to form stable reverse micelles solution. The presence of salt is also important to reduce the repulsive forces between two surfactant heads which will interfere with the electrostatic interaction between surfactant head group and bio-molecules. The percentage of erythromycin transferred to micellar phase was found to increase in KCl concentration from 2.0 until 10.0 g/L.

According to Zhao *et al.* [19], KCl salt is water structure forming and also causes lesser ‘screening’ of solutes, therefore is favourable for forward extraction in reverse micelle and hence has been chosen for use in the study. The erythromycin transferred of to micellar phase became lower when the KCl salt was raised from 12.0 to 16.0 g/L which could be explained by size exclusion effects. As the ionic strength increased, the electric double layer

adjacent to the hydrophilic headgroups became thinned which resulted in the reduction of the electrostatic repulsion force between the charged head groups of the surfactants [20]. This effect increased the tendency of smaller reverse micelles droplets in the organic phase (squeezing out effect) and thus the bio-molecules with a larger size were excluded [20], which is usually known as size exclusion effect.

4. CONCLUSIONS

Experiments on the solubilisation of erythromycin into mixed reverse micelles of AOT and SB3-18 have been carried out. Forward extraction was perfomed with MRMS and resulted in good solubilisation of erythromycin in term of percentage of erythromycin transferred. The results remark that surfactant concentration is the important parameter affecting the solubilisation of erythromycin into mixed reverse micelle phase. We found that extracting erythromycin using the AOT reverse micelle system, will not occur if insufficient AOT and SB3-18 concentration was used. In this study, 20.00 g/L was the minimum amount of AOT required. The maximum erythromycin transferred (98.13%) was obtained at 60.0 g/L AOT concentration. The present findings proved that erythromycin was successfully solubilised in MRMS which is valuable for next step of backward extraction (recovery process).

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