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SEPARATION OF PHARMACEUTICAL PRODUCTS WITH REVERSE MICELLES

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ABSTRACT

Reverse micelle extraction has received considerable attention in recent years due to its ability to selectively solubilise solutes from an aqueous phase and to maintain their biological activities. This paper presents the results from studies on extraction of penicillin G from aqueous solution (forward extraction) and from reverse micelles to a new aqueous solution (backward extraction), by employing the principle of liquid-liquid extraction with reverse micelles. AOT is used as a surfactant and isooctane is used as an organic phase. The effect of pH, salt concentration, AOT concentration, and initial penicillin concentration on the penicillin forward and backward transfer at 21°C was studied. The results show that the solubilisation of penicillin in the forward extraction is dependent on both pH and surfactant concentration. The backward extraction is dependent on ionic strength, surfactant concentration and the initial amount of penicillin in the organic phase before backward extraction. Despite successful results in the backward extraction, the overall extraction yield of penicillin is found to be less than the initial amount of penicillin before forward extraction due to difficulties during the forward extraction process.

KEYWORDS

Antibiotic; Surfactant; Extraction; Partition coefficient; Reverse micelle; Solubilisation

INTRODUCTION

The production of antibiotics typically involves a fermentation process and is usually capital intensive because large and complex fermentors and extensive equipment for multi-step downstream processing are required to handle large volume fermentation broths with a low product concentration [1]. In the separation of penicillin G from fermentation broth, a solvent extraction method has been used by industry for many years. Nabais and Cardoso [2] noted that the biggest concern in current solvent separation technique is the frequent formation of stable emulsions, which are difficult to treat with conventional techniques such as gravitation or centrifugation. These problems lead to others, such as contamination of the final product, low extraction yield, high solvent losses, and clogging of the equipment. The reverse micelle extraction technique has potential to be an alternative to conventional liquid-liquid extraction for the separation and purification of penicillin. In recent years it has been used successfully and widely in the separation of proteins [3], amino acids [4] and enzymes [5]. The overall reverse micelle liquid-liquid extraction process consists of two fundamental steps: 1) a forward extraction in which penicillin G is transferred from an aqueous solution containing KCl into a reverse micelle organic phase containing AOT and isooctane; and, 2) a back extraction in which the penicillin G in the reverse micelle is released into a fresh aqueous phase containing KCl. In this paper, we investigate the parameters such as pH, salt concentration, surfactant concentration, and initial penicillin concentration that affect solubilisation of penicillin molecules in the forward and backward extraction processes.

METHOD

Reagent grade sodium di-2-ethylhexyl sulfosuccinate (AOT) was used as the surfactant, the organic solvent used for the reversed micelles was analytical grade isooctane, and the biomolecule was penicillin G sodium salt. CaCl_2 and KCl were used as salts in the aqueous phase. All chemicals were supplied from Sigma Aldrich UK and were used as received. All experiments were conducted at a temperature of $23 \pm 1^\circ\text{C}$. 5 ml of aqueous solution containing penicillin and KCl was contacted with an equal volume of isooctane containing AOT. The solutions were then mixed for 10 minutes using a magnetic stirrer at 400 rpm and was left for the phases to separate before the organic phase was removed. All experiments were replicated from two to four times in order to assess the reproducibility of the results. The concentration of penicillin in the separated organic and aqueous phases was measured using the Kjeldahl method. Surface tension measurements were performed using a Wilhelmy Ring connected to a tensiometer (White Electrical Instrument Co. Ltd). In backward extraction, the organic phase solution from forward extraction was used to contact with a new aqueous phase. The solution was mixed for 10 minutes using a magnetic stirrer before analysis.

RESULTS AND DISCUSSION

AOT is used as the surfactant due to its ease of forming reverse micelles, its stability in comparison with other surfactants, and has been used in many published studies of other systems with success [6]. The critical micelle concentration (CMC) of the AOT in isooctane is 40 g/l.

Forward Extraction of Penicillin G Using Reverse Micelles

At different AOT concentrations, there was an increase in the volume of the organic phase that was proportionate to a decrease of the aqueous phase volume. The volume of the organic phase increased when concentrations of AOT >40 g/l were used. A sought after effect of the AOT concentration on penicillin extraction is to increase the amount of penicillin transferred to the reverse micellar phase. Surfactant concentration controls the number of reverse micelles and hence the capacity to solubilise biomolecules into the reverse micelle pool [10]. Figure 1 shows that increasing the concentration of AOT increases the amount of penicillin in the organic phase. There is no optimum AOT concentration as the amount of penicillin extracted was almost in proportion to the amount of AOT used. However, use of AOT concentration >445 g/l produced an emulsion that was stable. Figure 2 shows the relation between the concentration of penicillin in the organic phase and the pH value at the equilibrium state. For all concentrations of AOT, the results show a maximum penicillin concentration in the reverse micellar phase at low pH values due to the high electrostatic attraction between AOT anions and the positively charged penicillin aggregates at $\text{pH} \approx 1$. The salt concentration effect was investigated by varying the KCl concentration in the aqueous phase at fixed pH 7.6. When the $[\text{KCl}] > 10$ g/l the penicillin concentration was decreased; increasing $[\text{KCl}]$ from 0 to 10 g/l showed a significant increase of penicillin in the organic phase. At $[\text{KCl}] < 5$ g/l almost all of the surfactant migrated to the aqueous phase and no reverse micelles were formed in the organic phase. We found that the concentration of penicillin in the reverse micellar phase is higher when using CaCl_2 compared to KCl, since divalent salts lead to smaller reverse micelle droplets and the ability to solubilise more penicillin is increased.

Backward Extraction Process

Backward extraction is known to be improved with addition of alcohol as a co-surfactant, but we found almost all penicillin was transferred into an aqueous phase without alcohol addition. The pH was fixed at pH 7 and the initial amount of penicillin G in the organic phase before backward extraction ($P_{rm+o.i.bw}$) was 21.8 mg, which is the maximum concentration of penicillin G solubilised in the organic phase in the forward extraction at AOT concentration of 267 g/l. High ionic strength in the backward aqueous phase is important to promote penicillin release from the organic phase. Figure 3 shows that backward extraction depends on the initial concentration of penicillin before forward extraction ($P_{aq.i.fw}$) and AOT concentration used. The solubilisation of penicillin in the

backward aqueous phase increases when the AOT concentration is increased. The higher amount of penicillin in the backward aqueous phase ($P_{aq.f.bw}$) at an AOT concentration of 445 g/l is due to higher amount of penicillin G in the organic phase before backward extraction. Figure 4 shows the overall process of the penicillin extraction, and is a plot of the initial amount of penicillin in the aqueous phase before forward extraction ($P_{aq.i.fw}$) to the final amount in the aqueous phase after backward extraction process ($P_{aq.f.bw}$). The nearness of the plot to the 45° line indicates a better overall process, as the 45° line shows the amount of penicillin transferred in the backward aqueous phase is the same as the initial amount at the beginning the process before forward extraction. The results show that at all tested AOT concentrations the plot is far from the 45° line; this is because the effectiveness of the overall extraction process is complex and largely determined by the forward process that is affected by many variables, notwithstanding the highly effective backward process.

CONCLUSIONS

The results show that the solubilisation of the penicillin into the organic phase is favoured at higher AOT concentrations, low pH and low salt concentration. The backward extraction successfully solubilises penicillin G at high ionic strength at any pH.

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FIGURES

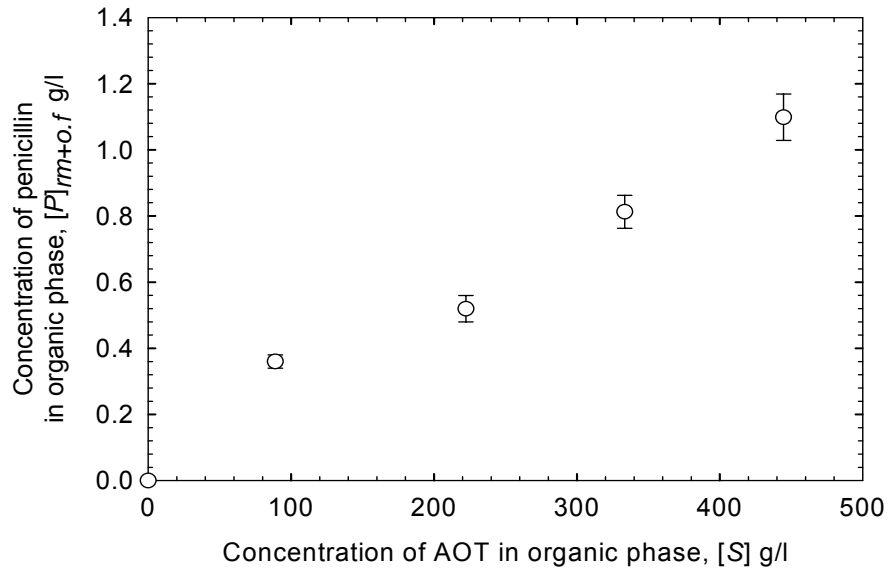


Figure 1: Concentration of penicillin G in the organic phase at different AOT concentrations.

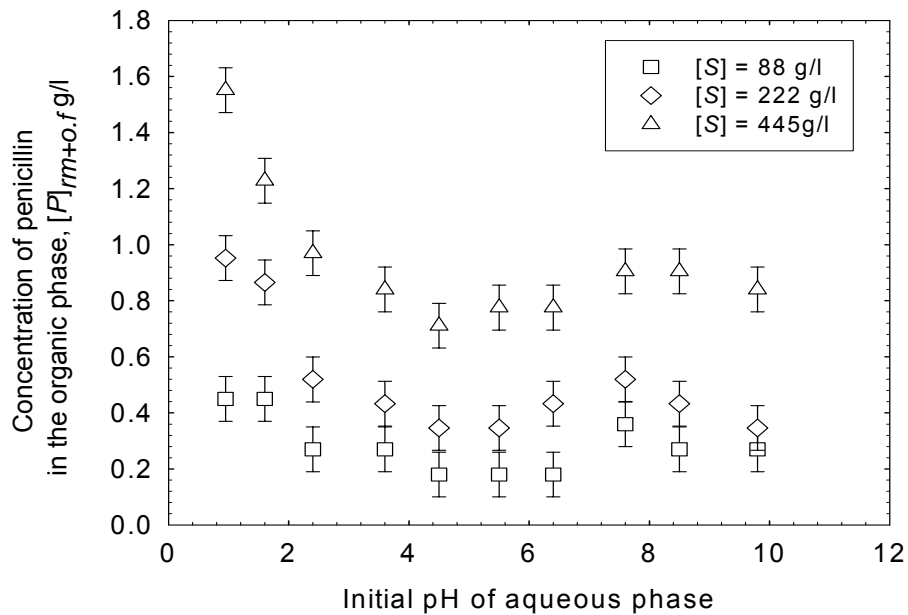


Figure 2: The effects of pH and AOT concentration on the amount of penicillin extracted into the micellar phases.

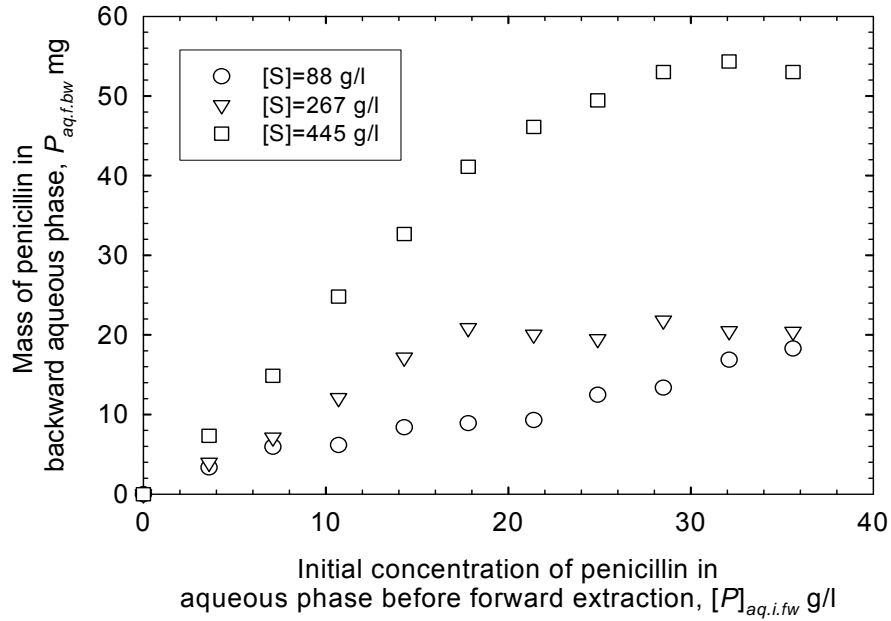


Figure 3: Effect of initial concentration of penicillin G before forward extraction to the amount of penicillin G in the backward aqueous phase.

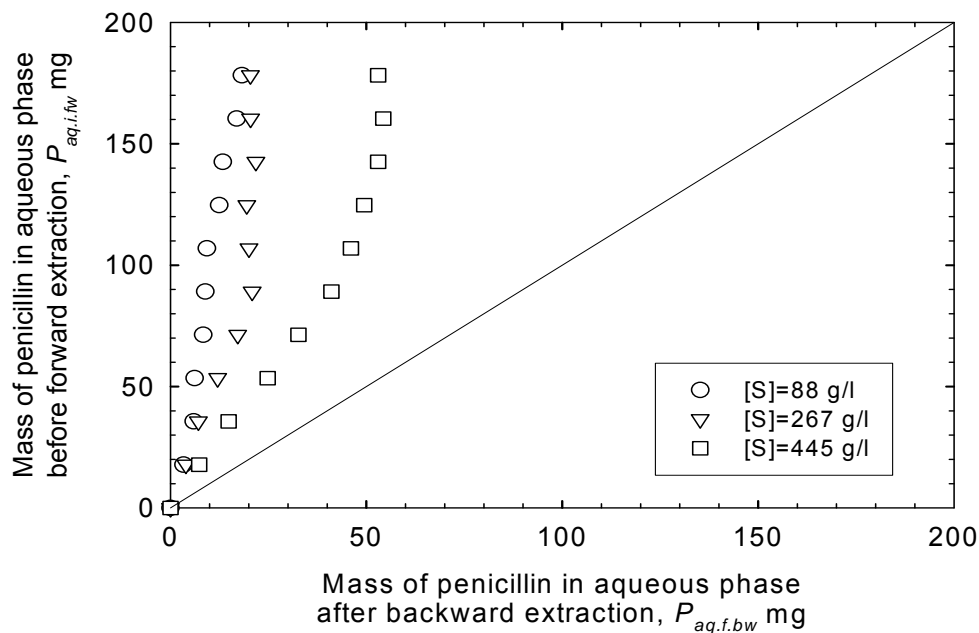


Figure 4: Overall extraction of penicillin G.