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Kinetic analysis on membrane-based reverse micellar extraction of lysozyme from aqueous solutions

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Abstract

The extraction of lysozyme from aqueous solutions through a flat-sheet microporous membrane (pore size 0.45 μm, thickness 147 μm, porosity 0.75) into an isooctane solution of sodium bis(2-ethylhexyl)sulfosuccinate (AOT) reverse micelles was examined. Batch liquid–liquid extraction experiments were first conducted at different lysozyme concentrations (250–1000 mg L^{-1}), KCl concentrations (0.1–1.2 M), pH (2–12) and AOT concentrations (0.01–0.1 M) to obtain equilibrium relationships. Effective extraction of lysozyme was achieved in the KCl concentration range of 0.1–0.4 M and pH range of 4–9. More than 90% of lysozyme could be stripped to an aqueous phase of high alkalinity (pH 11.5) and high KCl concentration (1.5 M). A mass transfer model was proposed that considers all diffusion in the aqueous stagnant layer, membrane and reverse micellar stagnant layer to predict the transport flux of lysozyme in the present membrane-based extraction process. The solubilization of lysozyme from aqueous phase to the AOT/isooctane reverse micelles was assumed to attain equilibrium instantaneously. A good agreement between the calculated and measured fluxes was obtained under the ranges studied (standard deviation, 11%).
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Keywords: Reverse micellar extraction; Lysozyme; AOT; Membrane-based extraction process; Mass transfer modeling

1. Introduction

In recent years, many separation techniques have been developed in biotechnology to achieve a highly efficient and economical process. One novel technique with the ability to be scaled up easily, to be operated continuously, and to be highly selective is liquid–liquid extraction using microemulsions [1–4]. The aggregates of surfactant molecules are spontaneously generated in organic solvents as a result of molecular self-assembly. These aggregates can solubilize water in their polar cores giving rise to water-in-oil microemulsions, commonly referred to as reverse micelles. It has been proven that proteins can be solubilized within these reverse micelles in active form [5]. This method is more suitable for separating proteins than conventional liquid–liquid extraction or other methods that were used in the past because the transfer of proteins into solvents often results in irreversible denaturation and loss of biological activity [4].

Traditional liquid–liquid reverse micellar extraction processes have been operated in devices, such as spray column, rotating disc contactor, packed towers, etc. [6–8], which seek to maximize contact area for mass transfer. The mild or intimate mixing that usually occurs in these devices might lead to the possible formation of emulsions of the two phases, thereby inhibiting phase separation and product recovery. These shortcomings could be minimized or eliminated using non-dispersive solid membrane-based processes, such as membrane contactors and supported liquid membranes [9,10]. They use a flat-sheet or hollow fiber microporous membranes soaked with the organic carriers to separate the aqueous feed phase and the organic phase or even the aqueous strip phase. Besides the non-dispersive nature, hollow fiber membrane-based extractions are substantially faster than those possible in traditional equipments and the extraction is not compromised by flooding and loading because the flows of reverse micelles and raffinate are almost completely independent [9].

In this work, the reverse micellar extraction of proteins from aqueous solutions using a flat-sheet microporous membrane was experimentally and theoretically examined. The system of lysozyme-bis(2-ethylhexyl)sulfosuccinate (AOT)/isooctane

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was exemplified. In contrast to a large number of studies investigating liquid–liquid extraction of lysozyme [11–17], little attention has been paid to examining reverse micellar extraction in solid membrane-based processes [9,18]. For example, Tsai et al. [18] have studied the extraction of α-chymotrysin by supported liquid membranes using AOT reverse micelles as carriers. Only a phenomenological model was proposed to describe the mass transfer. In fact, the extraction and separation of lysozyme with AOT reverse micellar the so-called bulk liquid membrane, a process without solid membrane support, have been reported [19,20].

The chemistry involved in membrane-based reverse micellar extraction process is essentially the same as that in liquid–liquid extraction process, and the overall membrane process is governed not only by equilibrium parameters but also by kinetic parameters. It is thus possible to model such membrane process from a good knowledge of extraction chemistry and transport properties of the relevant geometry [21]. In practice, the extraction equilibrium behavior is rather complicated. The distribution of proteins between the micellar phase and aqueous phase is largely determined by the environments of bulk aqueous phase, i.e., pH, ionic strength and type of salt. Parameters related to the organic phase also affect the distribution of protein, such as type and concentration of surfactant, presence of co-surfactant and type of solvent [1–5]. By controlling these parameters, the extraction efficiency can be varied via the variations of protein-micelle electrostatic, hydrophobic and steric interactions. Among these, electrostatic interaction is considered as the dominant driving force especially in forward extraction process [1].

To obtain consistent results and convincible conclusions under controlled conditions, batch liquid–liquid reverse micellar extraction experiments were first made to get equilibrium relationships. The membrane-based extraction experiments were then performed, in which the membrane and bulk organic phases were the same as the organic phase used in liquid–liquid extraction systems. A mass transfer model was proposed that takes into account diffusion in the aqueous-phase stagnant layer, membrane and micelle-phase stagnant layer to describe the extraction of lysozyme in the present membrane-based process. The validity of the proposed model was justified by comparing with the measured transport fluxes of lysozyme under various conditions.

2. Modeling of the mass transfer

2.1. Mass transfer in the membrane extraction process

For the kinetic studies on the extraction of lysozyme (1000 mg L^{-1}) by the AOT-isooctane reverse micelles across a flat liquid–liquid interface, Nishiki et al. [14] have indicated that the rate constant of lysozyme release (i.e., the dissociation of lysozyme-AOT complex) at the aqueous–organic interface is two orders smaller than that of lysozyme solubilization (i.e., the formation of lysozyme-AOT complex). They found that the release process at the interface is rate limiting for the stripping of lysozyme from reverse micellar phase to an aqueous phase of higher KCl concentration. On the other hand, the extraction rate from 0.1 M KCl solution of pH 6.5 to a reverse micellar phase was governed by diffusion in the aqueous film and the solubilization at the aqueous–organic interface. Using a similar device, Kinugasa et al. [11] have reported that in the stripping of lysozyme from the micellar solution there is a main resistance to leave it at the organic–aqueous interface; however, they indicated that the forward extraction rate of lysozyme into AOT reverse micellar phase at pH values below its p*I* is limited only by diffusion through the boundary layers. Although the role of solubilization reaction during the extraction is inconsistent in these two studies, the resistance of lysozyme solubilization at the interface to the overall extraction process is assumed to be negligibly small in the present flat-sheet microporous membrane-based extraction system. This is particularly valid in this case because of the use of a thicker membrane support (thickness 147 μm) here [21], in which membrane diffusion would play a crucial role.

The concentration profiles of the specie near and within the microporous membrane are shown in Fig. 1. If all the concentration profiles are linear and the diffusion process is described by Fick's equation, the transport flux of each step is given as follows:

1. Diffusion of lysozyme from the feed bulk phase through aqueous layer to the aqueous-membrane interface, in which lysozyme instantaneously solubilizes into AOT reverse micelles,

$$J_1 = k_{a,Lys}([Lys]_a - [Lys]_{ai}) \quad (1)$$

2. Diffusion of lysozyme present in the AOT reverse micelles through the membrane,

$$J_2 = k_{m,micelle}([\overline{Lys}]^m_{ai} - [\overline{Lys}]^m_{oi}) \quad (2)$$

where the overbar refers to the membrane (reverse micellar) phase and the superscript m refers to the membrane side adjacent to the aqueous- or organic-membrane interface. That is, $\overline{Lys}$ denotes the lysozyme that solubilizes in the AOT reverse micelles for simplicity.

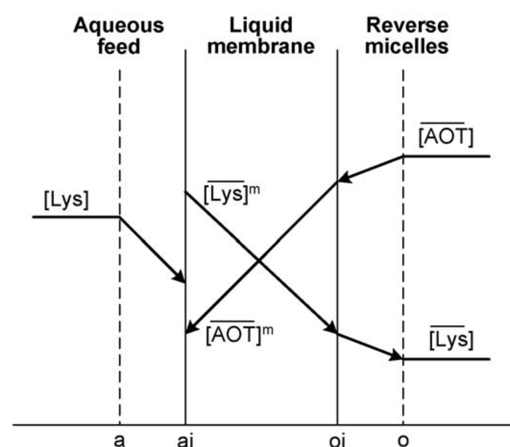

Fig. 1. Concentration profiles of lysozyme and its AOT reverse micelle near and within a flat microporous membrane support.

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