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MASS-TRANSFER OF LYSOZYME ACROSS A FLAT SURFACE WITH REVERSE MICELLES

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ABSTRACT

Reverse micelles are an effective way to separate and purify proteins by solvent extraction. Optimization of the process requires knowledge of the interfacial mass-transfer rates. In this study, individual mass-transfer coefficients were determined in a cylindrical stirred diffusion cell for the transfer of lysozyme between aqueous 0.1 *M* KCl solutions containing sucrose or starch, and *i*-octane containing AOT surfactant. Forward extraction rates were correlated well by the equation, $Sh_1 = 0.558 Re_1^{0.554} Sc_1^{0.205}$. Of particular novelty was the measurement of extraction kinetics in the presence of varying concentrations of sucrose and starch. Individual mass-transfer coefficients increased with sucrose concentration, by contrast, the converse was

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observed for starch. The influence of sucrose and kinetics of the extraction process are discussed.

Key Words: Mass-transfer; Surfactant; AOT; Reverse micelles; Lysozyme

INTRODUCTION

It has been demonstrated that under certain conditions proteins can be transferred from an aqueous to a reverse micellar phase and back.[1,3,5,6,7,13,14,16] The partitioning of protein between the two phases depends on several factors. Those that have been identified include: pH, ionic strength, nature and concentration of electrolyte, type and concentration of surfactant, charge on protein, solvent, the presence of co-solvents, temperature, and the degree of agitation.

In general, proteins are only transferred into a reverse micelle at a pH where the charge on the protein is opposite to that of the surfactant head-groups.[4] The difference between the pH, at which transfer occurs, and the iso-electric point (pI) of the protein depends on the size of the protein molecule.[18] Since electrostatic interactions between the protein and the reversed micelle are important, the distribution coefficient of protein in these systems is determined by the pH and ionic strength of the aqueous phase.

To apply the reverse micellar extraction technique to the recovery of proteins, a simultaneous forward and back extraction process is used. Moreover, the application of a reverse micellar liquid membrane has been demonstrated.[12] Nevertheless, the mechanisms for extraction and stripping (back extraction) of proteins in reversed micellar systems are still obscure, and understanding is complicated by the solubilization characteristics of water and proteins in surfactant systems.

Lye et al.[14] studied the reverse micellar mass-transfer of lysozyme in both a spray column and a stirred cell and found that the predicted overall mass-transfer coefficients were 2–10 times greater than observed, a result which they attributed to a large concentration of surfactant in the reverse micelle phase. Goto et al.[9] used a mixed AOT (dioctyl sulfosuccinate)–DOLPA (dioleyl phosphoric acid) reverse micelle system to extract chymotrypsin. They showed that the activity of chymotrypsin from mixed reversed micelles was higher than that of the initial protein.

Recently Kelly et al.[19] reported a novel method for the recovery of nisin, varicin, and carnocin from fermentation broths by extraction with toluene, which probably involves a reverse micelle mechanism. They posit that the extraction mechanism involves concentration of the surface-active peptides in reverse

micelles formed at the aqueous–organic interface. Although, they look briefly at the kinetics of extraction, they are more concerned with nisin decomposition than with mass-transfer, and so aim for close approach to equilibrium in their work.

In an interesting study involving the solvent extraction of bacteriocins from aqueous cultures, Burianek and Yousef[20] noticed that the use of chloroform generated a precipitate containing high activity at the interface, but little in the organic layer. It would appear that the activity was concentrated in the micelles created at the interface by the small surface activity of the chloroform. The high surface area of the micelles and small retention of organic within them resulted in very effective accumulation of the bacteriocin, lacidin. They further comment that surfactant Tween-80 was required for lacidin production or its release from the *Lactobacillus acidophilus* cells.

Kinugasa et al.[10] studied the extraction of lysozyme from aqueous solution using reversed micelles in an *i*-octane–AOT system. They found that the micellar solution when saturated with proteins had a limiting concentration that depended only on water content of the organic phase. Spirovska and Chaudhuri[17] investigated the effect of the simple sugars, glucose and sucrose, on the extraction of protein by AOT–*i*-octane reversed micelles. They observed that sucrose was taken up into the reverse micelles and suggested that it resulted in preferential hydration of protein and so reduced protein–surfactant interactions.

In the present study, we explore the action of sucrose further. The equilibrium distribution of protein into a reverse micellar solution was measured for lysozyme, a typical low molecular weight protein, using an anionic surfactant at a pH below its iso-electric point. The objective being to systematically determine the effects of sucrose and starch concentration, and stirring speeds on interfacial mass-transfer rates, whilst covering a wide range of property values, and to obtain a reliable correlation of the mass-transfer coefficients.

EXPERIMENTAL

Chemicals

Lysozyme (E.C. 3.2.1.17), from the mucopeptide *N*-acetyl muramyl hydrolase was obtained from Sigma Chemical Co. The AOT (bis-2-ethylhexyl sodium sulfosuccinate) was obtained from Aldrich Co. The AR grade *i*-octane (2,2,4-trimethylpentane) was obtained from Junsei Chemical Co. All other chemicals were obtained from Sigma.

An aqueous protein solution was prepared by dissolving an appropriate amount of lysozyme in distilled water containing potassium chloride for ionic strength adjustment. The pH of the aqueous solution was adjusted by direct

addition of 0.1 *M* HCl or NaOH as required. Density and viscosity were adjusted using sucrose (0.2, 0.4, and 0.8 *M* solutions) and starch (0.04, 0.16, and 0.3 g).

Analysis

Lysozyme concentrations were determined from measurements of absorbance at 280 nm by a UV-con 933 spectrometer.

Equilibrium Tests

To extract lysozyme from the aqueous phase, a 10 mL sample containing 50 mM AOT was withdrawn from the system, mixed with an equal volume of organic solvent and shaken for 10 min. The mixture was then centrifuged and the phases separated. The organic phase was mixed with a further equal volume of the prepared aqueous solution for back extraction. After centrifuging, the phases were again separated and analyzed.

Mass-Transfer Experiments

Mass-transfer rates for the extraction of protein were measured in a cylindrical stirred vessel, based on a design by Dekker et al.,[3] and shown in Fig. 1. The cell was a 60 mm i.d. glass cylinder, 130 mm high, containing four equi-spaced longitudinal baffles. A water-jacket controlled the temperature. Two impellers were used, each co-axial with the cell and independently driven. In all experiments, the impellers rotated in the same direction but at independent speeds. The interfacial area was 2.827×10^{-3} m^2.

During preliminary experiments, it was found that if the stirrer paddles rotated in opposite directions, under certain combinations of stirrer speeds, the mass-transfer coefficients were similar to those obtained when the interface as a whole was not rotating. Under these circumstances, it was difficult to find a reasonable correlation for the data. Thus, for the work reported here, the stirrers were run in the same directions resulting in observations that are more consistent.

To start, the vessel was charged with 200 mL of aqueous solution followed by 200 mL of organic and then agitated. One milliliter aliquots were withdrawn from the organic phase for analysis. Impeller speeds ranged from 100 to 300 rpm with care taken to maintain a quiescent interface. All experiments were carried out at 25°C in batch mode.

Physical properties of the systems are listed in Table 1. These show a wide range of coverage. The diffusivities of solutes in the aqueous solutions were

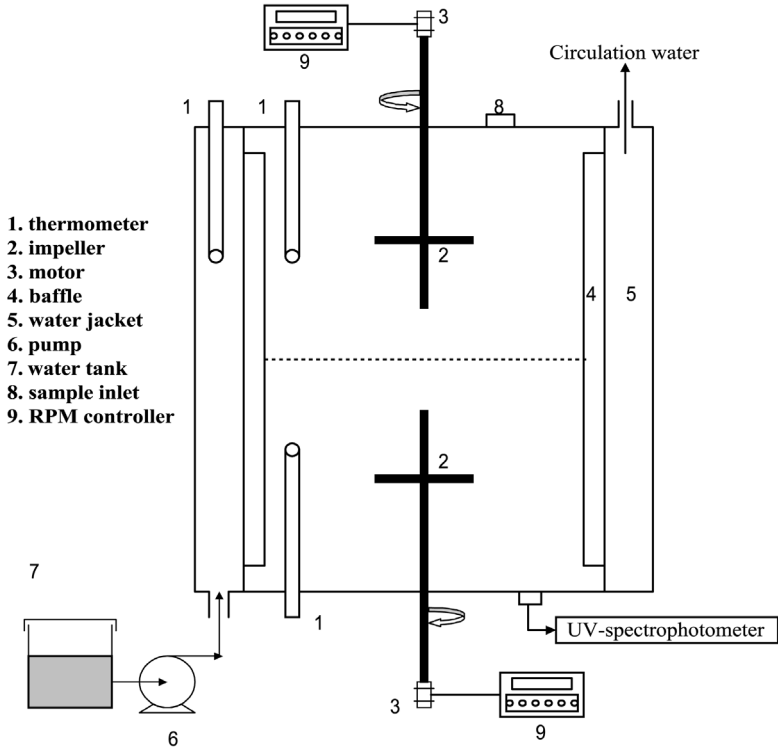

Figure 1. Schematic diagram of apparatus for lysozyme extraction with reverse micelles.

Table 1. Table Listing Physical Properties of Aqueous Solutions Used

System	ρ_w (kg/m^3)	μ_w (mN sec/m^2)	$D_w \times 10^{11}$ (m^2/sec)
Water	1002.3	0.0009	19
0.2 *M* sucrose	1366.7	0.0015	7.9
0.4 *M* sucrose	1379.2	0.0017	6.9
0.8 *M* sucrose	1620.8	0.0030	3.9
0.04 *g* starch	998.2	0.0018	6.5
0.16 *g* starch	998.3	0.0038	3.0
0.3 *g* starch	998.5	0.0059	1.9

System conditions: 25°C; lysozyme, 0.2 g/L; KCl, 0.1 *M*; *i*-octane + 50 m*M* AOT.

estimated from values for pure water with corrections for the presence of solute (i.e., starch or sucrose). The values refer to the liquid in equilibrium with the other phase and containing solutes at the same concentrations used during the extraction processes. Viscosities and densities were measured by conventional techniques.

Mass-Transfer Theory

The overall mass-transfer rate during liquid–liquid extraction is generally determined by three resistances. During forward extraction, protein molecules diffuse from the bulk of the aqueous phase to the interface. At the interface, the protein becomes encapsulated within a shell of surfactant molecules, so forming a protein-filled reversed micelle. The reverse micelle then diffuses from the interface into the bulk of the organic phase. During backward extraction, the processes reverse.

For most extraction systems, the interfacial concentrations of the transferable compound are generally assumed to be in equilibrium. Therefore, the mass-transfer rate is controlled by the diffusion processes on each side of the interface. In some cases, an interfacial reaction of transferring compound with a carrier molecule is rate-limiting.

The rate of mass-transfer, R, of protein for the forward extraction can be expressed by:

$$R_1 = k_1 a(c_w - c_{i,w}) \tag{1}$$

and for the back extraction as:

$$R_2 = k_2 a(c_{i,org} - c_{org}) \tag{2}$$

where a is the interfacial area for mass-transfer, k the relevant mass-transfer coefficient, and c the concentration of protein. Subscripts "w" and "org" refer to the aqueous and organic phases, respectively, and "i" to the interfacial zone.

If H is the distribution coefficient of the protein between phases, then:

$$c_{i,org} = Hc_{i,w} \tag{3}$$

Combining Eqs. (3) and (1), and introducing the molar flux, J, of protein across the interface, we obtain:

$$J = R/a = (v_w/a)(dc/dt) = k_1(c_w - 1/Hc_{i,org}) \tag{4}$$

Recognizing that the amount of protein at the interface will be small, and assuming equilibrium at the interface, then its concentration at the interface will be small and negligible compared with the bulk concentration. Equation (2) can

then be integrated to give:

$$\ln(1 - c_{org}/c_o) = -ak_1t/v \quad (5)$$

The mass-transfer coefficient for the forward process, k_1 can be obtained from the slope of the $\ln(1 - c_{org}/c_o)$ vs. t curve.

RESULTS AND DISCUSSION

Equilibrium Tests

The effect of starch and sucrose concentrations on lysozyme recovery is shown in Table 2. In the absence of sucrose or starch, typically 95% of the protein was extracted from the reverse micelle phase; yet by adding 0.4 *M* sucrose, essentially 100% of the protein was recovered.

Sucrose increased the extent of both forward and back extraction. The sucrose is presumed to reduce interactions between surfactant and protein and so the protein will be recovered with reduced loss of activity.[8,11,17] The effect of starch was more complex. Small additions increased extraction but larger amounts reduced it, an observation as yet unexplained.

Mass-Transfer Studies

The rate of mass-transfer was determined at different differential stirring rates under conditions established from the equilibrium tests. The forward mass-transfer coefficients, k_1 were obtained from Eq. (5).

Table 2. Effect of Additions of Starch or Sucrose on Lysozyme Extraction into Reverse Micelles

System	Forward Extraction (%)	Backward Extraction (%)
Water	90.2	95.0
0.2 *M* sucrose	94.2	94.4
0.4 *M* sucrose	94.9	100.0
0.8 *M* sucrose	93.3	99.1
0.04 *g* starch	91.9	98.6
0.16 *g* starch	88.9	97.1
0.3 *g* starch	86.6	96.6

Forward extraction conditions: lysozyme 0.2 g/L, pH 6.3, KCl 0.1 *M*, 50 m*M* AOT, time 5 min, stirrer 500 rpm. Backward extraction: pH 12, KCl 1 *M*, time 10 min, 500 rpm.

Table 3 shows the effect of starch and sucrose concentrations on the values of both k_{1exp} and k_{1cal} indicating that the individual coefficients increased slightly with an increase in sucrose concentration. The opposite was observed for starch, where the coefficients decreased as starch concentration increased.

Equation (5) represents transfer in the forward direction. There is a corresponding expression for the reverse transport process. Fig. 2 shows how the mass-transfer coefficient was determined from the slope of the curve. As time increased, the mass-transfer coefficient increased, regardless of sucrose concentration.

Subsequent rearrangement of Eq. (5) led to the following simple correlation in its final form:

$$Sh_1 = 0.558 Re_1^{0.554} Sc_1^{0.205} \tag{6}$$

Figure 3 compares the values of Sh_1 obtained in the present work with those calculated from Eq. (6). The need to include interfacial Reynolds number is probably closely connected with surface wave motion.[2] It should be noted that apart from this inclusion, the dependence of Sh_1, on Re_1, Re_2, and Sc_1 is analogous to that in the familiar correlations for turbulent mass-transfer to a stationary or rotating rigid surface.[15]

CONCLUSIONS

Liquid–liquid mass-transfer across a geometrically simple flat interface was studied systematically over a wide range of physical properties. The rate of forward mass-transfer was found to be controlled by the degree of agitation of the aqueous phase and the equilibrium conditions at the aqueous–organic interface. In the organic phase, mass-transfer was largely independent of agitation.

Table 3. Measurements of Mass-Transfer Coefficient, k_{1exp}, Under Various Conditions

System	Re_1	Sc_1	k_{1exp} ($\times 10^7$)	k_{1cal} ($\times 10^7$)
Water + 0.2 *M* suc.	1,351	13,511	5.87	6.70
Water + 0.4 *M* suc.	1,183	17,784	6.12	5.97
Water + 0.8 *M* suc.	781	47,894	6.21	3.23
Water + 0.04 g sta.	813	27,246	7.90	5.02
Water + 0.16 g sta.	378	125,917	1.05	2.09
Water + 0.3 g sta.	242	307,451	5.28	1.26

Temp.: 25°C, lysozyme 0.2 g/L, KCl 0.1 *M*, AOT 50 m*M*, rpm_1 150, rpm_2 130, suc.: sucrose, sta.: starch.

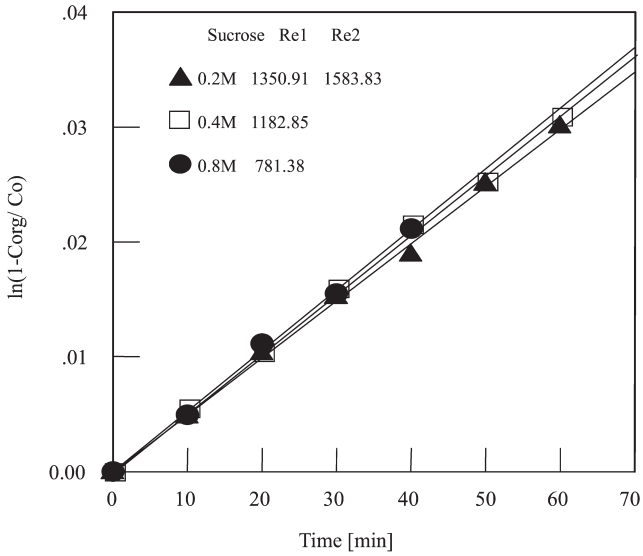

Figure 2. Ln(1 − c_{org}/c_o) vs. time at 25°C (0.2 g/L lysozyme, 50 m*M* AOT, pH 6.3, 0.1 *M* KCl).

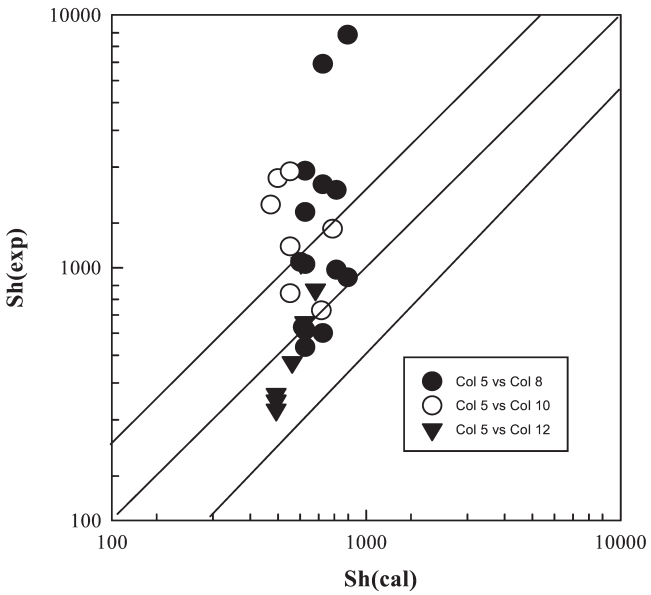

Figure 3. Sherwood number parity plot among sucrose, starch, and water solution systems.

Equation (6) correlated the data favorably for the aqueous/*i*-octane/AOT system in the absence of sucrose or starch.

The addition of sucrose was found to increase both the extents of extraction and the individual mass-transfer coefficients in forward and back extraction. In the cases where starch was added, coefficients decreased.

SYMBOLS

a	interfacial area, m^2
c	concentration of protein, mol/L
D	diffusion coefficient, m^2/sec
d	impeller diameter, m
H	distribution coefficient
J	molar flux of protein, mol/sec m^3
k	mass-transfer coefficient, L/sec m^2
L	length parameter, m
N	impeller speed, rpm
R	rate of mass-transfer, mol/sec
Re	Reynolds number, d^2N/ν
Sc	Schmidt number, ν/D
Sh	Sherwood number, k_1L/D
t	time, sec
μ	dynamic viscosity, N sec/m^2
ρ	density, kg/m^3
ν	kinematic viscosity, m^2/sec
v	aqueous phase volume, L

Subscripts

1	forward extraction
2	back extraction
i	interface
o	initial
org	organic phase
w	aqueous phase

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