Mass Transfer Coefficients in Two-phase Aqueous Extraction

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ABSTRACT

Mass transfer coefficients were measured in a 51 mm i.d. stirred cell with a plane interface. An aqueous solution of Dextran formed the heavy phase and an aqueous solution of polyethyleneglycol formed the light phase. The transfer of bovine serum albumin (BSA) was investigated. The effects of stirring speed, pH and phase concentrations on the mass transfer coefficient were studied. Values of the partition coefficient for BSA are reported at different values of pH and phase concentrations.

A new method is suggested for finding individual phase mass transfer coefficients, using the effect of pH on partition coefficient.

1. INTRODUCTION

Two-phase aqueous polymer–polymer and salt–polymer systems are used for the extraction and purification of proteins. Both phases can contain as much as 75% - 99% water, and as a result the interfacial tensions are very low. Hence equilibrium is reached quickly with little input of energy. These properties make the two-phase aqueous system biocompatible [1]. Any molecule will accumulate in the phase where the maximum interactions are possible and partition occurs in such a manner that the energy of the system is minimized.

The extraction of protein in a two-phase aqueous system is an interfacial phenomena. The important parameters that affect the partitioning are the molecular weights of the polymers constituting the phase, pH, temperature, phase concentration, biospecific ligands, interfacial potential etc. These parameters lead to a complex system where the effect of the interaction or interdependence of the said parameters is not well understood. Perhaps this is why systematic modelling–prediction of the behaviour of the majority of these systems has not been possible.

Organic compounds are not generally used for protein–enzyme separation as they tend to denature the proteins. On the other hand, aqueous solutions of polymers are reported as enhancing the stability of biologically active substances.

Perkins *et al.* [2] have studied the effect of pH, phase concentration and the molecular weight of the polymers on the partitioning of BSA and cytochrome *c* using dextran–polyethyleneglycol.

Sawant *et al.* [3] have studied the application of spray columns for this system and have measured dispersed phase hold-up, equilibrium partition coefficient m, and the product of overall mass transfer coefficient and interfacial area, $K_L a$.

Shanbhag [4] has measured the overall mass transfer coefficients of six proteins in the molecular weight range 13 000 - 290 000. Aqueous solutions of dextran and polyethyleneglycol formed the two phases in 25 and 75 ml stirred cells with a constant interface.

For the reliable design of extraction equipment, the effects of stirrer speed, phase concentration, pH etc. on the overall mass transfer coefficient are required. The present work is concerned with these aspects. Partition coefficients have been measured and an attempt has been made to obtain individual values of the phase transfer coefficients.

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2. MATERIALS USED

Dextran (506 000), polyethyleneglycol (8000) and bovine serum albumin (68 000) were all obtained from the Sigma Chemical Co., U.S.A., while monopotassium phosphate and dipotassium phosphate were procured from the British Drug House, Bombay. The quantities in parentheses indicate the molecular weight of the respective compound.

3. EXPERIMENTAL DETAILS

Details of the experimental arrangement are shown in Fig. 1. The stirred cell was made of Perspex having an i.d. of 51 mm and a capacity of 230 ml. It was provided with two impellers (one in each phase). Each impeller had two blades of 20 mm length and 10 mm width. Stirring speed could be varied between 0 and 13 revs min^{-1}.

Predetermined quantities of polyethyleneglycol (PEG) and dextran (DEX) were accurately weighed, and to this buffer solution of the required pH was added. The entire mixture was then stirred gently using a magnetic stirrer for 4 h. The phases were allowed to settle for another 4 h which led to distinct phase separation. However pure phases were obtained after centrifuging for 5–10 min.

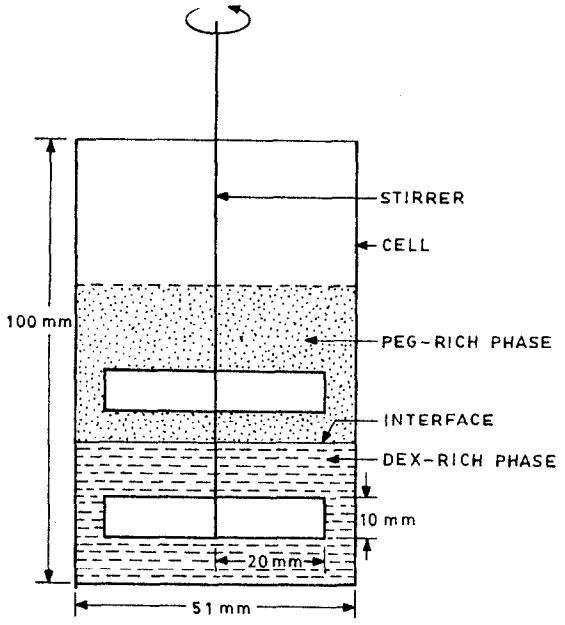

Fig. 1. The stirred cell.

A precisely weighed quantity of BSA was dissolved in a preweighed PEG-rich phase. A known amount of DEX-rich phase was added to the stirred cell such that the lower blade of the stirrer was positioned in the middle of the DEX-rich phase. To this the PEG-rich phase containing BSA was added so that the upper blade of the stirrer was located in the middle of the PEG-rich phase. During this addition care was taken not to cause any perturbations at the interface and beyond.

In a typical experimental run, after adding the phases to the cell, a sample was withdrawn and the clock was started. Thereafter samples were withdrawn every two min for the first ten min. Stirring was continued and the system was allowed to reach equilibrium. The samples were analysed for BSA on a Perkin-Elmer UV spectroscope at 280 nm.

4. RESULTS AND DISCUSSION

Figure 2 shows a phase diagram obtained for DEX and PEG at 20 °C [1] along with the other compositions above the critical point used in the present investigation. The compositions chosen were such that the points were away from the critical point and phase separation was achieved with ease. Physical properties of the phase compositions are briefly indicated in Table 1 [1].

The overall mass transfer coefficients K_L across the interface were calculated using the equation

$$K_L = \frac{W_L \Delta C_L}{\rho_L S t (C_L - m C_H)} \tag{1}$$

where W_L is the mass of the light (PEG-rich) phase, ΔC_L is the difference in the light phase concentration (mg g^{-1}) between adjacent time intervals, ρ_L is the density of the light phase (g cm^{-1}), S is the cross-sectional area (cm^2) of the cell, t is the time (s), while C_L and C_H are the average concentration of light and heavy phase respectively between adjacent time intervals. The equilibrium partition coefficient m is defined as the ratio of protein concentration in the light phase to that in the heavy phase at equilibrium. Therefore

$$m = C_{L_e}/C_{H_e} \tag{2}$$

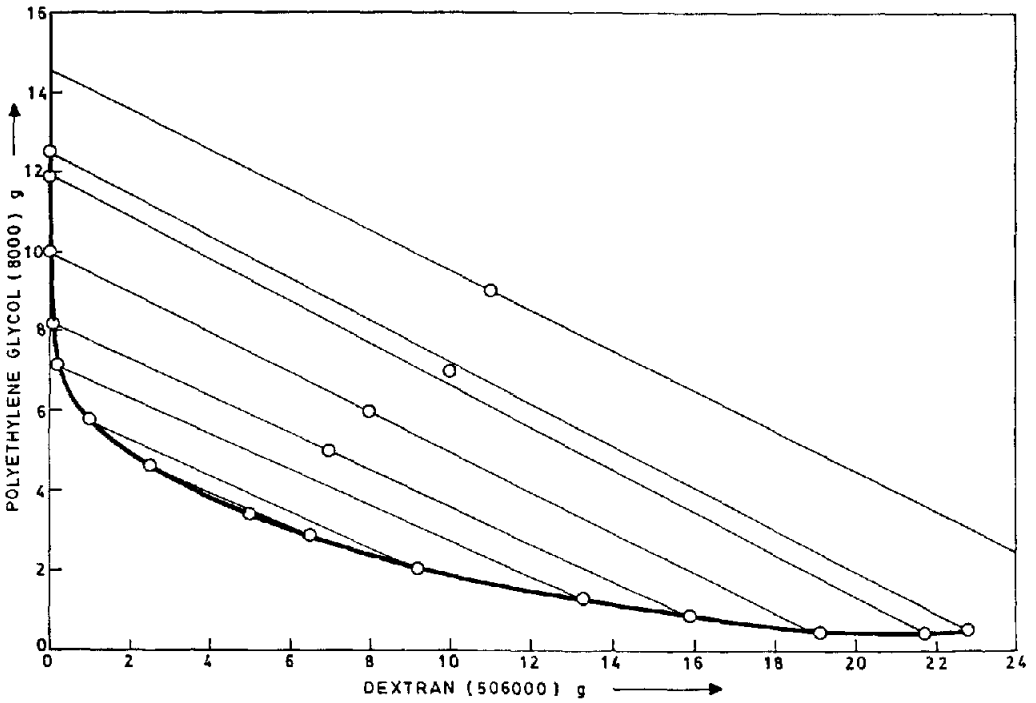

Fig. 2. Phase diagram for a two-phase aqueous system containing DEX (506 000) and PEG (8000).

TABLE 1

Physical properties of the phase composition

Phase composition DEX/PEG (wt.%)	Viscosity (mPa s)		Density (kg m^{-3})	
	μ_L	μ_H	ρ_L	ρ_H
7/5	4.4	95.7	1025.38	1076.73
8/6	3.61	1.51	1029.00	1077.9
10/7	3.18	248	1038.94	1107.08
11/9	2.89	292	1043.2	1120.0

Figure 3 indicates the influence of stirring speed on K_L. In the present study it was possible to operate the stirrer in the speed range 0 - 13.3 revs min^{-1}. In all the experiments the interface was kept horizontal. An increase in stirring speed resulted in an increase in K_L.

In Fig. 4, the overall mass transfer coefficient K_L *vs.* pH is shown, in which pH is varied in the range 5.8 - 7.8. K_L is seen to increase as the pH value increases until about pH 6.8 after which it declines. Whereas the equilibrium partition coefficient m in this entire range of pH was found to decrease from 1.32 at pH 5.8 to 0.26 at pH 7.8, as shown in Fig. 5.

It follows that alteration in the pH value of the medium brings about a change in the equilibrium partition coefficient of the protein and the transfer rates. This phenomenon probably results from coiling of the protein molecule, facilitating its distribution between the phases. It allows one the opportunity of altering the equilibrium partitioning of the protein easily and efficiently without varying any other operating conditions of the process. Hence there is a possibility of exploiting this property to obtain the individual values of mass transfer coefficient.

The overall mass transfer coefficient K_L and the equilibrium partition coefficient m have been measured for the DEX–PEG system in respect of stirring speed, the concentration of the polymers forming the phases and pH for extracting BSA. Thereby the individual mass transfer coefficients on either side of the interface can be obtained by application of the relations

$$\frac{1}{K_L} = \frac{1}{k_L} + \frac{m}{k_H}$$

$$\frac{1}{K_H} = \frac{1}{k_H} + \frac{1}{mk_L} \qquad (3)$$

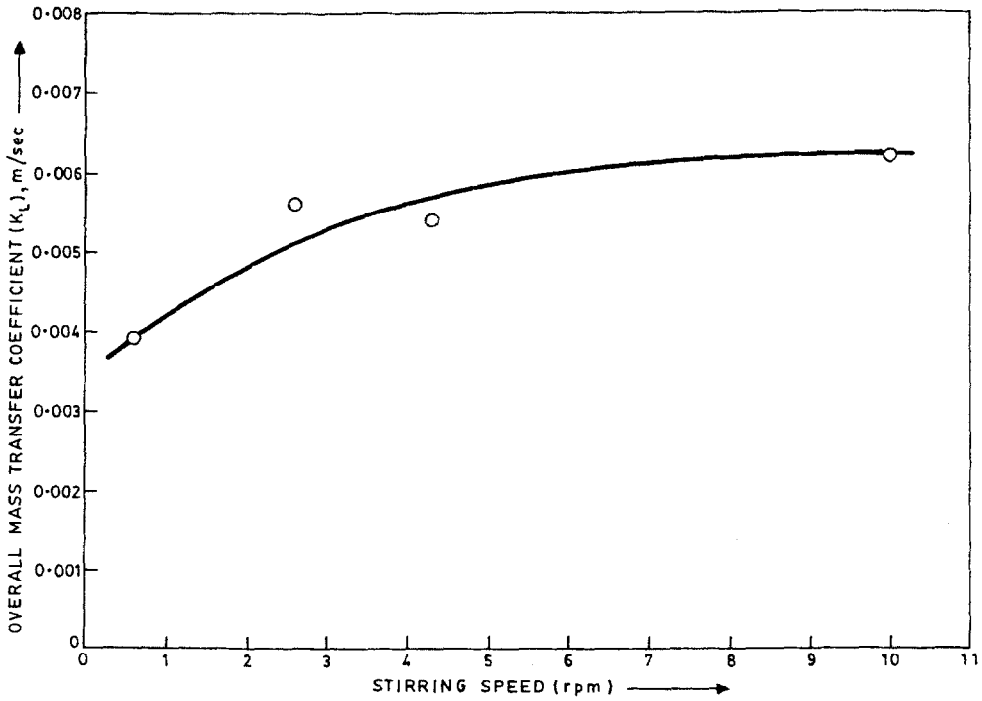

Fig. 3. Influence of stirring speed on K_L for BSA in 8 wt.% DEX and 6 wt.% PEG at 25 °C, pH 7.

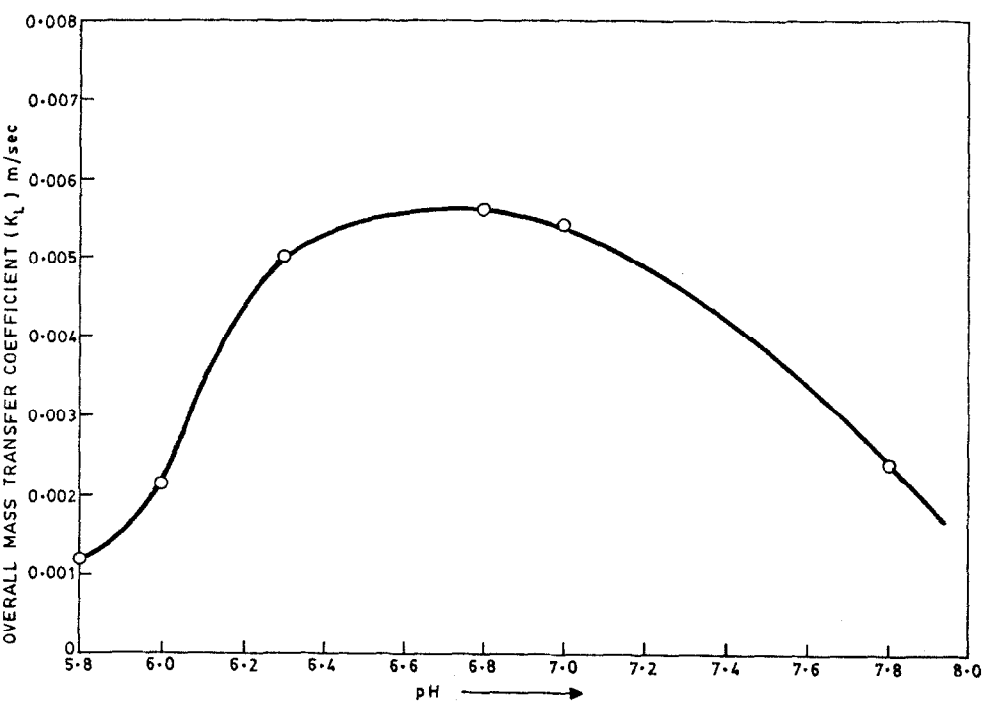

Fig. 4. Effect of pH on K_L for 8 wt.% DEX and 6 wt.% PEG at 25 °C, 2.6 revs. min^{-1}.

From the plot of the reciprocal of K_L against m, a straight line is obtained where the slope corresponds to the inverse of k_H, the mass transfer coefficient for protein transfer from the interface to the lower phase, and the intercept yields the inverse of k_L, the mass transfer coefficient for the protein transfer from upper phase to the lower phase.

From Fig. 6, the individual mass transfer coefficient for the light phase k_L is 2.8×10^{-3}

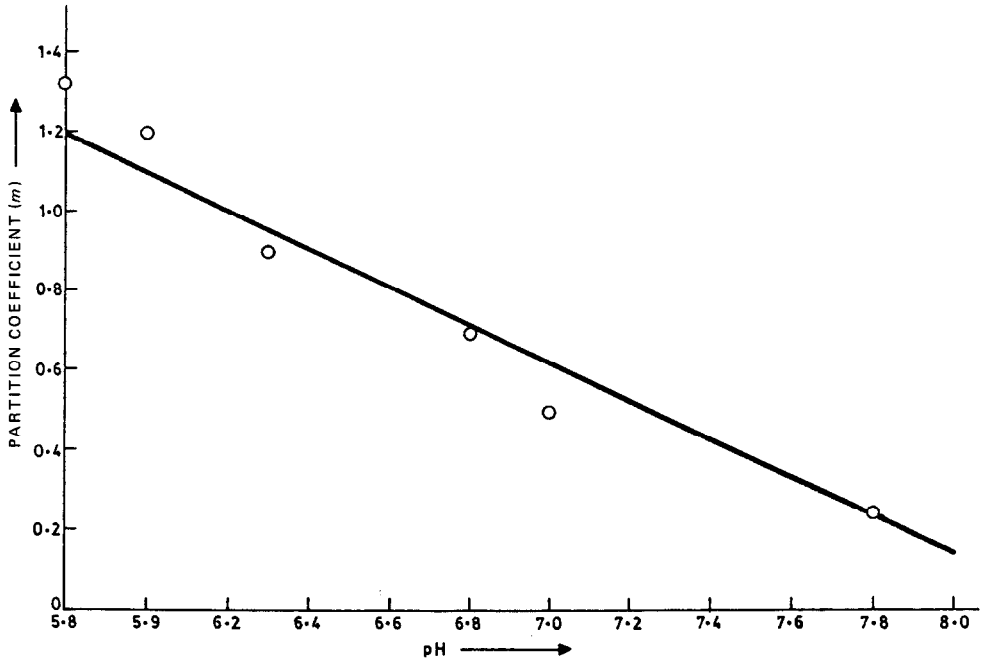

Fig. 5. Effect of pH on m for BSA in 8 wt.% DEX solution and 6 wt.% PEG solution at 25 °C, 2.6 revs min^{-1}.

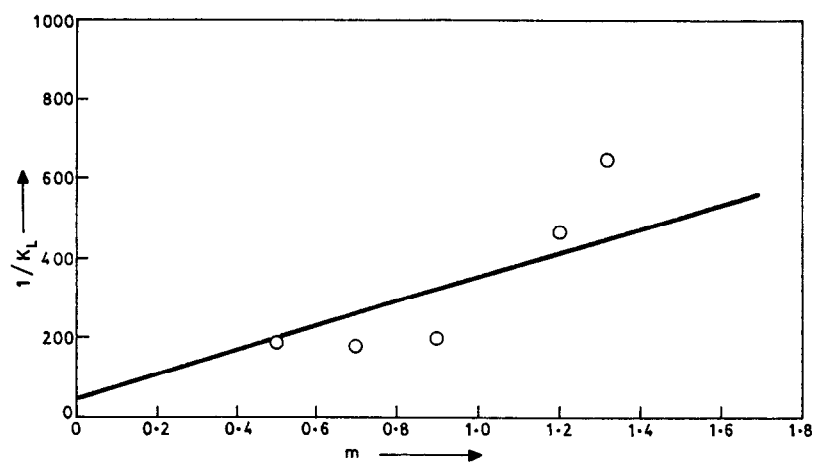

Fig. 6. Inverse of K_L *vs.* equilibrium partition coefficient for BSA in 8 wt.% DEX and 6 wt.% PEG at 25 °C, and 2.6 revs min^{-1} for various pH values.

m s^{-1} and for the heavy phase k_H is 2×10^{-2} m s^{-1} for the transfer of BSA in the present DEX–PEG system. Clearly the light phase transfer coefficient, being the smaller of the two, controls the overall transfer rate.

It was found that by increasing the concentration of phase components K_L increased and the partition coefficient varied in a sinusoidal manner. These results are displayed in Table 2. When the concentrations of both the phases were increased, it resulted in an increase in the viscosity of the liquid. Therefore, the diffusion coefficient would be expected to be reduced and the mass transfer coefficient to be decreased. Our experimental observations are contrary to these expectations.

5. CONCLUSIONS

For the extraction of BSA in a two-phase aqueous system, consisting of dextran and polyethyleneglycol solutions, the overall mass transfer coefficient K_L and partition coefficient m at equilibrium have been measured

TABLE 2

Effect of pH, stirring speed and phase concentration on mass transfer, rate and equilibrium parameters

pH	m	K_L ($\times 10^{-3}$ m s^{-1})
T = ? °C; *stirrer speed* = ? revs min^{-1}; *DEX/PEG* = ? wt.%		
5.8	1.3	?
6.0	1.2	2.14
6.3	0.9	5.0
6.8	0.7	5.6
7.0	0.5	5.4
Stirring speed (revs min^{-1})	m	K_L ($\times 10^{-3}$ m s^{-1})
T = ? °C, pH = ?; *DEX/PEG* = ? wt.%		
0.6	0.55	3.9
2.6	0.55	5.6
4.3	0.55	5.4
1.0	0.55	6.2
DEX/PEG (wt.%)	m	K_L ($\times 10^{-3}$ m s^{-1})
T = ? °C; *stirrer speed* = ? revs min^{-1}; pH = ?		
7/5	?	4.8
8/6	?	5.4
10/7	?	5.9
11/9	?	6.7

in laboratory stirred-cell experiments. The individual mass transfer coefficients on either side of the interface are estimated. The effect of stirring speed, pH and the concentrations of the polymers *i.e.* the effect of variation in the physical properties of the phases, has been quantified.

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APPENDIX A: NOMENCLATURE

a	interfacial area (m^2/m^3)
C_1, C_2	concentration of phase forming substances respectively (mg g^{-1})
C_H	BSA concentration in lower (DEX) phase (mg g^{-1})
C_L	BSA concentration in top (PEG) phase (mg g^{-1})
K_L	overall mass transfer coefficient (m s^{-1})
k_H	individual mass transfer coefficient on DEX side (m s^{-1})
k_L	individual mass transfer coefficient on PEG side (m s^{-1})
M	molecular weight
m	equilibrium partition coefficient
S	cross-sectional area (m^2)
T	absolute temperature (K)
t	time (s)
W	mass of top phase (kg)

Greek symbol

ρ	density (kg m^{-3})

Subscripts

e	equilibrium state
H	heavy phase (DEX)
L	light phase (PEG)