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Electroextraction

Two-Phase Electrophoresis

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ABSTRACT

Since both phases of aqueous two-phase systems are electrically conductive, application of electric fields in these systems gives rise to electrokinetic mass transfer of charged species. We have shown that an aqueous two-phase system can be used as a medium for electrophoretic separation with the liquid–liquid interface providing stability against convection and facilitating product recovery. Proteins have been directed into either the top or bottom phases of polyethylene glycol/dextran aqueous two-phase systems using 20–50 V/cm electric fields perpendicular to the phase interfaces. Binary protein mixtures have been separated in both batch and continuous modes by operating between isoelectric points and directing oppositely charged proteins into separate phases. Preliminary results on focusing *E. coli* cells into one phase while directing a proteinaceous product into the other phase indicate that two-phase electrophoresis may prove useful for commercial-scale recovery of proteins from fermentation broth.

Recent studies in our laboratory indicate that the electrostatic potential profile near the interface of conducting liquid–liquid systems can influence electrophoretic migration of charged species across the interface. We have observed that phase systems with high Donnan potentials hinder electrophoretic mass transfer across liquid–liquid interfaces, whereas phase systems with low Donnan potentials do not.

Index Entries: Electroextraction; two-phase electrophoresis; Donnan potential; aqueous two-phase systems; protein recovery.

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INTRODUCTION

Biological product recovery normally requires a series of steps beginning with large-capacity, inexpensive methods to reduce volume and partially purify a dilute product, and ending with more selective, more expensive methods to remove trace impurities from a concentrated product. Since valuable product is generally lost in each downstream step, it might be worthwhile to introduce more selective methods earlier in the process sequence and perhaps reduce the number of steps required. This article describes an attempt to introduce the selectivity of electrophoresis into an extractive separation process.

Liquid extraction is widely used in the chemical process industry for large-scale separations. Aqueous two-phase systems provide a biocompatible means of applying liquid extraction to biological product recovery, and utilization of them has begun on an industrial scale (*1–3*). Recently, several studies have been conducted to provide improved extractive separations via application of an electric field to both traditional organic solvent extractive systems and aqueous two-phase systems. Scott and Wham used an applied field to "create a high-surface-area emulsion, hold the emulsion in place against upward flow of the continuous phase, and induce coalescence" in a novel countercurrent solvent extraction device (*4*). They have also reported increased mass transfer in liquid–liquid systems owing to changing convection patterns within and around oscillating aqueous drops dispersed in a continuous, nonconducting, organic phase under the influence of a pulsing electric field (*5*). Brooks and Bamberger applied an electric field to an aqueous two-phase system in an attempt to facilitate droplet coalescence and phase separation in low gravity (*6*). They found that the speed of phase separation could be dramatically enhanced by application of relatively small electric fields. Rao and coworkers have made further studies toward reducing phase separation time in a process they call electrokinetic demixing (*7,8*). They obtained a threefold increase in demixing rate of polyethylene glycol (PEG)/dextran aqueous two-phase systems in phosphate buffer with an applied field of 15 V/cm. Enhanced demixing rates are important for industrial application of aqueous two-phase systems, since high-viscosity, low-interfacial tension, and similar phase densities make demixing and, hence, processing in these systems inherently slow.

Applied electric fields offer an excellent means of effecting selective separations owing to controlled electrokinetic migration of charged species. Well-developed electrokinetic separation processes include electrophoresis, electrodialysis, and isoelectric focusing. In addition to improving demixing rates, fields applied to extraction systems might give rise to electrokinetic migration of charged species across phase boundaries and increase mass-transfer rates provided both phases are electrically conductive. We have shown that aqueous two-phase systems can be used as a

medium for electrophoretic separation of proteins with the liquid–liquid interface providing stability against convection and facilitating product recovery (*9,10*). Similarly, Stichlmair and coworkers have shown that an applied field of 250 V/cm can be used to recover citric acid efficiently from water with water-saturated *n*-butanol as solvent in a continuous countercurrent electroextraction device (*11*). The high solubility of water in *n*-butanol renders the butanol-rich phase electrically conductive. A search of *Chemical Abstracts* uncovered about 50 papers or patents that use the term "electroextraction," but only those noted above appear to be related to combining liquid extraction with electrical fields.

In this article, we describe some of our work on aqueous two-phase electrophoretic separation of proteins (*10*) and present some new results on electrophoretic mass transfer across interfaces. Our recent work has been influenced by the fact that Levine and Bier have reported that electrophoretic transport of proteins across the interface of aqueous two-phase system is greatly impeded in one direction (*12,13*). They have shown that a protein will be readily transported across the interface when electrophoretic migration is from nonpreferred to preferred phase (preference refers to the equilibrium partitioning condition). They observed, however, that protein will not migrate across the phase boundary when directed from a preferred phase into a nonpreferred phase. We show here further evidence that proteins can be caused to migrate across the phase boundary in either direction.

TWO-PHASE ELECTROPHORETIC SEPARATION OF PROTEINS

We review here some of our previous results demonstrating the feasibility of achieving enhanced protein separations by applying an electric field directly to an aqueous two-phase system (*10*). Details of the experimental apparatus and procedure were presented in ref. (*10*). Single-protein experiments were made with 0.1 g/L hemoglobin to determine the effects of polarity, pH, electrophoresis time, field strength, and phase volume on two-phase electrophoresis. Binary protein separations were demonstrated with 0.2 g/L each of bovine serum albumin (BSA) and hemoglobin. Separation factors obtained with two-phase electrophoresis were compared to those obtained by partitioning alone in the same two-phase system and by electrophoresis in the same device using homogeneous buffer.

Buffers with pH between 4.5 and 9.5 were prepared by combining appropriate amounts (< 20 m*M*) of Bes free acid and Trizma base. Adding 5.5 g of dextran 500,000 and 3.8 g of PEG 8000 to 90 mL of buffer provided a two-phase system with nearly equal phase volumes. Electrophoresis was carried out in a 5.1-cm high by 3.8-cm diameter working chamber separated from similarly configured electrode chambers above and below

with ultrafiltration membranes. Normally, the electric field was applied as soon as a well-mixed two-phase system containing the proteins was loaded into the working chamber, before demixing had occurred. After a constant voltage was applied for a specified time, protein concentrations were determined spectrophotometrically or by HPLC for samples of top phase, interface, and bottom phase. Single-phase electrophoresis experiments were conducted similarly, except a homogeneous buffer was loaded and samples of known volume were removed from upper and lower portions of the working chamber after a run. For equilibrium partitioning experiments, phase systems were centrifuged to facilitate phase separation before measuring protein concentration in each phase.

We report two-phase electrophoresis results in terms of an effective partition coefficient, K_f, given as the concentration of a species in the top phase divided by that in the bottom phase after a specified field was applied for a specified time. It is also useful to define a top phase yield, Y, as the percent of the total protein in the two-phase system recovered in the top phase:

$$Y = K_f \epsilon / (1 + K_f \epsilon) \quad (1)$$

where ϵ is the ratio of the top phase-volume to the bottom-phase volume. For binary protein separations, we write the effective partition coefficients of albumin and hemoglobin as K_A and K_H, respectively, and define the separation factor, α_{AH}, as the ratio of the partition coefficient for albumin to that of hemoglobin and the purification factor, ϕ_{AH}, as the ratio of albumin yield to hemoglobin yield.

Figure 1 shows the effective partition coefficient of hemoglobin as a function of pH for two-phase electrophoresis for a fixed time of 30 min and a field strength of 25 V/cm. Hemoglobin was directed into either the top or the bottom phase, depending on the placement of the electrodes and the net charge on the protein. The equilibrium partitioning results are also shown in Fig. 1. The three curves for electrophoresis with the anode as the upper electrode, electrophoresis with the cathode as the upper electrode, and equilibrium partitioning cross at the protein isoelectric point.

The effective partition coefficient and top phase yield of hemoglobin as a function of electrophoresis time at pH 8 and a constant voltage of 25 V/cm are shown in Fig. 2. At these conditions, nearly all of the hemoglobin was recovered in the top phase after 100 min in the applied field.

The effect of varying the field strength with other factors held constant was also studied. Effective partition coefficient and yield increased with increasing field strength. For only 30 min of operation at pH 8, where hemoglobin is only slightly negatively charged, nearly all of the hemoglobin was recovered in the top phase at voltages above 50 V/cm.

The effect of varying the volume ratio, ϵ, on two-phase electrophoresis was also studied. It was observed that larger ϵ values resulted in higher K_f and Y values for a given time in a specified field. Since the volume, and

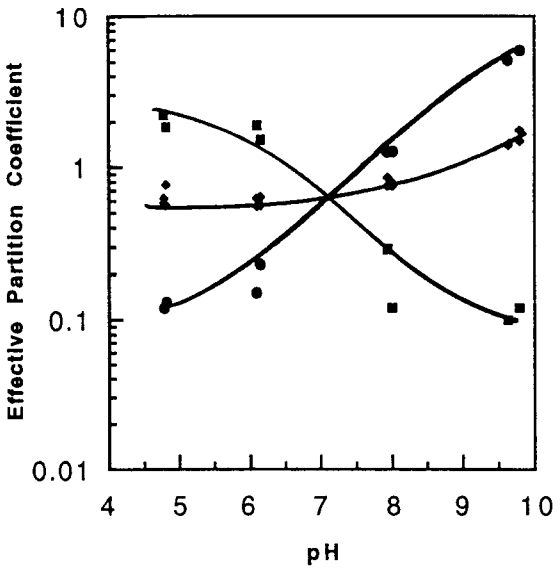

Fig. 1. Effective partition coefficient of hemoglobin as a function of pH after two-phase electrophoresis at 25 V/cm for 30 min: squares, cathode as upper electrode; circles, anode as upper electrode; diamonds, equilibrium partition coefficients (*10*).

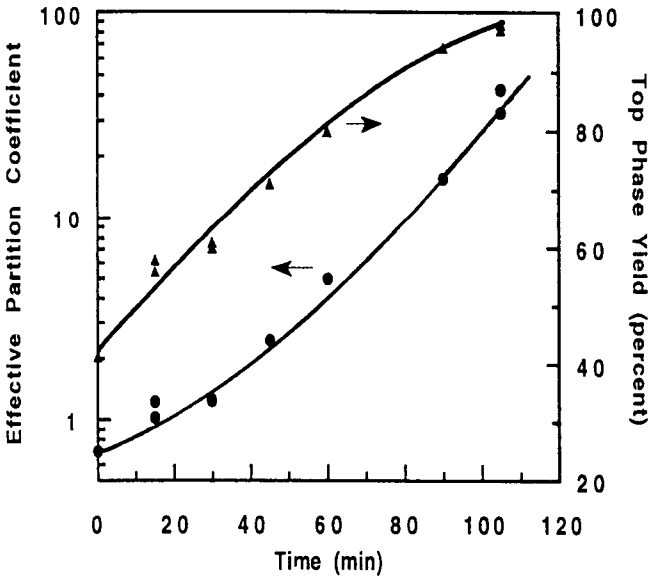

Fig. 2. Effective partition coefficient and top phase yield of hemoglobin as a function of time for two-phase electrophoresis at pH 8 and 25 V/cm (*10*).

Table 1
Separation of Albumin and Hemoglobin,
Equilibrium Partitioning, Two-Phase, and Homogeneous Electrophoresis

Field, V/cm	Time, min	K_A	K_H	α_{AH}	ϵ	Y_A	Y_H	ϕ_{AH}
No field equilibrium		0.62	0.61	1.02	1.0	0.38	0.38	1.0
50 two-phase	120	16.72	<0.004	>4180	1.15	0.95	<0.005	>190
50 homogeneous	120	0.72	0.008	90	4.8	0.78	0.037	21

Anode top, buffer = 10 m*M* Tris/Bes, pH = 6.

thus the height, of the more viscous bottom phase was smaller for larger ϵ, the bottom phase could be depleted of protein more easily as ϵ increased.

Binary protein separations of hemoglobin and BSA were made at pH 6, where the proteins are oppositely charged. Table 1 presents a comparison of separation results obtained using two-phase electrophoresis with those obtained from standard partitioning experiments in the absence of an applied field and from electrophoresis in homogeneous solution. For the experiments shown, the anode was the upper electrode. It can be seen that application of 50 V/cm for 2 h provided a good separation compared with essentially no separation owing to equilibrium partitioning in the same two-phase system. It can also be seen that electrophoresis in two-phase media gave better results than electrophoresis in homogeneous media under the same conditions. With a homogeneous buffer solution in the working chamber, samples withdrawn after electrophoresis are not separate phases, but merely samples from different regions in the device. We tried to obtain the best separation possible in the single phase run by collecting a larger top sample containing as much albumin as possible with little hemoglobin and a smaller bottom sample concentrated in hemoglobin. The separation and purification factors for the single-phase run would have compared even less favorably with those of the two-phase run if equal volume samples had been withdrawn.

The effect of polarity, pH, electrophoresis time, field strength, and volume ratio on the two-phase electrophoretic separation of hemoglobin and BSA was also studied, and the results obtained were consistent with those described above for two-phase electrophoresis of hemoglobin alone.

In a typical two-phase electrophoresis run, convective currents were observed in the top phase, but no convection across the phase boundary was observed. Thus, the two-phase system appears to have helped control convective mixing within the device. Moreover, product streams were readily recovered without contamination after two-phase electrophoresis, since about 90% of the top phase could be collected without fear of con-

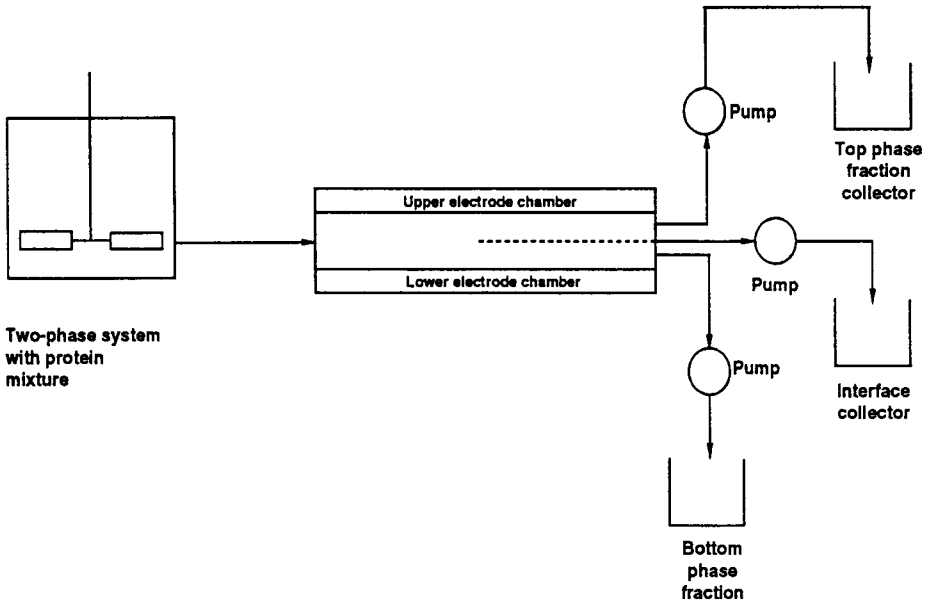

Fig. 3. Schematic diagram of continuous-flow two-phase electrophoresis device (*9*).

tamination from the bottom phase. Another potential advantage of two-phase electrophoresis is that initial conditions can be controlled to some extent by appropriate choice of the phase system and buffers used.

CONTINUOUS-FLOW TWO-PHASE ELECTROPHORESIS

For large-scale separations, a continuous-flow method is preferred over the batch procedure described above. We have constructed a prototype continuous-flow device with a 23 × 1.3 × 1.3 cm separation channel isolated from electrode channels on the top and bottom by ultrafiltration membranes. As shown schematically in Fig. 3 (*9*), a well-mixed two-phase system containing solutes to be separated flows in one end, while top, bottom, and interface fractions are pumped from the other end at controlled rates. Remixing and recirculating the buffer solutions through a refrigerated bath help remove electrolysis gas bubbles and maintain constant temperature and pH in the electrode channels. This flow device is similar in principle to other continuous-flow systems, including the density gradient stabilized flow device of Mel (*14*) and the electrical field flow fractionation devices used for split-flow field flow fractionation (*15*) and focusing field flow fractionation (*16*), except that none of these methods take advantage of a stable liquid–liquid interface to limit convective mixing between regions in the device. The electroextraction device of Stichlmair and coworkers appears to be several cocurrent flow devices similar to ours connected in a countercurrent series (*11*).

Preliminary experiments with our prototype flow device indicate that results similar to those reported above using the batch process can be obtained with flow rates of about 2 mL/min each for the top, bottom, and interface fractions. It was observed, however, that for some flow and electric field conditions, proteins were retained within the device. In those cases, even though good separation was achieved, the continuous recovery of purified protein was not optimized.

Solute retention within the device results, because the solute is driven against the membrane by the electric field. The problem is more likely to occur for solutes driven to the top phase, since the electrophoretic mobility is higher in the top phase than in the more viscous bottom phase. The solute will stay near the membrane if the force holding it there dominates the tendency to diffuse back into the bulk solution. Since fluid velocity approaches zero near a wall, solutes congregating at the membrane may not be washed out of the device, even if they do not adsorb to the membrane.

We are currently pursuing several potential solutions to this problem. One option is to increase the buffer chamber pressure slightly above that of the working chamber generating a flow through the membranes opposing electrophoretic migration near the membranes. It should be possible to control this flow so that the main result is the creation of slip at the wall. Another option is to switch polarity of the electric field periodically during operation. Thus, solutes trapped near the membrane could be periodically directed into a flowing region.

RECOVERY OF PROTEIN FROM FERMENTATION BROTH

Although the separation of two proteins of opposite charge in a low-ionic-strength buffer solution can be accomplished with two-phase electrophoresis, as we have demonstrated above, such separations will be of limited commercial interest. Separation of multiple components should be possible with a multi-step procedure, adjusting pH in each step to direct contaminants away from the desired product. It may also be possible to utilize two-phase electrophoresis early in the separation scheme to isolate the desired product from cell debris and any oppositely charged species. Product recovery directly from fermentation broth is unlikely because of high ionic strength. High ionic strength means high conductivity, which translates into excessive ohmic heating at the voltages of interest for electrophoretic separations. Two-phase electrophoresis may be useful, however, for recovery of intracellular or periplasmically located proteins from cell broth after initial cell washing and protein release steps. Cell mass would first be isolated by centrifugation or filtration. The cells would be washed, resuspended, and disrupted in an appropriate low-ionic-strength buffer. Cell debris might then be isolated in one phase of a two-phase system, while the desired product could be directed into the other. The

cells might also be caused to migrate under the effect of electrophoresis and could be sent in the direction opposite to that of the desired product. Adjustment of the phase volume ratio could allow concentration of the product as well.

In principle, a two-phase electrophoresis step could be used in a protein recovery process to eliminate a filtration or centrifugation step for cell debris removal while removing all oppositely charged contaminants and concentrating the product. This goal has not yet been achieved, but preliminary studies have shown that prewashed *E. coli* cells added to the two-phase system could be directed into one phase, while the protein hemoglobin could be directed into the other phase with an applied field (*17*). Another important question for commercial application of any separation techniques is whether or not the product remains stable and active during the recovery process. We have shown that β-galactosidase remains active after two-phase electrophoretic separation (*18*).

ELECTROPHORETIC MASS TRANSFER AT INTERFACES

Our previous studies have indicated that two-phase electrophoresis proceeds according to the basic principles of electrophoresis. We have observed no significant limitation to transport imposed by the two-phase system. However, Levine and Bier have observed an accumulation of protein at the interface in their studies on hemoglobin migration through an aqueous two-phase system in a U-tube electrophoresis device (*12*). This phenomenon was directional in that migration from preferred phase to nonpreferred phase yielded an accumulation, whereas migration from nonpreferred to preferred phase appeared unaffected by the phase boundary. Levine and Bier concluded that a partition coefficient dependence to electrophoretic migration was established by the thermodynamic properties of the phase system. It should be noted that Stichlmair and coworkers reported no difficulty in directing charged dyes and citric acid across the phase boundary from preferred to nonpreferred phase in their electroextraction experiments (*11*).

Since a significant limitation to interfacial transport will influence two-phase electrophoretic separations, we are investigating this phenomenon further. Using the apparatus and procedures described in ref. (*10*), we have made a series of studies to determine under what conditions protein can and cannot be forced to migrate across the interface from preferred to nonpreferred phase. First we considered altering the composition of the phase-partitioning system. Levine and Bier suggested that electrophoretic transport can be controlled by modifying the affinity of a protein for a phase. The affinity of a protein for a phase is described by the equilibrium partition coefficient, K_o. For a protein with $K_o < 1$, increasing the polymer concentrations in an aqueous two-phase system yields a lower

Table 2
Effect of Tie Line Length
on Effective Partition Coefficient, K_f

TLL = 7		TLL = 15	
K_o	K_f	K_o	K_f
0.62	1.5	0.24	0.45

T = 25°C, field = 20 V/cm, cathode top, time = 30 min, buffer = 10 m*M* Tris/Bes, pH = 6.

Table 3
Effect of Initial Phase Settling Condition
on Effective Partition Coefficient

Well mixed		Settled	
K_o	K_f	K_o	K_f
0.66	4.2	0.59	3.8

T = 25°C, field = 45 V/cm, anode top, time = 30 min, buffer = 10 m*M* Tris/Bes, pH = 8, TLL = 7.

K_o, indicating a stronger affinity for the bottom phase. The tie line length (TLL) is a convenient means of characterizing the polymer concentrations in an aqueous two-phase system:

$$TLL^2 = (P' - P'')^2 + (D' - D'')^2 \quad (2)$$

where P and D are PEG and dextran concentrations in weight percent; and ′ and ″ denote top and bottom phase, respectively. According to Levine and Bier, interfacial migration should be hindered further at longer tie line lengths. Our studies reported above were at TLL = 7. We prepared a two-phase system of TLL = 15 by adding 10 g dextran 500,000 and 6 g PEG 8000 to 84 g of buffer. Two-phase electrophoresis experiments with hemoglobin in Tris/Bes buffer at pH 8 at the two different tie line lengths are compared in Table 2. The equilibrium partition coefficients, K_o, indicate that indeed hemoglobin prefers the bottom phase more at the longer tie line. The effective partition coefficients obtained after electrophoresis for 30 min at 20 V/cm indicate that hemoglobin does migrate across the phase interface even at the longer tie line. Some accumulation at the interface may have occurred in one or both of these runs, but none was observed.

We next considered the initial settling condition of the phase systems. Our experiments began with a well-mixed two-phase system that settled during the course of a run. Levine and Bier's studies began with equilibrium phases presettled. Table 3 shows the effect of initial settling on two-phase electrophoresis in our device. It can be seen that protein migrated

Table 4
Effect of Temperature on Effective Partition Coefficient

25°C		5°C	
K_o	K_f	K_o	K_f
0.15	0.95	0.13	0.36

Field = 20 V/cm, anode top, time = 120 min, buffer = 10 m*M* Tris/Bes, pH = 8, TLL = 15.

Table 5
Effect of Buffer Type on Effective Partition Coefficient

Tris/Bes, 20 V/cm		Phosphate, 7 V/cm	
K_o	K_f	K_o	K_f
0.24	0.45	0.09	0.14

T = 25°C, cathode top, time = 30 min, TLL = 15, buffers = 10 m*M*, pH = 6, field shown as 20 or 7 V/cm.

readily across the phase interface from preferred to nonpreferred phase regardless of initial settling conditions. The difference between the two K_f values is probably within the uncertainties in their measurements.

We also studied the effect of temperature on migration across the phase interface. It can be seen in Table 4 that hemoglobin still moves across the interface to the nonpreferred phase at 5°C. The fact that less protein crossed the interface at the lower temperature can be explained by the fact that electrical conductivities are lower at lower temperature. Again, there may have also been some undetected accumulation at the interface.

Finally, we studied the effect of buffer type on interfacial migration. A 10 m*M* phosphate buffer was prepared by combining appropriate amounts of mono- and dibasic potassium phosphates. It can be seen in Table 5 that hemoglobin migrates across the phase interface from preferred to nonpreferred phase in this buffer as well. The voltage applied in the phosphate system was only 7 V/cm to avoid excessive heating owing to the relatively high conductivity of this buffer system.

Having established that proteins can be caused to migrate from preferred to nonpreferred phase under a variety of conditions, we have begun looking more deeply into the question of accumulation at the interface. We have measured interfacial concentrations and performed detailed mass balances, accounting for migration into a phase from the other phase and out of a phase owing to accumulation at the membrane boundary to determine if an interfacial accumulation can be detected. Although there is still

Table 6
Measured Donnan Potentials and Evidence
of Interfacial Accumulation for Several Two-Phase Systems

Buffer	pH	Donnan potential, mV	Hemoglobin charge	Accumulation t-b	Accumulation b-t	Opposing potential t-b	Opposing potential b-t
Tris/Bes	8	0.3	–	N	N	δ	ζ
Tris/Bes	6	0.3	+	N	N	ζ	δ
Phosphate	8	2.2	–	Y	N	δ	ζ
Phosphate	6	1.5	+	N	Y	ζ	δ

T = 25°C, TLL = 15, buffers = 10 m*M*, b-t = protein directed from bottom to top, Y = accumulation detected, N = accumulation not detected, δ = interfacial potential, ζ = zeta potential.

some uncertainty in our results, we have found evidence of accumulation in systems with phosphate buffer, known to provide a significant Donnan potential in these systems (*19*). We therefore measured Donnan potentials in our phase systems and attempted to correlate the evidence of accumulation with Donnan potential. Donnan potentials were measured according to the method of Brooks et al. (*20*) as discussed by Haynes et al. (*19*). Table 6 shows measured Donnan potentials in Tris/Bes and phosphate-buffered systems at pH 6 and 8 at 25°C and TLL = 15.

A qualitative measure of accumulation of hemoglobin being directed from the upper nonpreferred phase to the lower preferred phase (denoted t-b) and vice versa (denoted b-t) is given in the table as Y for accumulation detected and N for accumulation not detected. We also show in Table 6 an attempt to explain these results in terms of a simplified description of the potential profile across the interface first proposed by S. Levine (*21*). Baygents and Saville (*22*) have recently presented a more complete analysis than that of Levine. According to Levine, measured values of electrophoretic mobility for drops of top phase in a continuous bottom phase could be explained with a potential profile like that shown in Fig. 4 (*20*). Uneven partitioning of buffer ions gives rise to an electrostatic potential difference across the interface with electrical double layers formed on both sides. The bulk phase potential difference is the Donnan potential we measured, ψ. To account for rapid migration of phase droplets in directions opposite to those expected from the sign of the bulk phase, zeta potentials, ζ_t and ζ_b, representing the potential between the bulk and the interface for each phase, were oriented as shown, making the interfacial potential, δ, larger than the Donnan potential. Measured Donnan potentials give no indication of the ζ values, but do give a measure of δ, since larger ψ correspond to larger δ.

Positive species tend to move in a direction from high potential to low potential, whereas negative species move from low potential to high potential (*23*). For systems with the top phase at a higher potential than the bottom phase, as shown, a negative species would readily move against

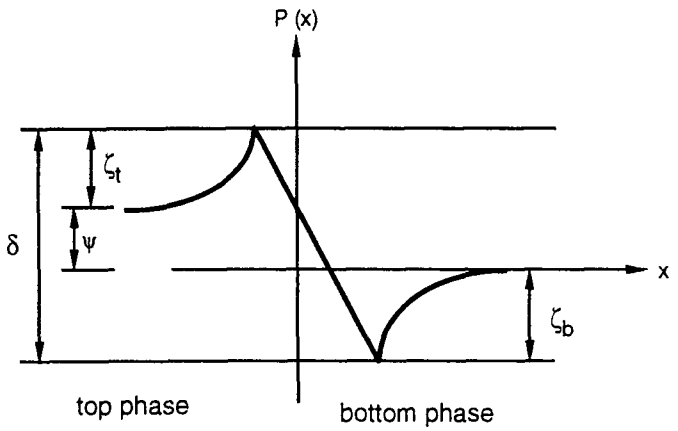

Fig. 4. Potential profile consistent with electrokinetic and equilibrium measurements in aqueous two-phase systems, adapted from (*20*).

the ζ potentials, but would be opposed by the interfacial potential, δ, in going from the top phase to the bottom phase. Similarly, a positive species would be opposed by the ζ potentials, but would readily migrate against the interfacial potential. We summarize this argument in Table 6 by noting which potentials of Fig. 4 will be expected to oppose the electrophoretic migration at the various conditions. Tris/Bes buffer yields relatively low Donnan potential and, therefore, relatively low interfacial potential. Interfacial accumulation was not detected at any of the conditions studied for this system. Measured Donnan potentials for the phosphate buffer were relatively large and consistent with those reported in the literature (*19*). Accumulation was detected in phosphate-buffered phase systems when the large interfacial potential (necessarily greater than or equal to ψ) opposed the migration of the protein. No accumulation was detected when the ζ-potentials opposed migration.

It appears that the electrostatic potential profile near the interface of conducting liquid–liquid systems can influence electrophoretic migration of charged species across the interface. Although material did migrate across the interface in all cases, we detected interfacial accumulation for migration in both directions: from preferred to nonpreferred phase and from nonpreferred to preferred phase. A simplistic view of the electrical phenomena at the interface showing that large interfacial potentials will oppose migration appears to be consistent with the observed results.

Electrical phenomena at interfaces should be investigated further. Unfortunately, in both our device and the device of Levine and Bier, it is not possible to measure accurately solute concentration as a function of position. In their device, significant accumulation can be visually observed, but concentrations cannot be measured, and it is difficult to determine if any protein is migrating across an interface. In our device, mixing-cup-phase concentrations are measured after electrophoresis for a specified time, and migration across the interface can be quantified. It is difficult to

detect accumulation and virtually impossible to quantify it in our device. Our reported effective partition coefficients obviously do not tell the whole story. Mass balances on material leaving one phase and entering the other generally closed to within 10%. Material at the interface could account for the discrepancy in some cases, but so could material accumulating at the membranes combined with uncertainties in concentration and phase volume measurements. The fact that protein does cross the interface from the preferred dextran-rich phase to the nonpreferred PEG-rich phase is clear from our data. The top-phase concentration does increase with increasing time and field strength. We have reported detecting accumulation only when mass balances that included estimates of the amount of material accumulating at the membranes were outside of the range of our expected experimental error and when interfacial samples showed higher concentrations than expected from a similar balance. Therefore, when accumulation was reported, it probably did occur. On the other hand, the lack of detection by our method does not mean there was no accumulation. A noninvasive method for the measurement of concentration as a function of time and position is being sought. Interferometric techniques can be used for such measurements (*24*), but light scattering from the polymers may hinder their utility in these systems. Interferometric measurements have revealed persistent density gradients in PEG/salt aqueous two-phase systems (*25*).

CONCLUSIONS

Proteins can be directed across the interface of an aqueous two-phase system by electrophoresis in either direction: from preferred to nonpreferred and from nonpreferred to preferred phase. Although proteins migrated across the interface in all cases studied, evidence of accumulation at the interface has been found in systems with large Donnan potentials. Two-phase electrophoresis separation of proteins was accomplished by directing two oppositely charged proteins into separate phases of a phase system with a small Donnan potential. Electroextraction may prove useful for commercial-scale electrophoretic separations, since it offers the following advantages over free solution methods: controlling starting compositions, limiting convective mixing, and facilitating product isolation.

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