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Two-Phase Electrophoresis of Proteins

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ABSTRACT

Proteins have been directed into either the top or bottom phase of a polyethylene glycol/dextran aqueous two-phase system by applying an electric field perpendicular to the phase interface. Protein migration across the interface was manipulated by varying polarity, pH, electrophoresis time, field strength, and phase volume ratio. Mixtures of hemoglobin and albumin were separated by operating between isoelectric points and directing oppositely charged proteins into separate phases. Applying 50 V/cm for 2 hours to 58 mL of an equal phase volume two-phase system containing 0.2 g/L of each protein at pH 6 resulted in a bottom phase containing 99% of the hemoglobin and a top phase containing 95% of the albumin. This represents a significant improvement over the separation obtained either by partitioning in the same two-phase system with no applied field or by electrophoresis under the same conditions in homogeneous buffer. The two-phase system divides the electrophoresis device into two distinct regions, providing stability against convective mixing and facilitating product isolation.

INTRODUCTION

Aqueous two-phase systems can be formed when appropriate small amounts of two incompatible polymers, such as polyethylene glycol (PEG) and dextran, are dissolved in water causing the system to split into two liquid phases separated by a sharp stable interface. The use of such phase systems for extractive separations of biological materials is well documented (1, 2). Unlike the aqueous/organic phase systems of traditional solvent extraction, both phases of these aqueous two-phase systems are predominantly water and hence biocompatible. Although the two phases are also electrically conductive, few applications with electric fields imposed

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on these systems have been made (3–7). Brooks and Bamberger first applied an electric field to an aqueous two-phase system in an attempt to improve the rate of the extraction process by speeding the phase settling step (3). Todd and coworkers further pursued this electrically enhanced phase demixing concept (4, 5). Levine and Bier have studied the electrophoretic mobility of a protein in an aqueous two-phase system by using a U-tube electrophoresis device, and they noted an impediment to electrophoretic transport across the phase interface (6). We have pursued protein separations by applying a field perpendicular to the phase interface to direct oppositely charged species into separate phases (7). Our goal is to utilize the phase interface to prevent convective mixing and to facilitate product isolation during electrophoresis, thereby allowing development of a new commercial scale electrophoretic separation method. In this paper we report the effects of polarity, pH, electrophoresis time, field strength, and phase volume ratio on the migration of albumin and hemoglobin across the phase interface under the influence of an applied field. The separation of the two proteins obtained using two-phase electrophoresis is compared to that obtained by equilibrium partitioning in the same two-phase system with no applied field and by electrophoresis in the same device in a homogeneous buffer.

MATERIALS AND METHODS

Proteins of bovine serum albumin (BSA) and hemoglobin, phase forming polymers of 500,000 average molecular weight dextran and 8000 average molecular weight PEG, and buffers BES free acid and Trizma base were obtained from Sigma Chemical Company. Deionized water was further purified with a Millipore Milli-Q water treatment unit before use. Low ionic strength buffers in the pH range between 4.5 and 9.5 were prepared by combining appropriate amounts of BES free acid and Trizma base. The compositions used were: 20 mM BES for pH 4.5; 0.5 mM Trizma and 9.5 mM BES for pH 6.0; 17 mM Trizma and 2.4 mM BES for pH 8.0; and 20 mM Trizma for pH 9.5. Two-phase systems with nearly equal phase volumes were prepared by adding 5.5 g dextran and 3.8 g PEG to 90 mL buffer. According to a published phase diagram (1), the resulting top phase contained 2 wt% dextran and 5.6 wt% PEG while the bottom phase contained 8.3 wt% dextran and 2.4 wt% PEG. Separating and recombining these equilibrium phases in appropriate amounts provided phase systems with controlled, uneven phase volumes. Single protein experiments were made with 0.1 g/L of either BSA or hemoglobin dissolved in the phase system. Binary protein separation experiments were made with 0.2 g/L of each protein.

To demonstrate the aqueous two-phase electrophoresis concept, we constructed the vertical electrophoresis device shown schematically in Fig. 1. The Plexiglas device consisted of three cylindrical chambers separated by ultrafiltration membranes. A small peristaltic pump was used to circulate buffer solution through the upper and lower chambers containing platinum wire electrodes. Buffers used in the electrode chambers contained no proteins nor phase forming polymers. The inner, working chamber was filled with a protein mixture in an aqueous two-phase system prepared in the same buffer as that in the electrode chambers. Millipore PLGC 10,000 NMWL membranes retained the protein mixture and phase forming poly-

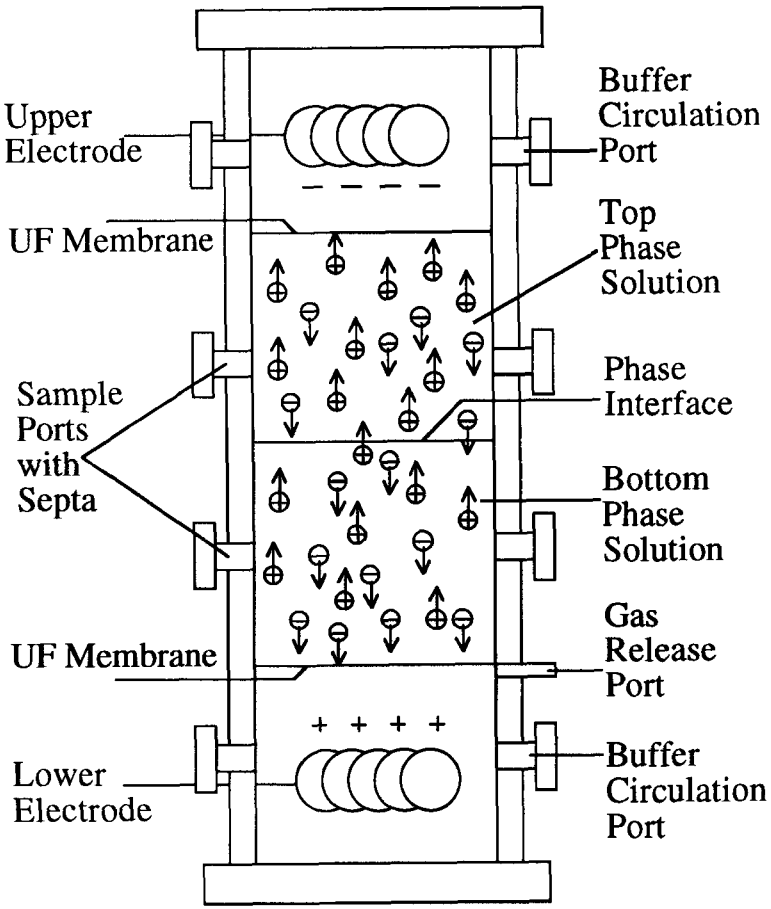

FIG. 1 Schematic diagram of the two-phase electrophoresis device.

mers, but allowed passage of electrical current. Electrolysis gases formed at the lower electrode were removed periodically through an outlet port just below the membrane covering the electrode chamber. A slight tilt of the device toward the outlet port facilitated gas removal. The 58-mL working chamber was 5.1 cm high and 3.8 cm in diameter and it contained four sample ports with septa: two near the top and two near the bottom. Either a Hoefer Scientific PS 500 XT or a Bio-rad 3000Xi power supply provided constant voltage across the interelectrode distance of 10 cm.

For each experimental run, an initially well mixed two-phase system of known protein concentration was introduced into the working chamber and subjected to a specified constant voltage for a specified time. Normally the electric field was applied as soon as the well-mixed system was loaded, before phase demixing had occurred. Some experiments were also made in which the phases were allowed to settle until a sharp interface was formed near the center of the working chamber before applying the electric field. For all experiments, heights and, thus, volumes of the final separated phases were determined before samples of each phase were collected and analyzed. As much as possible of the top phase was withdrawn through one of the upper sample ports by using a syringe while the other upper port served as a vent. An interface fraction containing the remaining top phase and a small portion of the bottom phase was then withdrawn. Finally, the remaining bottom phase was collected. Since concentration gradients were established within the device, as much as possible of each phase was collected and vortexed before measuring the average protein concentration in each phase.

A standard partitioning measurement in the absence of an applied field was also made for each experiment. Before loading the working chamber, a portion of the two-phase system was collected and centrifuged to facilitate phase separation in a test tube. Volume and protein concentration were determined for each phase.

Electrophoresis experiments using homogeneous buffers were also conducted by following a procedure similar to that of the two-phase electrophoresis experiments. After a run, samples of known volume were removed from the upper and lower portions of the inner working chamber. The brown color of hemoglobin in solution served as an indicator of how much sample to withdraw from each location to obtain the best results. Buffers with 10 wt% dextran added were used as well as those with no added polymer.

For single protein experiments using hemoglobin, protein concentrations in each phase were measured spectrophotometrically at 410 nm against blanks of the appropriate phase. For BSA experiments and binary separation experiments, protein concentrations were determined using HPLC.

A Millipore-Waters gradient HPLC system with UV detection and an Alltech Macrosphere 300 Å C4 reverse phase column, 15 cm long and 4.6 mm in diameter, was used. The elution gradient at 1 mL/min was 15 to 55% solvent B over 12 minutes; solvent A was 0.15% trifluoroacetic acid in water and solvent B was 0.15% trifluoroacetic acid in 95% acetonitrile, 5% water.

RESULTS AND DISCUSSION

Two-Phase Electrophoresis of Hemoglobin

Polarity and pH. Two-phase electrophoresis results are reported here in terms of an effective partition coefficient defined 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. For a standard partitioning experiment, the partition coefficient is defined similarly to the effective partition coefficient but only the former is an equilibrium value. The effective partition coefficients obtained as a function of polarity and pH for two-phase electrophoresis of hemoglobin for a fixed time of 30 minutes and a fixed field of 25 V/cm and using an equal volume two-phase system are shown in Fig. 2. The standard partitioning results are also shown in Fig. 2 where it can be seen that hemoglobin partitions slightly more toward the bottom phase at pH values below 9 and distributes evenly between the phase at pH 9.5 with no applied field. It can be seen that upon application of the field, hemoglobin can be directed into either the top or the bottom phase depending on the placement of the electrodes and the net charge on the protein. The curves representing standard partitioning, electrophoresis with the anode (positive electrode) in the upper chamber, and electrophoresis with the cathode (negative electrode) in the upper chamber cross near pH 7, the isoelectric point of hemoglobin. These results are at least qualitatively consistent with the basic principles of electrophoresis. Neglecting the effects of gravity and diffusion, the velocity, v, of a solute migrating perpendicular to the phase interface in an applied field depends on the field strength, E, and the solute electrophoretic mobility, u:

$$v = uE \tag{1}$$

The electrophoretic mobility of a protein depends on its size and net charge as well as the solution viscosity. The net charge on the protein depends on the pH; hemoglobin is positively charged below and negatively charged above pH 7, and the magnitude of the charge increases with increasing distance from the isoelectric pH. Reversing the electrode location changes the sign of the electric field and thus reverses the direction of protein migration across the interface.

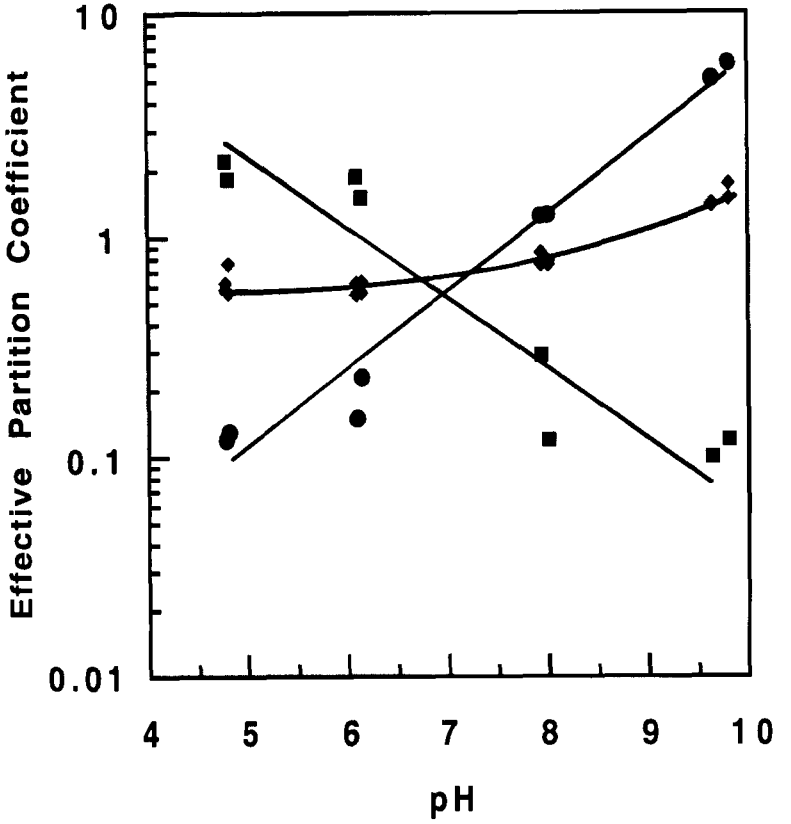

FIG. 2 Effective partition coefficient of hemoglobin as a function of polarity and pH after two-phase electrophoresis at 25 V/cm for 30 minutes: (■) cathode as upper electrode; (●) anode as upper electrode; (◆) equilibrium partition coefficients.

Time. Figure 3 shows how the effective partition coefficient of hemoglobin increases with increasing electrophoresis time at pH 8 and a constant field of 25 V/cm. With the anode as the upper electrode, more time in the applied field allowed more of the negatively charged hemoglobin to move across the phase interface. This behavior is again at least qualitatively consistent with Eq. (1) since for a given velocity on each protein molecule, longer times would allow more protein to move far enough to cross the interface. Also shown in Fig. 3 is the top phase yield, Y, defined as the percent of the total protein in the two-phase system recovered in the top phase:

$$Y = K_f\varepsilon/(1 + K_f\varepsilon) \quad (2)$$

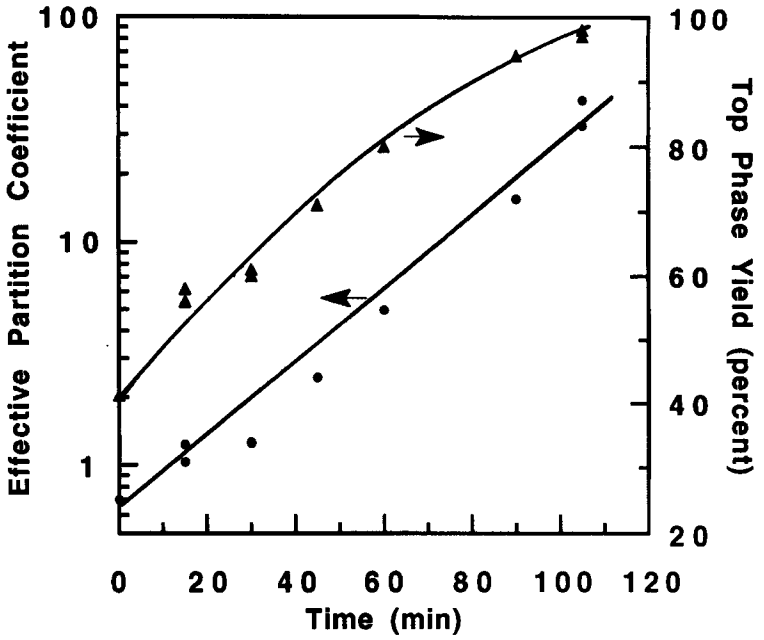

FIG. 3 Effective partition coefficient and top-phase yield of hemoglobin as a function of time for two-phase electrophoresis at pH 8 and 25 V/cm.

where K_f is the effective partition coefficient and ε is the ratio of the top phase volume to the bottom phase volume. At 25 V/cm with a nearly equal phase volume two-phase system, nearly all of the hemoglobin was recovered in the top phase after 100 minutes in the applied field. The volume ratio varied slightly from 1 for some runs since partial phase separation occurred while loading a well stirred two-phase system into the working chamber. The top phase was preferentially loaded upon transfer from a flask to the working chamber due to the higher viscosity of the bottom phase.

Electric Field. Figure 4 shows how the effective partition coefficient for hemoglobin increases with increasing voltage at pH 8 and a fixed time of 30 minutes. The observed increase in K_f for a slightly negatively charged protein with the anode as the upper electrode was again qualitatively consistent with Eq. (1) until an unpredicted maximum was obtained at about 55 V/cm. The decline in K_f at higher voltages can be explained by the observation that convective currents within the device began to disturb the phase interface at voltages above 45 V/cm. Below 45 V/cm, convective currents could be seen in the top phase but little or no convection was

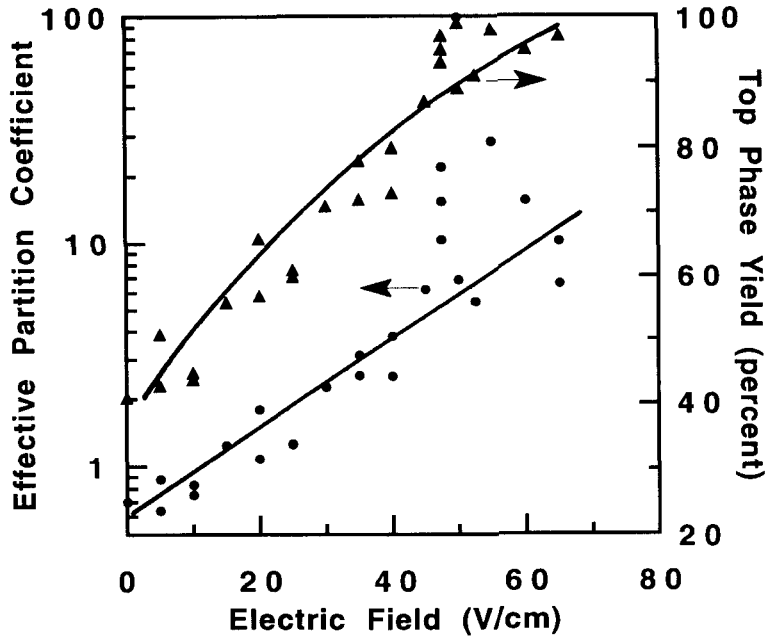

FIG. 4 Effective partition coefficient and top-phase yield of hemoglobin as a function of field strength after two-phase electrophoresis for 30 minutes at pH 8.

observed in the bottom phase and the interface remained stable. Above 45 V/cm, slight disturbances of the interface were observed as it appeared to be pulled slightly upward in the center. At 65 V/cm, convective currents were observed throughout the device and a stable interface was not observed until after the field was turned off.

It should be noted that in order to observe the effect of convection on the phase interface, no temperature control was imposed. Consequently, the temperature of the two-phase and buffer solutions increased from room temperature to as much as 40°C during some of the higher voltage runs. Although we have observed even more serious heating problems and obvious aggregation of denatured hemoglobin in some experiments with other buffers, none of the experiments reported here showed signs of protein denaturation. We have not yet made detailed studies of protein activity after two-phase electrophoresis, however. Cooling of the device or the circulating buffer solution would minimize this temperature increase but would not eliminate convection induced by local temperature gradients.

The yields of hemoglobin in the top phase are also presented in Fig. 4. Even for 30 minutes 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.

Phase Volume. The effect of varying the volume ratio, ε, on the effective partition coefficient and top phase yields obtained after 45 V/cm was applied with the anode as the upper electrode for 30 minutes at pH 8 is shown in Fig. 5. Larger ε values resulted in higher K_f values for a given time in a specified field. Since the volume, and thus the height, of the bottom phase was smaller for larger ε, the bottom phase could be depleted of protein more easily as ε increased. The top phase yield increased accordingly with increasing volume ratio.

Comparison to Previous Studies. From the results and discussion above, it appears that two-phase electrophoresis follows the basic principles of electrophoresis with no significant limitation to transport imposed by the two-phase system. However, in studying the migration of hemoglobin across the phase interface of an aqueous two-phase system in a U-tube electrophoresis device, Levine and Bier observed an impediment to electrophoretic transport from the preferred phase to the nonpreferred phase

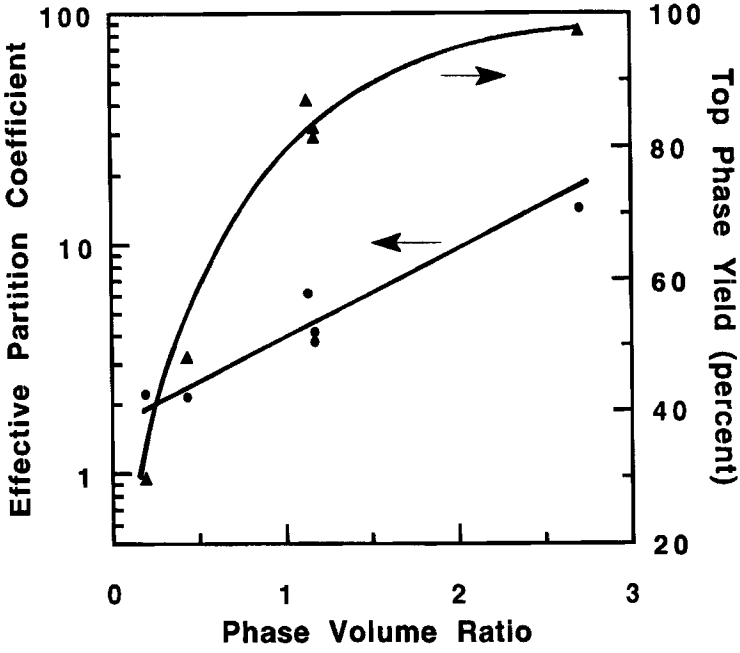

FIG. 5 Effective partition coefficient and top-phase yield of hemoglobin as a function of phase volume ratio after two-phase electrophoresis for 30 minutes at pH 8 and 45 V/cm.

(6). That is, in a phase system in which hemoglobin partitioned toward the bottom phase in the absence of an applied field, an accumulation of hemoglobin was visually observed at the phase interface when it was directed toward the top phase under the influence of an applied field. On the other hand, Levine and Bier observed that hemoglobin was readily directed from the top nonpreferred phase to the bottom preferred phase when the electrodes were reversed and that hemoglobin would also migrate across the interface in both directions in a system where it distributed evenly at equilibrium. Building on earlier studies by Davies on diffusion across interfaces (8), Levine and Bier concluded that the thermodynamic properties of the phase system introduced a partition coefficient dependence to electrophoretic migration across the interface.

We are currently making detailed studies of the apparent discrepancy between our results and those of Levine and Bier. At this point we can offer the following comments on the subject. It seems reasonable that a finite impediment to transfer across the interface would be presented by the phase partitioning thermodynamics and that the transport rates in the forward and reverse direction might be different. We do not, however, expect an infinite impediment to transport, but rather one that could be overcome with sufficient electrophoretic force applied for a sufficient time. The magnitude of the required force should depend on the equilibrium partition coefficient, the phase viscosities, the charge on the protein, the buffer type, and electrical double layer effects at the interface among other factors. Apparently all of our experiments were at conditions where the required force was exceeded while Levine and Bier's experiments were at conditions where the required force was not reached. We chose a phase system near the critical mixing point to insure that the phases were similar to one another and, therefore, that the equilibrium partition coefficients of all solutes would be close to 1. Levine and Bier's experiments were conducted in a phase system further from the critical point, providing more extreme partitioning of solutes. Other differences between our studies and those of Levine and Bier include different temperatures, different buffers, a different apparatus and applied field, and different phase settling conditions when the field was applied. We are studying all of these differences to determine when the phenomena of accumulation at the interface occurs and when solutes migrate freely across the interface.

Levine and Bier applied their field to settled phases while our experiments were conducted on initially well mixed phases which settled during application of the field. We did study the effect of settling in our two-phase electrophoresis system and found essentially no difference between K_f and Y results for two experiments conducted at 45 V/cm in an equal phase volume two-phase system at pH 8. In one experiment the phase system

was initially well mixed when the field was applied for 30 minutes and in the other experiment the phases were allowed to settle for 45 minutes before applying the field for 30 minutes. A phase interface formed within 15 minutes in the former experiment and it might, therefore, be expected that any difference between mixed and settled two-phase electrophoresis experiments would be seen only for times shorter than 30 minutes. Since Levine and Bier's experiments were for 2 hours, it appears that settling will not be a major factor in explaining when proteins are caused to accumulate at the interface rather than migrating across.

Protein Separations via Two-Phase Electrophoresis

Binary protein separations of hemoglobin and BSA were made by using two-phase electrophoresis. Figure 6 shows a cross plot obtained for BSA at 25 V/cm and 30 minutes. It can be seen that BSA behaves similarly to hemoglobin except that the curves cross near BSAs isoelectric point of pH 4.9. Superposition of Fig. 6 on Fig. 2 allows prediction of separations of the two proteins that could be made at various pH values for an applied field of 25 V/cm and 30 minutes. Presumably better separations would be made at higher voltages and longer times. Clearly, the best separation is expected when operating at pH 6 between the isoelectric points of the proteins. With the anode in the upper buffer chamber, for example, negatively charged albumin molecules will be directed into the upper phase while positively charged hemoglobin will be directed into the lower phase at pH 6.

Results of a series of two-phase elecrophoretic separations are shown in Table 1. Results of standard partitioning experiments in the absence of an applied field and of a single-phase electrophoretic separation are also presented in Table 1 for comparison with the two-phase electrophoresis results. Several terms must be defined before discussing these results. The column labeled polarity indicates electrode placement: + for anode at the top and − for cathode at the top. K_A and K_H are partition coefficients of albumin and hemoglobin, respectively. These values represent nonequilibrium effective partition coefficients or equilibrium partition coefficients depending on whether or not a field was applied. The separation factor, α_{AH}, is the ratio of the partition coefficient for albumin to that of hemoglobin and is a convenient measure of the separating ability under the specified conditions. The volume ratio, ε, and the top phase yield, Y, have been defined above. A measure of separating ability which includes the effect of volume ratio is given by the purification factor, ϕ_{AH}, defined as the ratio of albumin yield to hemoglobin yield.

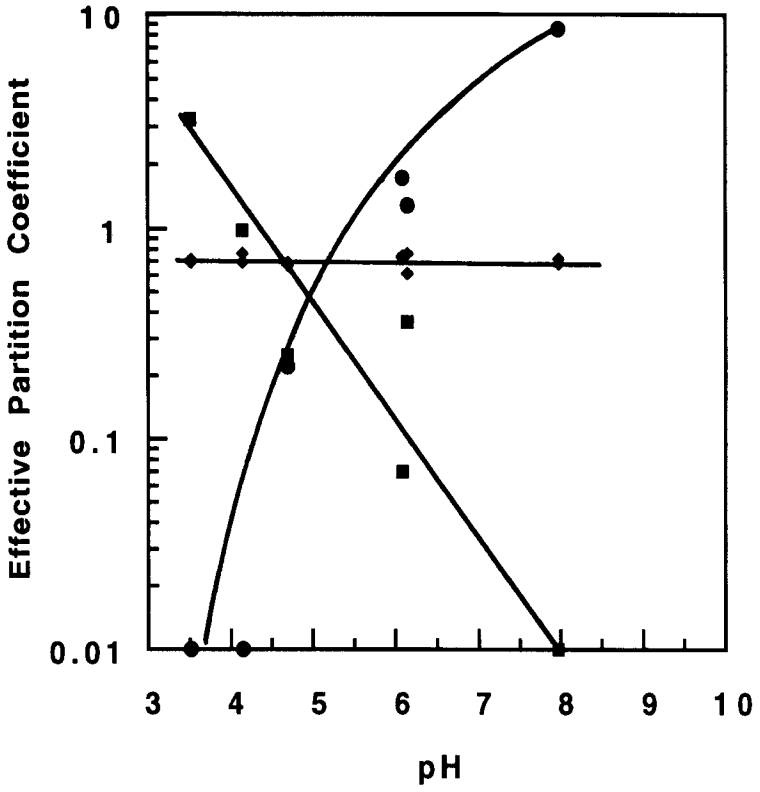

FIG. 6 Effective partition coefficient of BSA as a function of polarity and pH after two-phase electrophoresis at 25 V/cm for 30 minutes; (■) cathode as upper electrode; (●) anode as upper electrode; (♦) equilibrium partition coefficients.

Polarity, Time, and Field. Run 1 of Table 1 shows partitioning results for albumin and hemoglobin in the two-phase system at pH 6 in the absence of an electric field. The partition coefficients of 0.6 indicate that both albumin and hemoglobin have a preference for the bottom phase. With equal phase volumes the top phase contained 0.15 g/L while the bottom phase contained 0.25 g/L of each protein. Hence, only 38% of each protein was recovered in the top phase and no separation or purification was achieved. Comparison of Run 1 with Run 2, where 25 V/cm was applied for 30 minutes, shows the effect of the electric field on the separation at pH 6. With the anode at the top, albumin moved from the bottom phase to the top phase as indicated by a partition coefficient of 1.29. At the same time, hemoglobin moved to the bottom phase, resulting in an impressive

TABLE 1
Partition Coefficients, Separation Factors, Top-Phase Yields, and Purification Factors for Several Electrophoretic Separations of Albumin and Hemoglobin

Run	Field (V/cm)	Time (min)	Polarity	pH	K_A	K_H	α_{AH}	ε	Y_A	Y_H	ϕ_{AH}
1	No field			6.0	0.62	0.61	1.02	1.0	0.38	0.38	1.0
2	25	30	+	6.2	1.29	0.07	18.4	1.1	0.59	0.07	8.4
3	25	30	−	6.1	0.26	2.05	0.13	1.1	0.22	0.69	0.32
4	25	90	+	6.0	2.52	0.017	150	1.1	0.73	0.018	41
5	45	30	+	6.0	1.97	0.014	141	1.0	0.66	0.014	47
6	50	120	+	5.7	16.72	<0.004	>4180	1.15	0.95	<0.005	>190
7	No field			6.0	0.62	0.61	1.02	3.8	0.70	0.70	1.0
8	50	60	+	6.0	7.31	0.008	914	3.8	0.97	0.030	32
9	No field			7.9	0.68	0.80	0.85	1.0	0.40	0.44	0.91
10	15	30	+	7.9	1.85	0.91	2.03	1.15	0.68	0.51	1.3
11	50	120	+	5.7	0.72	0.008	90	4.8	0.78	0.037	21
	Homogeneous buffer										

increase in the separation factor above that with no applied field. With the volume ratio accounted for, 59% of the albumin and only 7% of the hemoglobin appeared in the top phase, yielding a purification factor for albumin in the top phase of 8.4. Thus, 93% of the hemoglobin was present in the bottom phase contaminated by 41% of the albumin. Reversing the electrode location directs the negatively charged albumin to the bottom phase and the positively charged hemoglobin to the top phase as shown by Run 3.

Comparison of Run 4 with Run 2 indicates the effect of increasing the electrophoresis time on the separation; albumin concentrated further in the top phase while hemoglobin concentrated further in the bottom phase. With 25 V/cm applied for 90 minutes, a purification factor of 41 was obtained since 73% of the albumin was recovered in the top phase contaminated by only 1.8% of the hemoglobin. Comparing Run 5 with Run 2 shows a similar effect upon increasing the electric field from 25 to 45 V/cm applied for 30 minutes.

By increasing the time to 2 hours and the field to 50 V/cm, we obtained a reasonably good separation of the two proteins, as shown in Run 6. The concentration of hemoglobin in the top phase was below our detection limit of 1 mg/L, yielding a purification factor greater than 190. Thus, 95% of the originally loaded 11.6 mg albumin was recovered in 31 mL of top phase with less than 0.06 mg of contaminating hemoglobin. Moreover, more than 99% of the original 11.6 mg hemoglobin was recovered in the bottom phase with only 0.6 mg albumin.

Phase Volume and pH. The volume ratio was also controlled in two-phase electrophoresis experiments to provide simultaneous concentration and purification of a desired product. In Run 8, 12 mL of bottom phase containing nearly 0.9 g/L hemoglobin and only 0.03 g/L albumin was recovered after 1 hour at 50 V/cm. Run 8 showed a significant improvement over Run 7 which yielded 12 mL of bottom phase containing 0.29 g/L hemoglobin and 0.29 g/L albumin with no field applied.

Runs 9 and 10 shown in Table 1 were made at pH 8 where albumin has a greater negative charge than hemoglobin. Applying 15 V/cm for 30 minutes increased the effective partition coefficient of albumin more than that of hemoglobin as shown in Run 10. This run demonstrates that some separation can be achieved with proteins moving at different rates in the same direction. More separation than that achieved at pH 8 would result at pH 7, where hemoglobin would not move, but clearly operation between isoelectric points is preferred.

Single-Phase Electrophoresis. We also investigated the possibility that a binary electrophoretic separation equivalent to that obtained with two-phase electrophoresis could be obtained in the absence of the two-phase system under similar 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. As indicated by the partition coefficients of Run 11, most of the hemoglobin appeared in the bottom sample while the albumin distributed more evenly 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. A volume ratio of 4.8 was obtained which resulted in 78% of the albumin being recovered in 48 mL of top sample and 96.3% of the hemoglobin concentrated in 10 mL of bottom sample. In both the two-phase Run 6 and the single phase Run 11, nearly all the hemoglobin was directed into the bottom, leaving purified albumin in the top. In the two-phase run, however, the albumin was also concentrated in the top phase, leaving purified hemoglobin in the bottom phase. In the single-phase run, albumin distributed throughout the device and contaminated the hemoglobin collected at the bottom. 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.

A qualitative comparison between Runs 6 and 11 should also be made. At the end of the two-phase run, the top phase looked clear while the bottom phase looked light brown with a darker brown band at the bottom. The brown color indicates the presence of hemoglobin in the otherwise

colorless solutions. At the end of the single-phase run, and indeed after about 30 minutes into that run, a dark brown band was also observed at the bottom of the chamber. However, white clouds and flakes, presumed to be aggregations of albumin, swirled in a circulating pattern throughout the device. A similar phenomenon was observed when a single-phase separation was run with the electrodes switched. Although a light brown band appeared at the top of the chamber, light brown clouds of hemoglobin swirled throughout the device. Analysis of that run, not shown in Table 1, showed a high concentration of albumin at the bottom contaminated with a significant amount of hemoglobin. These results indicate that gravity helps control convective mixing for the protein directed toward the bottom but contributes to convective mixing for the protein directed to the top in the single-phase experiments.

Electrophoresis experiments were also conducted in homogeneous buffer with 10% dextran added to increase viscosity. With experimental conditions similar to those of Run 11, the albumin concentrated near the top membrane and appeared to form a separate protein-rich phase which remained stable after the field was turned off. We did not verify that a separate stable phase was actually formed but did note that protein/polymer two-phase systems have been studied previously (9). With the anode as the upper electrode, the separation achieved was similar to that of Run 6. However, when the 10% dextran buffer was used with the cathode as the upper electrode, the hemoglobin that had concentrated near the upper membrane distributed throughout the solution when the field was turned off. These experiments indicate that electrophoretic separations, comparable to those made in aqueous two-phase systems, can be made in high viscosity homogeneous buffers in some, but not all, cases.

Using an aqueous two-phase system as a medium for electrophoresis appears to have helped control convective mixing within the device. In a typical run, convective currents were observed in the top phase, but no convection across the phase boundary was observed. The more viscous dextran-rich phase, rather than the phase interface, may actually be controlling convection. Nevertheless, two-phase systems offer advantages over homogeneous dextran solution because only part of the device is filled with the highly viscous dextran-rich phase. A controlling factor in making a separation is moving the solute of interest through the high viscosity solution in the device. In accordance with our expectations, we found that, all else being equal, higher volume ratios gave better separations in less time. Filling the device with dextran solution gives a zero volume ratio and takes more time to achieve the same separation as would result in a two-phase system. Moreover, there is an economic advantage to minimizing the amount of expensive dextran polymer.

Another distinct advantage offered by the two-phase buffer systems is that produce streams can be readily recovered without contamination after an electrophoresis run. That is, about 90% of the top phase could be collected without fear of contamination from the bottom phase in the two-phase runs, while sampling the top portion of a single-phase experiment required care to avoid collecting unwanted protein from the bottom of the chamber. Another potential advantage of two-phase systems is that initial conditions for electrophoresis experiments can be controlled to some extent by appropriate choice and manipulation of the two-phase buffer used. That is, in some cases a partial separation of solutes can be achieved by equilibrium partitioning, and the electric field can be used to drive the solutes toward further separation.

Prospects for Biotechnological Separations. Although the two-phase electrophoresis technique has yet to be optimized, the results shown in Table 1 demonstrate the feasibility of manipulating protein distribution between phases of an aqueous two-phase system with an applied electric field and of utilizing an aqueous two-phase system to limit convection within an electrophoresis device. In principle, the technique could be adapted to a larger scale by increasing the size and number of batch devices or by developing a continuous flow device (7). Although they have not yet been tested in a two-phase electrophoresis device, inexpensive phase forming polymers are available (10) that should provide low cost substitutes for dextran in large-scale applications. Cell separations or separation of a protein from cell mass should also be possible. A multistep procedure should handle more than two components as long as the pH can be adjusted to direct one component at a time into the desired phase.

CONCLUSION

Two-phase electrophoresis, using a PEG/dextran aqueous two-phase system as a medium for electrophoretic transport, was found to follow the same basic principles as electrophoresis in homogeneous media. Proteins were focused into either the PEG-rich upper phase or the dextran-rich lower phase depending on the protein's net charge and the electrode placement. Increases in the net charge on a protein, the electrophoresis time, the applied electric field, and the phase volume ratio with other parameters held constant all resulted in further directing a protein into one of the phases. Protein separations were made by directing two oppositely charged proteins into separate phases. The two-phase media offers several advantages over free solution that may prove useful for commercial scale electrophoretic separations. These advantages include controlling starting com-

positions, limiting convective mixing, and facilitating product isolation and recovery.

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