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K. S. M. S. Raghava Rao[a,b]; Robin Stewart[a]; Paul Todd[a]
[a] NATIONAL INSTITUTE OF STANDARDS AND TECHNOLOGY, BOULDER, COLORADO [b] Department of Chemical Technology, University of Bombay, Bombay, India

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Electrokinetic Demixing of Two-Phase Aqueous Polymer Systems. II. Separation Rates of Polyethylene Glycol–Maltodextrin Mixtures

K. S. M. S. RAGHAVA RAO,* ROBIN STEWART,
and PAUL TODD†
NATIONAL INSTITUTE OF STANDARDS AND TECHNOLOGY
325 BROADWAY, BOULDER, COLORADO 80303

Abstract

Aqueous two-phase extraction techniques have been successfully applied to the purification of enzymes and cells. However, due to their similar physical properties, immiscible aqueous phases do not separate rapidly. A method for enhanced demixing of aqueous two-phase systems in a thermostated vertical electrophoresis column was therefore studied. The effects of the electric field strength, field polarity, temperature, phase composition, and buffer concentration on demixing rates of a polyethylene glycol–maltodextrin (PEG–MDX) system were quantitatively measured. At normal electrical polarity (anode at the top of the column), using a maximum practicable field strength of 26.4 V/cm, the demixing rate was twice that in zero electric field at 25 ± 2°C. With polarity reversed (anode at the bottom, electric field opposing gravitational settling) at a field of 26.4 V/cm, demixing was 5.5 times as fast as in zero field. Reduction of the temperature from 25 to 14°C caused an increase in demixing rate in the absence of an applied field, while reduced temperature did not change the rate when using electric fields of either normal or reverse polarity. Increased phosphate buffer concentration substantially increased the demixing rate for PEG–MDX mixtures.

INTRODUCTION

The aqueous two-phase extraction technique has been successfully applied to the extraction and purification of enzymes and cell organelles (*1*–

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*Present address: Department of Chemical Technology, University of Bombay, Matunga, Bombay 400019, India.

†To whom correspondence should be addressed.

3). Although similar in principle to conventional liquid–liquid extraction, aqueous two-phase extraction has not yet seen widespread commercial applications. With the recent development of low cost aqueous two-phase systems, such as polyethylene glycol–maltodextrin (PEG–MDX) developed recently (*4*), this extraction method could be very attractive. However, the similar physical properties of the two aqueous phases makes the separation of the mixture very slow. Recent studies on the use of spray columns (*5*) as a replacement for commercial centrifugation have indicated that low interfacial tension of PEG–MDX systems makes phase separation slower than desired, so there is a need for further research on phase contacting and/or rapid demixing methods.

The application of an electric field to PEG–dextran–phosphate dispersions enhances the demixing rate of these systems (*6*, *7*). When PEG and dextran are dissolved in aqueous buffer solutions at concentrations that cause phase separation, certain dissolved ions (used as buffers) such as phosphate are unequally distributed between the two phases, leading to an electrochemical (Donnan) potential across the interface (*8*) and an electrokinetic (zeta) potential at the interface (*6*, *9*, *10*). Thus, droplets of one phase move within the dispersion or within the other phase when an external electric field is applied.

The primary objective of the present study is to characterize electrokinetic demixing of PEG–MDX dispersions in a vertical electrophoresis column. In principle, electrophoresis columns can be scaled up for phase disengagement and could be a feasible alternative to spray columns for two-polymer systems. In order to determine whether or not the application of an electric field enhances demixing of the PEG–MDX–phosphate system, a vertical electrophoresis column was used to measure the demixing rate of PEG-rich phases from MDX-rich phases.

Phase separation of the dispersion occurs due to the enlargement ("ripening") and coalescence of the individual PEG-rich and MDX-rich droplets within the continuous phase. The velocity of a droplet, either rising or falling (due to the density difference), is directly proportional to the difference between its density and that of the continuous phase and to the square of its average (or equivalent spherical drop) diameter. As droplets enlarge and absorb solute from the mixed phase, they move more rapidly. The application of an external force such as an electric field may enhance or retard coalescence and in turn the demixing rate, depending on factors such as field strength, ionic strength, and phase composition. The effects of these factors were examined in the experiments discussed below.

MATERIALS AND METHODS

Phase System

The aqueous two-phase system used in these experiments was composed of polyethylene glycol (PEG) (average MW = 8000) and maltodextrin (MDX) (average MW = 1800). The phase system was made by weighing the required amounts of PEG and MDX and adding them to measured quantities of buffer. The buffer consisted of doubly distilled water to which stock solutions of potassium phosphate were added to produce the desired concentration of phosphate buffer, pH 6.9–7.1. These solutions were stirred for approximately 4 h to form a fully mixed ("continuous") phase. The mixture was then allowed to settle in a conical separatory funnel for approximately 12 h at room temperature (25°C, unless otherwise specified) to obtain clear separation of two phases. Aliquots of each phase were collected without disturbing the interface (by pipetting the upper phase and draining the lower phase). The phase compositions of PEG-rich and MDX-rich phases in the three systems studied are shown in Table 1.

Electrophoresis Column

A water jacketed all-glass column, 2.2 cm in diameter and 22 cm in height, with side-arm electrodes, was used. Details concerning the apparatus and its assembly are given elsewhere (*7*, *11*).

Electrokinetic Phase Demixing

One hundred millimeters of the PEG–MDX dispersion (a mixture of 50 mL PEG-rich phase and 50 mL MDX-rich phase) was placed into the column. This volume completely filled the column. Field strength was

TABLE 1
Compositions and Physical Properties of the Phase-Forming Mixtures

	Viscosity (MPa · s)		Density (kg/m^3)		Composition	
System	PEG-rich	MDX-rich	PEG-rich	MDX-rich	PEG	MDX
1	20.60	66.0	1057.70	1176.70	8.40	28.57
2	8.83	28.5	1070.51	1161.10	4.93	23.95
3	7.66	17.3	1078.51	1120.80	3.75	23.79
	PEG-rich	DX-rich	PEG-rich	DX-rich	PEG	DX
4	3.11	27.5	1010.10	1041.10	3.51	6.52

varied by regulating the current and by using a constant phosphate concentration of 0.01 *M*. Field strength was calculated by using the measured conductivity of the dispersion.

Separation or demixing of the dispersion started with visible coalescence of the individual droplets. The height of the PEG-rich layer formed at the top was then recorded as a function of time. Phase separation took place from both top and bottom, with the final interface forming at the middle of the column. Temperatures at the top and bottom of the phase systems were recorded at the end of the experiment. Conductivities of the dispersion and the individual phases were recorded before and after the experiment, respectively. In each experiment, fresh aliquot parts of each phase were used.

As stated in the previous study of PEG–dextran, the lower phase droplets are positively charged when dispersed in PEG-rich phase in phosphate buffer (*8*), so the following convention was adopted: "normal" polarity corresponds to holding the upper electrode positive, while in "reverse" polarity the upper electrode is held negative (the sign of the charge of preequilibrated droplets has been reported to be opposite to the sign of the potential between the phases (*9*, *10*).

RESULTS AND DISCUSSION

Effect of Field Strength

The height of the PEG-rich phase that formed at the top of a dispersion of phase system 1 (Table 1) is plotted as a function of time in Figs. 1(a) and 1(b). In the case of normal polarity (defined above), the phase demixing was retarded by the electric field in the lower range of field strength (1–7.9 V/cm). At higher field strengths (13.2–26.4 V/cm), phase separation occurred much faster than at zero field. The results shown in Fig. 1(a) are similar to those observed using the PEG–dextran systems in the same column (7).

In the case of reverse polarity, as with the PEG–dextran system, increased field strength resulted in increased demixing rate, with the rate of demixing being fastest at 26.4 V/cm, and faster than in the case of normal polarity.

Above 26.4 V/cm using reverse polarity, complete demixing did not occur due to churning of the phase system (observed visually). Two phase boundaries appeared after 2 h of field application, but the phases remained turbid. The general behavior was the same as that of the PEG–dextran system at field strengths above 14.6 V/cm. In addition to the upper PEG-rich phase, a PEG-rich phase was trapped under the MDX phase at the

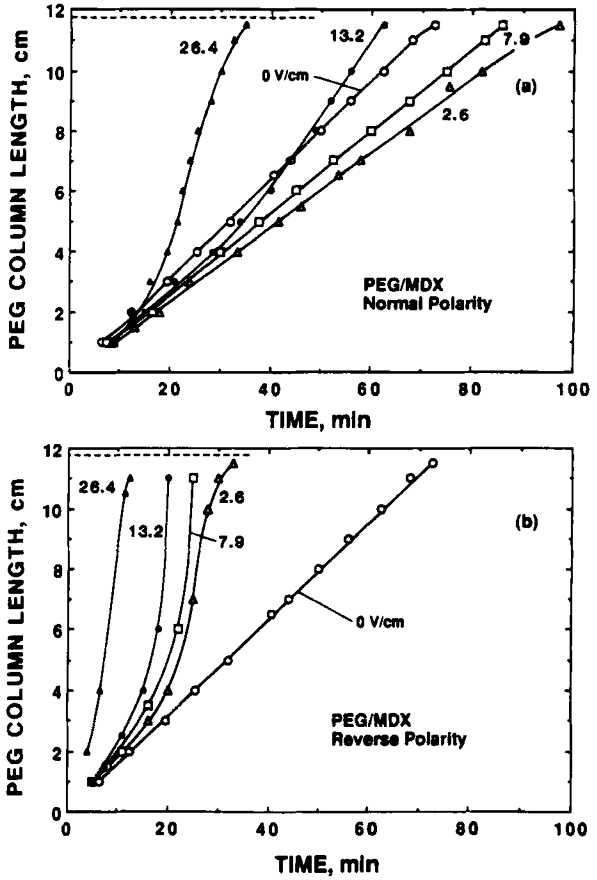

FIG. 1. Migration plots indicating the progress of the phase boundary from the top of the column as a function of time of electric field application using normal (a) and reverse (b) polarity, applied to phase system 1 (Table 1). The field strength, in V/cm, is indicated for each plot.

bottom of the column, confirming that PEG droplets are negatively charged.

The time required for a 10-cm column of PEG to form by demixing (inversely related to demixing rate) is plotted as a function of electric field in Fig. 2. In the case of reverse polarity, the time required decreased monotonically with an increase in field strength up to 26.4 V/cm. At normal polarity, the required time increased at low field strength (2.6 V/cm), then decreased to follow the same pattern as that for reverse polarity. This

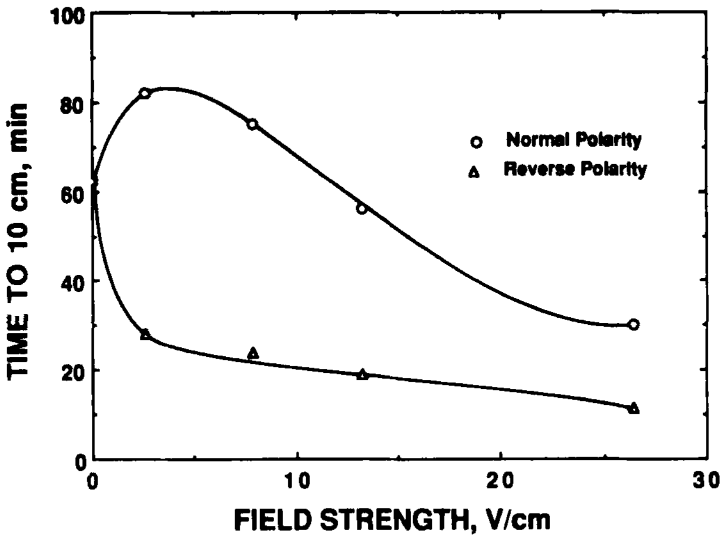

FIG. 2. Effect of field strength and its sign and magnitude on the time to form a 10-cm column of PEG. The time on the ordinate is inversely related to the migration velocity of the phase boundary.

increase in demixing rate at high field strengths for normal polarity is attributed to very rapid migration of droplets.

Effect of Phase Composition

The demixing rate of PEG–MDX was higher than that of PEG–dextran, which was previously reported (7). PEG–MDX systems had higher viscosity, density difference, and polymer concentration than did PEG–dextran (Table 1). Viscosity and density are related to phase composition, so the effect of phase composition for PEG–MDX was studied. Figures 3(a) and 3(b) show the migration plots of three PEG–MDX systems having the different compositions given in Table 1.

Table 1 and Fig. 3 show that System 2 had a higher demixing rate than System 1, despite the greater difference in density between the phases in System 1. System 1 had the higher viscosity. When the viscosities were of comparable magnitude (Systems 2 and 3), the system with higher density differences, System 2, demixed more rapidly. Similar results were observed in the presence of the electric field as shown in Fig. 3(b). In consideration of its physical properties, System 2 was chosen for the investigations that follow.

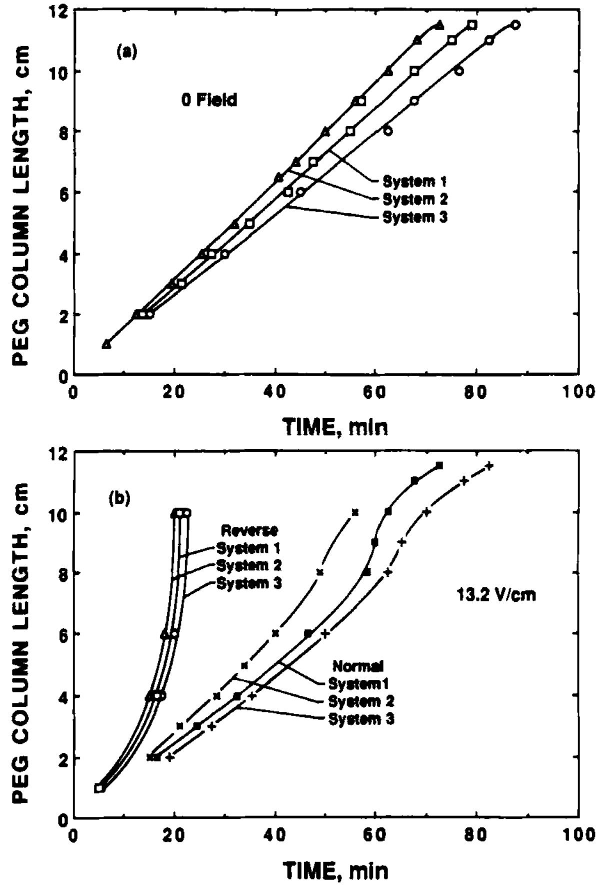

FIG. 3. Effect of composition of the phase-forming mixture on migration of the PEG phase boundary with no electric field (a) and with an applied field of 13.2 V/cm (b) at normal and reverse polarity. The phase compositions were (see Table 1): (1) 8.4% PEG, 28.57% MDX; (2) 4.93% PEG, 23.95% MDX; (3) 3.75% PEG, 23.79% MDX.

Effect of Temperature

Dispersion of equal volumes of top and bottom phases of System 2 were made and equilibrated at 25°C. The time required for a 10-cm column of PEG-rich phase to form is plotted as a function of the average final temperature of the phase dispersion in Fig. 4. When temperature was reduced, the volume ratio increased, and there was an increase in demixing rate at zero field, as shown in Fig. 4. When a field of 13.2 V/cm was applied in either reverse or normal polarity, the effect of temperature was not as

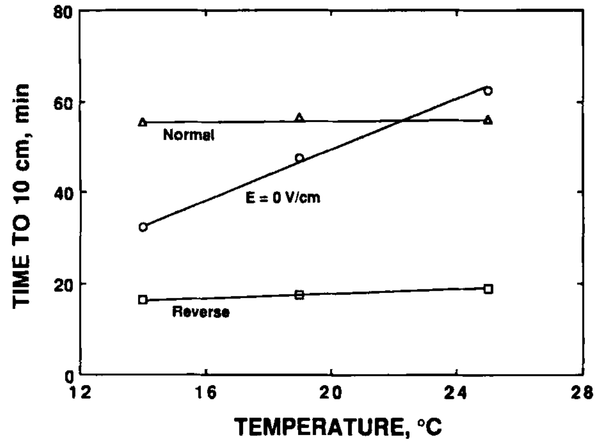

FIG. 4. Effect of temperature on the time to form a 10-cm column of PEG.

pronounced as it was in the absence of a field. However, as in the case of colloidal particles (*12*), the increase in effective field strengths (16.4 vs 13.2 V/cm) due to a decrease in conductivity at 8°C (conductivity decreases with decreasing temperature) added little to the demixing rate, presumably due to the increase of viscosity at lower temperatures.

Effect of Phase Equilibration Temperature

The phase mixture was equilibrated at three different temperatures by using a refrigerated centrifuge, and the electrophoresis experiments were carried out at three different temperatures. The results are summarized in Table 2. When the electrophoresis temperature was lower than the equil-

TABLE 2

Effect of Phase Equilibration Temperature on Demixing Time of Phase System 2

Temperature (°C)		Time to form 10 cm of PEG		
Equilibration	Run	Normal, +13.2 V/cm	Zero	Reverse, −13.2 V/cm
25	25	56.0	62.5	19.0
25	15	56.5	47.5	17.5
25	8	55.5	32.5	16.5
15	15	28.5	30.0	14.0
8	8	33.0	35.0	15.5

ibration temperature, separation of the phases was more rapid, because when the temperature was lowered the equilibrium composition of the phases (tie line length and slope) was disturbed (*13*). The PEG content increased in the PEG-rich phase and the MDX content in the lower phase remained essentially the same, while the total composition remained unchanged. The volume ratio of the phases also increased, but a volume ratio of 1.0 was used in preparing the column dispersion. The combined effect of these changes made the phases separate more rapidly.

When the phase mixture was equilibrated at 15°C (by centrifuging), the reequilibration was complete, and the density difference increased to a greater extent, and made the separation faster yet. However, when the phase mixture was equilibrated at 8°C, the viscosity increased along with the density difference. As already mentioned, the role of viscosity became prominent and separation was slow, as seen by comparing the last two rows of Table 2.

Effect of Buffer Concentration

The time for migration of the PEG interface is plotted as a function of phosphate concentration in Fig. 5. As the phosphate concentration increased, the conductivity (k) increased. To keep the effective field strength (I/kA) constant, the current density (I/A) was increased to the required value. Migration time decreased with increased buffer concentrations. Similar results were obtained with the PEG–dextran system (*7*) and in the electrophoresis of phase drops at equilibrium (*9*).

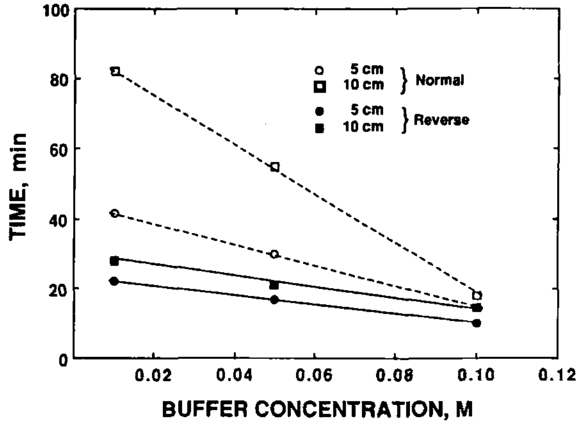

FIG. 5. Effect of phosphate concentration on the time to form a 5-cm or a 10-cm column of PEG.

CONCLUSIONS

The rate of demixing of phase dispersions of PEG and maltodextrin increased in an electric field under several conditions. At the maximum field strength studied (26.4 V/cm for normal as well as reverse polarity), demixing was most rapid—2 times the rate at zero field for normal and 5.5 times for reverse polarity. Very high field strengths using reverse polarity caused remixing of the dispersion instead of separation.

Reduction of temperature during phase separation resulted in a substantial increase in demixing rate at zero electric field due to a change in the equilibrium phase composition. When equilibration and demixing occurred at 15°C, the demixing rate was substantially reduced in normal field, while at 8°C, the increased viscosity caused a decrease in the demixing rate.

High phosphate concentration increased the demixing rate. There was an increase in the power required to achieve this result.

The electrokinetic demixing technique, coupled with low cost polymer systems, is potentially an economic method for the separation of bioproducts.

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