Separation Science and Technology
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713708471

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To cite this Article Rao, K. S. M. S. Raghava , Stewart, Robin M. and Todd, Paul(1990) 'Electrokinetic Demixing of Two-Phase Aqueous Polymer Systems. I. Separation Rates of Polyethylene Glycol-Dextran Mixtures', Separation Science and Technology, 25: 9, 985 — 996

To link to this Article: DOI: 10.1080/01496399008050379

URL: http://dx.doi.org/10.1080/01496399008050379

SEPARATION SCIENCE AND TECHNOLOGY, 25(9 & 10), pp. 985–996, 1990

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

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Abstract

The separation of biomolecules and cells using aqueous two-phase systems provides a mild, nontoxic extraction medium, in contrast with conventional organic–aqueous phase extraction. However, due to their similar physical properties, immiscible aqueous phases do not separate rapidly. Because a net surface potential occurs on phase droplets due to the unequal partitioning of certain dissolved ions, a study was undertaken in which the resulting motion in an electric field (electrophoresis) was explored as a possible method for rapid demixing of aqueous two-phase systems in a vertical electrophoresis column. The effects of electric field strength, buffer concentration, and field polarity on the demixing rate of mixtures of polyethylene glycol and dextran in phosphate buffer were measured. It was found that an optimum field strength of around 29.2 V/cm exists at which demixing is most rapid and occurs at about twice the rate in zero field at 25 ± 2°C using normal polarity (anode at the top of the column). With reverse polarity (anode at the bottom; electric field opposing gravitational settling) at a field strength of 14.6 V/cm the rate was 3 times as fast as in zero field. Strong convection was observed at high field strengths. Increasing the phosphate concentration increased the demixing rate.

INTRODUCTION

Organic–aqueous phase extraction is one of the most popular purification methods used in the chemical industry today. It has found limited popularity in bioprocessing owing to the damaging effects of organic solvents on biomolecules and cells, whereas aqueous two-phase systems, due to

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their high water content and nontoxicity of the polymers, are biocompatible (*1*). Moreover, these systems are reported to have provided stability to biologically active substances, such as enzymes (*2*). Despite some 800 papers on this subject (*3*), large-scale commercial applications are not widespread. The high cost and low speed of the process deter its widespread use. Cost reductions were recently achieved by the introduction of low-cost polymer–aqueous phase systems (*4*). The similarity of the physical properties of the two phases causes them to separate slowly in the absence of added physical force, such as provided by centrifugation. An alternative approach was addressed by the exploration of electrokinetic demixing methods in the well-characterized PEG–dextran system.

When two polymers are dissolved in aqueous solution at concentrations that cause phase separation, certain dissolved ions such as phosphate are unequally partitioned between the phases, leading to a Donnan potential across the interface (*5*) and an electrokinetic (zeta) potential at the interface (*6–8*). As a consequence of the zeta potential, droplets of one phase move in the other phase in the presence of an externally applied electric field. The use of electric fields in solvent extraction has been studied (*9–11*), and a related process has been applied on an industrial scale for the extraction of separands from emulsion liquid membranes (*12*). It should be possible to control demixing rates, and enhance demixing of phases with small density differences, by applying an electric field to two-phase polymer dispersions (*7*). The rate of demixing of PEG-rich phases from dextran-rich phases was measured in a vertical electrophoresis column. It was found that the rate of demixing of dispersions was increased severalfold in an electric field.

MATERIALS AND METHODS

Phase System

The aqueous two-phase system employed in our experiments was composed of polyethylene glycol (average MW = 8000) and dextran (average MW = 480,000).

The binodial phase diagram of the PEG–dextran–water system at 25°C is shown in Fig. 1. PEG and dextran were weighed and added to measured quantities of doubly distilled water to which stock solutions of potassium phosphate had been added to produce the desired strength of phosphate buffer, pH 6.9–7.1. These solutions were stirred for approximately 4 h to form a fully mixed ("continuous") phase. Phases were then allowed to separate at room temperature for approximately 12 h in a conical separatory funnel. Aliquot parts of each phase were removed without disturbing the interface. Unless otherwise specified, the composition of the top phase at

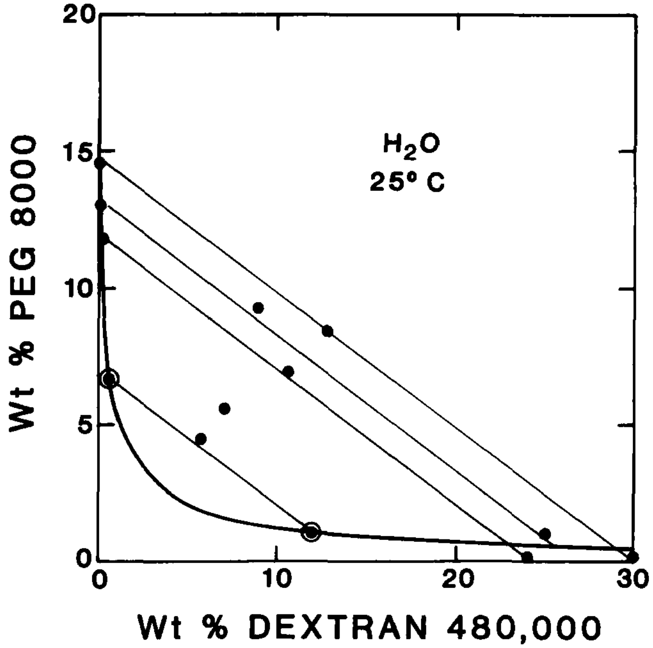

FIG. 1. PEG–dextran binodial curve. Circled points indicate phase compositions used in these experiments.

25°C and 0.01 *M* phosphate was 6.56% PEG and 0.31% dextran, and the corresponding bottom-phase composition was 0.94% PEG and 11.9% dextran. The locations of these points are shown on the binodial phase diagram. The top- and bottom-phase densities were 1.0164 and 1.1059 g/cm^3, respectively, and the corresponding viscosities were 0.00569 and 0.460 kg/m · s (5.69 and 460 cP).

Electrophoresis Columns

A schematic drawing of the experimental apparatus is shown in Fig. 2. The dispersion volume was 100 mL within a water-jacketed electrophoresis column, which provided direct electrical contact with the PEG–dextran solutions. The column had side-arm electrodes which support and contact the phase system through plugs of 15% polyacrylamide gel ("G" in the diagram). These plugs were made by the user and can be used for a number of experiments before needing replacement. More details on the assembly of the apparatus and formation of gel plugs are given elsewhere (*13*).

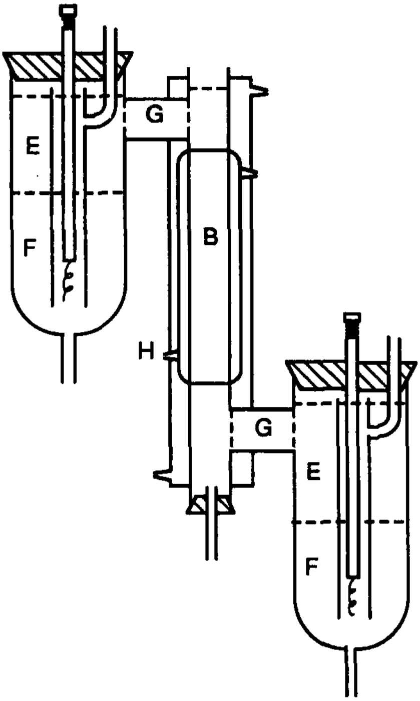

FIG. 2. Diagram of electrophoresis column systems for demixing studies. Adaptation of modular cell electrophoresis column (*13*). Solution B is described in the text. E is potassium phosphate buffer matching that used in the column, F is saturated sodium chloride, G is a 15% polyacrylamide gel plug, and H is the entrance to the cooling jacket. This is an all-glass column (*14*), to which an auxiliary cooling jacket, fabricated of Plexiglas was added for effective cooling of the sidearm areas.

Electrokinetic Phase Demixing

The electrophoresis column was completely filled with dispersion, and the height of the PEG-rich layer that formed at the top of the column was recorded as a function of time. The temperatures at the top and bottom of the jacketed area were recorded at the end of the experiment. The electrical conductivities of the phases were also recorded before and after the experiment. Fresh aliquot parts of each phase were prepared for each experiment.

The dextran-rich (lower) phase droplets are positively charged when dispersed in the PEG-rich phase in phosphate buffer (*5*), 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 (*6*, *8*)].

Experiment Design

An attempt was made in this work to demix the dispersions in a manner that would resemble the configuration of a pilot plant. A thermostated vertical cylinder is a proven configuration for both density-gradient electrophoresis and for two-phase separations.

Phase separation of the dispersion occurs due to the enlargement and coalescence of the individual phase droplets. The velocity of a droplet, either rising or falling, is proportional, by Stokes' law, to the square of its average (or equivalent spherical drop) diameter, and these droplets coalesce and ripen as they move. Consequently, as the droplets ripen (i.e., absorb solute from the mixed phase) or coalesce (combine with other droplets), they migrate more rapidly. The addition of another force that acts on phase drops, such as an electric field, may enhance or retard the overall process of phase demixing, depending on many other factors. The following experiments were designed to investigate some of these factors.

RESULTS

Effect of Field Strength

The height of the PEG-rich phase that formed at the top of the dispersion is plotted as a function of time in Fig. 3 (migration plots). Field strength was varied by regulating the current and using a constant phosphate concentration of 0.01 *M*.

In the case of normal polarity (electrophoretic migration in the same direction as density-driven settling), phase demixing is slowed by the electric field in the lower range (below 8.8 V/cm) of field strength. At fields at and above 8.8 V/cm, the demixing rate was increased when compared to the rate at zero field.

When fields of reverse polarity were applied (Fig. 3B), increasing the field strength resulted in an increased demixing rate, with the rate of demixing being fastest at 14.6 V/cm. At this field strength, demixing was faster than in normal polarity. In this case the electrophoretic force presumably acts in the opposite direction to the buoyancy force on the PEG-rich droplets. At the highest field strengths, convective currents (which were observed visually) rose at the center and fell in streamers along the column walls, due to cooling at the walls. At field strengths above 14.6 V/cm, vigorous churning was noted, and complete demixing never occurred while the phases remained turbid. This process merits a detailed description

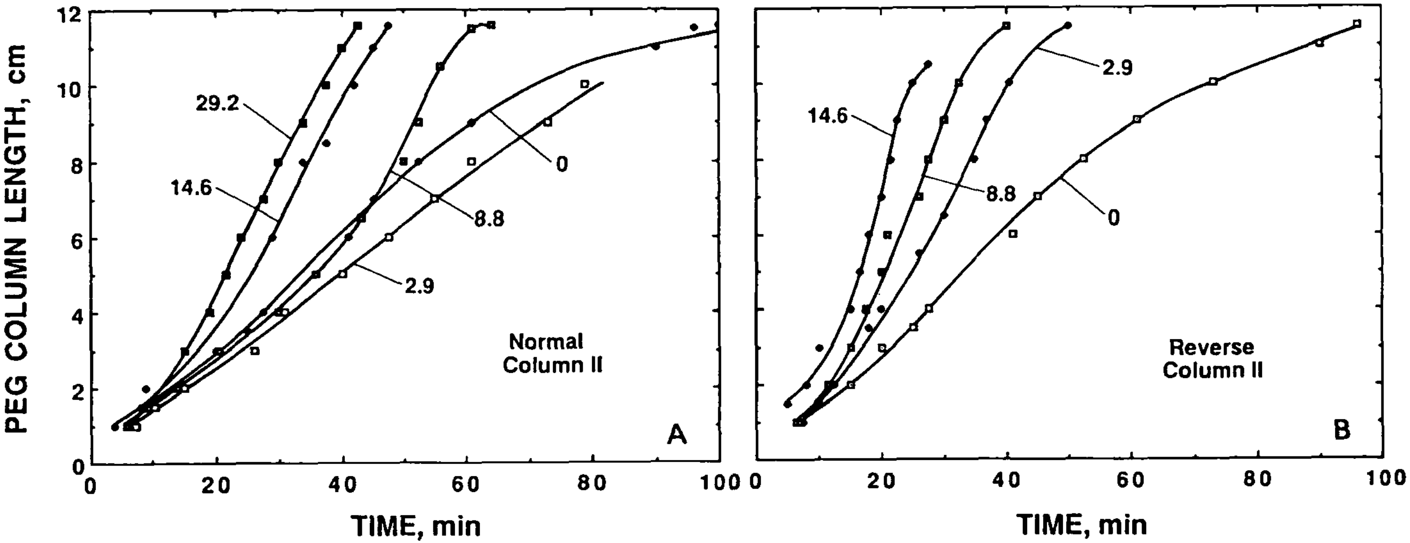

FIG. 3. Migration plots indicating the progress of the upper phase boundary from the top of the column as a function of time of electric field application using normal (A) and reverse (B) polarity.

because it may provide insight into the many events that occur when an electric field is applied.

As soon as the current was switched on, a very vigorous motion began with the formation of convective cells having a diameter of approximately 0.5 cm. There was no evidence of a phase boundary developing near the top of the column. This motion continued for about 5 min, after which 3 zones of very different activity developed over the total column height (25 cm). The uppermost 5 cm consisted of 2-way traffic of streamers of droplets about 400 μm in diameter traveling in clean vertical lines or "streamers" resembling a fingering instability. The 2-way traffic was observed to be uniformly distributed across the column. In the middle (vertically) 15 cm, classical convective pluming occurred: fluid rose slowly in the center of the column and fell gradually at the wall. The bottom 6 cm of the column contained slow streamers. No horizontal phase boundaries were visible by 12 min.

After 12 min the lower two-thirds of the column was clearer than the rest, while a great deal of churning motion and vertical convection cells persisted in the upper third of the column. A faint boundary was visible about 11 cm from the bottom of the column. Suddenly (in a period of a few seconds), a large convective bulge developed, replacing the faint phase boundary at this location. The bulge of the convecting boundary collapsed rapidly, and at 14 min the phase boundary was flat. By 17 min a quasi-steady state was reached; the droplet migration progressed slowly toward a final separation, which did not have the typical appearance of a thermodynamic steady state. The top phase remained turbid and contained dextran-rich drops that were obviously being drawn upward by electrophoresis only to fall back. Meanwhile, the dextran-rich phase was clear. Clearing of the top phase was very slow, so more drops may have been returning to the top phase by electrophoresis than the number being captured by the dextran-rich phase below. Below the lower electrode, a separate PEG-rich phase formed at the bottom, contrary to its lower density than the fluid above it, but consistent with its charge. This phase was restrained in the field-free zone at the bottom of the column by the electric field above it. Normally it should escape to the top, where it would travel by buoyancy. At any given time a few drops of this PEG-rich layer could be seen leaving through the bottom of the lower phase and rising, obviously by buoyancy and counter to the electric field, to the interface, where they would join the upper PEG-rich phase.

At about 30 min these processes were still occurring, but the rising drops of PEG were larger, rising at specific locations in the column, and moving upward like chains of pearls attached to one another. At 65 min a full separation of phases had not occurred, and the upper phase remained turbid and populated with dextran-rich drops in vertical motion.

By 90 min the top phase had clarified only slightly, and the motion of the dextran-rich droplets was considerably reduced. A new phenomenon developed at the top of the bottom phase. Some PEG was being dragged below the phase boundary into the top few millimeters of the bottom phase and then recycled back to the top phase through the boundary. This was happening only near the central axis of the column, where the local density of the bottom phase was reduced due to Joule heating. Otherwise, there was almost no motion of the boundary for more than 1 h. The field was then turned off.

Nonmonotonic field dependence was observed with normal polarity. The time required for a 10-cm column of PEG-rich phase to demix was chosen to represent the curves in Figs. 3A and 3B. This is plotted in Fig. 4. As described earlier, the rate of demixing increased with an increase in the reverse polarity field strength up to 14.6 V/cm, with further increases in field strength decreasing the demixing rate. Normal polarity field slightly retarded demixing at low field strength and enhanced demixing at higher fields with a field dependence parallel to that found at reverse polarity.

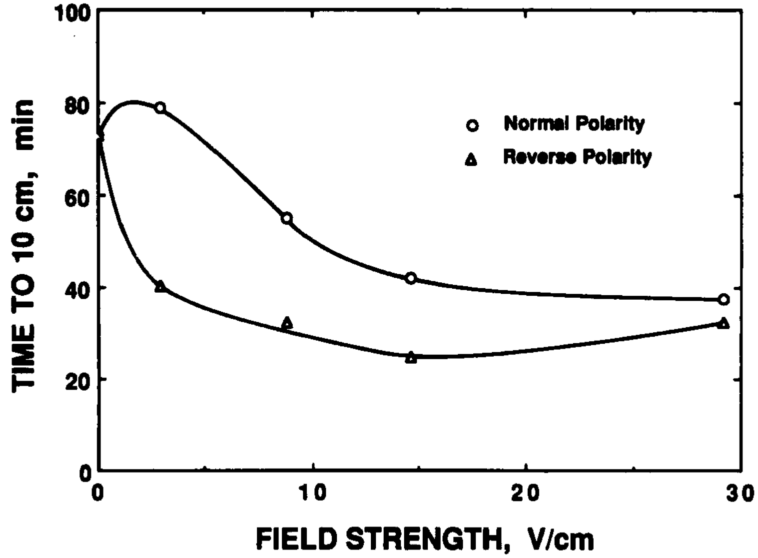

FIG. 4. The effect of field strength, its sign and magnitude, expressed as the time to form a 10-cm column of PEG-rich phase. The time on the ordinate is inversely related to the migration velocity of the phase boundary.

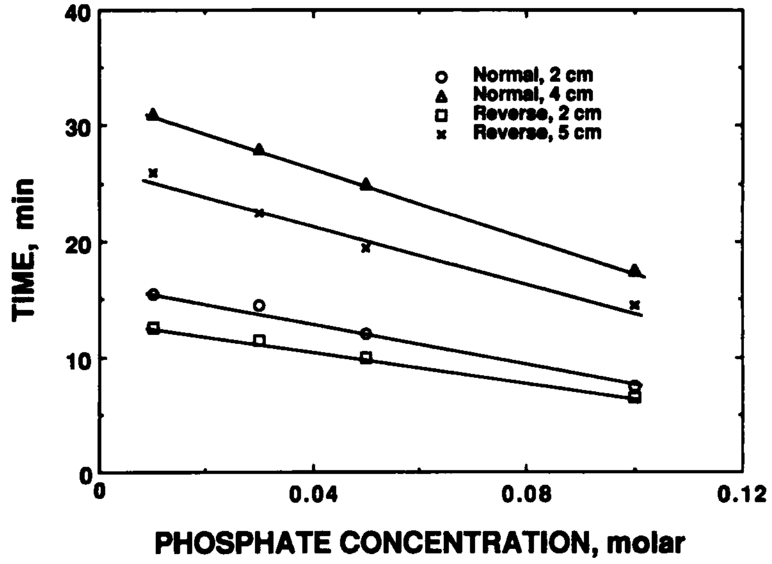

FIG. 5. Phosphate concentration dependence of demixing rate, expressed as the time required to form a column of PEG-rich phase of specified length in normal and reverse fields.

Effect of Phosphate Buffer Concentration

The migration time for a fixed distance is plotted as a function of phosphate concentration in Fig. 5. As the phosphate concentration increases, the conductivity increases. To keep the effective field strength constant, the current was increased to the required value, determined on the basis of measured conductivity.

This figure shows that the migration time decreased with increased buffer concentration. Presumably the droplet mobility increased with increasing buffer concentration (7). This effect would cause an increase in the demixing rate.

DISCUSSION

The observation that normal-polarity fields can retard demixing at low field strength and hasten it at high field strength appears enigmatic, as does the observation that reverse-polarity fields always increase the demixing rate. A quantitative model that explains these observations cannot be derived from the data set developed in these experiments.

The rate of demixing depends on the rate of droplet migration and the rate of droplet growth. Assuming, for example, that PEG drops are negative (*5*, *6*), they will move upward due to a normal-polarity field (with velocity proportional to their diameter) (*6*, *8*) and upward due to buoyancy (with velocity proportional to the square of their diameter). While moving upward they will grow by ripening and coalescence. Increasing the upward velocity by the addition of an electric field allows less time (distance/velocity) for drop growth. Unless the velocity exceeds some critical value, less drop growth, and hence less rapid demixing, can be expected, as unripened drops (drops that have not reached equilibrium composition or full size) would be brought to the upper phase boundary prematurely. If the drop migration velocity exceeds a critical value, as at higher field strength, less time is required for all PEG-rich drops to reach the top of the dispersion boundary, so the total process is faster than at zero field.

On the other hand, fields of reverse polarity oppose the upward buoyant motion of PEG-rich drops. This allows more ripening time in the continuous phase. The resulting increased drop diameter increases the buoyant velocity according to the square of the drop diameter. The downward electrophoretic velocity is only proportional to the first power of the diameter (*5*, *6*, *8*). Thus a reverse-polarity field, by increasing the drop diameter due to increased ripening, can increase the buoyant velocity of drops despite the opposing upward motion with an electric field.

Detailed quantitative testing of this hypothesis will require further experimentation, and alternative explanations may exist. For example, the sign of the charge on droplets being formed in the continuous phase may not be the same as that reported (*5*, *6*) on drops at equilibrium in a phase at equilibrium. As phase droplets are not the same as colloidal particles, field-induced ion motion leading to droplet polarization and internal fields cannot be overlooked (*8*). Any effects of the dc electric fields of the magnitudes used in this study on internal flows within droplets would have to be assessed microscopically (*9*, *11*, *15*).

CONCLUSIONS

The rate of demixing of phase dispersions was found to increase in an electric field. It was found that a maximally effective field strength exists (29.2 V/cm for normal and 14.6 V/cm for reverse polarity), at which demixing is most rapid, 2 and 3 times as fast, respectively, as zero-field demixing.

Very high field strengths with reverse polarity caused remixing during phase separation, resulting in a dynamic, rather than static, phase equilibrium.

Increased phosphate concentration increased the demixing rate; however, due to the increased conductivity, increased current densities were required to achieve the required field strengths.

This electrokinetic demixing method appears to be a promising method for the rapid demixing of aqueous two-phase systems. Further work is needed to characterize and quantitate the process; to determine its applicability to low-cost polymer solutions; to understand the roles of convection, phosphate ions, and gravitational acceleration; and to evaluate the effects on and of separand molecules.

Acknowledgments

We thank Mr. David C. Szlag and Dr. Subhas K. Sikdar for research guidance and Drs. Gary Hardin and Scott Rudge for critically reviewing the manuscript.

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Received by editor October 25, 1989