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Zonal Density Gradient Electrophoresis of Serum Albumin and Bacteriophages M13, φX174, and MS2. Electrophoretic Mobility Measurement

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Abstract

A method based on zonal density gradient electrophoresis is presented which permits rapid separation of proteins and bacteriophages and the estimation of their electrophoretic mobility. The electrophoretic mobility of serum albumin for different buffer solutions and pH values has been measured and found to be in good agreement with previous values from the literature. The electrophoretic mobility of albumin has also been measured in the presence of two neutral water-soluble polymers (polyvinyl alcohol (PVA), polyethylene oxide (Polyox)), and a charged polymer (hydrolyzed ethylene maleic anhydride (EMA)). The electrophoretic mobility of three bacteriophages (M13, MS2, φX174) has been estimated in a phosphate/glycine buffer at pH = 7.4 as well as in the presence of bovine serum albumin and bovine γ-globulin. The electrophoretic separation of M13 and φX174 bacteriophages was accomplished.

INTRODUCTION

Zonal Density Gradient Electrophoresis

In zone electrophoresis the separation column is filled with a single electrolyte system and the mixture of substances to be separated is applied to a certain location as a narrow layer. After application of an electric field to the system, the mixture substances will be separated if they differ in isoelectric point or mobility.

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The stabilization of the column by a density gradient of electrically neutral solutes was first advanced by Philpot (*1*). An excellent review of this technique is given by Svensson (*2*). The preparation of the density gradient can be carried out with gradient mixers or manually, and optical methods, radioactive and/or biological activities can be used for concentration recording. Zonal density gradient electrophoresis has been successfully used for a number of years for serum protein separation (*2, 3*). The first use for purification of viruses was reported in a short note by Brakke (*4*). Since that time this technique has been used for virus purification by other researchers, among them Cramer (*5*), Van Regenmortel (*6*), and Mandel (*7*). More recently Boltz and Todd (*8, 9*) and Catsimpoolas and Griffith (*10*) performed zonal density-gradient electrophoresis of cells with an isoosmotic density gradient made of solutions of Ficoll and sucrose.

Mobility

The electrophoretic mobility of a particle is related to the net charge present on the particle. However, when the particle is suspended in an ionic medium, ions of opposite sign are attracted from the environment and form an ionic cloud around the particle. In the concept of the electrical double layer, the charged areas on the particle surface attract a layer of tightly bound ions, referred to as the Stern layer. Extending outward from the Stern layer is a layer of somewhat loosely bound ions, known as the Gouy layer (*11*). When the particle moves about in an electric field, all of the Stern layer ions and some of the Gouy layer ions move with it, creating a shear plane within the diffuse Gouy layer. The potential at this slippage boundary is known as the zeta potential. Henry (*12*) derived the following expression for electrophoretic mobility:

$$U = \varepsilon\xi f(Kr)/6\pi\eta \qquad (1)$$

where U = electrophoretic mobility
ε = dielectric constant
ξ = zeta potential
η = viscosity of the medium
K = Debye-Hückel constant
r = radius of the particle
$f(Kr)$ = Henry factor

Overbeek (*13*) has provided a table of values of the Henry factor. Van Oss

(*14*) has established conversion factors for U for water at different temperatures and thus at different values of ε and η. Hunter (*15*) reviewed different electrophoretic mobility expressions and studies of zeta potential.

The concentration and valence of the electrolytes influence the double-layer thickness and therefore the electrophoretic mobility. Hannig (*16, 17*) listed numerous buffer solutions used by different workers for electrophoresis of many biological materials, proteins, cells, viruses, phages, etc.

Some authors have investigated the influence of adsorbed neutral polymers on the zeta potential. Fleer (*18*) and Koopal and Lyklema (*19*) investigated this phenomenon on AgI particles in the presence of polyvinyl alcohol (PVA). They showed that the initial effect of adsorbed polymer was to move the isoelectric point to higher values with little effect on the maximum value of ξ. However, Brooks (*20*) and Seaman (*21*) observed large increases in the absolute value of the zeta potential of biological (red blood cell), organic (polystyrene latex), and inorganic (SiO_2) surfaces in the presence of neutral polymers such as dextran, polyethylene glycol, methyl cellulose, and pluronic (block copolymer of polypropylene and polyethylene oxide). Brooks set up a model and showed that, even if the adsorption of the polymer did not affect the surface charge and the plane of shear, an increase in ξ was predicted due to the change in ion distribution in the diffuse double layer. An increase in zeta potential in the presence of water-soluble polymers means that the observed electrophoretic mobility of a particle is higher than the mobility predicted from the Henry equation, despite the increase in the bulk viscosity of the suspending medium.

Stability

The stability of the column is of prime importance for a good separation and/or electrophoretic mobility estimation. The undesirable effects of certain transport processes, such as convection currents due to unequal heating and dissipation of heat generated on the passage of current, can be limited by forming a density gradient. The solute most commonly employed for the gradient is sucrose. The obvious stability condition is that the density of the solution must increase downward in the column. Svensson (*2*) discussed different possible gradient profiles in detail. The permissible electric load depends on many factors such as the value of the density gradient, the conductance of the solution, the means of dissipating the Joule heat, etc. Svensson estimated this electric load to

be 12 W for a density gradient of 0.2 g/mL per 40 cm length, with buffer solutions of ionic strength 0.025 *M* and with air cooling.

Instability of the initial sample zone can occur despite careful adjustment to proper density, and this can prevent any separation. It was found experimentally that initial zones of proteins or cells may form small droplets which sediment quickly in the column. A full explanation of this phenomenon was presented by Brakke (*22*). If diffusion of sucrose into the initial zone is not balanced by a corresponding diffusion of proteins out of the zone, the net result is an increase in the density of the initial zone and droplets of higher density are formed. In support of this explanation, Svensson (*3, 23*) reported that no droplet sedimentation occurred in a protein separation experiment in a dextran gradient because dextran diffuses at approximately the same rate as proteins. Theoretical analysis of the maximum mass of solute and number of suspended particles per unit volume have been derived by Svensson (*24*) and Mason (*25*). Boltz (*8*) and Catsimpoolas (*10*) recommended a concentration limit for cell suspensions of around 10^7 cells/mL. However, there is no lower limit. The smaller the amount of material to be separated, the more stable the column will be.

Finally, electroosmotic flow can impair resolution. As a result of the ionic double layer at the electrophoresis cell wall, electric forces set in motion the electrolyte adjacent to it and, in a closed system, a flow in the opposite direction results in the central region of the column to ensure zero net fluid transfer. Boltz et al. (*8*) estimated the electroosmotic velocity at a glass wall to be around 10^{-6} cm/s and that in chambers of diameter larger than those used in microscopy electrophoresis (0.1 mm in diameter), electroosmosis is not a serious problem.

EXPERIMENTS

Apparatus

The form of the electrophoresis cell used in our study is shown in Fig. 1. The cell is made from a 5-mL pipette having a length of 18 cm. The utilization of gel bridges composed of 3.0 wt% agarose, containing the appropriate electrolyte, eliminates fluid connections. The gel bridge at the bottom of the cell effectively seals it while permitting passage of current. To prevent convection, a linear sucrose gradient from 40 wt% at the bottom to 4 wt% at the top (0.16 g/mL per 18 cm) is superimposed on the electrolyte. The sucrose gradient is formed either manually, by adding

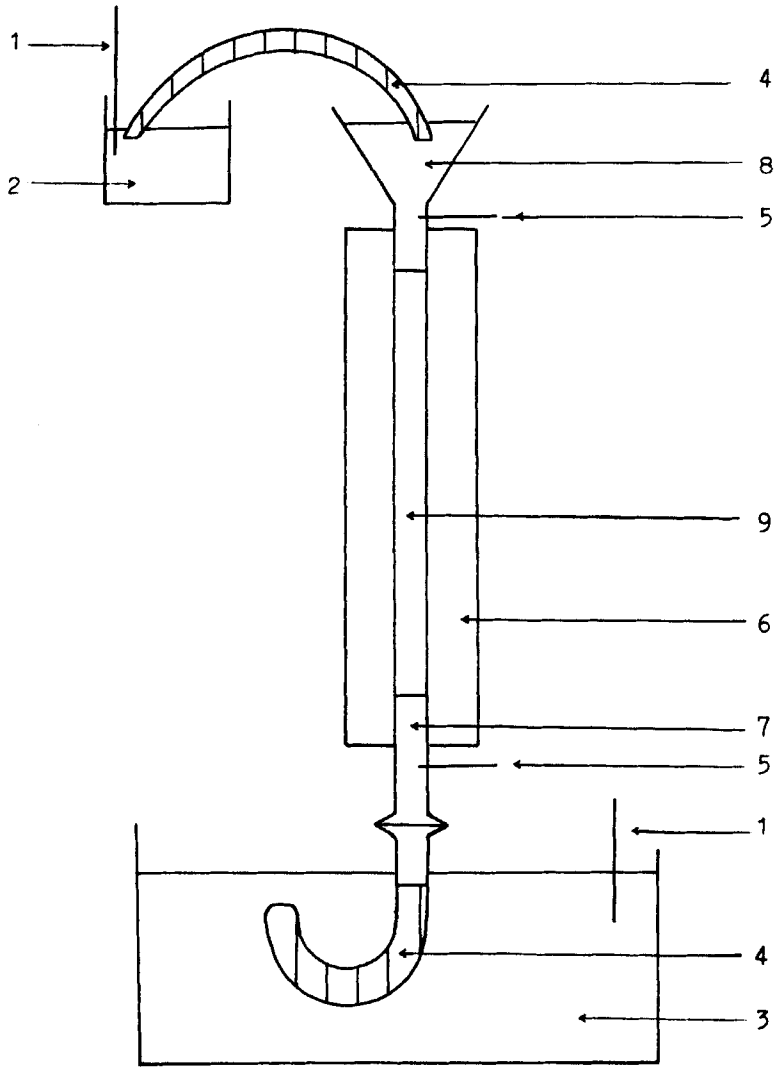

FIG. 1. Zonal density gradient electrophoresis apparatus. (1) Electrode; (2) 10 X buffer at 0% sucrose concentration; (3) 10 X buffer at 40 wt% sucrose concentration; (4) U-tube filled with agarose gel; (5) platinum post for voltage measurement; (6) water cooling jacket; (7) contact fluid, 1 X buffer at 40 wt% sucrose concentration; (8) contact fluid, 1 X buffer at 0 wt% sucrose concentration; (9) 1 X buffer density gradient.

twenty-five 0.2 mL fluid increments to the cell starting at the bottom position, or automatically by using pumps. Three cells are normally run in parallel with one power supply. This allows the proper controls to be run in parallel with the test cells and permits efficient study of the effects of variables important to this problem (buffer type, ionic strength, pH).

The pH and ionic strength are homogeneous throughout the column. All ions present in the solution were considered in the calculation of the ionic strength. It has been found experimentally that, to prevent changes in voltage across the part of the cell containing the gradient during electrophoresis, the composition (concentration and ionic strength) of the electrolyte of contact fluids and gradient buffer have to be similar.

The temperature is controlled by a water jacket surrounding the cell. All runs were made at 4°C because the proteins are more stable at low temperature and the stability of the column is enhanced by the increased viscosity of the sucrose solution.

Dyes (phenol red, methylene blue, etc.) can be used as markers to detect discrepancies in migration from one column to the other due to leaks etc. However, some dyes affect the mobility of particles and should not be mixed with the sample to be separated. They should be added a few fractions above or below, depending on the direction of migration.

Linear Sucrose Density Gradient Manually Formed

In the course of this work, only linear sucrose density gradients have been used.

a. Preparation of the Density Gradient

The following procedure was used to prepare the fractions for layering in the electrophoresis column.

1. Prepare 300 mL of buffer solution at 4 wt% and 300 mL of buffer solution at 40 wt% sucrose.
2. Use 150 mL of each solution to make up gradient in 25 fractions according to Table 1.
3. Use the 150 mL left of each buffer solution to prepare additional fraction volume, for instance, 1, 5, 10, 15, 20, and 25, to measure the electric conductivity and the viscosity.
4. Check gradient fractions by measuring the refractive index.

TABLE 1

Fraction	Milliliters, 4 wt%	Milliliters, 40 wt%	Fraction	Milliliters, 4 wt%	Milliliters, 40 wt%
1	12.0	0	14	5.5	6.5
2	11.5	0.5	15	5.0	7.0
3	11.0	1.0	16	4.5	7.5
4	10.5	1.5	17	4.0	8.0
5	10.0	2.0	18	3.5	8.5
6	9.5	2.5	19	3.0	9.0
7	9.0	3.0	20	2.5	9.5
8	8.5	3.5	21	2.0	10.0
9	8.0	4.0	22	1.5	10.5
10	7.5	4.5	23	1.0	11.0
11	7.0	5.0	24	0.5	11.5
12	6.5	5.5	25	0.0	12.0
13	6.0	6.0			

b. Gel Preparation

1. Heat 1 g of agarose in a 100-mL stirred flask on a steam bath. Add 30 mL of a pH 7 phosphate buffer of 0.2 *M* ionic strength and 40 wt% sucrose concentration.
2. Stir until a clear solution results.
3. Fill U-tubes and allow the gel to solidify in 6 to 10 min.
4. Immerse the bottom U-tubes in 40 wt% sucrose buffer solution and the top U-tubes in 0 wt% sucrose buffer solution. Store at 4°C.

c. Layering of the Electrophoresis Column

The following procedure was used in layering of the electrophoresis column.

1. Cool the column to 4°C with chilled water flowing through the column cooling jacket. Maintain cooling throughout the experiment.
2. Prepare a sample fraction of the material to separate.
3. Clamp the gel bridge to the base of the column after greasing the joint. Fill the column with a syringe and a long needle to the 5-mL mark with 40 wt% sucrose buffer solution. Avoid contaminating the sides of the column with the buffer solution.

4. Using a syringe, layer gradient in 0.2 mL fractions beginning with Fraction 25. Avoid mixing of layers and contamination of the column sides. Continue in this manner up to the sample fraction.
5. Layer the sample fraction in 0.1 mL. Complete layering of the column with 0.2 mL fractions and 0.3 mL for the top fraction.
6. Fill the remainder of the column with 0 wt% sucrose buffer solution. Attach a funnel to the top of the column and fill with buffer solution containing no sucrose.
7. Connect the funnels with the gel bridges to the electrode reservoir which is filled with a buffer solution containing no sucrose. Place the other electrode reservoir filled with 40 wt% sucrose buffer solution over the gel bridges at the base of the column.
8. Place the electrodes in reservoirs with the right choice of polarity depending on the desired direction of migration. Apply 150–250 V; experiment runs 1½ to 6 h depending on the buffer used.

d. Processing of Fractions

The following procedure is used to process fractions from the electrophoresis column.

1. Record the column voltage drop and the current intensity flowing through at 1-h intervals or less in fast separations.
2. After completion of the run, aspirate the column down to Fraction 25. Using a vacuum system, aspirate twenty-five 0.2 mL fractions into separate numbered vials. Drain and rinse the column.
3. Measure the concentration, the activity, etc. of the species in each fraction.
4. Estimate the electrophoretic mobility.

Automatic Layering of the Density Gradient

Figure 2 shows schematically the mixing device for automatic density gradient layering. An initial volume V_0 of sucrose solution at concentration C_0 is placed in the stirred chamber. As some of the fluid is pumped out of the chamber at flow rate q, a sucrose solution of concentration C_i is pumped in at a flow rate q_i. The density gradient in the column is related to the change in sucrose concentration in the mixing chamber. The

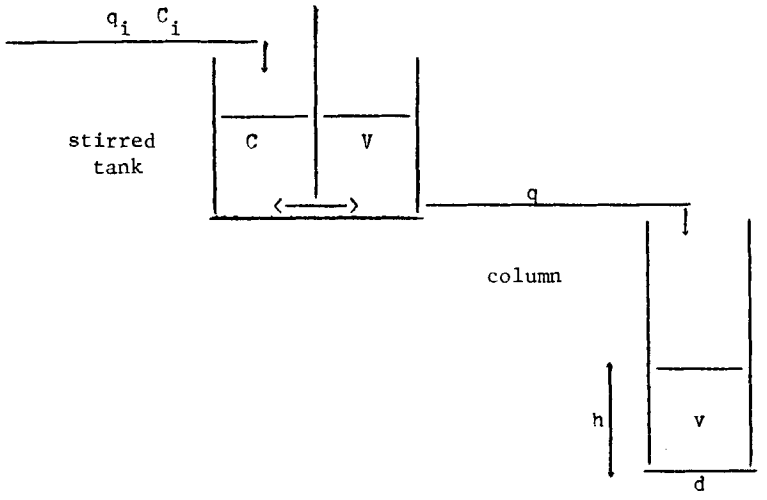

FIG. 2. Schematic representation of automatic density gradient layering system.

following balances allow the dependence of the concentration C on q, q_i, C_i, and V_0 to be established.

The sucrose mass balance over the stirred chamber leads to

$$d(VC)/dt = C_i q_i - Cq \tag{2}$$

where i = inlet
C = sucrose concentration
q = flow rate
V = volume of sucrose solution in the stirred chamber

The volume change of the sucrose solution in the stirred chamber leads to

$$dV/dt = q_i - q \tag{3}$$

and the condition $V = V_0$ at $t = 0$. Integrating Eq. (3) from $t = 0$ yields,

$$V = (q_i - q)t + V_0 \tag{4}$$

The volume of layered gradient in the column at time t is v, hence:

$$v = qt = hS = h(\pi d^2/4) \tag{5}$$

where d = diameter of the column
S = cross-sectional surface area of the column
h = height of fluid in the column

Combining Eqs. (2), (4), and (5) and solving for C with the condition that at $t = 0$, $C = C_0$, yields

$$\bar{C} = \frac{C - C_i}{C_0 - C_i} = \left[\frac{(q_i/q - 1)Sh + V_0}{V_0}\right]^{q_i/(q-q_i)} \tag{6}$$

where $\bar{C}$ is a dimensionless concentration number such that $\bar{C} = 0$ and $\bar{C} = 1$ at the top and at the bottom of the column, respectively. Therefore, by adjusting q_i and q, different density gradient profiles are obtained. For a linear gradient, the exponent takes the value of 1 and,

$$q = 2q_i \tag{7}$$

thus,

$$\bar{C} = (-Sh/2V_0) + 1 \tag{8}$$

To make up the linear gradients used in our study, cassette pump drive and pumping cassettes from Manostat were used. To check the gradient, the columns were delayered in 0.2 mL fractions and the index of refraction was measured for each fraction, Fig. 3. The initial and inlet concentrations were 40 and 4 wt% sucrose solution, respectively, with an initial volume V_0 of 2.5 mL. The fraction processing, concentration recording, etc. is similar to the previous section. Automatic layering of the columns is fast and easily achieved when the sample (migrating zone) is layered at the bottom or at the top of the sucrose gradient.

Band Spreading

After the band of material has migrated for 2 to 5 h, the liquid is removed in 0.2 mL portions from the column and analyzed. There are 25 fractions. The concentration of particles in each fraction is recorded and the initially sharp band of 0.1 mL appears as shown in Fig. 4. Spreading of the migrating zone can be due to the effects of a weak gradient, distribution of mobilities, instability of the initial zone, diffusion, electroosmosis, and adsorption on the column wall. Stability and

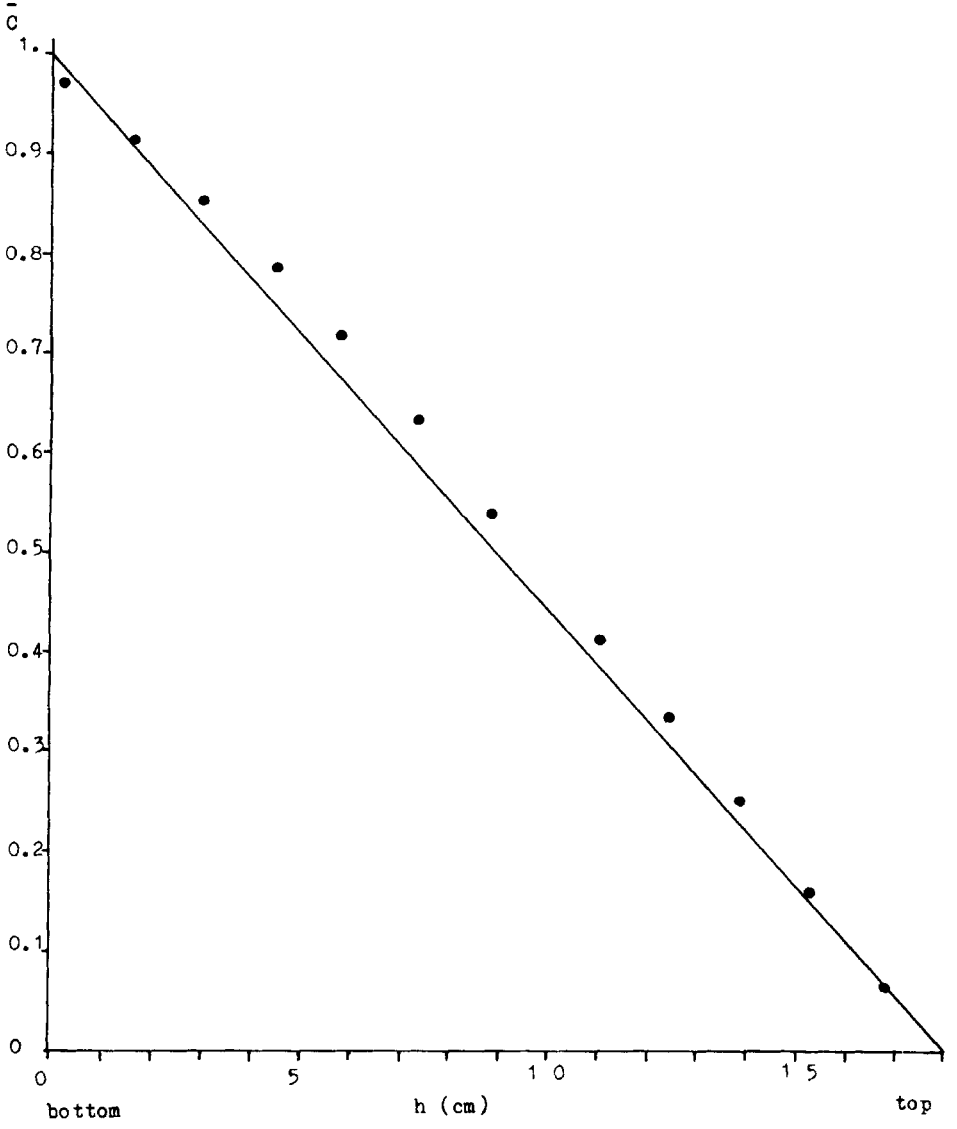

FIG. 3. Sucrose density gradient layered with pumps. Dimensionless $\bar{C}$ at different positions along the column. (•) Measured $\bar{C}$, (——) $\bar{C}$ profile predicted from Eq. (8).

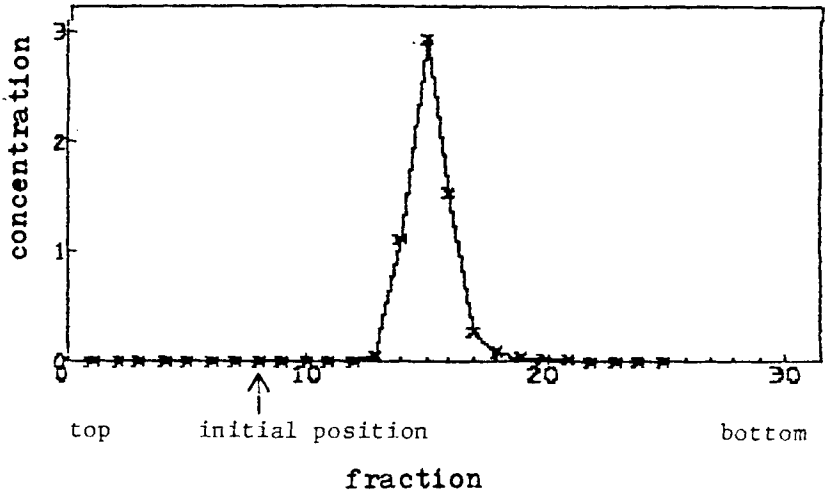

FIG. 4. Spreading of the migrating zone after 4 h electrophoresis. Serum albumin sample in barbital buffer of ionic strength 0.02 *M* and pH 8.6.

electroosmosis have been discussed earlier. Since the recorded concentration always shows a bell-shaped curve and no trailing tail, the effect of adsorption on spreading is negligible. The spreading from diffusion is also negligible owing to small diffusion coefficients of the species being separated (around 6×10^{-7} cm^2/s for albumin). Well-layered samples and a sufficient density gradient minimize spreading. Figure 4 is characteristic of the concentration curves obtained.

Electrophoretic Mobility

The general expression used to calculate the mobility is

$$U = v/E = (L/t)/E \tag{9}$$

where U = electrophoretic mobility
v = electrophoretic velocity
L = length of migration
t = time
E = voltage gradient = I/kS
I = current intensity

k = conductivity
S = cross-sectional area of the column

The density gradient is necessarily accompanied by other gradients (viscosity, conductivity, dielectric constant). The dielectric constant of electrolytes whose concentrations are of the order of 1 *M* were found to be lower than that of pure water by a few percent (*26, 27*), and the dielectric constant of aqueous solutions at different sucrose concentrations has been measured by Akerlof (*28*). Since the dielectric constant changes only by 10% over the whole column, the dielectric constant variation was not taken in consideration to estimate the electrophoretic mobility. The conductivity k and the viscosity η of the gradient solution were measured at different sucrose concentrations, corresponding to different positions along the column. Plots of $k(x)$ and $\eta(x)$ were made and a polynomial regression model (usually second or third order) was used to fit the data. For constant current and cross-sectional area of the column, an average voltage gradient and an average viscosity over the length of migration could then be computed. For instance,

$$<E> = [I/S(b - a)] \int_a^b 1/k(x)\, dx \tag{10}$$

where $<E>$ = average voltage gradient
a, b = initial and final positions

This permitted estimation of the mean experimental mobility $<U>$ for an average viscosity over the length of migration,

$$<U> = [L/t]/<E> \tag{11}$$

From the relation shown by Seaman (*29*),

$$U_a\eta_a = U_b\eta_b \tag{12}$$

$<U>$ is extrapolated to 0% sucrose concentration or calculated for any position along the column.

Materials

The electrophoretic mobility of albumin in the presence of a sucrose density gradient was estimated under different conditions.

a. In the presence of barbital, phosphate, MOPS (3-(*N*-morpholino) propanesulfonic acid)-imidazole, citrate, and succinic buffer solutions for different pH values.
b. In the presence of two neutral polymers (PVA, polyethylene oxide). (Polyvinyl alcohol (PVA), Gelvatol grade 20-30 (Monsanto Inc.) at 3.5 g/100 mL of solution. Polyethylene oxide, Polyox grade WR (Union Carbide Corp.) at 0.6 g/100 mL of solution.) Phosphate buffer of ionic strength 0.02 *M* and pH 7.4 was used as the supporting electrolyte for PVA and Polyox.
c. In the presence of a charged polymer (hydrolyzed ethylene maleic anhydride (EMA)). (Hydrolyzed ethylene maleic anhydride (EMA), grade 100 000 (Monsanto, Inc.).) EMA buffer at 0.02 *M* ionic strength was used.

The sample zone was composed of 0.1 mL of a preparation of 10 μL radiolabeled human serum albumin (Mallinckrodt, Inc., St. Louis, Missouri) + 10 μL of 20 wt% bovine serum albumin, BSA (Sigma Chemical Company, St. Louis, Missouri) in 2 mL of a density gradient fraction.

The electrophoretic mobility measurement of three bacteriophages (M123, ϕX174, MS2) and the electrophoretic separation of two bacteriophages (M13, ϕX174) were also studied. The bacteriophage M13 is a slender flexible rod approximately 8000 Å in length and 60 Å in width, containing single-stranded DNA (*30*). ϕX174 bacteriophage is a spherical phage of isometric capsid with icosahedral symmetry. It contains single-stranded DNA and has a diameter of 30 nm (*31*). MS2 phage is an icosahedral particle of 23-25 nm in diameter and contains single-stranded RNA (*32*). Phosphate/glycine buffer (pH = 7.4), described by Hannig (*17*), was used. The concentration of bacteriophages was measured by the plaque assay technique described by Douglas (*33*). The sample zone was 0.1 mL of a preparation of 10 μL of approximately 10^6 plaque-forming units (pfu/mL) of phages in 2 mL of the appropriate density gradient fraction.

RESULTS AND DISCUSSION

a. Electrophoretic Mobility of Serum Albumin

Figure 5 shows the mobility values of serum albumin from extrapolation to η_0 (viscosity of the buffer solution at 0% sucrose concentration and

4°C) compared to data obtained by Stenhagen (*34*) and Schlessinger (*35*). The comparison shows good agreement with the literature values.

b. Electrophoretic Mobility of Serum Albumin in the Presence of a Neutral Water-Soluble Polymer

Polyethylene oxide and PVA had similar effects. The measured electrophoretic mobility of albumin was two times higher (3.5×10^{-5} cm^2/V · s) than the expected mobility based on the viscosity of the solution (from Fig. 5). This increase in electrophoretic mobility, and therefore in zeta potential, in the presence of a neutral water-soluble polymer is in agreement with the observations made by Brooks (*20*).

c. Electrophoretic Mobility of Serum Albumin in the Presence of a Charged Water-Soluble Polymer

EMA greatly increased the mobility of albumin. The mobility was increased 8 times at pH 5 and 5 times at pH 6 over the mobility in aqueous buffer solution (from Fig. 5).

d. Electrophoretic Mobility of Phages (M13,φX174,MS2)

The mobilities of the phages, extrapolated to 0% sucrose concentration in phosphate/glycine buffer at pH = 7.4, were

Phage	U (($cm^2/V \cdot s$) × 10^5)
φX174	2
M13	15
MS2	15

Control columns, plaque-assayed for each run, indicate no loss of phage activity after electrophoresis.

e. Electrophoretic Mobility of MS2 and M13 Phages in the Presence of BSA and γ-Globulin

Electrophoretic mobility measurements at different protein concentrations were also made with a phosphate/glycine buffer (pH 7.4). As shown

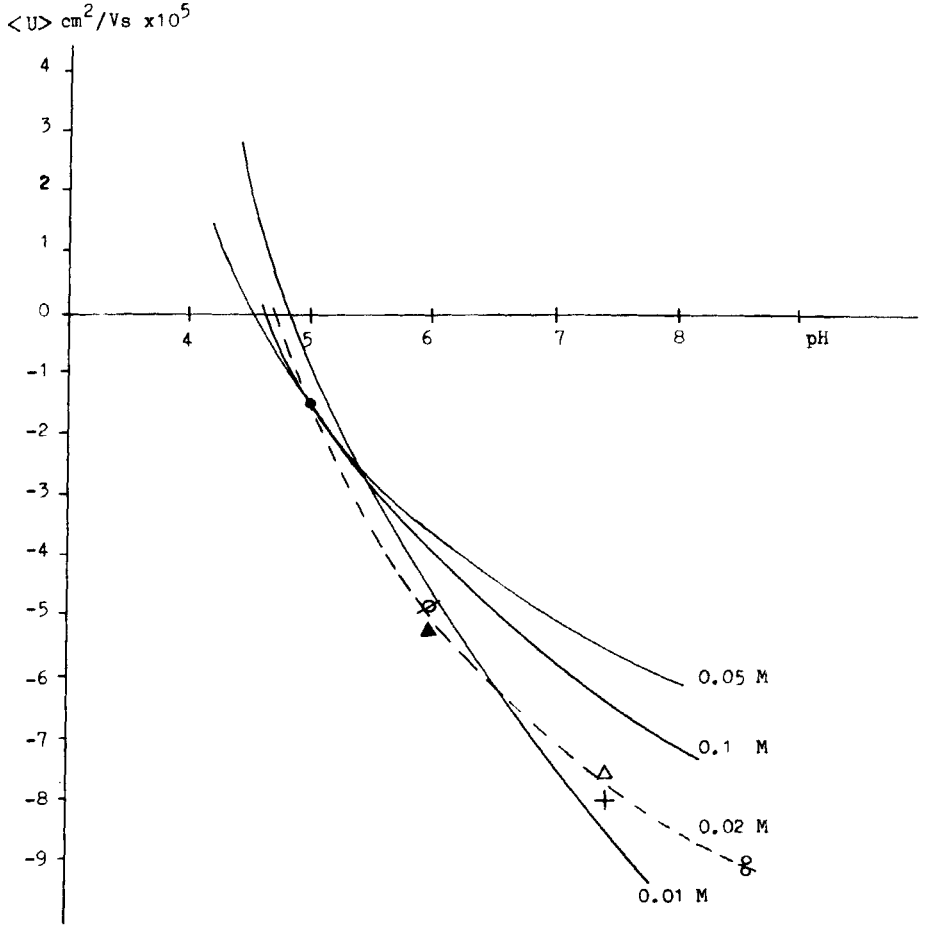

FIG. 5. Electrophoretic mobility of serum albumin for different buffer solutions and pH values:

	Buffer	pH	Ionic strength
●	Succinic	5.0	0.02 *M*
∅	Succinic	6.0	"
▲	Citrate	6.0	"
△	MOPS-imidazole	7.4	"
+	Phosphate	7.4	"
○	Barbital	8.6	"

Stenhagen (*34*) ionic strength 0.1 *M*; Schlessinger (*35*) ionic strength 0.05 and 0.01 *M*.

in Fig. 6, the mobility of both phages was reduced toward the mobility of the protein present in the buffer solution. In the presence of γ-globulin (Bovine Cohn fraction II, Sigma Chemical Company), the decrease was pronounced even at low protein concentration. This may indicate a phage-protein interaction.

f. Electrophoretic Separation of φX174 and M13 Phages

Figure 7 shows complete separation of φX174 and M13 phages initially mixed and layered at Position 14. Phosphate/glycine buffer (pH = 7.4) was used.

Discussion

A practical method is presented for measurement of electrophoretic mobilities of proteins and phages in a column stabilized by a density gradient.

The electrophoretic mobility of albumin for different buffers and pH values was measured and found to be in good agreement with comparable data in the literature.

The electrophoresis of serum albumin in the presence of a neutral water-soluble polymer showed an increase in mobility and appears to confirm Brooks' observations of an increase in zeta potential.

The effect of neutral and charged water-soluble synthetic polymers on the mobility was investigated because it was hoped that such polymers might enhance differences in the mobility of proteins facilitating electrophoretic separations. This could occur through polymer-polymer interactions in solution. Negatively charged carboxyl groups of hydrolyzed ethylene maleic anhydride (EMA) could interact with the positively charged guanidinium or amino groups on albumin or γ-globulin. Hydrophobic interactions are also possible with some polymers. Indeed, hydrolyzed EMA caused a large increase in the electrophoretic mobility (zeta potential) of serum albumin at pH 5 and pH 6. We are presently investigating if EMA shows any selective effect over different macromolecules. Preliminary results on serum albumin mobility show that the anions of EMA appear to interact with the protein, causing it to have a strong negative charge at pH values of 7, 6, 5, and 3, which apparently is still above the isoelectric pH.

Electrophoretic mobility of phages M13, φX174, and MS2 were

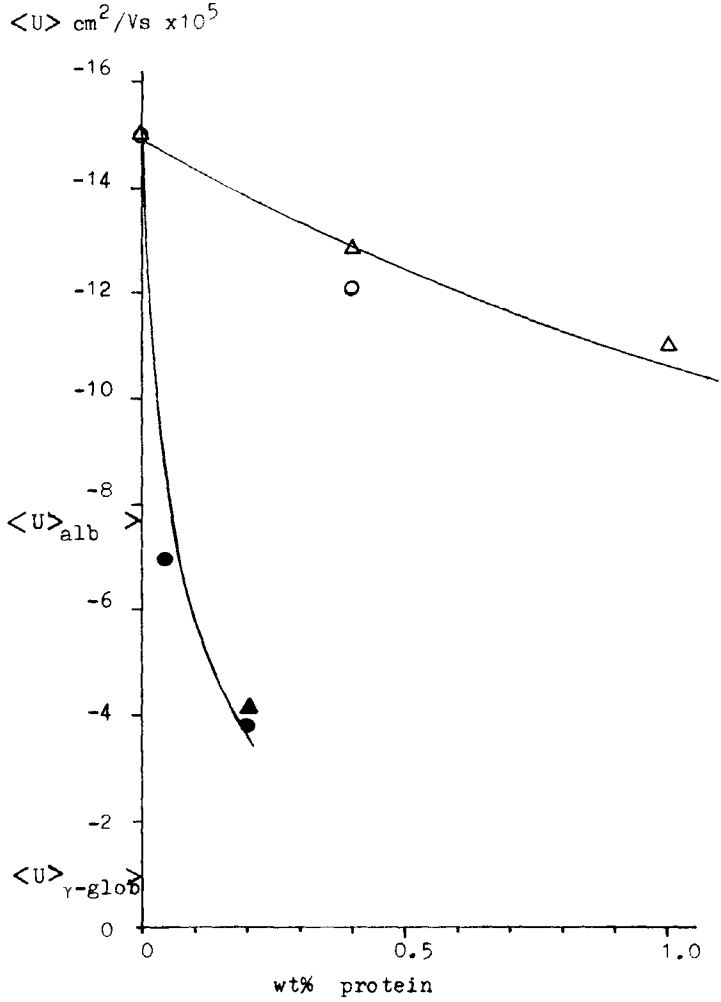

FIG. 6. Electrophoretic mobility of M13 and MS2 phages in the presence of bovine serum albumin (BSA) and γ-globulin in phosphage/glycine buffer at pH 7.4 (*17*).

Phage	BSA	γ-Globulin
MS2	○	●
M13	△	▲

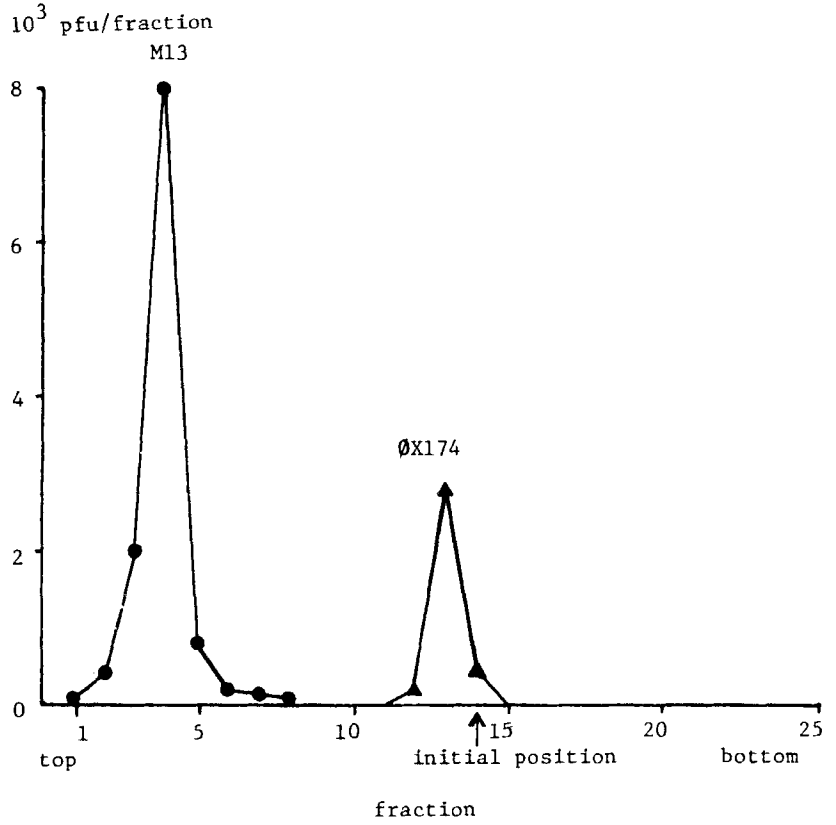

FIG. 7. Separation of phages ϕX174 and M13 after 3 h, 45 min in phosphate/glycine buffer of pH 7.4 (*17*).

measured in phosphate/glycine buffer at pH 7.4. M13 phage was found to migrate as fast as MS2 phage even though they differ by almost an order of magnitude in total surface area. MS2 and M13 phages were also found to migrate much faster than ϕX174. This may indicate a large difference in the surface charge of phages due to differences in the coat protein primary sequence or in the manner in which the coat protein is arranged in the particle. In the presence of different concentrations of serum albumin and γ-globulin, the mobility of the phages was decreased toward the protein mobility. This may indicate a phage-protein interaction which shows that a phage-protein separation at high protein concentration would be difficult to carry out.

Finally, the separation of phages M13 and ϕX174 was realized in less than 4 h in a phosphate/glycine buffer at pH 7.4.

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