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BUFFER-FOCUSING CHROMATOGRAPHY USING MULTICOMPONENT ELECTROLYTE ELUTION SYSTEMS

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SUMMARY

Procedures for the generation of internal pH gradients on columns of ion-exchange resins using multicomponent eluent systems of defined chemical composition, comprised of common amphoteric and non-amphoteric buffers, are described. The influence of buffer concentration, pH and resin characteristics on pH gradient formation have been examined. The physicochemical basis of the pH gradient formation under these chromatographic conditions has been discussed in terms of dissociation theory for polyelectrolytes which highlights the similarity with the formation of natural pH gradients by buffer electrofocusing procedures. Applications of this chromatographic technique for the separation of proteins are described.

INTRODUCTION

Ion-exchange chromatography has proved to be a versatile technique for the fractionation of peptides, proteins and other ionogenic biological molecules. Ion exchangers based on derivatised cellulosic resins have gained very wide acceptance particularly for the separation of protein mixtures due to their relatively high sample capacities compared to other types of resins and the ease of elution of the adsorbed proteins using step-wise or differential gradients of ionic strength and/or pH. Two approaches are feasible for the formation of pH gradients used in ion-exchange separations. The most widely used method employs a mixing device to progressively change the pH of the initial buffer by dilution with a second buffer of different pH, the mixer effluent being continuously introduced onto the column. In, for example, an anion-exchange separation, proteins are applied to the column, which has been equilibrated to an initially high pH, and eluted with a descending gradient formed from a buffer of lower pH. The pH gradient formed by this method has conventionally been considered to be an "externally" generated pH gradient. In this elution mode, a protein component will remain adsorbed to the resin until the pH of the incoming buffer is slightly below the p*I* of that protein.

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It is also possible to form a pH gradient directly on a column of a suitable ion-exchange resin. In this case, the resin, previously equilibrated to a certain pH, is eluted with a buffer of fixed composition but at a different initial pH. The formation of this type of pH gradient, which can be described as "internally" generated, will depend on the dissociation equilibria established between the hydrated, or partially hydrated, ionised buffer components and charged groups on the resin. If mobile phases based on multicomponent polyelectrolyte buffer systems are used, internally generated pH gradients will form because some buffer components preferentially migrate more rapidly than others down the ion-exchange column. As a consequence, these types of pH gradients will be particularly responsive to changes in the chemical composition and buffer capacities of both the eluent and the ion exchanger. Several studies have shown[1–5] that solutions of random mixtures of proprietary carrier ampholytes such as the Ampholines or Pharmalytes can be effectively used to form internally generated pH gradients on ion-exchange resins. Selective elution of proteins under mild desorption conditions at pH values proximal to the solutes' p*I* values is one of the desirable features of this chromatographic ampholyte titration technique. In addition, with the proprietary polyamino–polycarboxylic ampholytes, it is possible to produce focusing effects in the ion-exchange chromatographic separation of proteins similar to the effects observed with isoelectric focusing methods.

In recent publications[6–11] from this and other laboratories, methods for electrofocusing in granulated polysaccharide gels and polyacrylamide gels have been described using multicomponent systems of defined chemical composition comprised of common amphoteric and non-amphoteric buffers, to generate the required natural pH gradient. Depending on the choice of the component reagents and the pH of the terminating anolyte and catholyte, linear or cascade-step pH gradients can be formed. Under these electrophoretic conditions, proteins focus to sharp boundaries which have relative positions in accord with their intrinsic p*I* values. The present paper reports an investigation on the characteristics of pH gradients internally generated on ion-exchange resins using a multicomponent buffer system, previously shown to generate a stable pH gradient over the range pH 3–10 under electrofocusing conditions. The application of this technique to the fractionation of protein mixtures is also described.

EXPERIMENTAL

Materials

The ion exchangers DEAE-Sephadex A-25, QAE-Sephadex A-25 and CM-Sephadex A-25 were obtained from Pharmacia (Uppsala, Sweden), DEAE-cellulose type A17 was from Phoenix (Nelson, New Zealand) and TETA-cellulose was a gift from Dr. J. Ayers, Massey University, Palmerston North, New Zealand. The small ion capacities of these gels were stated by the manufacturer to be 3.5, 3.0, 4.5, 1.0 and 1.8 mequiv./g, respectively. The buffers used in the preparation of the wide pH range eluent system were obtained from Sigma (St. Louis, MO, U.S.A.). A 20 m*M* stock solution of this multicomponent buffer system was prepared as described previously[5,6] for use with electrofocusing procedures and can be stored at 4°C for at least six months. This buffer system is also available ready for use from Pierce (Rockford, IL, U.S.A.) as part of their Buffalyte range of electrofocusing reagents (Buffalyte WR

3–10). Human serum albumin, sperm whale myoglobin, bovine serum albumin, bovine haemoglobin, lysozyme, transferin and catalase were commercial samples from Sigma. Antisera against serum and other proteins were either raised in this laboratory or purchased from Behringwerke (Marburg, G.F.R.).

Chromatographic equipment and procedures

The ion-exchange resins were precycled as recommended by the manufacturer and then equilibrated to the initial buffer condition which was generally 5 m*M* Na_2HPO_4–NaOH, pH 9.5, or 5 m*M* Tris–HCl, pH 9.8. Columns were slurry packed using about one part of equilibrated, swollen gel to one part of equilibration buffer and the bed stabilised by eluting with *ca.* 50 column volumes of the initial buffer. Flow-rates were controlled with Multiperpex pumps (LKB, Bromma, Sweden) and column eluents were collected with a LKB Ultrarac 7000 fraction collector. The pH of the collected fractions was measured with a combination glass electrode on a Radiometer PHM64 pH meter. Protein samples were applied to the equilibrated ion exchangers in the initial buffer and subsequently eluted with a solution of the multi-component buffer system at an appropriate concentration and pH. Proteins not eluted under these conditions were recovered by elution with a gradient of 1 *M* NaCl in the final eluting buffer. Proteins in the column effluents and concentrates were detected by their UV extinction 254 and 280 nm on a Uvicord III monitor and by the Bradford assay[12]. Analysis of specific proteins was carried out by standard immunodiffusion, immunoelectrophoresis or SDS–polyacrylamide gel procedures.

RESULTS AND DISCUSSION

As a solution containing ampholytes of defined chemical composition passes through an ion-exchange column each buffer component will exhibit a different affinity for the ion exchanger. This will result in a continuous sequence of dynamic replacement events of bound zones of buffer counter-ions by new buffer ions entering the column. Individual buffer components will selectively migrate through the column according to their ionisation state, weakly bound buffer components being progressively displaced by buffer components with higher affinities for the resin. Due to the buffering action of the ion exchanger, a continuous change in the pH of the mobile phase will thus occur as it moves down the column until a limiting pH value is reached, the slope of the pH gradient so formed being controlled by the buffering capacity of the ion-exchange resin and the eluent. By considering the ion-exchange column to be made up of *n*-sections of an interactive stationary phase which exhibits a range of apparent p*K* values, the physicochemical basis for the continuous readjustment of the pH and composition of each aliquot of the mobile phase as it passes down the column can be evaluated in terms of the various dissociation equilibria established by the participating ionised and unionised species. According to the detailed theoretical studies of Sluyterman and Elgersma[3], the pH at any point in an ion-exchange column eluted with an amphoteric buffer of low ionic strength can be given by

$$pH_i = \frac{pH_{m,i} + \frac{a_s}{a_m} pH_{s,i}}{1 + \frac{a_s}{a_m}} \quad (1)$$

where pH_m and pH_s are the pH values of the mobile phase and the stationary phase and a_m and a_s are the buffer capacities per unit of column length of the mobile phase and the stationary phase, respectively. Furthermore, if the pH difference between two adjacent column sections, i and j, is ΔpH, then the pH change, ΔpH_j, in the section j caused by advancing the mobile phase over this section can be expressed by

$$\Delta pH_j = \frac{\Delta pH}{1 + \frac{a_s}{a_m}} \tag{2}$$

The rate of advance of an individual buffer component, B_1, down the column under conditions of ideal linear chromatography can be given by[13]

$$\left(\frac{dc'}{dt}\right)_{B_1} \cdot \left(1 + K_{B_1} \frac{A_s}{A_m}\right) = \mu \left(\frac{dc'}{dx}\right)_{B_1} \tag{3}$$

where $(dc'/dt)_{B_1}$, is the change in the buffer component, B_1 concentration in the initial aliquot of the mobile phase as it passes through a controlled section of ion exchangers, of dimensions x, K_{B_1} is the equilibrium distribution constant ($= c_{s,B_1}/c_{m,B_1}$), A_s and A_m are the cross-sectional areas of the stationary and the mobile phases respectively, and μ is the linear flow velocity. If we assume that the charge, Q, on the buffer component confined within a zone a distance x along a column is proportional to pI–pH for ampholytes and pK–pH for monopoles, then the equilibrium distribution constant can be evaluated for each buffer component from

$$\ln K = -\frac{dQ}{dpH} \cdot \frac{dpH}{dx} \cdot \chi \cdot x \tag{4}$$

where χ is the appropriate normalised Donnan coefficient and dQ/dpH is the buffering capacity term. Provided the participating chemical equilibria are rapid relative to the transport processes, the effects of radial and longitudinal temperature gradients can be omitted from eqn. 4. In addition, conditions of electroneutrality will prevail. Under these conditions, the $K \cdot A_s/A_m$ term in eqn. 3 can be related to the buffer capacity per unit volume of the ion-exchange resin, a_e, and the eluent, a_b, respectively and the velocity of the pH gradient can be given by

$$\left(\frac{dx}{dt}\right)_{pH} = \left(\frac{dx}{dt}\right)_m \left(\frac{1}{\frac{A_s}{A_m} \cdot \frac{a_e}{a_b} + \frac{a_e}{a_b} + \frac{A_s}{A_m} + 1}\right) \tag{5}$$

At all times the pH of the column effluent will be equal to the pH of the last section of the column, whilst the instant the first aliquot of the multicomponent buffer system emerges from the column outlet the complete pH gradient can be considered to be present in the column.

Proteins passing through an ion-exchange column in an internally generated pH gradient will be subjected to the same selective migration effects as the buffer components. The rate of advance of a protein or similar biological polyelectrolyte will

be identical to the rate of advance of a buffer component of the same p*I* and charge characteristics. In practice, proteins will be confined to zones by buffer components of lower and higher p*I*/p*K* values. The migration of each protein will thus be controlled by its ionisation state relative to the adjacent sections of the ion-exchange resin and be bounded by mobile phase aliquots with pH values slightly higher and slightly lower than the p*I* of the protein. These processes will result in a converging zone for each protein which will be emerged from the column at a pH value near to its p*I* value.

The selective migration of individual buffer components on ion-exchange supports bears some similarity to the separation of amphoteric buffers under electrofocusing conditions. In the latter technique, buffer ampholytes are initially distributed throughout the support, but during the electrolysis individual components migrate and accumulate in zones corresponding to their isoelectric points. As a consequence of its buffering capacity, each focused ampholyte generates a pH gradient upon the immediate vicinity in the support medium, with the pH gradient increasing monotonously from anode to cathode. The pH gradient stability in these systems is dependent on the buffer capacity, d*Q*/dpH, of each ampholyte. Furthermore, the velocity of pH gradient formation under electrofocusing conditions is also a function of the buffering capacity, and particularly the relative concentrations and flux mobilities of the different buffer species.

In common with pH gradients formed by electrofocusing methods, shallow pH gradients, *i.e.* when the ΔpH/Δx term in eqn. 4 involves small incremental pH charges per unit column length, internally generated on ion-exchange resins will favour higher resolution in the separation of protein mixtures. According to the outlined theoretical considerations, for any given ion-exchange column, the slope of the internally generated pH gradient will depend on the concentration, composition and pH of the eluent buffer system. For different types of ion-exchange resins, eluted with a common multicomponent buffer system under the same conditions, the pH gradient shape will depend on the buffer capacity per unit volume of the ion exchanger, *i.e.* the pH and surface coverage of charged groups of the resin. These conclusions were verified with a variety of experiments using the wide range buffer system, Buffalytes WR 3–10, and different ion-exchange resins. Typical results from these experiments are summarised below.

Effect of concentration of buffer eluent

The influence of buffer concentration on the pH gradient shape was evaluated using the various resins, packed into columns of the same dimensions. In typical experiments, columns (12 × 1.6 cm) containing the pre-equilibrated DEAE-cellulose type A17 resin (pH 9.8) were eluted with aqueous solutions containing 1, 2, 5, 10 and 20 m*M* of the multicomponent buffer system, Buffalyte WR 3–10. The plots of the column effluent pH *versus* elution volume are shown in Figs. 1 and 2. These results clearly demonstrate the synergistic dependence of pH gradient slope on the concentration of the buffer species in the eluent. During the early part of pH gradient formation, most of the buffer species will be bound to the anion exchanger. As an increasing number of the ionic groups on the resin are titrated, more buffer species will move through the column, initially the most basic component followed by the other components in order of decreasing basicity. This movement of buffer species with concomitant internal pH gradient formation, results in a progressive increase in

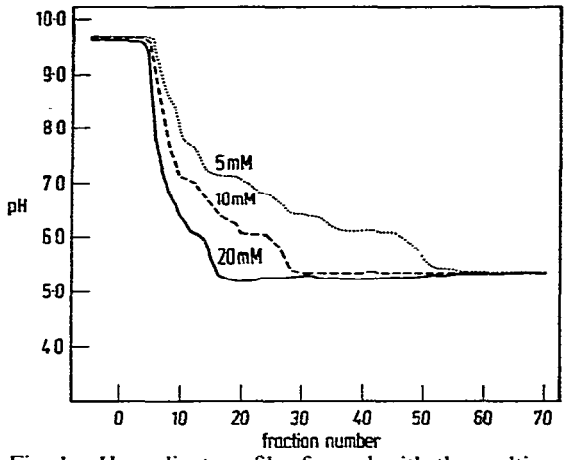

Fig. 1. pH gradient profiles formed with the multicomponent buffer system, Buffalyte WR 3–10, on a DEAE-cellulose column as a function of buffer concentrations. Column dimensions, 12 × 1.6 cm; flow-rate, 45 ml/h; resin pH 9.8; buffer eluent pH 5.3.

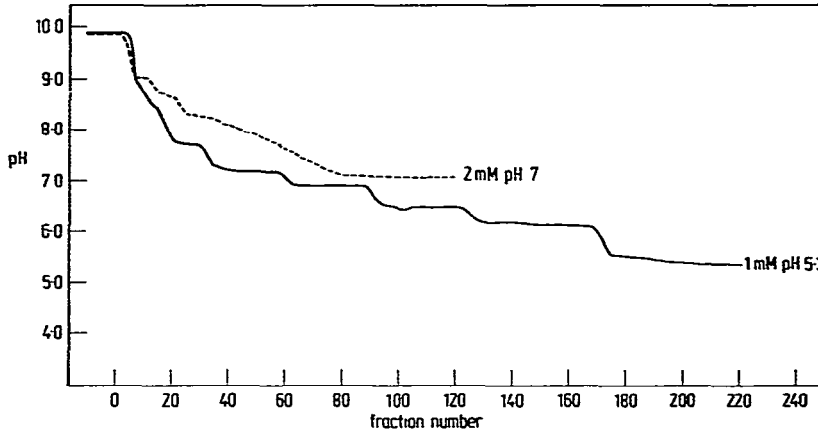

Fig. 2. pH gradient profiles formed with the multicomponent buffer system, Buffalyte WR 3–10, on a DEAE-cellulose column as a function of buffer concentration and pH. Column dimensions and flow-rate as in Fig. 1.

ionic strength throughout the elution. Compared to conventional elution conditions, the ionic strengths and buffer concentrations employed in these experiments are low relative to the total capacity of the ion exchanger. It is worth recalling that low buffer concentrations and large Donnan potentials will favour resolution with ion-exchange separations under buffer focusing elution conditions. Because of practical limitations with very shallow pH gradients which will lead to large dilution factors, a compromise between buffer concentration, pH gradient slope and resolution must be achieved. With the ion exchangers examined in this study, buffer concentrations in the range of 2.5–5 m*M* (*i.e. ca.* 0.0005–0.001 mmol/pH unit/ml eluent) were found to provide convenient pH gradients (in terms of analysis time, elution volume and resolution) with columns containing 20–300 ml swollen resin. Furthermore, an acceptable com-

promise between resolution and solute dilution can be achieved when the total gradient volume was less than or equal to *ca.* 10 bed volumes. It is noteworthy that the internal pH gradients formed by this procedure with the Buffalyte WR 3–10 system show cascade pH steps at very low buffer concentrations. Similar pH gradient effects have been observed[7] in electrofocusing experiments with related buffer systems. For preparative separations of a particular protein, a pH plateau near the region of p*I* interest will be advantageous provided it creates a large difference in ionisation between adjacent protein zones. Furthermore, linear pH gradients are not essential with preparative loadings of proteins since it is unlikely that all protein zones will concommitantly migrate at their theoretical narrowest band width. With minor modification of the buffer compositions, it is possible to generate linear internal pH gradients at buffer concentration levels below 1 m*M*.

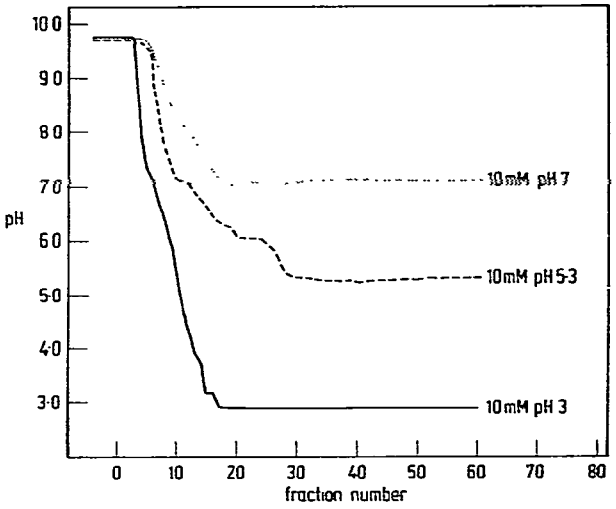

Fig. 3. Influence of buffer eluent pH on the internally generated pH gradients formed on a DEAE-cellulose column with the 10 m*M* buffer system eluent. Column dimensions and flow-rates as in Fig. 1.

Effect of the pH of the buffer eluent

According to theory, the terminating pH value of an internally generated pH gradient should be identical to the pH of the buffer eluent. Shown in Fig. 3 are the plots of the column effluent pH *versus* elution volume using the 10 m*M* buffer eluent at its intrinsic pH (pH 5.3) and at pH 7.0 and pH 3.0. In these experiments, DEAE-cellulose column of identical bed dimensions were first equilibrated to pH 9.8 and then eluted with the buffer system adjusted to the appropriate pH. As is evident from these experiments, and from comparisons with the data shown in Figs. 1 and 2, the slope of the pH gradient is affected by changes in buffer concentrations but in each case the terminating pH value of the column effluent ultimately reaches the limit set by the pH of the eluent. The choice of the eluent pH used with this, or other, buffer systems can thus be dictated in preparative protein separations by the operational isoelectric point range of the sample. Since the majority of proteins have isoelectric

points[14] which encompass the range pH 4–7.5, an eluent at pH 4 or below would be required for most applications. When the eluent pH is higher than the p*I* value of a particular protein in a mixture, then that protein will not elute from an anion exchanger. For example, with the Buffalyte WR 3–10 (pH 3.0) eluent, serum proteins including albumin, eluted near to their respective p*I* values with good sample recoveries. However, with the same system but at pH 5.5, albumin and other proteins with isoelectric point values below 5.5 could not be eluted without a salt gradient or an eluent pH change.

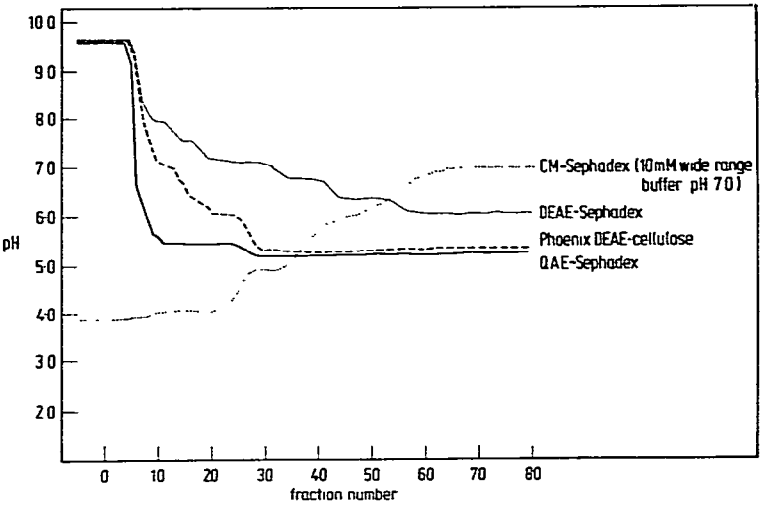

Fig. 4. Plots of the pH gradients formed on the anion exchangers DEAE-Sephadex, DEAE-cellulose and QAE-Sephadex using the 10 m*M* multicomponent buffer system. The resins were initially equilibrated to pH 9.8 and eluted with the same volume (500 ml) of the buffer system at pH 5.3. Also shown is the plot of the pH gradient formed on the cation exchanger, CM-Sephadex equilibrated to pH 4.0 and eluted with the 10 m*M* multicomponent buffer system adjusted to pH 7.0.

Effect of the buffering capacity of ion-exchange supports

In Fig. 4 are shown plots of the pH gradients formed with four different ion-exchange resins, packed into columns of identical bed dimensions, using as a common eluent the 10 m*M* multicomponent buffer system. As anticipated from a comparison of the reported properties of the various resins, the shape of the pH gradient is dependent on the charge functionality and buffering capacity of the ion exchanger. Many commercial cellulose anion exchangers show acceptable buffering capacities only over the range of *ca.* pH 6.5–10.0. To be effective in buffer focusing chromatographic experiments, the ion exchanger should exhibit an extended titration curve, which ideally should cover the range pH 3.0–11.0, and a uniform buffer capacity across this range. Furthermore, the resin should exhibit large penetration constants with macroglobulins, *i.e.* the fraction of the internal surface area available to both high- and low-molecular-weight species is comparable, and have a high surface coverage of charged groups. To meet these requirements, large-pore high-capacity ion-exchange resins are needed. Several recently described resins, for example the cross-

linked, fully porous Sepharose 6B PBE ion exchangers, appear to satisfy most of these criteria.

With anion-exchange resins, the charge on the resin increases as the column pH decreases during the buffer elution. It is thus essential to select the initial pH condition for the sorbent such that the most basic protein in the sample is weakly adsorbed. Typically this pH value was chosen to be *ca.* 0.5 pH units above the p*I* value of the most basic protein. For cation exchangers, the opposite effects apply and a resin pH below the p*I* value of the most acidic protein must be chosen. With two resins of similar charge functionality but different buffering capacities the shallower pH gradient will result under similar chromatographic conditions with the resin of higher capacity. The small ion capacities of different ion exchangers can be used to assess which resin will exhibit the more favourable distribution ratio for multi-component buffer eluents of low ionic strength and hence form the more appropriate pH gradient shape. Besides the influence of resin characteristics, the slope of the pH gradient can also be manipulated by changing the column bed volume. For example, an increase in column dimensions using the same resin and elution conditions resulted in a more shallow pH gradient. Finally, titration of the anion-exchange resins to lower initial pH values caused more shallow gradients to form under otherwise identical chromatographic conditions. However, it has been our experience that resin titration is not always appropriate in protein separations, *e.g.* when the resin pH and protein p*I* values are similar.

Protein separations using multicomponent buffer systems

The separation of two proteins, P_1 and P_2 with isoelectric points at pH 9.0 and pH 7.0, respectively, on an anion exchanger initially equilibrated to pH 10.0 and eluted with a multicomponent buffer system at pH 4.0 can be considered in terms of the respective distribution equilibria. Due to the Donnan effect, the pH inside the pores of the matrix will always be slightly higher than the pH of the surrounding mobile phase environment. When the pH of the resin falls to pH 9.0 and the charge on P_1 becomes zero, P_1 will diffuse away from the charged surface of the pore into a mobile phase environment which has a pH value slightly lower than the p*I* value of P_1. As a consequence, P_1 will be protonated and move with a mobile phase zone at a pH slightly lower than its p*I* value. The migration of P_2 will follow in a similar fashion when the resin pH reaches 7.0. Selectivity under buffer focusing chromatographic conditions will thus arise due to differences between the participating equilibrium distribution processes for P_1 and P_2, which for each protein can be represented by a equilibrium distribution constant. In the general case, the equilibrium distribution constant will take the form explicit to eqn. 4. Resolution will thus depend, *inter alia*, on the slope of the pH gradient (dpH/dx) and the rate of change of charge on the protein with pH, (dQ/dpH). The involvement of the latter term, which reflects the ionisation rate of a protein as the pH of the environment is varied, has important ramifications in both the buffer focusing chromatographic and electrophoretic separation modes. For examples, if the two proteins P_1 and P_2 have the same p*I* value but different values for dQ/dpH, that is different titration curves, it may be possible to resolve them under certain experimental conditions. Proteins with steep dQ/dpH slopes are expected to show narrower band widths than proteins with flatter depedencies of charge on pH.

Since the dpH/dx term in eqn. 4 is dependent on the concentration of buffer species, resolution is anticipated to decrease as the buffer concentration increases. Fig. 5 illustrates an example of this effect obtained with a crude myoglobin preparation using two different concentrations of the Buffalyte WR 3–10 system with a DEAE-cellulose column. Similar concentration-dependent changes in resolution have been described[4] for proteins eluted from anion-exchange columns with mobile phases containing Ampholine systems.

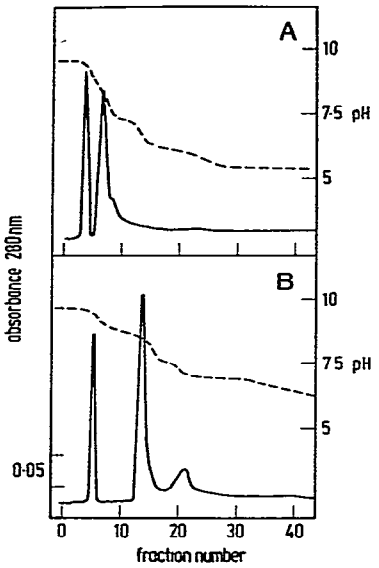

Fig. 5. Elution profiles for a crude myoglobin preparation on a column (25 × 1.6 cm) of DEAE-cellulose adjusted to pH 9.8. The elution buffers for A and B were respectively the 10 mM and the 2 mM multicomponent buffer systems, pH 5.3. Flow-rate 40 ml/h.

In Fig. 6A is shown the pH gradient and elution profile for a mixture of standard proteins (lysozyme, myoglobin, haemoglobin and human serum albumin) on a DEAE-cellulose column using a 5 mM Buffalyte WR 3–10 system at pH 3.0. In each case the proteins eluted in order of their isoelectric points at a pH value slightly lower than the pI value as measured by buffer electrofocusing[6]. Examination of the recovered fractions by SDS–polyacrylamide gel electrophoresis on 10% slab gels (Fig. 7), immunodiffusion, against specific antisera and by analytical buffer electrofocusing on 5% polyacrylamide slab gels[15], confirmed that the individual proteins can be recovered with a high degree of purity. The elution profile obtained with the same protein mixture on a TETA-cellulose column eluted with the same buffer eluent at pH 5.5 is shown in Fig. 6B. With the buffer system at this terminating pH a 0–1 M NaCl gradient was required to elute the human serum albumin. In subsequent studies, similar buffer focusing chromatographic methods have been utilised in the purification of human transferin, complement component C_3 and human thyroid autoantibodies. Compared to existing ion-exchange methods for the fractionation of these

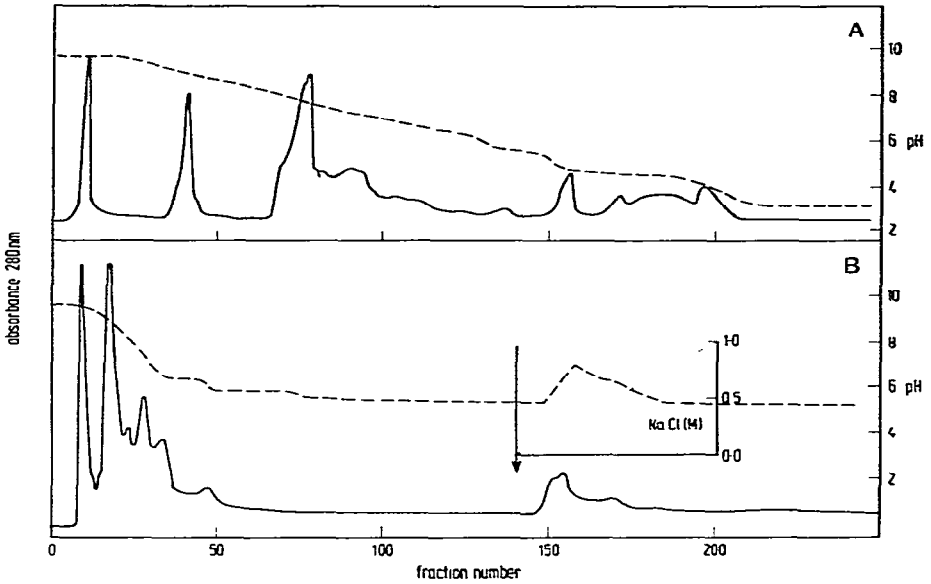

Fig. 6. A. Separation of protein mixture containing 50 mg each of chick lysozyme, sperm whale myoglobin, haemoglobin and human serum albumin on a DEAE-cellulose column (40 × 2.6 cm) eluted with the 5 m*M* Buffalyte WR 3–10 system, pH 3.0. The column was adjusted to pH 9.8 before application of the proteins. Flow-rate 40 ml/h. B. Separation of the same protein mixture on a TETA-cellulose column (40 × 2.6 cm) eluted with the same buffer system at pH 5.5. A salt gradient (0–1 *M* NaCl) was commenced at the point indicated.

proteins from suitable human sera, these buffer focusing chromatographic techniques permitted excellent resolution under preparative conditions, *i.e.* sample loadings greater than 500 mg.

In summary, this study has shown that internally generated pH gradients can be formed on ion-exchange resins with multicomponent buffer systems. Under these conditions proteins are resolved essentially according to their p*I* values. The separation mode bears close physicochemical similarities to the resolution of proteins under buffer electrofocusing conditions on granulated polysaccharide gels. These chromatographic procedures lend themselves to relatively large sample sizes because of the focusing effect to which solutes are subjected during their migration. In contrast to some synthetic polymeric ampholyte systems, the amphoteric and non-amphoteric components of the Buffalyte WR 3–10 system typically do not bind to proteins undergoing purification. In addition the buffer species can be easily removed from resolved proteins by, ultrafiltration or dialysis, gel filtration or other appropriate chromatographic methods[6,7]. From the array of low molecular weight buffers now available, clearly a large variety of multicomponent systems could be prepared for use in the generation of internal pH gradients on ion-exchange resins. Their selection will depend on further studies which establish the most appropriate combination of components of defined chemical composition for a particular application. With optimal pH gradient slope and precise control over the extent of solute ionisation, these buffer systems should find wide application in the separation of complex mixtures of pro-

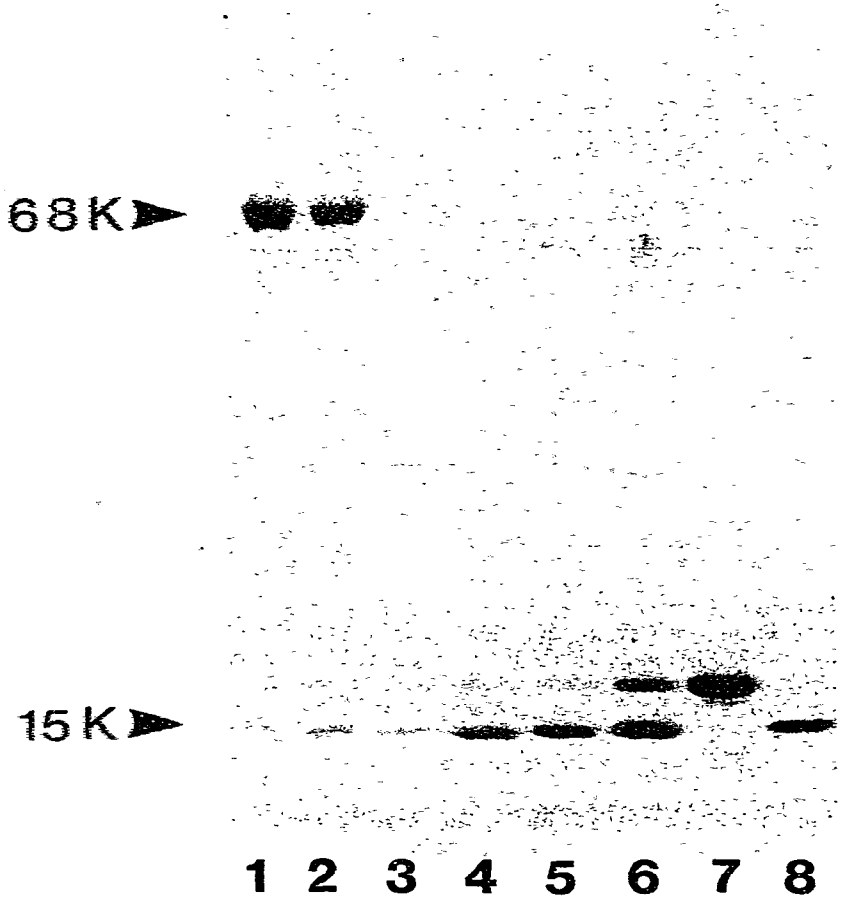

Fig. 7. Sodium dodecylsulphate–polyacrylamide slab gel electrophoresis of the recovered pooled fractions from the separation shown in Fig. 6A. The concentration of acrylamide and sodium dodecylsulphate in the gel were 10 and 0.1%, respectively. The slab well numbers corresponding to the following pooled chromatographic fractions from the DEAE-cellulose separation: 1, 161–175; 2, 146–160; 3, 121–145; 4, 101–120; 5, 86–100; 6, 81–85; 7, 66–80; 8, 37–43. Under the electrophoretic conditions used, haemoglobin exhibits an apparent molecular weight of 15,500. The predominant protein as assessed by immunodiffusion in the pooled fractions was: 1, albumin; 2, albumin; 3–5, haemoglobin; 6, haemoglobin–myoglobin; 7, myoglobin and 8, lysozyme. Pooled chromatographic fractions 8–15 also contained only lysozyme.

teins and other biological polyelectrolytes. These aspects together with further potentials of this method, including its extension to high performance ion-exchange chromatographic systems, are under investigation.

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