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New approaches to concentration on a microliter scale of dilute samples, particularly biopolymers with special reference to analysis of peptides and proteins by capillary electrophoresis II[☆]. Applications

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Abstract

On theoretical grounds several approaches for the concentration of ionic solutes are suggested in ref. 1. The methods are of general applicability. In this paper we describe how they can be employed in high-performance capillary electrophoresis (HPCE) for 1000-fold manual and automated on-line concentration from microliters down to nanoliters. The risk that the solutes may escape detection —the obvious disadvantage of HPCE— is, accordingly, reduced considerably. Briefly, the whole capillary (and for more than 1000-fold concentration also an electrode vessel or a vessel in contact with an electrode vessel) is filled with the dilute sample solution. The sample becomes concentrated as it migrates toward a pH gradient, a small-pore gel, a dialysis tubing attached to one end of the capillary, or by displacement electrophoresis combined with a hydrodynamic counterflow. These concentration steps (except the last) require a short mobilization step to prevent the narrow, concentrated sample zone from broadening in the initial phase of the final analysis step (free zone electrophoresis).

1. Introduction

For any analytical or preparative separation method to work satisfactorily the solute concentration must be above some threshold value, characteristic of the particular method. The reason is not only (1) detection difficulties at extremely low concentration but also (2) adsorptive losses of solutes on the surfaces of containers, filters, spatulas, plastic columns, etc., and (3) contaminants from these items and from air, hands, gloves, etc. The latter two problems may very well turn out to be more severe than the first one, at least for macromolecules such as proteins, which often are adsorbed by a strong multi-point attachment. Owing to surface adsorption the concentrations of the free solutes may differ from those in the original sample, giving rise to erroneous analysis data, or even fall below the detection limit.

There is, accordingly, a need for efficient concentration methods. In the preceding article [1] we have treated theoretically several new methods which were developed especially for minute-volume sample solutions of peptides and

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[☆] For Part I see ref. 1.

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proteins to be analyzed by capillary electrophoresis. In this article we present a few applications.

This paper is written in such a way that the reader does not need to be acquainted with the theory discussed in ref. 1 in order to employ the concentration methods described in this paper. However, to those who are interested in the theory behind a particular concentration technique we refer to the section or the figure in ref. 1, where that technique is presented.

2. Materials and methods

Fused-silica tubing from Polymicro Technologies (Phoenix, AZ, USA) and glass tubes from Modulohm (Herlev, Denmark) were coated with polyacrylamide to suppress electroendosmosis and adsorption as described in ref. 2, with the difference that γ-methacryloxypropyltrimethoxysilane was used as a 50% (v/v) solution in acetone. Glycine and chemicals used for the preparation of coating and gel plugs of polyacrylamide were electrophoresis purity reagents from Bio-Rad (Hercules, CA, USA). Diaminopimelic acid (DAPA) and N-2-hydroxyethylpiperazine propane sulfonic acid (EPPS) were from Sigma (St. Louis, MO, USA). All standard proteins used were commercial products except *R*-phycoerythrin, which was prepared as described in ref. 3.

3. Experiments and results

The theoretical background is given in ref. 1.

3.1. Concentration of proteins by electrophoresis toward a steep, non-buffering pH gradient (isoelectric focusing: step I, alternative a in ref. 1) and mobilization by displacement electrophoresis (step II, alternative a in ref. 1) followed by zone electrophoresis (step III, alternative b in ref. 1).

The sample consisted of 20 μg of each of the proteins phycoerythrin (Pe), ovalbumin (Ova), human transferrin (Tf), human hemoglobin (Hb) and carbonic anhydrase (CA) dissolved in 1 ml of 0.01 *M* Tris–HCl, pH 8.5. For rapid generation of the pH gradient this buffer should have a relatively low buffering capacity, *i.e.*, a low molarity or/and a pH 0.5–0.7 pH units lower than the p*K* value of the buffering constituent. The same buffer was also used as catholyte in the concentration step. A 0.5 *M* Tris–HCl solution, pH 2.5, served as anolyte. The fused-silica tubing [130(115) × 0.05 mm I.D., where 115 mm is the length of the capillary from the cathodic end to the detector] was filled with the sample solution. A voltage of 1500 V (0.8 μA) was applied during 12 min in order to concentrate the proteins into a narrow zone in the pH gradient (see Fig. 1a in ref. 1). The polarity of the electrodes was then reversed following an exchange of the 0.5 *M* Tris–HCl solution, pH 2.5, for 0.03 *M* glycine–NaOH, pH 10.0 At a voltage of 1500 V for 2.5 min a sharp zone was obtained by displacement electrophoresis (chloride was the leading ion and glycine the terminating ion; *cf.* Fig. 2b, step II in ref. 1). The glycine buffer was then replaced by 0.01 *M* Tris–HCl, pH 8.5 and an analysis of the sample by zone electrophoresis (see Fig. 2b, step III in ref. 1) was performed at 3000 V, corresponding to 1.6 μA (without the above displacement step the starting zone in the zone electrophoresis step became blurred, as revealed by visual inspection during an experiment done in a transparent glass tube). The electropherogram is presented in Fig. 1b. The detection was done at 220 nm. A control experiment was performed without concentrating the sample and with a starting zone 3–4 mm wide with the hope to detect at least the main peaks (Fig. 1a). A comparison between Figs. 1a and 1b shows the efficiency of the concentration technique. The width of the starting zone in the zone electrophoresis step was about 0.2 mm (visual observation in transparent glass capillaries). From the length of the capillary and this zone width one can conclude that we obtained approximately a 500-fold concentration of the sample. The recovery was difficult to determine quantitatively because of the low protein con-

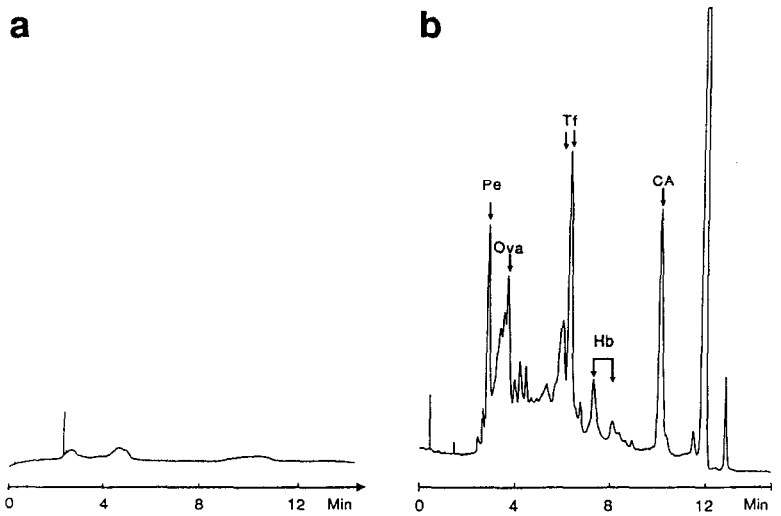

Fig. 1. High-performance capillary zone electrophoresis of model proteins. (a) Prior to concentration (concentration of each protein: 20 μg/ml), (b) following concentration toward a steep, non-buffering pH gradient and a short mobilization by displacement electrophoresis. The large peak at 12 min may correspond to a moving boundary. The width of the applied sample zone was in (a) 3–4 mm and in (b) 130 mm (= the length of the capillary). Following concentration, the zone width in (b) was about 1 mm, but became much narrower in the mobilization step.

tent. However, it should be close to 100% as judged from the fact that no residue of the strongly colored phycoerythrin was observed after completion of a parallel experiment done in a transparent glass tube.

The above buffer system is designed for acidic peptides and proteins and other ampholytes. For basic ampholytes we have used the following buffers.

I. The concentration step (isoelectric focusing): 0.05 *M* N-hydroxyethyl-piperazine propane sulfonic acid (EPPS), pH 7.5, in the left electrode vessel (anolyte) and in the capillary; 0.25 *M* EPPS, pH 11.0, in the right electrode vessel. EPPS was titrated to the desired pH with sodium hydroxide.

II. The mobilization (displacement) step: 0.05 *M* EPPS, pH 7.5, in the left electrode vessel (now catholyte) and in the capillary; 0.1 *M* Bis–Tris (titrated to pH 2.0 with HCl) in the right electrode vessel.

III. The free zone electrophoresis step: 0.05 *M* EPPS, pH 7.5, in both electrode vessels and in the capillary (the polarity the same as in step II).

3.2. Concentration of proteins by electrophoresis toward a small-pore polyacrylamide gel (step I, alternative b in ref. 1) and mobilization by displacement electrophoresis (step II, alternative a in ref. 1) followed by zone electrophoresis (step III, alternative b in ref. 1).

The gel is prepared in a glass tube with an inside diameter of about 1 mm. The pores of the gel must be so small that the solutes cannot penetrate the gel. In our studies we have used crosslinked polyacrylamide gels with the total concentration $T > 20\%$ (v/w) and the crosslinking concentration $C = 3\%$ (w/w); the parameters C and T are defined in ref. 4. Observe that the pores of a polyacrylamide gel decrease when T increases if $C \leq 3\%$ and that these C values give transparent gels, independently of the T values. For C values $> 5\%$ the pore size increases when C increases and the gels become white and non-transparent [5]. A short plug of the gel is introduced into one end of the fused-silica tubing (filled with sample solution) by pressing the tubing into the gel in the glass tube. The tubing should be drawn up slowly to avoid

detachment of the gel plug from the tubing wall. Upon electrophoresis the analytes cease to migrate for steric reasons when they come into contact with the gel plug (see Fig. 1b in ref. 1).

The capillary [140 (125) × 0.05 mm I.D.] was filled with a solution of the same proteins as were used in the experiment presented in Fig. 1. They were dissolved in the same buffer (0.01 *M* Tris–HCl, pH 8.5) at the same concentrations (20 μg/ml). This buffer was also employed as catholyte in step I (the concentration) and step III (the free zone electrophoresis). The catholyte in step II (the terminating solution in the displacement electrophoresis) was 0.03 *M* diaminopimelic acid, titrated to pH 9.2 with Tris [6]. The experiment is outlined in Fig. 2b in ref. 1.

With a polyacrylamide gel of the composition $T = 60\%$ (v/w) and $C = 3\%$ (w/w) at the anodic end of the capillary the concentration of the proteins toward the gel plug was completed in 10 min at 2000 V (anolyte: 0.01 *M* Tris–HCl, pH 8.5). The mobilization by displacement electrophoresis of the very narrow, concentrated protein zone took place at 2000 V for 1.5 min following reversal of the polarity of the electrodes (see Fig. 2b, step II, in ref. 1). This displacement electrophoresis step was introduced to avoid a broad starting zone in the subsequent zone electrophoresis, which was performed at 3000 V after replacing the diaminopimelic acid–Tris buffer in the cathode vessel by 0.01 *M* Tris–HCl, pH 8.5 (Fig. 2b).

The experiment was repeated, with the difference that the analysis was performed without concentration of the sample. The width of the starting zone was 3–4 mm. The 0.01 *M* Tris–HCl buffer (pH 8.5) was used both in the capillary and in the anode and cathode vessels. The striking difference between the electropherogram obtained (Fig. 2a) and that in Fig. 2b shows the importance of having access to a method for in-tube concentration of dilute protein solutions. For a discussion of the degree of concentration obtained, see 3.1.

An analysis similar to that in Fig. 2b was performed with a urine sample from a diabetic patient. The essential differences in the experimental conditions were that (1) the gel plug had the composition $T = 40\%$ (w/v), $C = 3\%$ (w/w), (2) the capillary was 130 (120) mm long and made of glass, and the terminator was a 0.03 *M* glycine–NaOH solution, pH 10. The urine was diluted 10-fold with 0.01 *M* Tris–HCl, pH

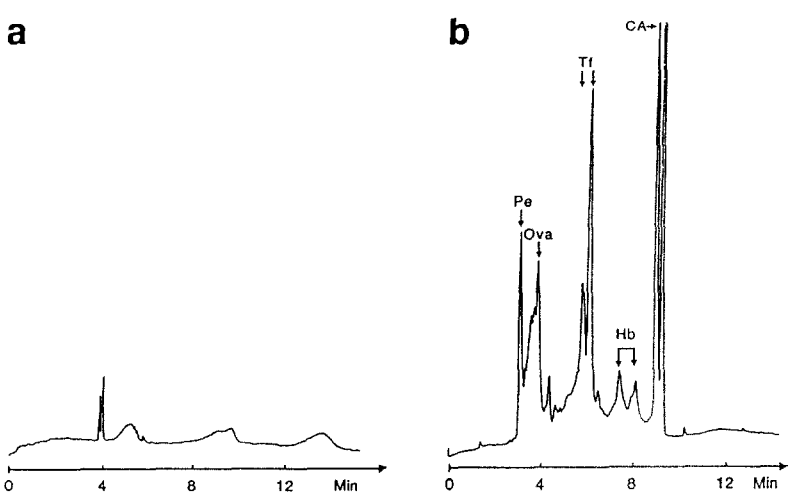

Fig. 2. High-performance capillary zone electrophoresis of model proteins. (a) Prior to concentration (concentration of each protein: 20 μg/ml), (b) following concentration toward a small-pore polyacrylamide gel and a short mobilization by displacement electrophoresis. The width of the applied sample zone was in (a) 3–4 mm and in (b) 140 mm (= the length of the capillary). Following concentration the zone width in (b) was about 0.2 mm.

8.0, before it was sucked into the capillary. The concentration required 15 min at about 1200 V, the mobilization 50 s at about 3000 V with 0.03 *M* glycine NaOH, pH 10, as terminator and the zone electrophoresis in 0.01 *M* Tris–HCl, pH 8.5, 25 min at 3000 V. The detection was done at 280 nm, where proteins have an absorption maximum, albeit with an absorption coefficient much smaller than that at 220 nm. In spite of this, the protein zones were easily detected (Fig. 3). No peaks were recorded with the non-concentrated 10-fold diluted urine sample.

3.3. Concentration of proteins and peptides by a combination of displacement electrophoresis and a hydrodynamic counterflow (step I, alternative e in ref. 1) followed by zone electrophoresis (step III, alternative c in ref. 1).

The concentration method is outlined in Fig. 2e in ref. 1.

As leading buffer (anolyte) we chose 0.015 *M* HCl, titrated to pH 8.5 with Tris, and as terminator (catholyte) 0.1 *M* glycine titrated to pH 8.5 with NaOH. The electrophoresis tube [155 (145) × 0.05 mm I.D.] was filled with the sample solution (about 20 μg/ml of each of the proteins phycoerythrin (Pe), human serum albumin (Alb), human transferrin (Tf), human hemoglobin (Hb) and carbonic anhydrase (CA)) dissolved in the leading buffer. The liquid level in the anode vessel was 2 cm higher than that in the cathode vessel. The buffer flow thus created in the capillary gave a virtually stationary boundary between the leading chloride ions and the terminating glycine ions 1–2 cm from the cathodic end of the capillary at a voltage of 500 V. The proteins gathered between these ion species in a very narrow zone. Following a 15-min concentration, the glycine–NaOH solution was replaced by the HCl–Tris buffer, and a zone electrophoretic analysis was performed at 3000 V for 18 min with no difference in the liquid levels (see Fig. 4b). The experiment was then repeated with the difference that not only the capillary was filled with the sample solution but also the anode vessel and that the duration of the concentration step was 22 min (Fig. 4c). A blank experiment was also performed (Fig. 4a), *i.e.*, a zone electrophoresis in the HCl–Tris buffer with a 5 mm wide starting zone (without concentration). A comparison between Fig. 4b and Fig. 4c shows that a continuous concentration with the sample solution in the anode vessel (in addition to the capillary) is the method of choice for very dilute samples.

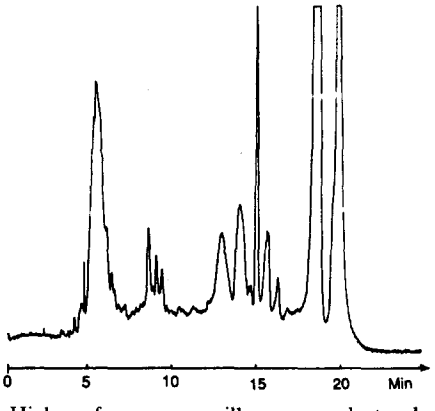

Fig. 3. High-performance capillary zone electrophoresis of urine following concentration toward a small-pore polyacrylamide gel and a short mobilization by displacement electrophoresis. The experimental conditions were similar, but not identical, to those used in the experiment presented in Fig. 2b. For details, see text.

The following two experiments illustrate that precautions should be taken in order to avoid some loss of the most rapidly and most slowly migrating proteins during the concentration procedure.

A commercial HPCE apparatus, BioFocus 3000, from Bio-Rad (Hercules, CA, USA) was employed in these experiments. Eppendorf 0.7-ml plastic vials served as electrode vessels. The leading buffer was the same as that used in the experiment presented in Fig. 4. The anode vessel contained 600 μl of this buffer. A 200-μl volume of 0.01 *M* glycine, titrated with NaOH to pH 9.0, was employed as terminator (catholyte). The resulting difference in buffer levels (1–2 cm) provided the counterflow required to give a stationary boundary between the leading chloride ions and the terminating glycinate ions in the concentration step. About 1.5 μg of each of the proteins β-lactoglobulin A (β), α-lactal-

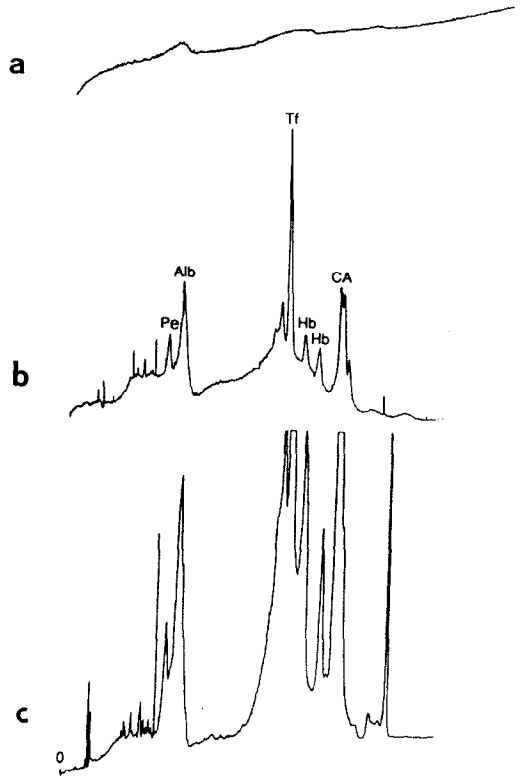

Fig. 4. High-performance capillary zone electrophoresis of model proteins. (a) Prior to concentration (concentration of each protein: 20 μg/ml). The width of the applied sample zone was 5 mm. (b) Following concentration by a combination of displacement electrophoresis and a hydrodynamic counterflow. The whole capillary was filled with the sample solution. (c) = (b) with the difference that not only the capillary was filled with sample solution but also the anode vessel.

bumin (α), and hemoglobin A (Hb A) was dissolved in the terminator diluted 5-fold with water. The capillary [250 (203) × 0.1 mm I.D. mm] was filled with this sample solution. The concentration took place at 3000 V for 12 min (see Figs. 1e and 2e in ref. 1). The distance from the cathodic end of the capillary to the stationary boundary was 1–2 cm. Prior to the final zone electrophoresis step the inlet carousel moved to a position where the vial contained 600 μl of 0.015 *M* HCl, titrated with Tris to pH 8.5. Without counterflow, the zone electrophoresis was conducted at 8000 V for 9 min. The temperature of the carousel was 15°C and that of the coolant 20°C. All operations were performed automatically and gave the electropherogram in Fig. 5a upon detection at 220 nm. Part of the slowly migrating proteins moved out of the capillary when the sample was dissolved in the non-diluted terminator, resulting in a reduced peak of hemoglobin A (not shown herein).

The run shown in Fig. 5b was performed as that presented in Fig. 5a, although the experimental conditions were as follows. Dimensions of the coated capillary: 260 (213) × 0.1 mm I.D.; leading buffer: 0.015 *M* HCl, titrated to pH 8.8 with Tris; terminating buffer: 0.1 *M* alanine, titrated to pH 8.8 with Tris. The sample (1.5 μg/ml of each protein) was dissolved in the leading buffer. Buffer level difference: 19 mm. Concentration step: 2000 V, 20 min; zone electrophoresis step: 10 000 V, 17 min. Detection wavelength: 220 nm. Temperature of the carousel: 15°C; temperature of the coolant: 20°C. Observe that the first two peaks are smaller than in Fig. 5a because the sample was dissolved in the leading, rather than the terminating buffer. This loss of protein can be avoided by diluting the leading buffer in the capillary (but not that in the anodic vessel) 10-fold.

The above automated experiment was repeated, although with basic proteins (cytochrome *c* (Cyt C), ribonuclease A (Rib A) and α-chymotrypsinogen A (α-chy)) which required other experimental conditions. Each of the proteins was dissolved in 700 μl of the leading solution (0.02 *M* ammonium acetate, titrated to pH 4.5 with acetic acid) to a concentration as low as 50 ng/ml. The main purpose of this experiment was to demonstrate that the concentration technique permits detection of proteins even at this very low concentration. The capillary [260 (213) × 0.1 mm I.D.] was filled with this sample solution. A 0.01 *M* solution of acetic acid (100 μl) was chosen as terminator (anolyte). The buffer level in the cathode vessel was 2 cm higher than that in the anode vessel. Following concentration at 2000 V for 20 min with the stationary boundary

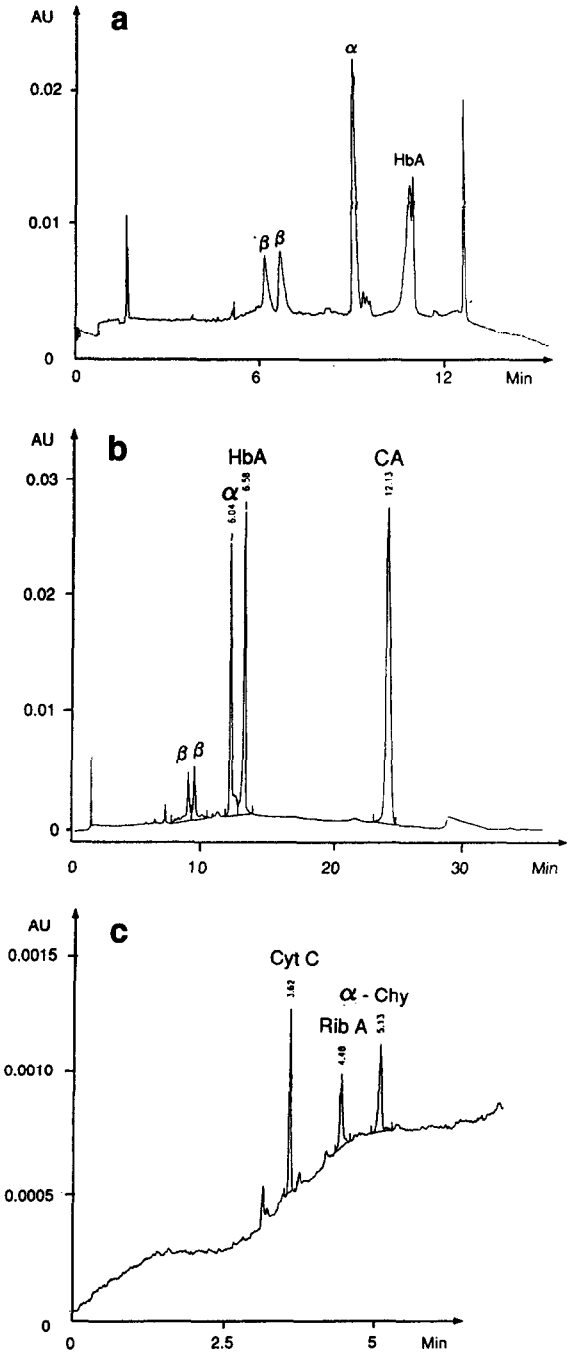

Fig. 5. Automated high-performance capillary zone electrophoresis of model proteins following concentration by a combination of displacement electrophoresis and a hydrodynamic counter flow. (a, b) acidic proteins, (c) basic proteins.

about 1–2 cm from the anodic end of the capillary the acetic acid in the anode vessel was replaced by the ammonium acetate solution. The subsequent zone electrophoresis at 10 000 V gave the electropherogram shown in Fig. 5c. The monitoring was done at 220 nm at a sensitivity of 0.002 AUFS to get relatively large peaks in spite of the extremely low concentrations of the proteins in the applied sample. Variations in the composition of the buffer and in its concentration in different sections of the capillary are detectable at this high sensitivity and account for the stepped form of the base line. In experiments where the protein concentration was lower than 50 ng/ml the adsorption of the proteins to the sample vial was significant. Several approaches to decrease this adsorption are mentioned in Discussion.

A tryptic digest of bovine albumin was concentrated in-tube and subjected to electrophoretic analysis essentially as described for the experiment presented in Fig. 4b. The electropherogram in Fig. 6 shows that the concentration method is applicable also in the concentration of peptides, *i.e.* substances of relatively low molecular weight.

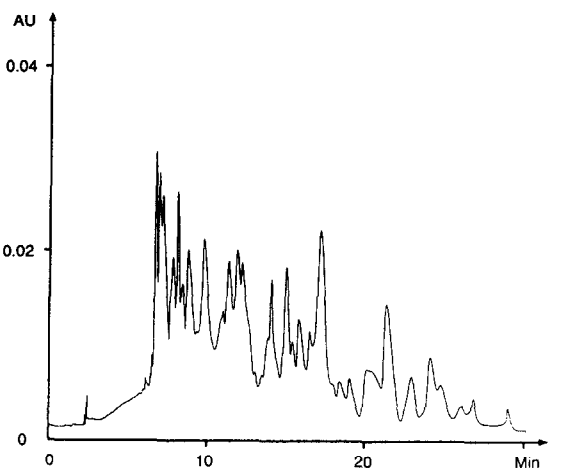

Fig. 6. Automated high-performance capillary electrophoresis of trypsin-digested bovine albumin following concentration by a combination of displacement electrophoresis and a hydrodynamic counterflow.

3.4. Concentration of proteins by electrophoresis toward a piece of dialysis tubing (= gradient in cross sectional area) (step I, alternative c in ref. 1) followed by zone electrophoresis (see Fig. 2c in ref. 1).

The fused-silica tubing [150 (135) × 0.1 mm I.D.] was coated with polyacrylamide to suppress adsorption of solutes and electroendosmosis [2].

The dialysis tubing was prepared as follows. A single hair with a diameter only slightly smaller than that of the inside diameter of the fused-silica tubing was inserted into the tubing, which was then placed in the mold shown in Fig. 7. The groove G was filled with a deaerated aqueous solution of acrylamide (276 mg/ml), N,N'-methylenebisacrylamide (24 mg/ml) and ammonium persulfate (2 mg/ml). Accordingly, the total concentration of the monomer (T) was 30% (w/v) and the crosslinking concentration (C) was 8% (w/w). Upon heating at 60°C a gel formed which was transparent and had very small pores. The single hair was then cautiously removed.

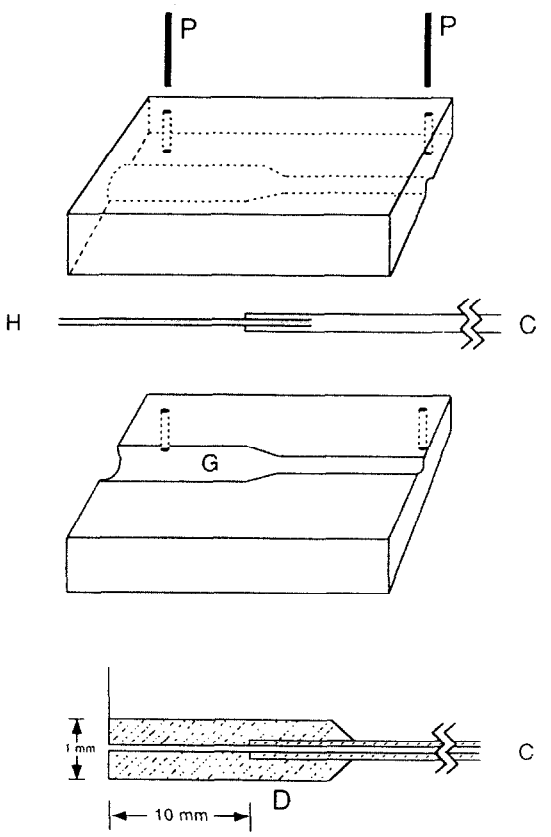

Fig. 7. Preparation of dialysis tubing (D) attached to the capillary (C). G, groove; H, single hair; P, guide pins.

The silica tubing was filled with 0.1 *M* Tris–HCl, pH 8.5 containing the sample (15 μg/ml of each of the proteins β-lactoglobulin A, β-lactoglobulin B and α-lactalbumin). The cathode vessel (at the left) was filled with the same buffer and the anode vessel with 0.01 *M* Tris–HCl, pH 8.5, *i.e.*, a buffer of low conductivity to create a sharp starting zone for the subsequent electrophoretic analysis. Upon electrophoresis for 16 min at 2000 V all proteins became concentrated into a sharp band in the dialysis tubing (see Fig. 1c in ref. 1). The concentrated zone was moved into the capillary by a hydrodynamic flow to a position 3 mm from the end of the capillary (*cf.* Fig. 2c, step II in ref. 1). A free zone electrophoresis was then performed at 3000 V (cf. Fig. 2c, step III in ref. 1) following exchange of the 0.01 *M* Tris buffer in the cathode vessel with the 0.1 *M* buffer to avoid the pH and concentration changes which may occur in dilute buffers by electrolysis (Fig. 8b). This step can be omitted when the volume of the cathode vessel is relatively large, particularly when only a few analy-

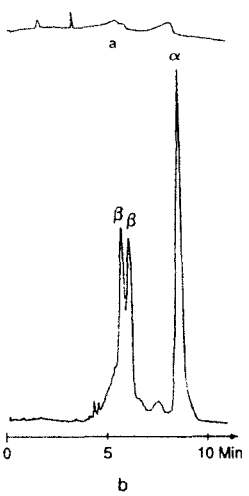

Fig. 8. Automated high-performance capillary zone electrophoresis of model proteins following concentration by electrophoresis toward a dialysis tubing.

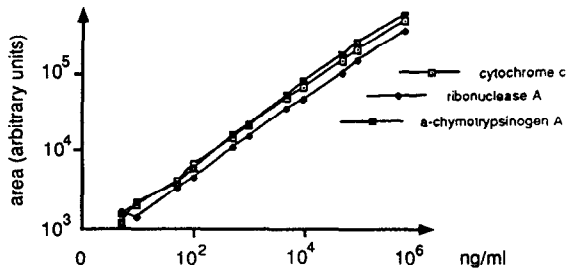

Fig. 9. A plot of peak area against sample concentration. Data from experiments similar to that presented in Fig. 5c.

ses are performed. The monitoring was done at 220 nm. A blank experiment was also performed (Fig. 8a), i.e. a zone electrophoresis without concentration of the sample.

3.5. The influence of sample concentration on peak area

The areas of the three main peaks in the electropherogram shown in Fig. 5c and similar electropherograms for other sample concentrations were used for the plot in Fig. 9. Obviously, a linear relationship between peak area and protein concentration was obtained over the whole concentration area 5 ng/ml–1 mg/ml.

4. Discussion

To better understand the design of the experiments described herein consult the theoretical treatment in ref. 1. Readers who do not study the theory in detail may find it difficult to tailor-make buffers for their own experiments. Therefore we have compiled in Table 1 several buffer systems from which one can easily select the appropriate one for any particular protein sample.

In this investigation we have generated a hydrodynamic counter flow in the capillary with the aid of different buffer levels in the two electrode vessels, although also other techniques, including the electroendosmotic pump [6], can be used to create a small pressure difference. Colored proteins and transparent glass tubes were used to establish experimentally the correct combination of flow rate and voltage to obtain a stationary boundary. Although one can, with the aid of an optical sensor, automatically and continuously adjust one of these parameters to a value that will keep the boundary stationary, we must point out that a moderate change in the flow rate affects the position of the boundary very little [1].

When the sample is dissolved in the leading buffer for concentration by displacement electrophoresis and a counterflow there is a risk that some part of the fastest migrating protein may migrate out of the capillary (Fig. 5b). To avoid this the concentration of the leading buffer in the electrode vessel should be higher than in the sample solution (see Table 1) or the sample should be dissolved in the terminator (Fig. 5a). Analogously, some part of the slowest protein can be lost when the sample is dissolved in the terminator. The loss can be eliminated if the terminator in the electrode vessel has a lower concentration than has the terminator in the sample solution.

As mentioned in ref. 1, high salt concentrations in the sample may eliminate the possibility to concentrate a sample by displacement electrophoresis. Different approaches to circumvent the problem were discussed therein.

The fact that the measuring points in the plot in Fig. 9 do fall on a straight line and are not scattered around it indicate that the concentration method described is highly reproducible.

Most solutes adsorb to some extent to most surfaces they come in contact with. This surface adsorption is very disturbing when the solute concentrations are extremely low, particularly when the sample consists of proteins with their tendency for multi-point attachment. It may very well happen that all of the sample molecules become adsorbed to pipette tips, the vial, the capillary, etc. and thereby escape detection. This surface adsorption combined with contamination from the surrounding milieu might be a greater hindrance to analysis of minute quantities of material than is the performance of the detectors. We have found that glass vials coated with methyl cellulose [7] exhibit less adsorption than

Table 1
Appropriate buffers for different concentration methods

Concentration method	Buffer system	I. Concentration	II. Mobilization	III. Free Zone Electrophoresis
Steep pH gradient (see Fig. 2a in ref. 1)	1 (P^-)	(+): 0.5 *M* Tris–HCl (pH 2.5) (S): 0.01 *M* Tris–HCl (pH 8.5) (−): 0.01 *M* Tris–HCl (pH 8.5)	(+): 0.01 *M* Tris–HCl (pH 8.5) (−): 0.01 *M* glycine–NaOH (pH 10.0) or 0.1 *M* NaOH	(+): 0.01 *M* Tris–HCl (pH 8.5) (−): 0.01 *M* Tris–HCl (pH 8.5)
	2 (P^+)	(+): 0.05 *M* EPPS–NaOH (pH 7.5) (S): 0.05 *M* EPPS–NaOH (pH 7.5) (−): 0.25 *M* EPPS–NaOH (pH 11.0)	(+): 0.1 *M* Bis–Tris–HCl (pH 2.0) or 1 *M* HCl (−): 0.05 EPPS–NaOH (pH 7.5)	(+): 0.05 *M* EPPS–NaOH (pH 7.5) (−): 0.05 *M* EPPS–NaOH (pH 7.5)
Small-pore gel ($T > 20\%$, $C = 3\%$) (see Fig. 2b in ref. 1)	(P^-)	(+): 0.01 *M* Tris–HCl (pH 8.5) or 0.03 *M* DAPA–Tris (pH 9.2) (S): 0.01 *M* Tris–HCl (pH 8.5) (−): 0.01 *M* Tris–HCl (pH 8.5)	(+): 0.01 *M* Tris–HCl (pH 8.5) (−): 0.03 *M* DAPA–Tris (pH 9.2) or 0.01 *M* glycine–NaOH (pH 10.0)	(+): 0.01 *M* Tris–HCl (pH 8.5) (−): 0.01 *M* Tris–HCl (pH 8.5)
Dialysis tubing (gradient in *q*) (see Fig. 2c in ref. 1)	P^-	(+): 0.01 *M* Tris–HCl (pH 8.5) (S): 0.1 *M* Tris–HCl (pH 8.5) (−): 0.1 *M* Tris–HCl (pH 8.5)		(+): 0.1 *M* Tris–HCl (pH 8.5) (−): 0.1 *M* Tris–HCl (pH 8.5)
Displacement electrophoresis + hydrodynamic counterflow (see Fig. 2e in ref. 1)	1 (P^-)	(+): 0.015 *M* HCl–Tris (pH 8.5) (S): 0.015 *M* HCl–Tris (pH 8.5) or 0.0015 *M* HCl–Tris (pH 8.5) or 0.002 *M* glycine–NaOH (pH 8.5) (−): 0.01 *M* glycine–NaOH (pH 8.5)		(+): 0.015 *M* HCl–Tris (pH 8.5) (−): 0.015 *M* HCl–Tris (pH 8.5)
	2 (P^-)	(+): 0.015 *M* HCl–Tris (pH 8.8) (S): 0.015 *M* HCl–Tris (pH 8.8) (−): 0.01 *M* alanine–Tris (pH 8.8)		(+): 0.015 *M* HCl–Tris (pH 8.8) (−): 0.015 *M* HCl–Tris (pH 8.8)
	3 (P^+)	(+): 0.01 *M* HAc (S): 0.02 *M* NH_4Ac–HAc (pH 4.5) (−): 0.02 *M* NH_4Ac–HAc (pH 4.5)		(+): 0.02 NH_4Ac–HAc (pH 4.5) (−): 0.02 NH_4Ac–HAc (pH 4.5)

(+), (S) and (−) refer to anolyte, sample buffer and catholyte, respectively. P^- and P^+ stand for buffer systems designed for acidic and basic solutes, respectively. EPPS = N-2-hydroxyethylpiperazine propane sulfonic acid. DAPA = diaminopimelic acid. The molarity of the buffers refers to the first mentioned constituent.

do plastic vials. Another alternative is to pretreat all contact surfaces with an aliquot of the sample prior to the analysis which, however, requires access to relatively large amounts of the sample. A variant of this coating method is to saturate all surfaces with solutes having a structure similar to that of the sample molecules and which are available in larger quantities.

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References

[1] S. Hjertén, J.-L. Liao and R. Zhang, *J. Chromatogr. A*, 676 (1994) 409.
[2] S. Hjertén, *J. Chromatogr.*, 347 (1985) 191–198.
[3] H. Kylin, *Z. Physiol. Chem.*, 69 (1910) 169–239.
[4] S. Hjertén, *Arch. Biochem. Biophys. Suppl.*, 1 (1962) 147–151.
[5] S. Hjertén, S. Jerstedt and A. Tiselius, *Anal. Biochem.*, 11 (1965) 219–223.
[6] S. Hjertén, *Electrophoresis*, 11 (1990) 665–690.
[7] S. Hjertén and K. Kubo, *Electrophoresis*, 14 (1993) 390–395.