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On-column transient and coupled column isotachophoretic preconcentration of protein samples in capillary zone electrophoresis

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ABSTRACT

Two strategies for the isotachophoretic preconcentration of samples were evaluated using a standard protein mixture as an example. In the first, on-column transient isotachophoretic migration permits the injection of relatively large volumes of sample into a commercial instrument. Depending on the composition, 30–50% of the column length can be filled with the sample while maintaining good resolution of the sample components. When proper electrolyte compositions are selected, the conventional single-column instrument can be used for an isotachophoretic sample preconcentration of 50-fold or more without any modification. In the second strategy, a coupled-column system was examined which, in principle, provides a higher degree of freedom in the selection of capillary zone electrophoretic running conditions and the possibility of injection of higher sample volumes. The gain in detection level is at least a factor of 1000 in the coupled-column arrangement; however, the instrumentation is more complicated.

INTRODUCTION

A main limitation of the current methodology for capillary electrophoresis is the relatively low sample detection level, *i.e.*, the minimum concentration of an analyte in a sample that can be detected. Especially when UV detection is used, the low detection level may exclude the use of capillary zone electrophoresis (CZE) for analyses of sample components in low concentrations. Several strategies to improve the detection limit in CZE have been described, including injection from low-conductivity sample matrices where the diluted sample components are preconcentrated during the stacking across the stationary concentration boundary between the sample and the background electrolyte [1–3], the use of an on-line packed precolumn [4], the variation of pH and the use of sample-induced stacking [5–7], and the use of on-line coupled column isotachophoresis (ITP)–CZE [8–13].

The use of ITP preconcentration would appear to be very promising especially when the sample is not dissolved in pure water or diluted buffer but also contains other ions which increase its conductivity. Moreover, at very dilute buffer concentrations, biopolymers may not be stable and such low-conductivity media also generate excess heat within the injected sample which can degrade thermally unstable species [14]. During the ITP step, the concentrations of all ionic components of the sample are rearranged according to the concentration and electrophoretic mobility of the leading electrolyte [15], *i.e.*, the highly concentrated components are diluted and trace components concentrated. Generally, the resulting concentration of the preconcentrated zone is in the millimolar range. From the instrumental point of view, on-line ITP preconcentration can be performed either on-column or in a coupled-column arrangement. Because in the on-column proce-

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dure the migration mode gradually changes from isotachophoresis to zone electrophoresis, we suggest the term on-column transient ITP preconcentration.

There are several basic arrangements of electrolytes for both on-column transient and coupled-column ITP–CZE for the preconcentration of species. The classification can be based on the relationship between the effective electrophoretic mobilities of the co-ion of the background electrolyte C, the sample constituents S, the leading ion L and the terminating ion T, where the co-ion represents the constituents of the background electrolyte with the same charge sign as the species to be separated.

In this paper, the feasibility of the ITP preconcentration method is demonstrated with protein samples; however, this approach is general and can be applied to the analysis of most ions. The basic instrumental approaches and electrolyte arrangements are described in the following section.

CLASSIFICATION OF ITP PRECONCENTRATION METHODS

On-column transient arrangement

In this method, the analysis is conducted such that both ITP preconcentration and CZE separation proceed in the same capillary. Two basic electrolyte systems can be envisioned (see Fig. 1), as follows.

Method A1. If the background electrolyte (BGE) is selected with the co-ion having a higher effective mobility than the sample ions, *i.e.*, $u_C > u_S$, ITP migration is achieved by using a suitable terminating electrolyte behind the sample zone ($u_S > u_T$) (see Fig. 1A). The sample is injected by pressure or gravity at time t_0 and, after adding the terminating electrolyte, ITP stacking of the sample takes place during time t_1. After on-column ITP preconcentration, the terminating electrolyte in the electrode vessel is replaced with the background electrolyte, and separation in the zone electrophoretic mode during time t_2, including the migration of a zone of terminating ions behind the sample zones, results.

Method A2. If, on the other hand, the capillary contains a background electrolyte with a lower effective mobility co-ion C, *i.e.*, $u_S > u_C$, the sample itself must be supplemented by a leading ion (such as NH_4^+, K^+ or Na^+ for cationic solutes) to maintain transient ITP migration (Fig. 1B). The co-ion, C, of the BGE then serves as a terminating ion. This option was recently confirmed by mathematical

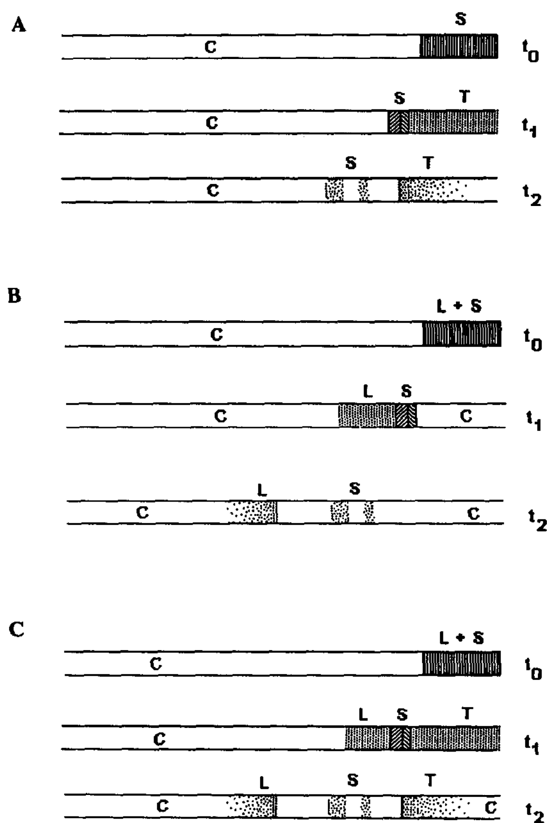

Fig. 1. Illustration of transient ITP preconcentration in a single-column arrangement. (A) The co-ion of the background electrolyte C has a mobility higher than those of the sample components S and can thus serve as the leading ion. The analysis starts with the sample injection at time t_0. When a suitable terminating electrolyte T is used, the sample can be isotachophoretically preconcentrated during the time t_1. Next, the terminating electrolyte is replaced with the background electrolyte C, and separation proceeds in the zone electrophoretic mode from time t_2. (B) When the mobility of the co-ion of the background electrolyte C is lower than that of the sample ions, a suitable leading ion L must be added to the sample. The separation starts in the isotachophoretic mode during t_1. As the concentration of the leading zone gradually decreases owing to its electromigration dispersion [14], the migration mode converts into zone electrophoresis. After the transient ITP preconcentration, the bands continue to move in a zone electrophoretic mode, t_2. (C) Both previous modes can be combined and this situation will always occur when method A is applied to a sample containing salts of highly mobile ions.

modeling [16,17]. Real samples often contain such high-mobility ions in sufficient amounts to permit ITP preconcentration. Alternatively, appropriate amounts of salt can be added, and this may additionally aid in the stabilization of protein sample components. Of course, both basic arrangements can be combined, as depicted in Fig. 1C, and this situation will always occur when method 1A is applied to a sample containing salts of highly mobile ions.

Method B: coupled column arrangement

In this instance the sample migrates isotachophoretically in the preconcentration capillary between the leading L and terminating T ions. The analytical capillary is connected on-line at the end of the preconcentration capillary, as described previously [11]. The overall instrument is shown in Fig. 2 and is described in the Experimental section. The expanded view of the interface of the coupled capillary columns shown schematically in Fig. 2 depicts the moment when the ITP preseparated zones arrive at the conductivity detector located in front of the entrance to the analytical capillary. This latter capillary is filled with a background electrolyte whose actual composition (concentration, pH) influences the effective electrophoretic mobilities of both the separating species and the terminating ion. After the sample zones have entered the analytical capillary, the separation continues in either the ITP or

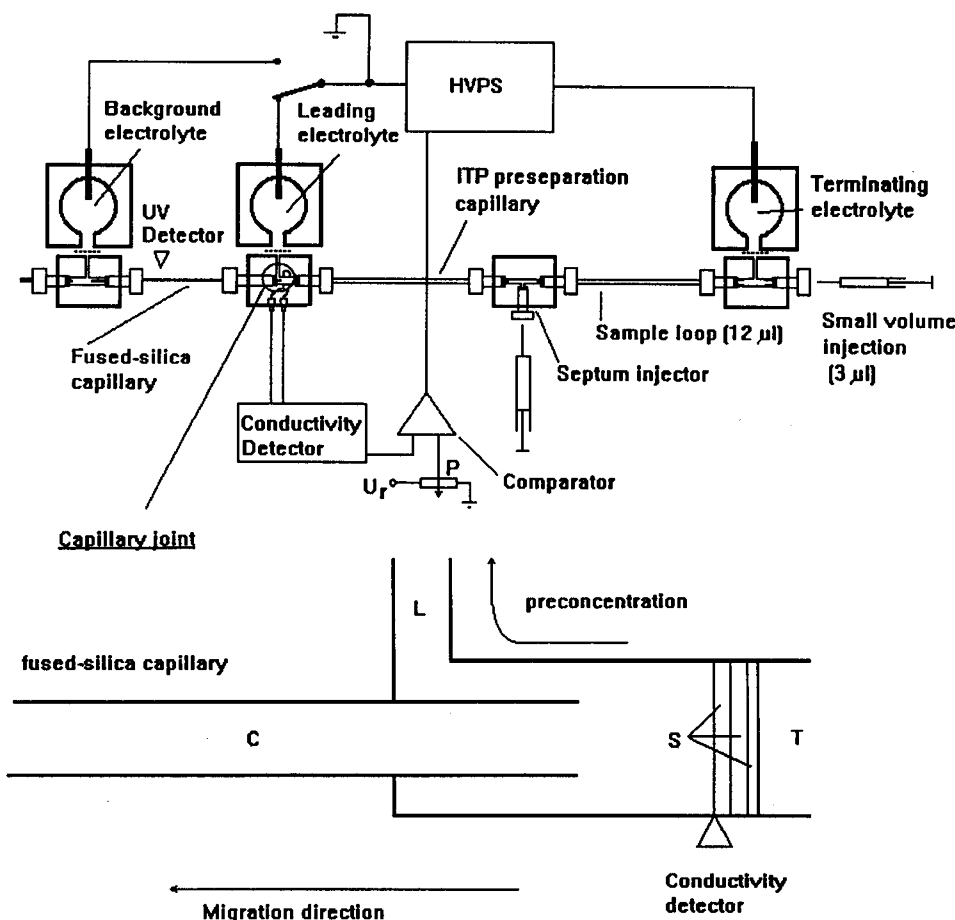

Fig. 2. Schematic diagram of the coupled column system with an expanded view of the capillary column connection. U_r = Reference voltage; P = potentiometer; C = co-ion of the background electrolyte; L = leading ion; S = sample bands; T = terminating electrolyte. Specific components are detailed in the Experimental section.

TABLE I

RELATIONSHIP BETWEEN THE MOBILITIES OF IONS IN THE COUPLED-COLUMN SYSTEM WITH ITP PRECONCENTRATION (METHOD 2) AND RESULTING MODES OF MIGRATION IN THE ANALYTICAL CAPILLARY

Co-ion C		Sample S		Terminator T	Mode of migration
u_C	>	u_S	>	u_T	ITP
u_C	>	u_S	<	u_T	CZE in T
u_C	<	u_S	<	u_T	CZE in C
u_C	<	u_S	>	u_T	CZE in C

CZE mode. The most important cases are depicted in the Table I. In addition, the terminating electrolyte in the preconcentration capillary can be replaced after ITP and migration continued in the analytical capillary without restriction on the electrophoretic mobilities [11].

EXPERIMENTAL

Instrumentation

A P/ACE 2100 capillary electrophoresis instrument controlled by System Gold software (Beckman, Fullerton, CA, USA) was used for experiments in the on-column transient ITP arrangement. Fused-silica capillaries, 27 cm (20 cm to the detector) × 75 μm I.D. and 47 cm (40 cm to detector) × 75 μm I.D., with the inner wall coated with linear polyacrylamide [18], were used as analytical separation columns.

For the ITP preconcentration approach of method B, a laboratory-made coupled column system, shown schematically in Fig. 2, was used. The ITP system consisted of 20 cm × 0.4 mm I.D. PTFE tubing as a preconcentration capillary connected to a 39 cm (30 cm to detector) × 75 μm fused-silica analytical capillary with the inside wall coated with linear polyacrylamide. All connection blocks and electrode chambers were made from Plexiglas. The electrode chambers were separated from the connection blocks by a semipermeable Cellophane membrane in order to prevent any liquid flow. As the apparatus was designed for the future coupling to a mass spectrometer, the capillaries were opened at both ends. When necessary, the system can be closed at both the injection and detector ends. A laboratory-made conductivity detector [19], equipped with a conductivity detection cell [20] molded from an acrylic resin (Castolite-AP; Castolite, Woodstock, IL, USA), was used to identify the front of the ITP migrating zone and to control the high-voltage power supply. The output voltage of the conductivity detector (proportional to the resistance of the isotachophoretic zones) was compared with a reference voltage U_r by means of an operational amplifier connected as an electronic comparator, and the resulting signal was used for the remote control of the high-voltage power supply. The actual level of the reference voltage could be set by a potentiometer P. Thus, the high-voltage power supply was automatically turned off exactly at the moment of the arrival of a selected ITP zone to the detection cell. At this point the high driving current (85 μA) used to speed up the ITP step was decreased to 12 μA, and the grounded electrode of the high-voltage power supply was connected to the electrode vessel close to the UV detector. The separation then proceeded in the fused-silica analytical capillary. A similar system was described for use in column switching isotachophoresis [21].

The sample was injected into a variable-volume sample loop. In the present experiments, the loop was formed by a piece of PTFE capillary (10 cm × 0.4 mm I.D.) which corresponds to a sample volume of *ca.* 12 μl. A Spectra 100 spectrophotometer (Spectra Physics, San Jose, CA, USA) was used for UV detection at 214 nm, and the driving current was supplied by a Series EH high-voltage power supply (Glassman High Voltage, Whitehouse Station, NJ, USA).

Chemicals

All chemicals used for the preparation of running buffers were of analytical-reagent grade and were supplied by Sigma (St. Louis, MO, USA). Lysozyme (chicken egg white), cytochrome *c* (bovine heart), ribonuclease A (bovine pancreas), trypsin

(bovine pancreas) and trypsinogen (bovine pancreas), also supplied by Sigma, were used as received. Doubly distilled water was employed for the preparation of all solutions. The running buffers were prepared by dissolving the appropriate amount of triethylamine or ε-aminocaproic acid in distilled water with the pH being set by the addition of the glacial acetic acid. When refrigerated, the electrolytes could be used for several weeks.

RESULTS AND DISCUSSION

Method A: on-column transient ITP preconcentration

As described in the introduction, samples dissolved in water can be effectively preconcentrated during migration across the stationary concentration boundary between the sample and background electrolyte [1,2]. In such cases no sample pretreatment is necessary and, owing to the initial sharpening of the sample zone, relatively high sample volumes can be injected. An example of such a separation is shown in Fig. 3, where 38 nl of a model mixture of basic proteins dissolved in water were electrophoresed in capillary free zone electrophoresis. However, as the conductivity of the sample plug is much lower than that of the background electrolyte, the initial voltage distribution along the capillary will not be uniform but will be mainly spread over the sample plug. This can result in excessive Joule heating of the injected sample and, as was shown recently, degradation of thermally labile sample components may occur.

When the above sample was diluted fourfold with the background electrolyte, the focusing effect was lost, and neither pressure nor electrokinetic injection permitted satisfactory separation (see Fig. 4). This situation always occurs when the sample is not dissolved in distilled water ions but also contains other or dilute electrolyte in sufficient amount. In these realistic cases, ITP may represent an optimum on-line preconcentration technique.

In the above example, triethylamine, the co-ion

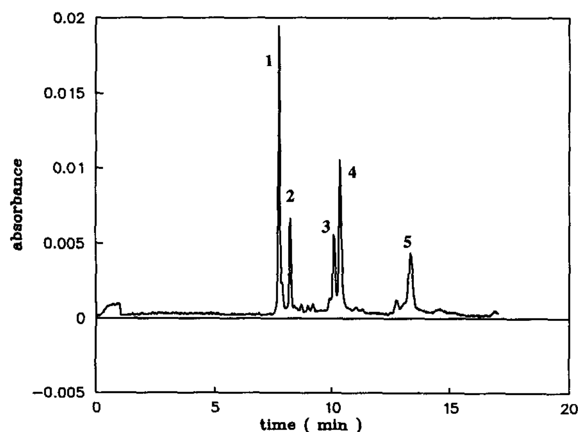

Fig. 3. CZE separation of proteins in the single capillary column system. The sample was dissolved in water. Background electrolyte 0.02 *M* triethylamine–acetic acid (pH = 4.4). Injection: pressure 3 s, 38 nl. The estimate of this volume is based on the measured time of the sample flow from the injection to detection end of the capillary when applying a constant sampling pressure difference (Δp = 50 kPa). Capillary: 27 cm (20 cm to detector) × 75 μm I.D. Current: 22 μA, 8.2 kV; UV detection at 214 nm. Concentration of the sample: 1 = lysozyme, 72 μg/ml ($5 \cdot 10^{-6}$ *M*); 2 = cytochrome *c*, 40 μg/ml ($3.3 \cdot 10^{-6}$ *M*); 3 = trypsin, 56 μg/ml ($2.3 \cdot 10^{-6}$ *M*; 4 = ribonuclease A, 54 μg/ml ($4 \cdot 10^{-6}$ *M*); 5 = α-chymotrypsinogen A, 56 μg/ml ($2.4 \cdot 10^{-6}$ *M*).

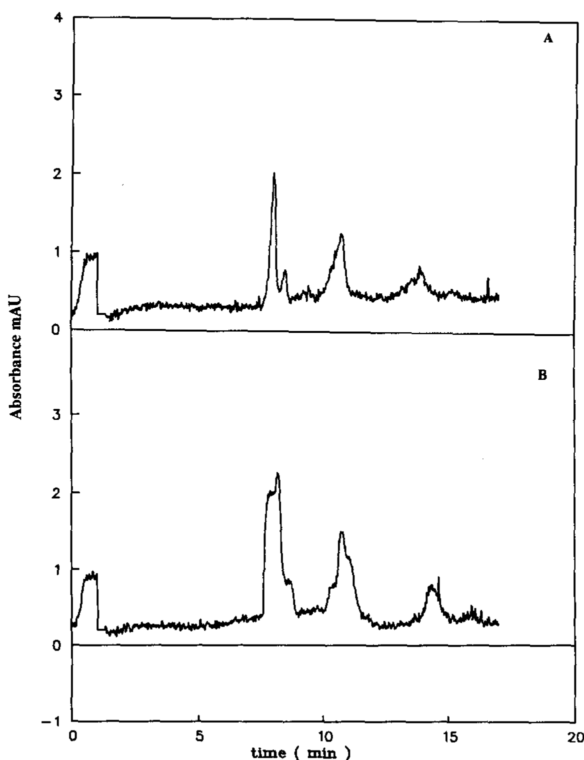

Fig. 4. CZE separation of the protein mixture used in Fig. 3 diluted fourfold with the running buffer. Injection: (A) pressure; (B) electrokinetic, 8 kV, 20 s. Other conditions as in Fig. 3.

in the background electrolyte, had an electrophoretic mobility of $33 \cdot 10^{-5}$ $cm^2/V \cdot s$ [22]. Measured mobilities of separated proteins were in the range $18 \cdot 10^{-5}$–$25 \cdot 10^{-5}$ $cm^2/V \cdot s$. Hence in this instance triethylamine can act as a leading ion for ITP migration. At the same time, for stable ITP migration, a terminating ion must be selected with an effective electrophoretic mobility lower than that of any sample component. When the counter ion of the leading electrolyte is a weak acid, the migrating front of hydrogen ions can be always used as a universal terminator for the ITP separation of cations [15]. In such a case, the extremely high electrophoretic mobility of H^+ ions ($360 \cdot 10^{-5}$ $cm^2/V \cdot s$) is slowed by a recombination reaction with the counter ion, and therefore a solution of any weak acid can be used as the terminating electrolyte. The mobility of terminating H^+ ions can be influenced by the concentration of the counter ion, its pK value and the pH of the leading electrolyte.

An example of the ITP separation of protein zones with UV detection is shown in Fig. 5. Here the proteins from an identical sample as in Fig. 4 migrate between the leading electrolyte (triethylamine) and the H^+ front forming the terminator. As all the proteins are focused into a very narrow zone, they could not be resolved by ITP. However,

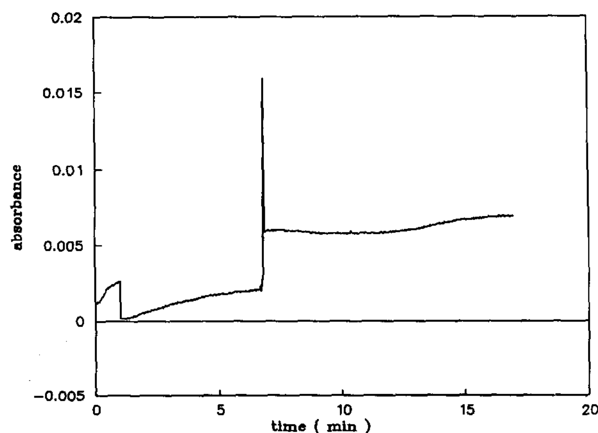

Fig. 5. ITP migration of the protein mixture diluted with the background electrolyte. Leading electrolyte, 0.02 M triethylamine–acetic acid (pH 4.4); terminating electrolyte, 0.01 M acetic acid. Injection: pressure, 30 s, 380 nl, i.e., 42% of the volume of the separation capillary (injection–detection). Other conditions as in Fig. 3.

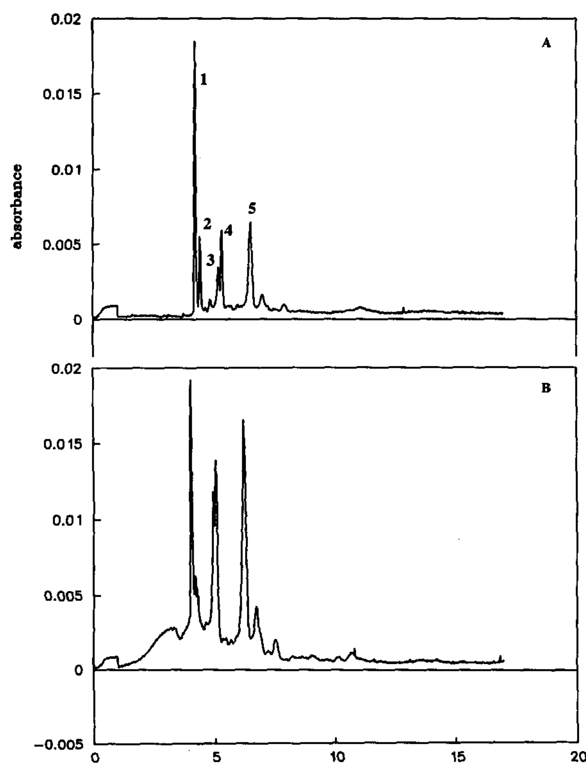

Fig. 6. CZE separation with transient ITP sample preconcentration in the column. After 90 s of ITP preconcentration in the electrolyte system from Fig. 5, the terminating electrolyte (acetic acid) was replaced with the leading electrolyte, serving now as the background electrolyte for CZE migration. Injected volume: (A) 127 nl; (B) 380 nl. Other conditions as in Fig. 3.

such a narrow zone represents an ideal starting point for the CZE analysis.

The use of an on-column transient ITP sample preconcentration step with CZE separation is shown in Fig. 6. In this example, the starting conditions were the same as in Fig. 5; however, after 90 s of the ITP migration, the terminating electrolyte in the electrode vessel was automatically replaced by the background electrolyte, and the separation was continued in the zone electrophoretic mode (see Fig. 1A). In Fig. 6A, the sample volume injected was 127 nl, *i.e.* 15% (3 cm) of the capillary was filled with the sample, and good resolution of protein zones was obtained. The separation is slightly compressed relative to that shown in Fig. 3 because a shorter length of the capillary was available for CZE, following the ITP step. When the injected

sample occupied 42% of the length of the capillary, as shown in Fig. 6B, the migration distance remaining after preconcentration was too short to complete the separation.

In order to increase the injection volume beyond that in Fig. 6 without a decrease in resolution, it is necessary to increase column length. This is demonstrated in Fig. 7 where the effective capillary length (length from injection to detection point) was doubled. The volume of the sample injected is now 1 μl, which represents 57% of the total effective volume of the capillary; however, the remaining migration distance is still sufficient for good resolution of protein zones. Although the migration distance left for the CZE separation after the transient ITP preconcentration was shorter than that without the ITP step, the resolution was still better as the proteins were focused into a very sharp zone during the ITP step. The reproducibilities of migration times when the separation column was thermostated at room temperature was better than 0.5% (relative standard deviation).

It is interesting to compare Figs. 7 with Fig. 3, where a typical CZE analysis was performed using a

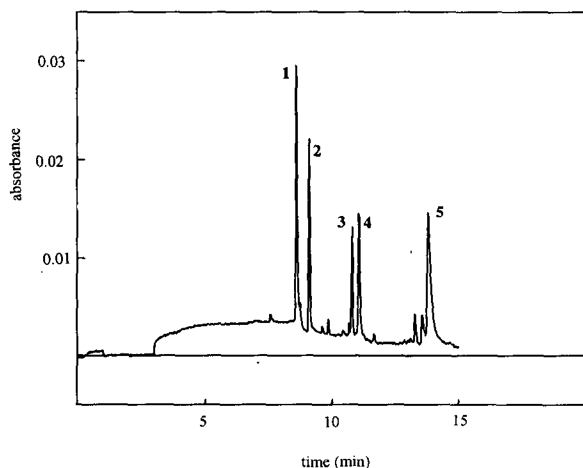

Fig. 7. CZE separation with transient ITP preconcentration in the column. Leading electrolyte, 0.02 *M* triethylamine–acetic acid (pH 4.4); terminating electrolyte, 0.01 *M* acetic acid. Capillary: 47 cm (40 cm to detector) × 75 μm. Concentration of the sample: 1 = lysozyme, 13.5 μg/ml ($1 \cdot 10^{-6}$ *M*); 2 = cytochrome *c*, 15 μg/ml ($1.2 \cdot 10^{-6}$ *M*); 3 = trypsin, 7 μg/ml ($3 \cdot 10^{-7}$ *M*); 4 = ribonuclease A, 7 μg/ml ($5.2 \cdot 10^{-7}$ *M*); 5 = α-chymotrypsinogen A, 7 μg/ml ($3 \cdot 10^{-7}$ *M*); dissolved in the leading electrolyte. Injection: 1 μl (57% of the effective length of the separation capillary). Current: 22 μA, 20 kV.

dilute buffer sample matrix. The injection volume in Fig. 3 was 38 nl, which is relatively high for this type of injection. As 1 μl was used in Fig. 7, the increase is 30-fold in volume with on-column ITP preconcentration. Further, the average signal-to-noise ratio for peaks 3–5 in Fig. 7 is *ca.* 30, which suggests that the concentration of proteins (*ca.* $3 \cdot 10^{-7}$ *M*) can in principle be further reduced by a factor of 10. Also, it can be noted that the separation time, *i.e.*, the time from the point at which the leading electrolyte enters the injection side of the capillary) for the longer column in Fig. 7 is similar to that in Fig. 3. The additional ITP focusing time of 2.5 min does not significantly increase this analysis time. This result means that in principle even higher volumes of the sample can be injected using longer capillaries with good resolution.

As has already been noted, if the co-ion of the background electrolyte posseses a lower electrophoretic mobility than the sample components, the co-ion can then serve as a terminating electrolyte during the transient ITP separation with leading electrolyte ions added to the sample itself. Fig. 8A shows a separation of the protein mixture in the background electrolyte containing ε-aminocaproic acid as the co-ion, with the effective mobility at this pH of *ca.* $15 \cdot 10^{-5}$ $cm^2/V \cdot s$ [22]. As the sample was dissolved in the background electrolyte, no sample preconcentration is evident. When the sample was dissolved in ammonium acetate, ammonium served as a leading ion (effective mobility *ca.* $79 \cdot 10^{-5}$ $cm^2/V \cdot s$) during the transient ITP migration, and the separation dramatically improved, as shown in Fig. 8B.

From the above examples, it is clear that on-column transient ITP migration can greatly improve concentration detection limits in the typical column system. The extent of enhancement is limited mainly by the volume of the sample which can be injected into the separation capillary. Depending on the length of the capillary, up to several microliters can in principle be injected with corresponding concentration detection limits in the nanomolar region.

Method B: coupled-column ITP preconcentration

The sample concentration detection level can be further increased with the aid of the coupled-column system where sample volumes in the 10-μl range or higher can effectively be preconcentrated

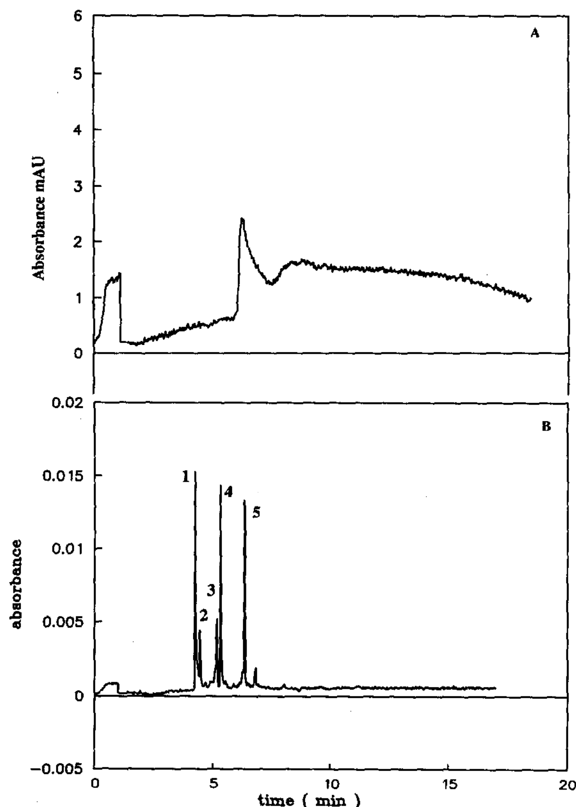

Fig. 8. CZE separation of the protein mixture in the background electrolyte with the co-ion possessing a low electrophoretic mobility. Background electrolyte: 0.02 M ε-aminocaproic acid–acetic acid (pH 4.4). (A) Without ITP preconcentration. Pressure injection, 10 s, 127 nl. Current: 22 μA, 13.9 kV. Sample: lysozyme, 22 μg/ml ($1.6 \cdot 10^{-6}$ M); cytochrome c, 12.5 μg/ml ($1 \cdot 10^{-6}$ M); trypsin, 16.7 μg/ml ($0.7 \cdot 10^{-6}$ M); ribonuclease A, 16.7 μg/ml ($1.2 \cdot 10^{-6}$ M); α-chymotrypsinogen A, 16.7 μg/ml ($0.7 \cdot 10^{-6}$ M); dissolved in 0.0075 M ε-aminocaproic acid–acetic acid (pH 4.4). (B) With on-column transient ITP sample preconcentration. Conditions as in Fig. 6A except that sample was dissolved in 0.003 M ammonium acetate. Ammonium served as the leading ion in the transient ITP migration.

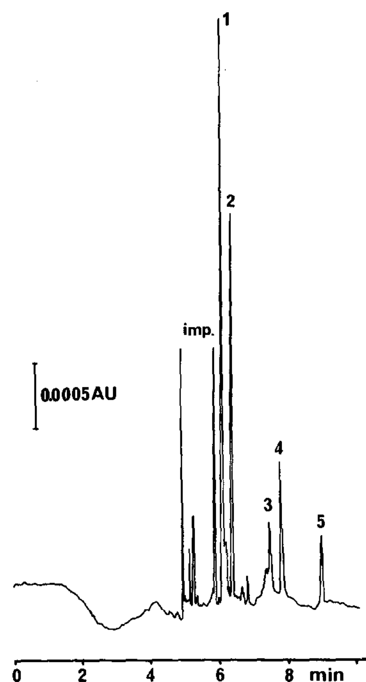

Fig. 9. CZE, separation of the protein mixture with ITP preconcentration in the coupled column apparatus. Electrolytes: (A) preseparation capillary, L = 0.01 M ammonium acetate–acetic acid (pH 4.8) containing 1% Triton X-100, T = 0.02 M ε-aminocaproic acid–acetic acid (pH 4.4); (B) analytical capillary, 0.02 M ε-aminocaproic acid–acetic acid (pH 4.4). Sample: 1 = lysozyme, 0.4 μg/ml ($2.9 \cdot 10^{-8}$ M); 2 = cytochrome c, 0.5 μg/ml ($4 \cdot 10^{-8}$ M); 3 = trypsin, 1 μg/ml ($4.2 \cdot 10^{-8}$ M); 4 = ribonuclease A, 0.6 μg/ml ($4.4 \cdot 10^{-8}$ M); 5 = α-chymotrypsinogen A, 1.0 μg/ml ($4.2 \cdot 10^{-8}$ M); dissolved in BGE. Volume: 12 μl. Current: ITP, 85 μA, 3–10 kV; CZE, 12 μA, 15 kV. Imp. = impurities accumulated from the terminating electrolyte during the ITP step.

in the ITP step. Moreover, the bulk amounts of ions present in the sample can be preseparated and, when desired, automatically directed out from the system prior to the subsequent CZE analysis, *i.e.*, column switching [9]. An example of the analysis performed with the coupled-column system is shown in Fig. 9. Here the concentrations of individual proteins were at the level of 10^{-8} M dissolved in the background electrolyte. Although the concentration of the background electrolyte in the sample is 10^6 higher than the concentration of the sample proteins, the ITP preconcentration step permits the detection of sample proteins with a signal-to-noise ratio of > 50. A main advantage of the coupled-column system stems from the possibility of injecting extremely large sample volumes which exceed the volume of the analytical separation capillary itself. The corresponding detection limit is then in the subnanomolar region. In the present case the injected volume was nine times higher than the effective volume of the analytical separation capillary itself.

The actual concentration of proteins in their ITP zones can be determined by calibration of the detector response with standard protein solutions. As

confirmed by separate experiments (data not shown), the sample proteins were preconcentrated from the level of 1 μg/ml to *ca.* 20 mg/ml during the ITP step. At this concentration the possibility of precipitation of proteins must be kept in mind. In our experiments we did not observe precipitation, probably due in part to the high positive charge of basic proteins which were separated at a pH well below their isoelectric points. This high charge can minimize aggregation due to coulombic repulsion. In cases where precipitation is an issue, the use of additives to the running buffer electrolytes, such as non-ionic detergents or carrier ampholytes, may reduce the problem [19]. In the present example, Triton X-100 at a level of 1% was added to the leading electrolyte, and this detergent may have helped reduce precipitation. The addition of a non-ionic detergent usually also suppresses the sorption of proteins on the wall of the Teflon preconcentration capillary, in addition to minimizing electroosmotic flow.

CONCLUSIONS

The use of ITP sample preconcentration is a useful approach to improve the detection of proteins in CZE analysis. This method will work well not only for small molecules but also, as shown in this work, for proteins. In the single-column arrangement, 1–2 orders of magnitude higher sample volumes can be injected than in the normal mode of CZE with little if any preconcentration. Furthermore, the sample need not to be dissolved in distilled water or dilute buffer and may contain an excess of other ions which may be useful for stabilization. The main prerequisite for successful ITP preconcentration is a knowledge of the range of solute mobilities in the sample. This can be estimated by a preliminary CZE run of the sample mixture. The appropriate leading L, terminating T and running C buffer ions can be chosen with the aid of tabulated data [22].

Method A1, which involves the change of the terminating electrolyte in the electrode vessel to the leading electrolyte after ITP preconcentration, is the most universal approach, as no sample pretreatment is necessary. Disadvantages from electrolyte change are minor when an automated instrument is used, where the change can easily be programmed. The duration of the ITP step depends on the sample composition and usually ITP will be completed within 1–5 min.

Method A2, where the background electrolyte itself serves as the terminating electrolyte for the transient ITP preconcentration, is useful mainly for samples containing salts of highly mobile ions which can serve as a source of leading ions. Moreover, the addition of a salt can be also expected to narrow the differences in conductivity and ionic strength between various samples. The optimum concentration of the leading electrolyte in the sample should generally be around 0.01 *M*.

The coupled-column approach (method 2) has several advantages, including the possibility of injecting large volumes of the sample, effective sample clean-up, and, with controlled current switching, selected ion analysis. Detection levels in the subnanomolar range can easily be achieved, and quantitative trace analysis is feasible, providing that possible sample losses during the preconcentration step are minimized [23]. Here especially, the sorption of the separating species on the wall of the ITP preconcentration capillary must be eliminated, because at protein concentrations below 10^{-7} *M* even residual adsorption can cause a significant loss of material during migration.

The electrolyte systems used here are suitable for cationic analyses; however, the basic rules for electrolyte selection are applicable also for the analysis of anions. Compositions of many suitable electrolytes for ITP separation can be found in the literature [15,19,22].

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