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Applicability of dynamic change of pH in the capillary zone electrophoresis of proteins

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ABSTRACT

A method to extend the separation power of CZE is described. The method is based on the separation of sample components at two different pH values during one separation run, and involves dynamic buffering of the pH inside a separation capillary by controlling the flow of H^+ ions from the anodic electrode chamber. By changing the anolyte in the chamber, a dynamic pH step is generated, which proceeds rapidly along the capillary and establishes the required new pH value. The use of the method has been demonstrated by the cationic separation of a model mixture of proteins.

INTRODUCTION

In capillary zone electrophoresis (CZE), it is often very difficult to find a suitable buffer composition with which both complete separation of all analyte substances and their reasonably rapid migration may be achieved. With acids and bases possessing similar ionic mobilities and a wide range of pK values, the finding of a suitable pH value is often almost impossible. With protein mixtures possessing a wide range of pI values, very low (<4)[1] or very high (>10)[2] pH values are frequently required, which causes difficulties in finding a suitable background electrolyte (BGE). Recently, it was established[3] that the buffering of pH may be effectively controlled by the regulated electromigratory flow of ions into the BGE in the capillary.

It has already been shown that various types of controlled changes are possible, namely, pH gradients[4], pulses of counter ions[5] and step changes of counter ions[6]. In previous work[7], we described the programmable formation of pH gradients by means of time-controlled pH changes in the anodic electrode chamber. Here we demonstrate the utilization of dynamic step changes of H^+ serving as the co-ion, which lead to the possibility of using two different pH values during one analytical run.

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Obviously, step changes of pH closely resemble the mobilization of substances after isoelectric focusing[8,9]. The formation of pH gradients for the improvement of the separation of proteins has recently also been demonstrated in free-flow electrophoresis[10].

The principle of the performance of stepwise changes of the pH of the BGE can be explained with the help of migration trajectories[6] (see Fig. 1). It can be seen in Fig. 1 that substances A and B are not separated at pH_1 as their trajectories are identical. The pair of substances A and C cannot be separated at pH_2 as their trajectories show the same slopes (which reflects the same mobilities); however, once separated, these substances migrate in parallel and do not mix. Further, it can be seen from Fig. 1 that the change from pH_1 to pH_2 moves very fast and soon reaches and passes the substances to be analysed.

To fulfil the requirement that the change from pH_1 to pH_2 moves sufficiently fast along the migration path L, unbuffered BGE must be used, where the migration of H^+ is not hindered by the formation of reaction moving boundaries. These reaction bounderies are well known in isotachophoresis, where their existence is employed to create H^+ terminating zones in cationic systems[11].

EXPERIMENTAL

A Bio-Rad HPE 100 electrophoretic analyser equipped with a 20 cm × 25 μm I.D. coated fused-silica capillary was used in all experiments. All chemicals were of analytical-reagent grade from Serva (Heidelberg, F.R.G.). A mixture containing cytochrome *c* (horse heart), lysozyme (chicken egg), myglobin (horse heart), carbonic anhydrase (bovine) and trypsinogen (bovine), all from Sigma (St. Louis, MO, U.S.A) was used as the model sample. All proteins were dissolved in 0.05 *M* Tris–HCl electrolyte at pH 3.0 and their concentration was 0.1 mg/ml.

The sample was introduced into the capillary by electromigration for 6 s at 7 kV and the analysis was performed at constant voltage of 8 kV.

RESULTS AND DISCUSSION

For the verification of the possibility of the dynamic control of pH, separations were performed at both a constant pH of the BGE and with pH changes during the

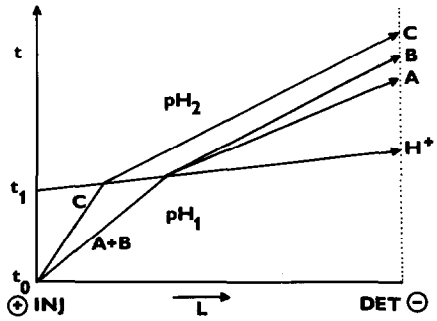

Fig. 1. Migration trajectories of cations A, B and C in CZE with a dynamic pH step. *t* = Time of migration; INJ = injection; DET = detection; L = migration path.

analysis. The separation records obtained at constant pH of the BGE (pH 3.0, 3.8 and 4.5) are shown in Fig. 2a, b and c, respectively.

It can be seen from Fig. 2a that at low pH (3.0) the proteins migrate rapidly and give symmetrical peaks; however, myoglobin, carbonic anhydrase and trypsinogen are separated only partially or not at all.

At pH 3.8 (Fig. 2b), carbonic anhydrase and trypsinogen are slower than myoglobin and they are separated from it, but their mutual separation is only partial.

At pH 4.5 (Fig. 2c), the mobility of lysozyme is reduced to a greater extent than that of myoglobin and the peaks of lysozyme and myoglobin are resolved only partially. The peaks of carbonic anhydrase and trypsinogen are fully resolved, but tailing of last three peaks is more pronounced. Obviously, for a good mutual separation of lysozyme and myoglobin, pH 3.0 or 3.8 should be selected, but this is not suitable for the mutual separation of carbonic anhydrase and trypsinogen. On the other hand, for a good separation of the last pair, pH 4.5 should be selected, but this is not suitable for the separation of myoglobin and lysozyme. Hence it is clear that any one of these pH values, if kept constant, does not provide a successful separation of the whole mixture.

For the reasons mentioned earlier, a step change of pH should be selected in such a way that $pH_2 < pH_1$ for cationic separations. Obviously, the propagation of the H^+ front is much faster than the migration of the sample zones. As is known from theory[12], when a front of a highly mobile cation penetrates electrophoretically into the BGE containing cations with a lower mobility, a moving concentration gradient is formed. Thus, in the case of an H^+ front a moving pH gradient is formed. This dynamic pH gradient also brings an inherent focusing effect which may decrease the tailing of the peaks.

The utilization of dynamically controlled pH is shown in Fig. 3, where a pH change from 4.5 to 3.0 was applied. Based on preliminary experiments, a period of

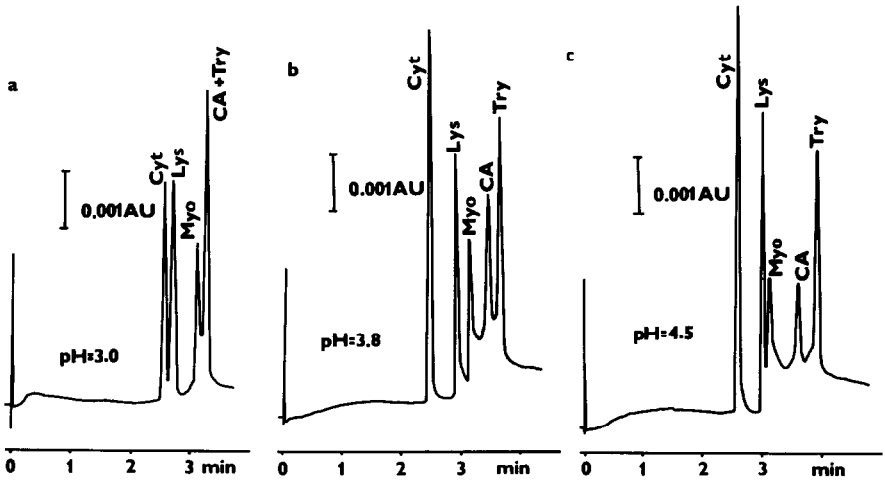

Fig. 2. Cationic separation of proteins at constant pH of the BGE: (a) pH 3.0 (8 kV, 13 μA); (b) pH 3.8 (8 kV, 12 μA); (c) pH 4.5 (8 kV, 12 μA). UV detection at 206 nm. Cyt = cytochrome; Lys = lysozyme; Myo = myoglobin; Try = trypsinogen; CA = carbonic anhydrase.

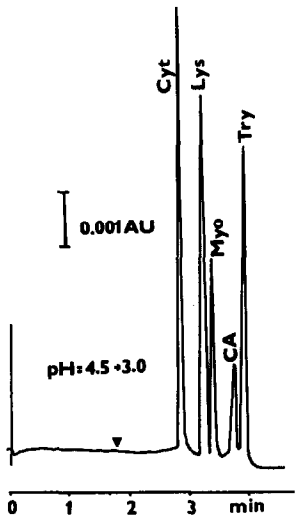

Fig. 3. Cationic separation of proteins using a dynamic pH step. pH change from 4.5 to 3.0. Other details as in Fig. 2.

2 min was elected as a suitable time interval for the first stage of separation at higher pH of the BGE. This combination resulted in complete separation and fairly symmetrical peaks.

We conclude that the use of a step change of pH in CZE can substantially improve the separation of protein mixtures. This improvement can be attributed both to the change in the selectivity during the separation and focusing of the rear boundary of migrating zones.

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