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Rapid protein analysis by capillary electrophoresis

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ABSTRACT

Rapid protein analysis by free-zone capillary electrophoresis in an untreated fused-silica column is presented. Separation of model proteins by the proposed method shows that the retention times of these proteins correlate well with each of their isoelectric points. By using a high voltage gradient, most proteins could be separated in 200 s with excellent reproducibility. Similarly, human serum proteins could be resolved and determined in less than 100 s.

INTRODUCTION

Zone electrophoresis on paper, starch gel, cellulose acetate, agarose and polyacrylamide gels has been used for the separation of proteins and polynucleotides during the last 40 years. It is one of the most important and versatile tools for the structural analysis of proteins and DNA. Analysis of the resulting gel required staining with a dye to reveal the molecules, or the molecules are labelled with radioisotopes or fluorphores. It is a labor-intensive, skill-dependent and a relative slow procedure. Recent developments with capillary columns represent an important technical advance in analytical zone electrophoresis [1]. It is well suited for automation with real-time data analysis. Sample amounts are in the nanoliter range, and microliters of buffer reagent are consumed for each run.

Earlier attempts to electrophorese proteins in untreated fused-silica capillaries resulted in broad and irreproducible migration of all sample zones [2]. Protein–silica surface interaction, and ion-exchange mechanism proposed by Lauer and McManigill [3] and Green and Jorgenson [4], is believed to be responsible for degrading the efficiency and reproducibility of protein separation. Thus, the separation of proteins by capillary electrophoresis (CE) requires the buffer pH to be above the isoelectric points of the sample proteins [3] or below 2.5 [5] or under neutral conditions with high ionic strength [2]. Chemical modification of the silica surface resulted in many successful high-efficiency protein separations under limited pH conditions [6–8]. The object of this work was to perform protein analysis by CE, reproducibly, with high efficiency, in an untreated fused-silica column. This paper summarizes our approach to rapid protein separations by CE.

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EXPERIMENTAL

Materials

All model proteins were obtained from Sigma Biochemicals (St. Louis, MO, USA) and Serva Biochemicals (Westbury, NY, USA). Protein samples were dissolved in buffer containing 75 m*M* sodium chloride, 20 m*M* potassium phosphate and 0.01% sodium azide (PBS). Each protein concentration was 200–500 μg/ml. Serum samples were diluted 1:20 in PBS. Dimethylformamide (DMF) (0.01%, v/v) was added to the sample diluent as a neutral marker.

Capillary electrophoresis system

A P/ACE 2000 system (Beckman, Palo Alto, CA, USA) was used with P/ACE system software controlled by an IBM PS/2 Model Z-50 computer. Data analysis was performed on System Gold™ software (Beckman, San Ramon, CA, USA). The P/ACE 2000 system contains built-in 200-, 206-, 214-, 280- and 340-nm narrow-band filters for on-line detection and quantification. Electrophoreses were performed in a fused-silica tube, 25 or 37 cm × 25 μm I.D., supplied by Polymicro Technologies (Phoenix, AZ, USA). The detection window is located at 6.5 cm from the column outlet. The capillary was assembled in the Beckman cartridge format.

Capillary electrophoresis procedures

Samples were placed on the inlet tray of the P/ACE 2000 instrument and introduced into the capillary by pressure injection for 8–10 s. The system can be programmed to run samples in a random access manner for up to ten different assay methods. Proteins were monitored at 200 or 206 nm. The temperature of the capillary during electrophoresis was kept constant at 22°C. The buffer used for model protein separation was 150 m*M* borate of pH 10.5 and 100 m*M* borate of 11.5. The buffer for human serum protein analysis was a Beckman proprietary buffer of pH 10.0. All buffers were filtered through a 0.45-μm filter. Between runs, the capillary was washed with 1.0 *M* sodium hydroxide solution and water, followed by reconditioning with running buffer. All the washing, rinsing and reconditioning procedures were performed on the P/ACE system.

RESULTS AND DISCUSSION

Protein analysis by CE under both high salt buffer and high pH conditions can achieve a fast separation without compromising resolution. Borate buffer of 150 m*M* (pH 10.5) allows the separation of protein species by CE with moderate success, as shown in Fig. 1. Peak-width analysis indicates significant protein–silica surface interactions. Titration of proteins such as ribonuclease showed that the pK_a of the side-chain amino group in the lysine residue is between 10 and 10.2 whereas that of the guanidyl group of arginine residues is well above 12.0 [9]. Hence, a significant portion of most protein contains positively charged moieties at a buffer pH of 10.5. Increasing the pH of the borate buffer to 11.5 reduces the number of positively charged side-chain lysine moieties sifnigicantly (by *ca.* one order of magnitude). A well resolved separation of the same protine mixture could be achieved simply by raising the pH of the borate buffer to 11.5, as shown in Fig. 2. Fig. 3A shows the separation of another

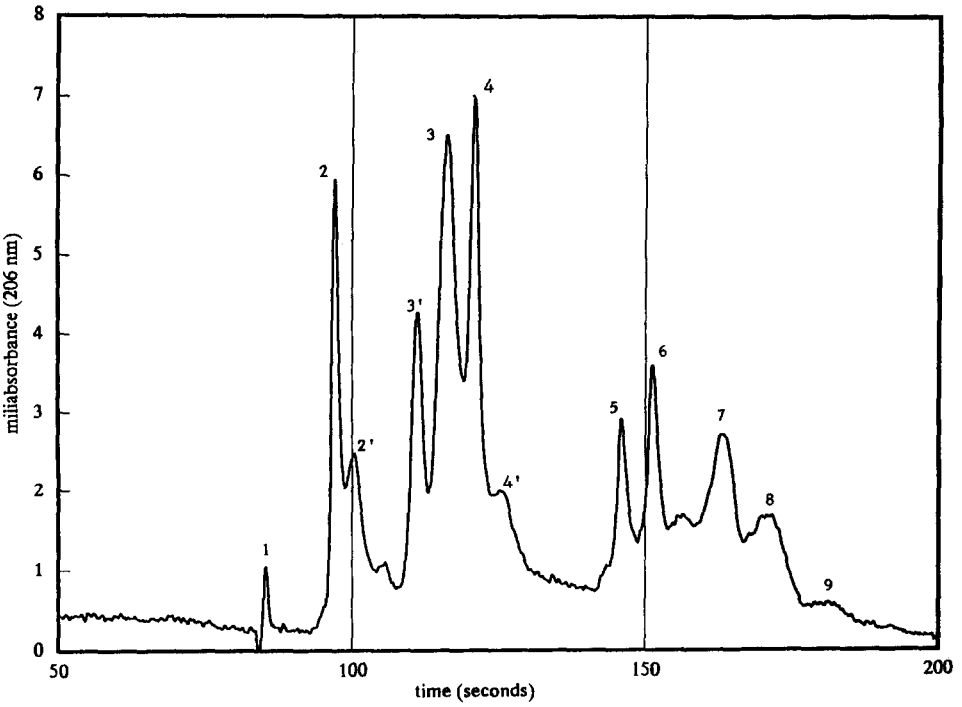

Fig. 1. Electropherogram of model proteins. Conditions: untreated fused-silica capillary, 25 cm × 25 μm I.D.; applied potential, 15 kV; buffer, 150 m*M* borate (pH 10.5). Peaks: 1 = DMF (neutral marker); 2 = bovine ribonuclease A; 3 = whale myoglobin; 4 = horse myoglobin; 5 = conalbumin; 6 = β-lactoglobulin; 7 = bovine serum albumin; 8 = ferritin; 9 = α-amyloglycosidase. Peaks 2′, 3′ and 4′ are impurities from 2, 3 and 4, respectively.

model protein mixture with a separation efficiency of more than 100 000 theoretical plates for all protein species. Addition of bovine ribonuclease to the above protine mixture (Fig. 3B) shows that its p*I* can be estimated to be 9.5.

Analysis of human serum protein by CE in an untreated capillary column has been attempted [10,11]. Our group has achieved a serum protein analysis by CE that reproduces the separation patterns obtained by using conventional agarose gel-based electrophoresis. With a high voltage gradient of 800 V/cm, a fast serum protein separation is achieved in less than 100 s without compromising the resolution (Fig. 4). Each of the serum protein fractions can be clearly identified; γ-globulin appears first, followed by β, α_2- and α_1-globulin, albumin and a minute amount of prealbumin.

A substantially better resolution of serum protein separation can be obtained with a higher ionic strength of the same buffer, as is evident from Fig. 5. Good resolution is evident for both the β- and α-globulin regions. Transferrin is resolved from β-lipoprotein and haptoglobin appears well above the shoulder of α_2-macroglobulin. The addition of a high salt concentration to the buffer appears to minimize protein–protein interactions which may be responsible for peak broadening in protein separation by CE in general.

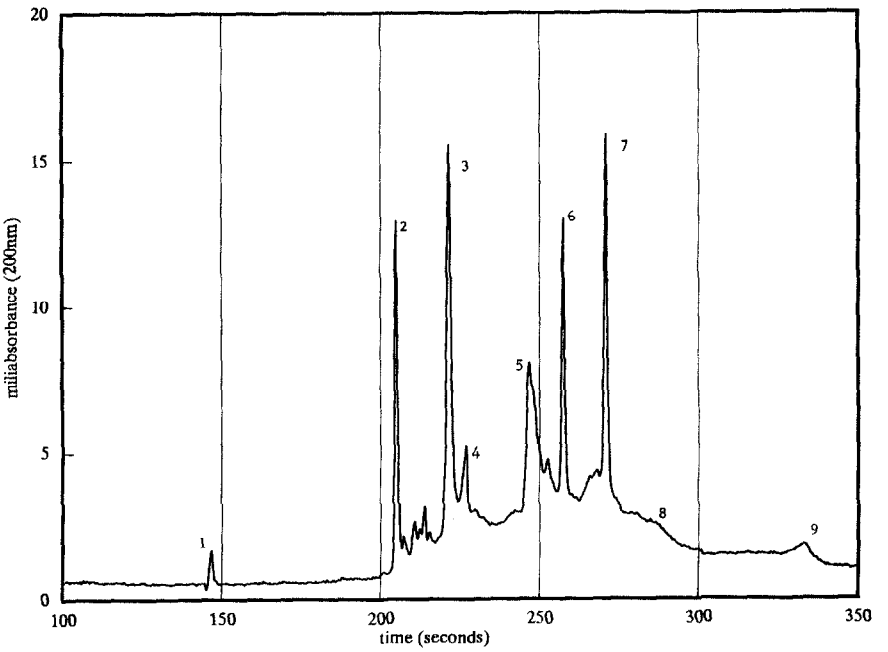

Fig. 2. Electropherogram of model proteins as in Fig. 1. Conditions: untreated fused-silica capillary 37 cm × 25 μm I.D.; applied potential, 12 kV; buffer, 100 m*M* borate (pH 11.5).

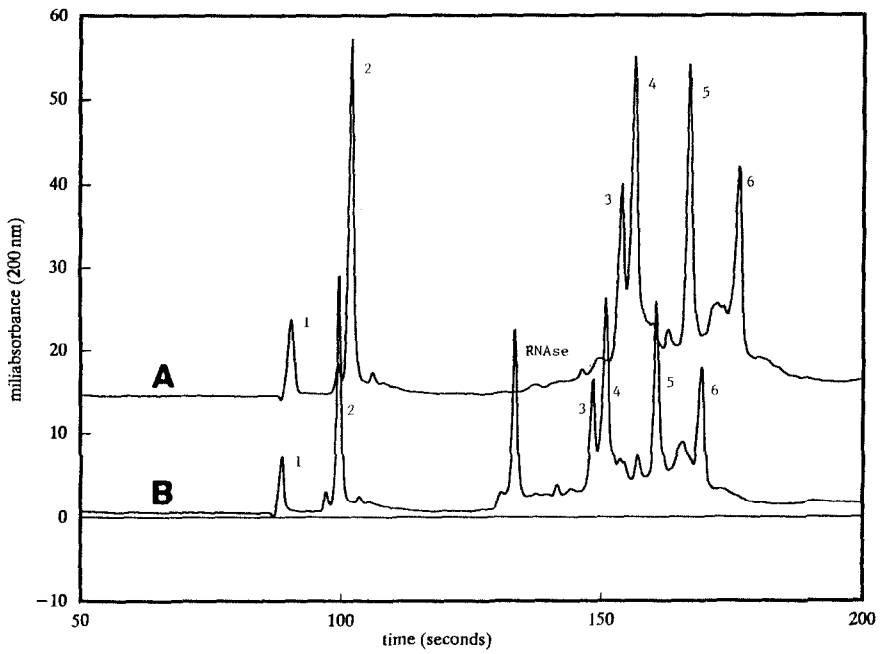

Fig. 3. Electropherogram of model proteins. Conditions: untreated fused-silica capillary, 25 cm × 25 μm I.D.; applied potential, 15 kV; buffer, 100 m*M* borate (pH 11.5). Peaks: (A) 1 = DMF; 2 = bovine trypsin inhibitor; 3 = whale myoglobin; 4 = human carbonic anhydrase; 5 = bovine carbonic anhydrase; 6 = soybean trypsin inhibitor; (B) same as (A) with addition of bovine ribonuclease A between peaks 2 and 3 at 130 s.

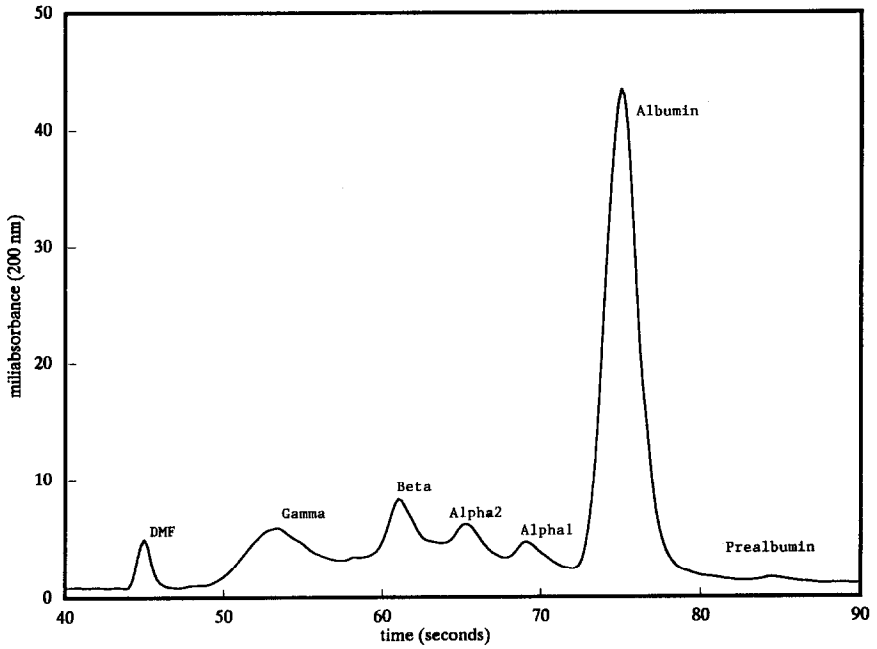

Fig. 4. Electropherogram of a normal control serum protein. Conditions: untreated fused-silica capillary, 25 cm × 25 μm I.D.; applied potential, 20 kV; buffer, pH 10.0.

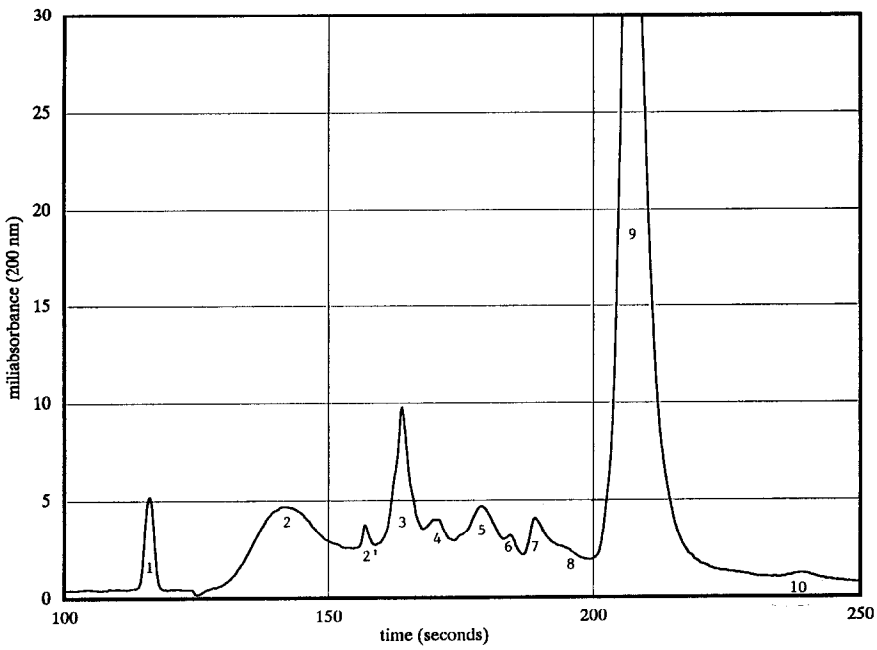

Fig. 5. Electropherogram of a normal control serum protein. Conditions: untreated fused-silica capillary 25 cm × 25 μm I.D. applied potential, 10 kV; buffer, as in Fig. 4 with a higher buffer strength (pH 10.0). Peaks: 1 = DMF; 2 = γ-globulin; 3 = transferrin; 4 = β-lipoproteins; 5 = haptoglobin; 6 = α_2-macroglobulin; 7 = α_1-antitrypsin; 8 = α_1-lipoproteins; 9 = albumin; 10 = prealbumin; 2′ = complements.

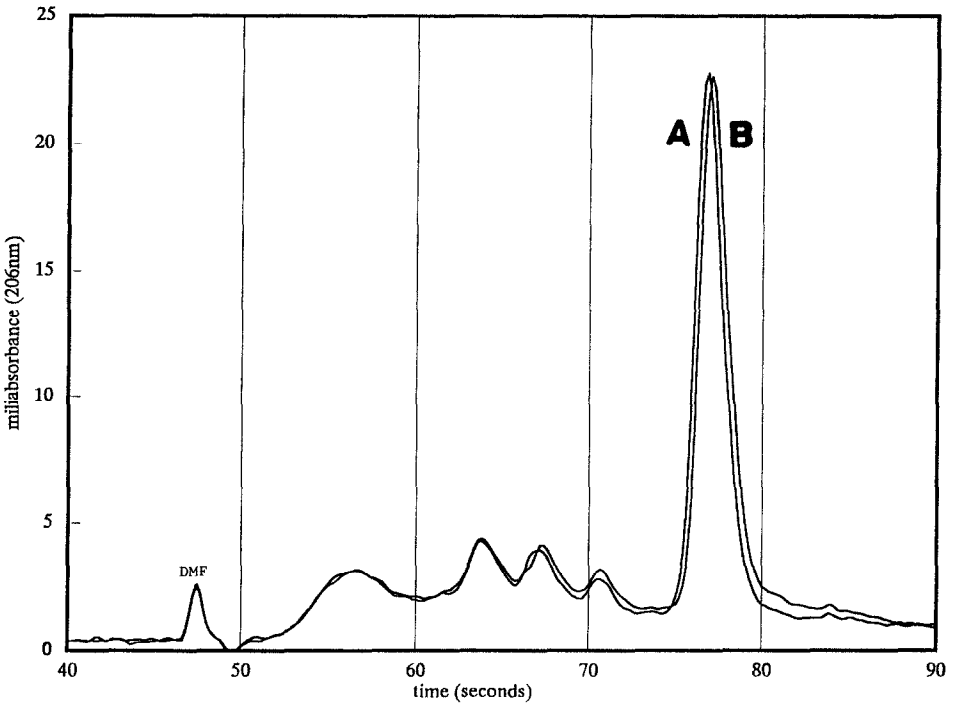

Fig. 6. Reproducibility of rapid capillary electrophoresis: (A) first run; (B) ninth run. Conditions as in Fig. 4.

The reproducibility of serum protein separation by capillary electrophoresis with nine-repeated runs is exhibited in Fig. 6. Between runs, the capillary was washed with base and water. The relative standard deviation (R.S.D.) of the migration time of each protein fraction is less than 1% regardless of the voltage gradient, as shown in Fig. 7. Washing of the capillary between runs ensures reproducibility. Without washing, however, the R.S.D. of the migration time of each protein fraction is significantly higher at a voltage gradient of 200 V/cm, as shown in Fig. 8A. Presumably a slow build-up of proteins on the capillary wall is responsible for the gradual increase in migration time. At a voltage gradient of 800 V/cm, the R.S.D. of the migration time of each protein fraction is less than 1% with nine repeated runs, regardless of post-run washing (Figs. 7B and 8B). Rapid serum protein separation results in a shorter residence time of protein species in the capillary, which may be responsible for reduced protein adsorption on the capillary wall.

In conclusion, the results presented demonstrate the utility of rapid protein analysis by capillary electrophoresis in an untreated fused-silica column. With a high salt concentration and a high pH buffer, the apparent isoelectric point of an unknown protein can be determined. A fast protein separation by CE has been achieved without compromising resolution. The potential of this method for clinical diagnostic applications, serum protein analysis in particular, is promising.

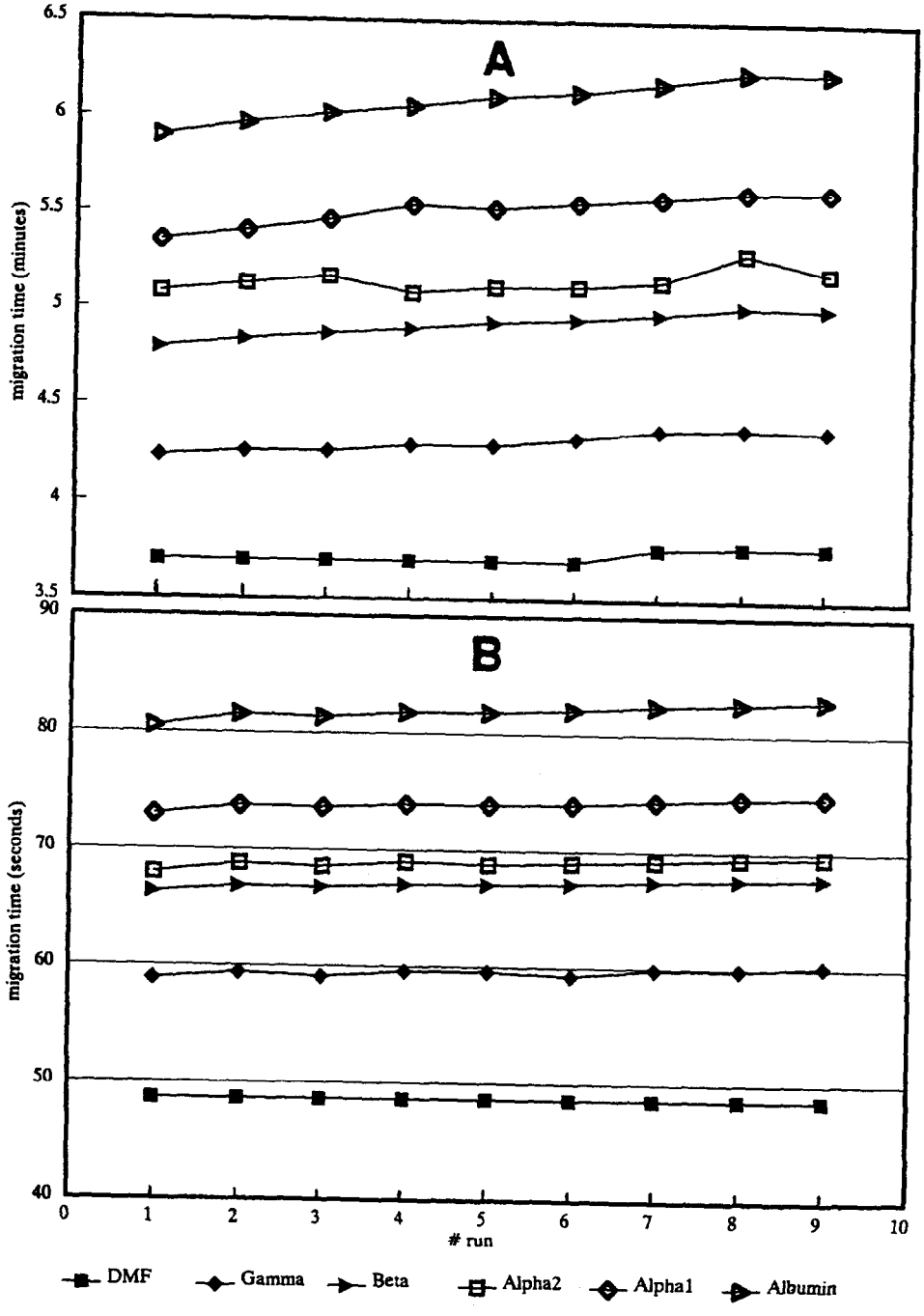

Fig. 7. Migration time of each protein fraction with washed capillary for nine repeated runs. Conditions as in Fig. 4 except the voltage gradients were (A) 200 and (B) 800 V/cm.

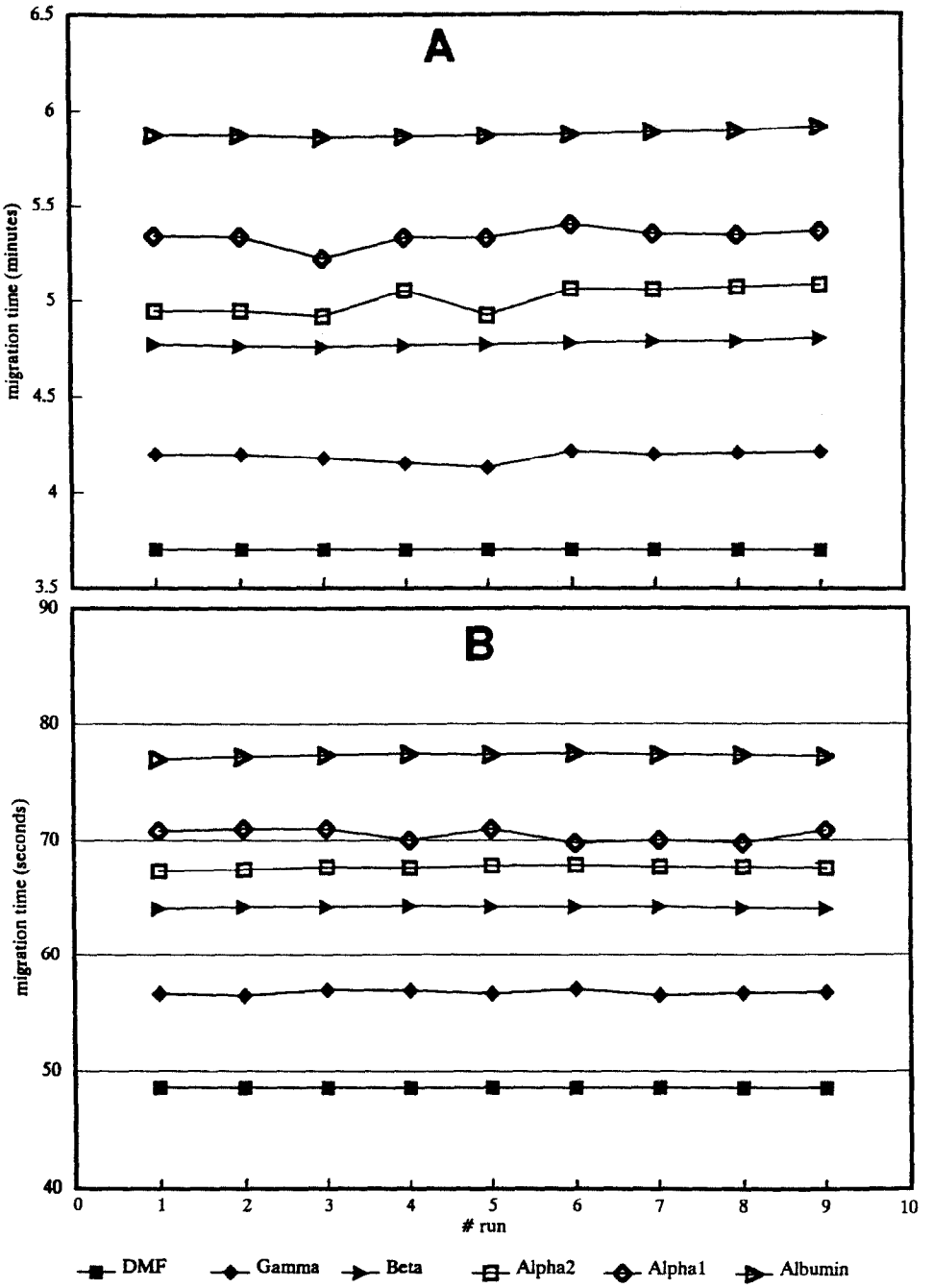

Fig. 8. Migration time of each protein fraction with unwashed capillary for nine repeated runs. Conditions as in Fig. 4 except the voltage gradients were (A) 200 and (B) 800 V/cm.

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REFERENCES

1 J. W. Jorgenson and K. D. Lukacs, *Science, (Washington, D.C.)*, 222 (1983) 266.
2 J. W. Jorgenson and K. D. Lukacs, *Anal. Chem.*, 53 (1981) 1298.
3 H. H. Lauer and D. McManigill, *Anal. Chem.*, 58 (1986) 166.
4 J. S. Green and J. W. Jorgenson, *J. Chromatogr.*, 478 (1989) 41.
5 R. McCormick, *Anal. Chem.*, 60 (1988) 2322.
6 S. Hjerten, *J. Chromatogr.*, 347 (1985) 191.
7 M. Novotny, K. A. Cobb and J. Liu, *Electrophoresis*, 11 (1990) 735.
8 J. K. Towns and F. Regnier, *J. Chromatogr.*, 516 (1990) 69.
9 C. Tanford and J. D. Hauenstein, *J. Am. Chem. Soc.*, 78 (1956) 5287.
10 F.-T. A. Chen, C. M. Liu, Y. Z. Hsieh and J. C. Sternberg, *Clin. Chem.*, 37 (1991) 14.
11 M. J. Gordon, K.-J. Lee, A. A. Arias and R. N. Zare, *Anal. Chem.*, 63 (1991) 69.