In conclusion, using the Cobas Fara II centrifugal analyzer, we produced a method that is quick, reliable, precise, and accurate and can be applied to technology that is available in most laboratories. The rapidity of the method allows large numbers of samples to be quickly processed, preventing degeneration of glutathione in storage in tissue samples that have high γ-glutamyltransferase contents, an enzyme that degrades glutathione.

We are grateful to the Special Trustees of St. Thomas' Hospital for their continuing support.

References

Quantitative Analysis of Serum Proteins Separated by Capillary Electrophoresis

Jong Wan Kim,1 Jeong Ho Park,1 Jong Woo Park,1 Hyeon Ju Doh,2 Gwi Suk Heo,2 and Kong-Joo Lee2,3

The possibility of open tubular capillary electrophoresis for clinical diagnostic use is examined. Capillary electrophoresis was performed in an untreated 50 μm (i.d.) × 100 cm (65 cm to detector) capillary with detection of absorbance at 200 nm. Conditions for the separation of serum proteins without adsorption to the capillary surface were established. Quantitative analyses of serum samples from 38 patients with liver cirrhosis, nephrotic syndrome, or polycyphal gammapathy by capillary electrophoresis were done and the results were compared with those by conventional agarose gel electrophoresis and sodium dodecyl sulfate-polyacrylamide gel electrophoresis. All samples were analyzed in duplicate. We evaluated linearity of response, within-run CV, and the correlation between capillary electrophoresis and agarose gel electrophoresis.

Indexing Terms: liver cirrhosis • kidney function • polyclonal gammapathy

Since the moving-boundary electrophoresis developed by Tiselius (1) was applied to the separation of serum proteins in 1937, electrophoresis has become one of the most important tools in biochemistry and clinical diagnostics. During the past 50 years, many forms of solid matrix media, including paper, agarose, cellulose acetate, and polyacrylamide, have been adopted for improving the resolution by preventing the convection of free solutions originating from Joule heating. Agarose gel electrophoresis, as it is now used in most clinical laboratories, is a qualitative screening method useful for detecting abnormalities of the major proteins in biological fluids. Here we apply the recent technology of capillary electrophoresis with free solution to analysis for serum proteins.

Capillary zone electrophoresis, first developed by Mikkers et al. (2), was extensively studied with fused silica columns for biological application by Jorgenson and Lukacs (3-5). Because the capillary column, which has a large surface-to-volume ratio (<100 μm (i.d.)), dissipates heat efficiently, the high applied voltages (>10 kV) used in capillary electrophoresis produce rapid separation (<10 min) and high separation efficiency (number of theoretical plates, 2.5 × 105 to 106). Also, capillary electrophoresis is simple and is easily automated with real-time data analysis. Many types of ionic small molecules can be separated quickly and efficiently with capillary electrophoresis. However, in the early stages of the development of capillary zone electrophoresis, many proteins had a tendency to stick to the walls.

CLINICAL CHEMISTRY, Vol. 39, No. 4, 1993
of the fused-silica capillary (3). The resulting slow adsorption and desorption kinetics caused extensive tailing in the protein peaks. Several strategies were developed to overcome the problems caused by adsorption of proteins to the negatively charged silanol surface of fused-silica capillary columns: (a) removing the negative charges of the capillary surface by using a pH lower than the pKₐ of silanol (3.0) (6), (b) using higher pH (>9) for the assay buffer so that most proteins would be negatively charged above their pI values (7–9), (c) coating the inner surface of the capillary with hydrophilic polymer (10, 11), and (d) adding alkali metal salts in buffer (12) to minimize the protein adsorption by removing the ionic interaction between silica surface and multicharged proteins. The advantages and disadvantages of the methods were described previously (9).

In this study, we separated serum proteins using a high-pH assay buffer, similar to previous methods (8, 9, 13) with untreated fused-silica columns. Abnormalities of serum proteins were routinely measured by agarose gel electrophoresis and were simultaneously analyzed by capillary electrophoresis and compared with results by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE).

Materials and Methods

Reagents. Low-molecular-mass standard proteins, SDS, acrylamide, glycine, N,N,N',N'-tetramethylethylenediamine, and Tris were purchased from Sigma (St. Louis, MO). Human standard serum, SRM 909, was obtained from the National Institute of Standards and Technology (NIST; Gaithersburg, MD).

Capillary electrophoresis system. The homemade capillary electrophoresis system was as described previously (3). A high-voltage power supply (0–40 kV; Glassman, Whitehouse Station, NJ; Model PS/EH 40R 2.5C7TR) was used to drive the electrophoretic process across the capillary. The platinum wires connected to the anode and the cathode of the power supply were immersed in 3 mL of buffer chambers. A straight fused-silica capillary (Polymicro Technologies, Phoenix, AZ), 100 cm long (63.5 cm to detector), 50 μm (i.d.), and 360 μm (o.d.), was used as a separation tube. Detection was performed at the anodic end by on-column measurement of absorbance at 200 nm with a CV4 ultraviolet detector (ISCO, Lincoln, NE) and a D-502A integrator (Young-In Scientific Co., Seoul, Korea) for data analysis. Before each run, the capillary was rinsed sequentially for 1 min each with 1 mol/L sodium hydroxide solution, distilled water, and assay buffer. After the capillary was filled with the assay buffer (30 mmol/L sodium borate buffer, pH 9.4), a sample was introduced by siphoning at 16 cm height. By this sample injection, 2.5 mL of sample solution was carried into the capillary. Analysis was performed by applying 30 kV in the constant voltage mode.

Sample preparation. Normal human serum from NIST and patients’ serum samples from Chungnam National University Hospital (Daejon, Republic of Korea) were diluted 40-fold with deionized distilled water.

Serum protein electrophoresis. Serum proteins were analyzed by an agarose gel electrophoresis system (Corning 720; Corning Medical, Medfield, MA). All procedures were performed according to the manufacturer’s instructions, with barbital buffer (pH 8.6) as the assay buffer and Ponceau S staining; the band density was scanned at 520 nm.

SDS-PAGE. Protein patterns of serum samples were analyzed by SDS-PAGE under nonreducing conditions. Proteins were separated on 13% polyacrylamide slab gel by the Laemmli procedure (14). The gels were stained with Coomassie Brilliant Blue.

Results and Discussion

Human serum proteins from various patients were separated with traditional agarose gel electrophoresis and capillary electrophoresis (Figure 1). Protein separations, which were performed within 8 min with capillary electrophoresis in an untreated fused-silica capillary column, show a similar pattern to that by agarose gel electrophoresis because the separation in both methods is based on the charge density of protein. In agarose gel electrophoresis, albumin, having a low pI and so being highly negatively charged at pH 8.6, migrates most quickly toward the anode, followed by α₁, α₂, β, and γ fractions. In capillary electrophoresis, the mobility of the solute is the sum of the vectors of electrophoretic mobility and electroosmotic mobility, as explained elsewhere (15). At pH 9.4 (the pH of the assay buffer in capillary electrophoresis), serum proteins are negatively charged; however, they migrate toward the cathode because the strong electroosmotic flow from the anode to the cathode overwhelms the electrophoretic mobility of negatively charged proteins toward the anode (5). Close to the electroosmotic flow front is the γ fraction, whose electrophoretic mobility toward the anode is small because of its high pI value. At decreasing pI values of proteins, the migration velocity is reduced. The order of migration toward the cathode is γ, β, α₂, α₁, and albumin fraction. Within this context, the separation profiles of serum proteins in agarose gel electrophoresis are comparable with those in capillary electrophoresis. SDS-PAGE separations under nonreducing conditions, based on the molecular mass of the protein, were performed to identify the abnormally increased or decreased protein in a patient’s serum (Figure 2).

Serum samples—NIST serum standard and samples from patients with various diseases—all exhibited almost identical patterns in both capillary and agarose gel electrophoreses systems (Figure 1), although the separation efficiency of capillary electrophoresis is better than that of agarose gel electrophoresis. Further studies are required for the clinical usage of finely resolved electropherograms. Four samples (panels 2–5) had abnormally high γ band content (identified in Figure 2 as mainly immunoglobulins) but presented comparable agreement between the two electrophoretic methods. The γ fraction shows a broad band because of the heterogeneity of immunoglobulins (mainly IgG with minor components of IgA and IgM). In capillary electrophoresis, the resulting slow adsorption and desorption kinetics caused extensive tailing in the protein peaks, several strategies were developed to overcome the problems caused by adsorption of proteins to the negatively charged silanol surface of fused-silica capillary columns: (a) removing the negative charges of the capillary surface by using a pH lower than the pKₐ of silanol (3.0) (6), (b) using higher pH (>9) for the assay buffer so that most proteins would be negatively charged above their pI values (7–9), (c) coating the inner surface of the capillary with hydrophilic polymer (10, 11), and (d) adding alkali metal salts in buffer (12) to minimize the protein adsorption by removing the ionic interaction between silica surface and multicharged proteins. The advantages and disadvantages of the methods were described previously (9).

In this study, we separated serum proteins using a high-pH assay buffer, similar to previous methods (8, 9, 13) with untreated fused-silica columns. Abnormalities of serum proteins were routinely measured by agarose gel electrophoresis and were simultaneously analyzed by capillary electrophoresis and compared with results by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE).

Reagents. Low-molecular-mass standard proteins, SDS, acrylamide, glycine, N,N,N',N'-tetramethylethylenediamine, and Tris were purchased from Sigma (St. Louis, MO). Human standard serum, SRM 909, was obtained from the National Institute of Standards and Technology (NIST; Gaithersburg, MD).

Capillary electrophoresis system. The homemade capillary electrophoresis system was as described previously (3). A high-voltage power supply (0–40 kV; Glassman, Whitehouse Station, NJ; Model PS/EH 40R 2.5C7TR) was used to drive the electrophoretic process across the capillary. The platinum wires connected to the anode and the cathode of the power supply were immersed in 3 mL of buffer chambers. A straight fused-silica capillary (Polymicro Technologies, Phoenix, AZ), 100 cm long (63.5 cm to detector), 50 μm (i.d.), and 360 μm (o.d.), was used as a separation tube. Detection was performed at the anodic end by on-column measurement of absorbance at 200 nm with a CV4 ultraviolet detector (ISCO, Lincoln, NE) and a D-502A integrator (Young-In Scientific Co., Seoul, Korea) for data analysis. Before each run, the capillary was rinsed sequentially for 1 min each with 1 mol/L sodium hydroxide solution, distilled water, and assay buffer. After the capillary was filled with the assay buffer (30 mmol/L sodium borate buffer, pH 9.4), a sample was introduced by siphoning at 16 cm height. By this sample injection, 2.5 mL of sample solution was carried into the capillary. Analysis was performed by applying 30 kV in the constant voltage mode.

Sample preparation. Normal human serum from NIST and patients' serum samples from Chungnam National University Hospital (Daejon, Republic of Korea) were diluted 40-fold with deionized distilled water.
Transferrin shows electrophoresis. the second panels distinguished, phoresis, (Figure 2). To plot the same amount of volume as described in Materials and Methods. Plotting the peak
trophoresis, one must have reproducible migration time because the peak area in capillary electrophoresis is dependent on the flow velocity. The reproducibilities of migration time and the relative peak area are examined in Table 1. The within-run CVs of migration time are <3.0%, which is sound reproducibility for quantifying each fraction; those of the relative peak area vary up to 16.5% for the β fraction, primarily because the relative area of α1, α2, or β is too small (<9.5%). However, the standard deviation of α1, α2, or β fraction determinations is reasonably low (<2.0%). The CV of relative peak area could be improved by increasing the number of determinations. For quantitative analysis with capillary electrophoresis, it is essential to establish the linearity of the absorbance response at 200 nm to the amount of analyte in the sample. To test whether capillary electrophoresis gives stoichiometric results for individual bands, we analyzed various dilutions of one serum (10-, 20-, 30-, and 80-fold) by capillary electrophoresis, injecting the same amount of volume as described in Materials and Methods. Plotting the peak
tropheresis, the γ fraction is divided into the front broad band of IgG and a second sharp shoulder peak, which is presumed to be IgA or IgM. These two fractions are well distinguished, depending on the patients (Figure 1, panels 2–5). Especially in sample 5, a distinct γ fraction with a slightly lower molecular mass than normal IgG (Figure 2) shows the sharp γ band dominating the second γ. Other examples are the serum samples from the patient with nephrotic syndrome (panel 6) and an unknown disorder (panel 7). In sample 6, the α2 and β fractions are dramatically increased in agarose gel electrophoresis; in contrast, only the α2 peak is increased in the finely resolved electropherogram with capillary electrophoresis. The increase of α2 and β in sample 7 shows consistency between the two methods.

To quantify each fraction resolved by capillary electrophoresis, the γ fraction is divided into the front broad band of IgG and a second sharp shoulder peak, which is presumed to be IgA or IgM. These two fractions are well distinguished, depending on the patients (Figure 1, panels 2–5). Especially in sample 5, a distinct γ fraction with a slightly lower molecular mass than normal IgG (Figure 2) shows the sharp γ band dominating the second γ. Other examples are the serum samples from the patient with nephrotic syndrome (panel 6) and an unknown disorder (panel 7). In sample 6, the α2 and β fractions are dramatically increased in agarose gel electrophoresis; in contrast, only the α2 peak is increased in the finely resolved electropherogram with capillary electrophoresis. The increase of α2 and β in sample 7 shows consistency between the two methods.

To quantify each fraction resolved by capillary electrophoresis, one must have reproducible migration time because the peak area in capillary electrophoresis is dependent on the flow velocity. The reproducibilities of migration time and the relative peak area are examined in Table 1. The within-run CVs of migration time are <3.0%, which is sound reproducibility for quantifying each fraction; those of the relative peak area vary up to 16.5% for the β fraction, primarily because the relative area of α1, α2, or β is too small (<9.5%). However, the standard deviation of α1, α2, or β fraction determinations is reasonably low (<2.0%). The CV of relative peak area could be improved by increasing the number of determinations. For quantitative analysis with capillary electrophoresis, it is essential to establish the linearity of the absorbance response at 200 nm to the amount of analyte in the sample. To test whether capillary electrophoresis gives stoichiometric results for individual bands, we analyzed various dilutions of one serum (10-, 20-, 30-, and 80-fold) by capillary electrophoresis, injecting the same amount of volume as described in Materials and Methods. Plotting the peak
trophoresis, the γ fraction is divided into the front broad band of IgG and a second sharp shoulder peak, which is presumed to be IgA or IgM. These two fractions are well distinguished, depending on the patients (Figure 1, panels 2–5). Especially in sample 5, a distinct γ fraction with a slightly lower molecular mass than normal IgG (Figure 2) shows the sharp γ band dominating the second γ. Other examples are the serum samples from the patient with nephrotic syndrome (panel 6) and an unknown disorder (panel 7). In sample 6, the α2 and β fractions are dramatically increased in agarose gel electrophoresis; in contrast, only the α2 peak is increased in the finely resolved electropherogram with capillary electrophoresis. The increase of α2 and β in sample 7 shows consistency between the two methods.

To quantify each fraction resolved by capillary ele

---

**Table 1. Within-Run Reproducibility of Migration Time and Relative Peak Area of Each Fraction by Capillary Electrophoresis**

<table>
<thead>
<tr>
<th>Component</th>
<th>Migration time</th>
<th>Relative peak area</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD, min</td>
<td>CV, %</td>
</tr>
<tr>
<td>Albumin</td>
<td>7.14 ± 0.17</td>
<td>2.43</td>
</tr>
<tr>
<td>α1 fraction</td>
<td>6.73 ± 0.10</td>
<td>1.54</td>
</tr>
<tr>
<td>α2 fraction</td>
<td>6.23 ± 0.16</td>
<td>2.91</td>
</tr>
<tr>
<td>β fraction</td>
<td>5.78 ± 0.13</td>
<td>2.25</td>
</tr>
<tr>
<td>γ fraction</td>
<td>5.06 ± 0.06</td>
<td>1.09</td>
</tr>
</tbody>
</table>

*n = 4 each.*
area of each fraction vs concentration in serum gave a
linear response for all five serum fractions under con-
ideration (Figure 3), indicating the validity of the
method for quantifying these fractions. Correlation co-
efficients for all five fractions were >0.997.

To compare the capillary electrophoresis method with
the traditional agarose gel electrophoresis method, we
analyzed 37 serum samples by both methods. As shown
in Table 2, albumin and γ fraction occupied the greatest
portion (~80%) of the serum results of capillary elec-
phoresis, in reasonable agreement with the results of
agarose electrophoresis (slope = 0.934 and r = 0.872 for
albumin, and slope = 0.964 and r = 0.907 for γ fraction).
α2, present as 10–30% of total protein, gave relatively
low correlation coefficients (slope = 0.731 and r = 0.893). The relative values obtained from capillary elec-
phoresis are comparable with those from agarose elec-
phoresis for albumin, γ, and α2. The lowest cor-
relation coefficients were for α1 (slope = 1.41 and r = 0.535) and β (slope = 0.197 and r = 0.434), as expected,
because these are minor components of serum proteins
(<15%). The β peak is overestimated in agarose gel elec-
phoresis because it overlaps with the γ fraction, as shown in Figure 1. Better resolution with capillary elec-
phoresis makes it possible for more accurate
quantitative analysis of serum proteins.

![Graph showing linear response for serum fractions](image)

**Table 2. Correlation of Capillary Electrophoresis Results (y) with Agarose Gel Electrophoresis Results (x)**

| Peak  | r    | Regression equation | Standard error, S
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin</td>
<td>0.872</td>
<td>y = 0.94x + 5.13</td>
<td>0.067</td>
</tr>
<tr>
<td>α1</td>
<td>0.535</td>
<td>y = 1.14x + 3.45</td>
<td>0.062</td>
</tr>
<tr>
<td>α2</td>
<td>0.893</td>
<td>y = 0.73x + 1.34</td>
<td>0.087</td>
</tr>
<tr>
<td>β</td>
<td>0.434</td>
<td>y = 0.20x + 3.59</td>
<td>0.223</td>
</tr>
<tr>
<td>γ</td>
<td>0.907</td>
<td>y = 0.96x + 1.70</td>
<td>0.066</td>
</tr>
</tbody>
</table>

n = 37 each.

In conclusion, we have demonstrated the possibility of
using capillary electrophoresis for analysis of serum
proteins. The optimum separations of serum proteins
were established by using an untreated fused-silica
capillary column, which is similar to the previous
method (8, 13). Analyses of serum proteins from various
patients by capillary electrophoresis, compared with
traditional agarose gel electrophoresis, indicate that
capillary electrophoresis could be a new feasible tech-
nique for the analysis of serum proteins because of its
high separation efficiency, on-line data analysis, quick
separation, and ease of automation. Moreover, the within-
rund CV, linearity of response, and the correlation
between capillary electrophoresis and agarose gel elec-
phoresis show that capillary electrophoresis is the
reliable and reproducible technique for clinical diagnos-
tics of serum protein abnormalities.

We acknowledge the financial support of the Korea Ministry of
Science and Technology.

References
2. Mikkers FEP, Everaers FM, Verheggen TPEM. High-performance
3. Lukacs KD, Jorgenson JW. Capillary zone electrophoresis.
4. Jorgenson JW, Lukacs KD. Zone electrophoresis in open-tubular
6. McCormick RM. Capillary zone electrophoretic separation of
peptides and protein using low pH buffers in modified silica
7. Lauer HH, McManigill D. Capillary zone electrophoresis of pro-
protein mixtures in capillary zone electrophoresis. Anal Chem
9. Lee K-J, Heo GS. Free solution capillary electrophoresis of
proteins using untreated fused silica capillary. J Chromatogr
10. Hjerten S. High-performance electrophoresis elimination of
of proteins under alkaline conditions. J Chromatogr
12. Green JS, Jorgenson JW. Minimizing adsorption of proteins on
fused silica in capillary zone electrophoresis by the addition of
14–9.
14. Leemlwi UK. Cleavage of structural proteins during the
15. Ewing AG, Wallingford RA, Olefrowicz TM. Capillary elec-