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Capillary Electrophoresis of Serum Proteins

Reproducibility, Comparison with Agarose Gel Electrophoresis and a Review of the Literature

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Summary: Conditions for serum protein analysis by capillary electrophoresis were optimized and within day, between day and between capillary variations were examined for both migration times and relative peak areas. For the five currently accepted zones, albumin, α_1, α_2, β and γ-globulin, reproducibilities of migration times were in the range of 2.3–3.1% (n = 200 measurements). Although variations in relative peak areas were slightly higher than those obtained by conventional agarose gel electrophoresis, from a resolution perspective, capillary electropherograms provided better detail than the densitometric scans of agarose gel electrophoresis. Precise localization of C3 and transferrin in capillary electrophoresis resulted in more accurate detection of the β-globulin fraction. When C3 appeared in the γ-fraction it was not detected as a separate peak in agarose gel electrophoresis, whereas it was in capillary electrophoresis.

In artificially prepared mixtures of highly purified albumin and γ-globulin preparations, best correspondence with theoretical values was found with capillary electrophoresis.

Inter-individual variations and reference values were obtained by measuring 140 samples from healthy controls (59 females, 81 males) with both techniques. For capillary electrophoresis the inter-individual variations of the albumin, α_1, α_2, β and γ fractions were respectively 6, 21, 19, 14 and 18% and for agarose gel electrophoresis 5, 20, 17, 18 and 22%. From these results it can be concluded that the more precise localization of the β- and γ-globulin fraction results in about 4% lower inter-individual variations in capillary electrophoresis compared to agarose gel electrophoresis. For the other fractions, comparable variations were obtained. Differences between males and females were not significant.

For patient samples, a good correlation was found between capillary electrophoresis and agarose gel electrophoresis data for all five protein fractions.

We conclude that separation efficiency of capillary electrophoresis is better than that of agarose gel electrophoresis and even weak monoclonal components can easily be distinguished with the capillary electropherogram. Capillary electrophoresis is a qualitatively good, cheap, fast and easy to perform alternative to agarose gel electrophoresis.

Introduction

Capillary electrophoresis has been suggested as a new tool for separation and quantification of serum proteins (1–14). It combines the separation principles of conventional electrophoresis with the advanced instrumental design of high-performance liquid or gas chromatography and capillary technology. The serum sample is introduced into a buffer-filled fused silica capillary (internal diameter 20 to 200 μm and lengths of 10–100 cm), either electrokinetically or hydrodynamically with pressure (fig. 1). The amount of the sample applied can be regulated by changing the injection time. For separation, both ends of the capillary are placed into a buffer solution that also contains the electrodes, and high voltage is applied to the system. The applied voltage causes the analytes to migrate through the capillary and past the detector window. Separation is based on differences in velocities of the charged particles (migration times). The data obtained in the electropherogram are collected, stored and interpreted with an appropriate data acquisition system. For each separation, only nanoliters of sample and microliters of buffer are used. The walls of untreated fused silica capillaries are negatively charged in aqueous solution from the ionization of surface silanol groups (pI = 1.5). The negatively charged silica surface attracts positively charged ions and cations from the buffer, creating an electrical double layer (fig. 2). When a voltage is applied across the capillary, cations in the diffuse portion of the double layer migrate in the direction of the cathode, carrying water with them. The result is a net flow of buffer solution in the direction of the

negative electrode – electro-osmotic flow. This flow is particularly important at alkaline pH, and a small change of pH can dramatically alter the separation pattern. Liquid cooling of the capillary allows excellent maintenance of temperature control. The final result of the protein separation is affected by capillary length and diameter, buffer composition and pH, sample injection mode, capillary thermostating (*Joule* heat), separation temperature, the electro-osmotic flow, solute concentration effects, wall-solute interactions and applied field.

Table 1 presents a review of the methods used for serum protein analysis by capillary electrophoresis presented in the literature to date. Although several methods are published, very few data are available on reproducibility of capillary electrophoresis and no data are available on variation between capillaries.

In most clinical laboratories agarose gel electrophoresis is used as a screening method for detection of abnormalities of the major proteins in biological fluids like serum, urine and cerebrospinal fluid. The results of serum protein agarose electrophoresis are quantified from peak area determination of the electrophoresis scanning pattern. Comparison of visual inspection of electropherograms with agarose electrophoresis has been performed by *Jenkins* et al. (13). They found that capillary electrophoresis was able to detect all monoclonal bands detected by high resolution agarose electrophoresis, and, in particular, better able to detect IgA monoclonal bands occurring in the beta region.

Reference values were determined only by *Klein* et al. (8), but exact description of the measurement conditions was not given.

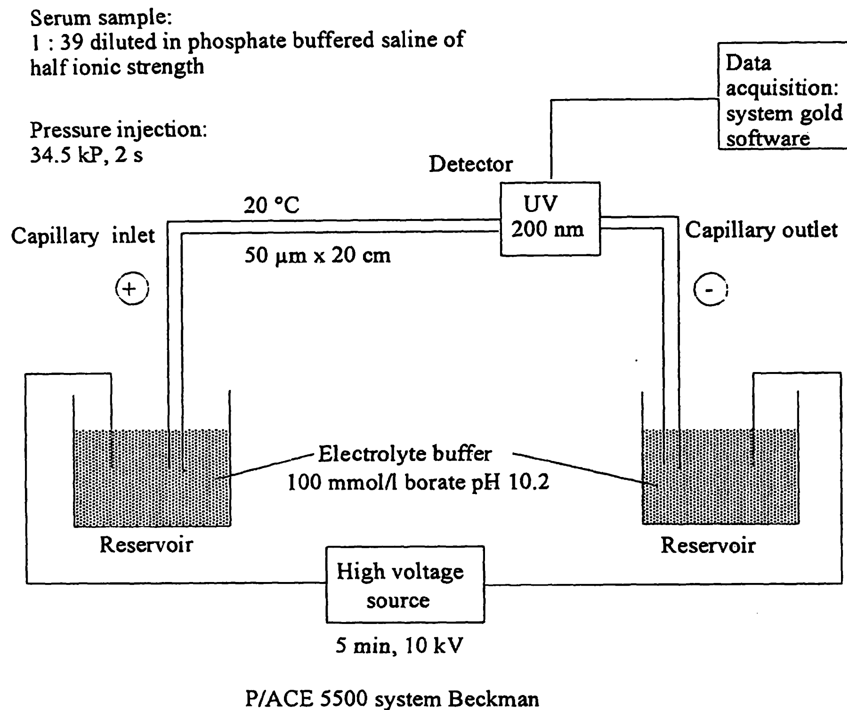


Fig. 1 Capillary electrophoresis system with configuration used in the text.

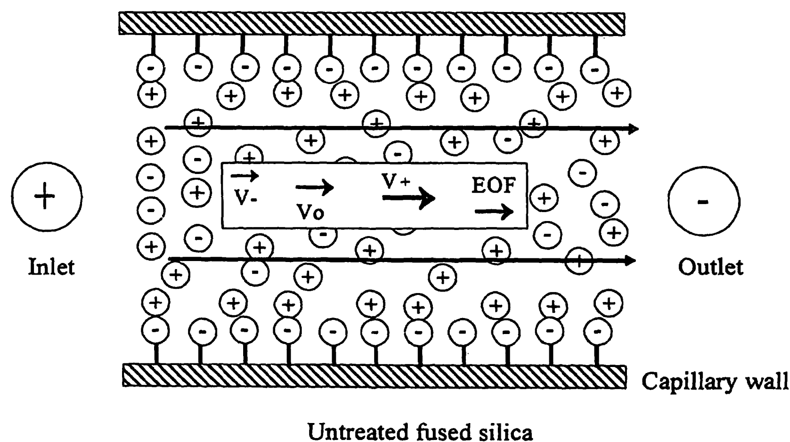


Fig. 2 Untreated fused silica capillary. The negatively charged silica surface attracts cations from the buffer, creating an electrical double layer. The result is a net flow of buffer in the direction of the negative electrode, electro-osmotic flow (EOF). V is the migration velocity of the different charged particles.

Tab. 1 Review of serum protein capillary electrophoresis methods presented in the literature.

Method	*Chen* et al. (1)	*Kim* et al. (6)	*Stob* et al. (7)	*Klein & Jolliff* (8)	*Landers* (12)	*Jenkins & Guerin* (12)	*Chen*, Beckman	This paper
Instrument	Capillary electrophoresis system Beckman	Home-made	Lauerlabs, PRINCE	Capillary electrophoresis system Beckman	–	Applied Biosystems capillary electro-phoresis system, Model 270A-HT	Beckman P/ACE	Beckman P/ACE 5500
Sample	Serum	Serum	Serum	Serum	Serum	Serum	Serum	Serum
Dilution	1 : 19 phosphate buffered saline	1 : 39 deionized distilled water	1 : 199 running buffer	1 : 10 phosphate buffered saline	1 : 9 phosphate buffered saline	1 : 49 running buffer	1 : 19 phosphate buffered saline with half the ionic strength	1 : 39 phosphate buffered saline with half the ionic strength
Capillary	Untreated fused silica 75 µm × 25 cm	Untreated fused silica 50 µm × 63.5 µm	– 75 µm × 56 cm	Fused silica 25 µm × 20 m	– 25 µm × 20 cm	Fused silica 50 µm × 72 cm	Untreated fused silica 20 µm × 20 cm	Untreated fused silica 50 µm × 20 cm
Buffer	Beckman proprietary buffer	Borate	CAPS* and TAPS** buffer, SIGMA	Borate	Borate	Boric acid containing 1 mmol/l calcium lactate	Borate	Borate
Molarity	–	30 mmol/l	46.3 mmol/l CAPS*, 3.7 mmol/l TAPS**	–	–	50 mmol/l	100–200 mmol/l	100 mmol/l
pH	10.0	9.4	9.9	–	8.3	9.7	10.2	10.2
Injection	Electrokinetic 1 kV	Siphoning at 15 cm height, 2.5 nl sample	Pressure 2 kPa, 15 nl sample	Pressure 34.5 kPa	Pressure 34.5 kPa	5 in. vacuum injection	Pressure 34.5 kPa	Pressure 34.5 kPa
Injection time	3–10 s	–	6 s	10 s	10 s	2 s	20 s	2 s
Temperature	–	–	33 °C	24 °C	24 °C	–	24 °C	20 °C
Separation	< 10 min 5 kV	– 30 kV	5 min 30 kV	8 min 10 kV	– 10 kV	15 min 18 kV	2 min 20 kV	5 min 10 kV
Detection	214 nm	200 nm	200 nm	214 nm	214 nm	200 nm	200 or 206 nm	200 nm
Rinse	–	1 min 1 mol/l NaOH 1 min distilled water 1 min running buffer	100 mmol/l NaOH running buffer 4 min total	NaOH distilled water	–	2 min 100 mmol/l NaOH 2 min distilled water 3 min running buffer	0.2 min 1 mol/l NaOH 0.2 min distilled water 1 min running buffer	1 min 100 mmol/l NaOH 1 min distilled water 2 min running buffer

* CAPS = 3-cyclohexylamino-1-propane-sulphonic acid.
** TAPS = 4-tris[hydroxymethyl]methyl-3-aminopropane sulphonic acid.

The objective of the present study was to present the state of the art in the literature, to establish the reproducibility of capillary electrophoresis and compare it with results from agarose electrophoresis. Differences between capillaries were also examined. Artificially prepared mixtures of purified protein preparations (albumin and γ-globulin) were used to check the quantification of peak areas.

Reference values were measured, especially for peaks that could not be detected separately with agarose electrophoresis like transthyretin (pre-albumin), transferrin and C3. The results were compared to results obtained with agarose electrophoresis.

Materials and Methods

Materials

Control sera: Beckman I. D.-Zone normal (BI 015-555985-AR) and abnormal (BI 015-555983-AP) were used for examination of variation and are indicated as normal control and abnormal control.

Seronorm protein, (Mat no. 1003405, batch no. 305024) obtained from Nycomed Pharma AS, Oslo, Norway and a pooled serum were used as control sera for agarose electrophoresis.

Cartridge coolant for the Beckman P/ACE system 2000 capillary cartridge coolant (No. 359976) was used as cooling fluid.

Albumin purified and essentially globulin-free, electrophoretic purity approximately 99% was obtained from Sigma (lot 109F93041, Zwijndrecht, NL).

γ-Globulins of electrophoretic purity approximately 99% were obtained from Sigma (lot 106F9315, Zwijndrecht, NL).

Methods

Capillary electrophoresis was performed with a P/ACE 5500 system (Beckman Instruments Inc., Mijdrecht, NL) with P/ACE System Gold software controlled by an IBM 330-450-DX computer (fig. 1). Post-run data analysis, like data integration or mobility/area correction, was performed with System Gold software (Beckman Instrument Inc.). A capillary column of 50 μm and 27 cm (20 cm to the detector window) was assembled in the P/ACE cartridge format (100 × 800 μm aperture) from Beckman.

Samples were placed on the inlet tray of the P/ACE 5500 system and introduced into the capillary by pressure injection (2 seconds, 34.5 kPa). Serum was diluted 1 : 39 in phosphate buffered saline with half ionic strength (diluted 1 + 1). Serum protein separations were carried out using an untreated fused silica capillary of 50 μm, at an oven temperature of 20 °C. The column was maintained at ambient temperature during electrophoresis with circulating coolant surrounding the column. Electrophoresis was performed for 5 minutes at 10 kV at 20 °C. Detection was made at the cathodic end by on-capillary UV absorbance measurements at 200 nm. The system contains built-in filters that can be changed. Quantification of the various fractions was obtained from the area under the curve by real-time data analysis. Before each run, the capillary was sequentially rinsed one minute with 0.1 mol/l NaOH and one minute with distilled water and two minutes with assay running buffer (100 mmol/l borate buffer pH 10.2). The method we used was a slight modification of the Beckman application (tab. 1).

Agarose gel electrophoresis was performed with the Paragon Serum Protein Electrophoresis kit from Beckman Instruments Inc., Mijdrecht NL (BI 015-556458-J). After electrophoresis, using a barbital buffer of pH 8.6, ionic strength 0.05, and staining with Paragon Blue Stain, the gels were scanned at 600 nm on the Beckman Appraise System. The fraction of each protein zone was calculated from the area under the curve. Serum protein electrophoresis gels were also visually interpreted for the presence of monoclonal bands or polyclonal gammopathy and were quantitated by densitometry. Agarose electrophoresis allowed discrimination of five protein fractions, i. e. albumin, α_1-, α_2-, β- (in some cases β_1- and β_2-) and γ-globulins. Total analysis time, electrophoresis (25 minutes) and staining, is about 90 minutes for 10 serum protein electrophoresis's on a gel.

Total protein and albumin were determined on a Synchron CX-7 analyzer (Beckman Instruments Inc, USA, California) using test-kits from Beckman Instruments Inc. For the determination of serum albumin, the bromcresol purple method (testkit 442765) was used. Determination of serum total protein occurred with a timed endpoint biuret-method (testkit 442740). Mean total protein and albumin content in the Beckman I. D.-zone normal control and abnormal control samples were determined by measuring the concentration of 20 different days.

Results

We investigated the within-day, between-day and between-capillary variation of migration times and relative peak areas using Beckman I. D.-Zone control sera normal (BI 015-555985-AR) and abnormal (BI 015-555983-AP). Within day variation was obtained by measuring the normal normal control and abnormal control ten times during one day and this was performed on five different days, giving a mean within-day variation. Between-day variation was obtained by measuring the control sera ten times a day on five different days (n = 50). Between capillary variation was obtained by

Tab. 2 Overall reproducibility of migration times for different control sera.

Fraction	Mean migration time (n = 100)				Mean migration time (n = 200)	
	Minutes (normal control)	CV % (normal control)	Minutes (abnormal control)	CV % (abnormal control)	Minutes (normal control and abnormal control)	CV % (normal control and abnormal control)
Transthyretin	4.09	4.1	4.04	5.1	4.07	4.7
Albumin	3.52	3.4	3.53	2.9	3.53	3.1
α_1-Globulins	3.36	3.3	3.36	2.7	3.36	3.0
α_2-Globulins	3.16	3.0	3.14	2.4	3.15	2.7
β_1-Globulins (transferrin)	2.89	2.7	2.89	2.3	2.89	2.5
β_2-Globulins (C3)	2.73	2.4	2.69	1.8	2.71	2.2
γ-Globulins	2.52	2.4	2.53	2.0	2.53	2.2

performing the same procedure on two different capillaries (n = 100). Table 2 presents the mean migration times for the normal control and for the abnormal control (n = 100) and the mean migration times for all normal control and abnormal control measurements (n = 200) performed. Variation in migration times on different days, using different capillaries and with different control sera appears less than 3.5% for all fractions, except for pre-albumin, where a variation of 4.7% is found.

Figure 3a, b presents capillary electropherogram and agarose gel of the normal (fig. 3a) and abnormal control (fig. 3b).

Variations in relative peak areas are presented in tables 3 and 4. For the five currently accepted zones, within day variations are below 5% (tab. 3). Overall variation, obtained by measuring relative peak areas on different days, using different capillaries, is higher. The high γ-globulin fraction makes quantification of the C3 less reliable, but the localization of the peak is precise. In agarose gel electrophoresis, C3 cannot be detected as a separate peak in samples with a high γ-globulin fraction, for instance in patients with polyclonal gammopathy. If in capillary electrophoresis the C3 is counted with the γ-globulin fraction, the CV for the β-globulin fraction becomes 5% instead of 18%, which is comparable to agarose gel electrophoresis. Variations for agarose gel electrophoresis were obtained by measuring the normal, abnormal control, Pool serum and Seronorm Protein on an agarose gel on 30 different days. Although variations in relative peak areas with capillary electrophoresis are a slightly higher than those obtained by conventional agarose gel electrophoresis, capillary electropherograms provided better detail than the densitometric scans of agarose electrophoresis from a resolution perspective.

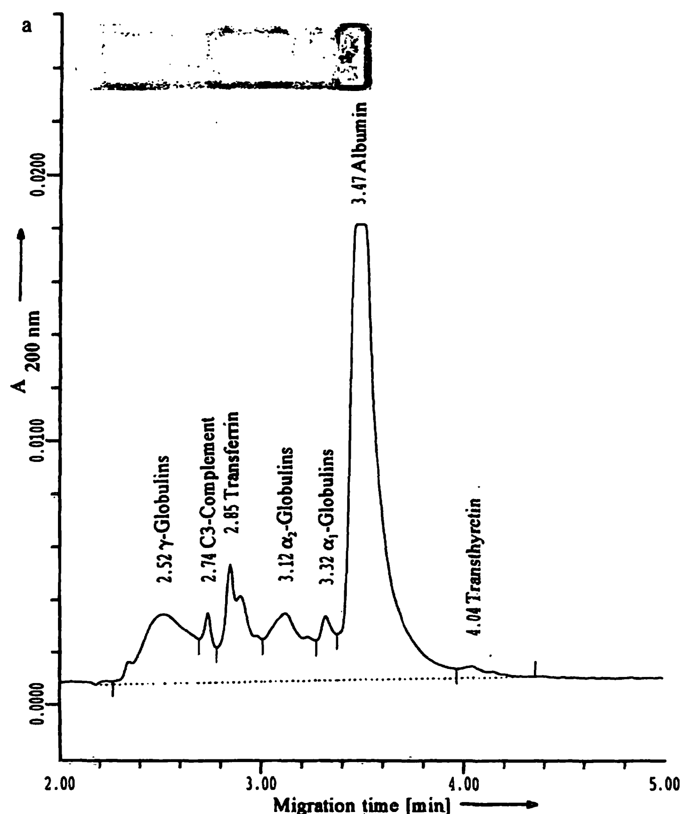
Table 5 presents capillary electrophoresis and agarose electrophoresis results of artificially prepared mixtures of albumin and γ-globulin preparations dissolved in phosphate buffered saline of half ionic strength to known concentrations. The results are compared with the theoretical values. Data are means of duplicate analysis.

Table 6 presents the inter-individual variations (reference values) obtained by measuring 140 serum samples from normal healthy controls.

Figures 4a–e, present the correlation of capillary electrophoresis result (y) with agarose gel electrophoresis (x). A good correlation was obtained for all fractions.

Special examples

Figure 5 shows an example of a serum sample, where Paragon serum protein electrophoresis showed a band on the application slot. This occurs when large molecules are kept in the agarose layer and cannot be separated. In capillary electrophoresis this artifact disappears and the band appears in the γ-globulin fraction.



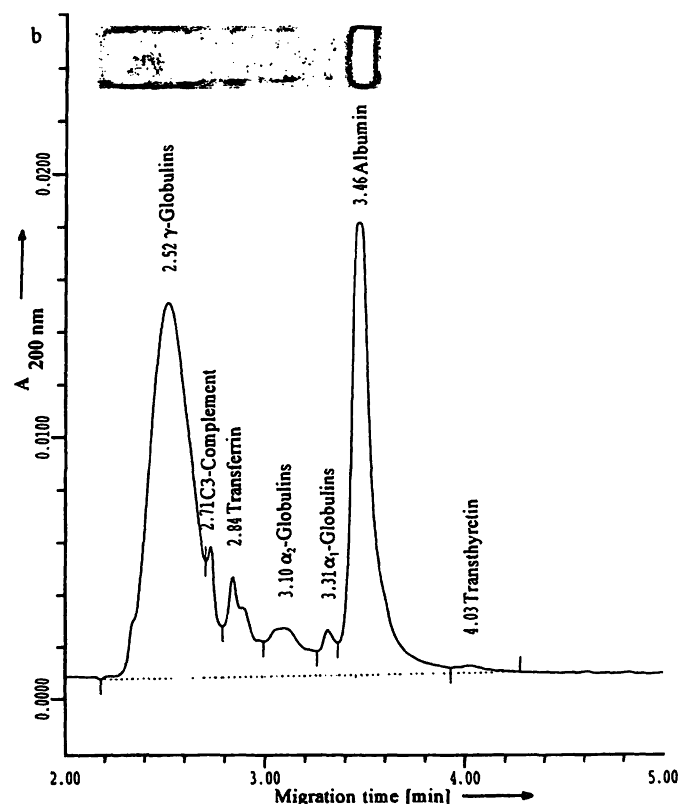


Fig. 3a, b Serum protein analysis by capillary electrophoresis and agarose gel electrophoresis for the normal (fig. 3a) and abnormal control (fig. 3b). For the abnormal control separate detection of C3 is only possible in capillary electrophoresis and not in agarose gel electrophoresis. The figures show the capillary electropherogram and the agarose gel.

Figure 6 shows that two weak M-components in the γ-globulin fraction can be discriminated even better in the capillary electropherogram, as compared to the visual inspection of the agarose gel.

Figure 7 shows an example of a clear-M-component both in the capillary electropherogram and on the agarose gel.

The figures also show that very clear detection of C3 and transferrin is possible, which allows better discrimination from weak M-components.

Discussion

Klein et al. (8) exhaustively evaluated the variables affecting the capillary electrophoresis separation of serum proteins. However, an exact description of the method advocated is not given. Therefore, in table 1 an overview of the methods and the differences between the methods is presented. In the present study, a slight modification of the method described by *Chen*, Beckman Company (no official reference available) was used. Serum samples were diluted 1 : 39 instead of 1 : 19 and capillary

Tab. 3 Reproducibility of relative peak areas by capillary electrophoresis.

Fractions	Overall mean relative peak area				Within-day variation cap 1		Between-day variation cap 1		Between-capillary variation (cap 1 and cap 2)	
	Normal control n = 100		Abnormal control n = 100		CV normal control mean of 5 days	CV abnormal control mean of 5 days	CV normal control n = 50	CV abnormal control n = 50	CV normal control n = 100	CV abnormal control n = 100
	%	g/l	%	g/l	%	%	%	%	%	%
Transthyretin	1.15	0.68	0.88	0.70	17.59	17.62	21.14	50.57	25.97	63.01
Albumin	55.14	32.6	32.34	25.6	1.51	0.86	3.45	2.59	4.16	4.28
"Albumin" (transthyretin + albumin)	56.29	33.3	33.21	26.3	1.31	0.83	3.31	1.81	4.19	3.92
α_1-Globulins	4.29	2.54	2.14	1.69	3.94	4.82	5.47	5.98	13.11	7.68
α_2-Globulins	11.05	6.54	6.27	4.96	2.35	2.55	6.16	4.03	8.42	5.12
β_1-Globulins (transferrin)	10.41	6.16	6.02	4.76	2.81	2.29	6.79	4.09	7.54	4.22
β_2-Globulins (C3)	5.06	3.00	7.73	6.11	4.98	5.10	13.08	23.97	14.75	30.70
β-Globulins (C3 + transferrin)	15.47	9.16	13.75	10.88	2.67	3.48	3.13	13.95	6.59	17.91
γ-Globulins	12.88	7.62	44.61	32.29	1.96	1.08	7.74	4.45	8.55	6.37

Total protein:
normal control = 59.2 g/l ± 1.8%;
abnormal control = 79.1 g/l ± 1.8%.

Albumin:
normal control = 35.6 g/l ± 1.5%;
abnormal control = 26.0 g/l ± 1.6% (mean over 20 days).

Tab. 4 Comparison of reproducibility of capillary electrophoresis and agarose electrophoresis.

Fraction	Capillary electrophoresis n = 100		Agarose electrophoresis n = 30			
	CV % (normal control)	CV % (abnormal control)	CV % (normal control)	CV % (abnormal control)	CV % (pooled serum)	CV % (seronorm protein)
Albumin	4.2	3.9	2.3	2.8	6.3	2.9
α_1-Globulins	13.1	7.7	4.6	6.7	5.2	4.8
α_2-Globulins	8.4	5.1	3.5	4.1	4.6	3.1
β-Globulins	6.6	17.9	7.4	4.7	5.2	4.3
γ-Globulins	8.6	6.4	7.3	1.9	6.9	7.1

Tab. 5 Recovery of artificially prepared protein mixtures.

Albumin : Globulin mixture theoretical values		Agarose electrophoresis		Capillary electrophoresis	
Albumin (%)	Globulins (%)	Albumin (%)	Globulins (%)	Albumin (%)	Globulins (%)
80	20	78.4	21.5	78.3	21.7
50	50	54.1	45.9	47.1	52.9
20	80	25.7	74.3	19.2	80.8

Tab. 6 Reference values for capillary electrophoresis and agarose gel electrophoresis.

Fractions	Capillary electrophoresis					Agarose electrophoresis				
	Mean		CV	Median	Reference range	Mean		CV	Median	Reference range
	%	g/l	%	%	%	%	g/l	%	%	%
Transthyretin	1.32	0.88	31.9	1.24	0.48–2.16					
Albumin	54.5	36.2	5.96	54.2	38.1–61.1					
"Albumin" (transthyretin + albumin)	55.9	37.1	5.98	55.6	49.2–62.6	63.3	42.1	5.19	63.4	56.7–69.8
α_1-Globulins	5.62	3.73	21.2	5.60	3.24–8.01	3.39	2.25	19.9	3.3	2.04–4.74
α_2-Globulins	8.79	5.84	18.5	8.74	5.53–12.1	8.48	5.64	17.1	8.4	6.58–10.4
β_1-Globulins (transferrin)	10.1	6.71	9.32	10.1	8.29–12.0					
β_2-Globulins (C3)	5.15	3.42	30.9	5.06	1.97–8.32					
β-Globulins (C3 + transferrin)	15.3	10.2	13.7	15.3	11.1–19.5	13.5	8.98	18.1	13.5	8.57–18.3
γ-Globulins	14.4	9.58	18.4	14.4	9.08–19.7	11.4	7.58	21.8	11.5	6.43–16.4

Total protein:
66.5 ± 3.5 g/l (mean ± SD), range 56.5–75.6 g/l.

Albumin:
39.7 ± 2.7 g/l (mean ± SD), range 32.4–45.3 g/l.

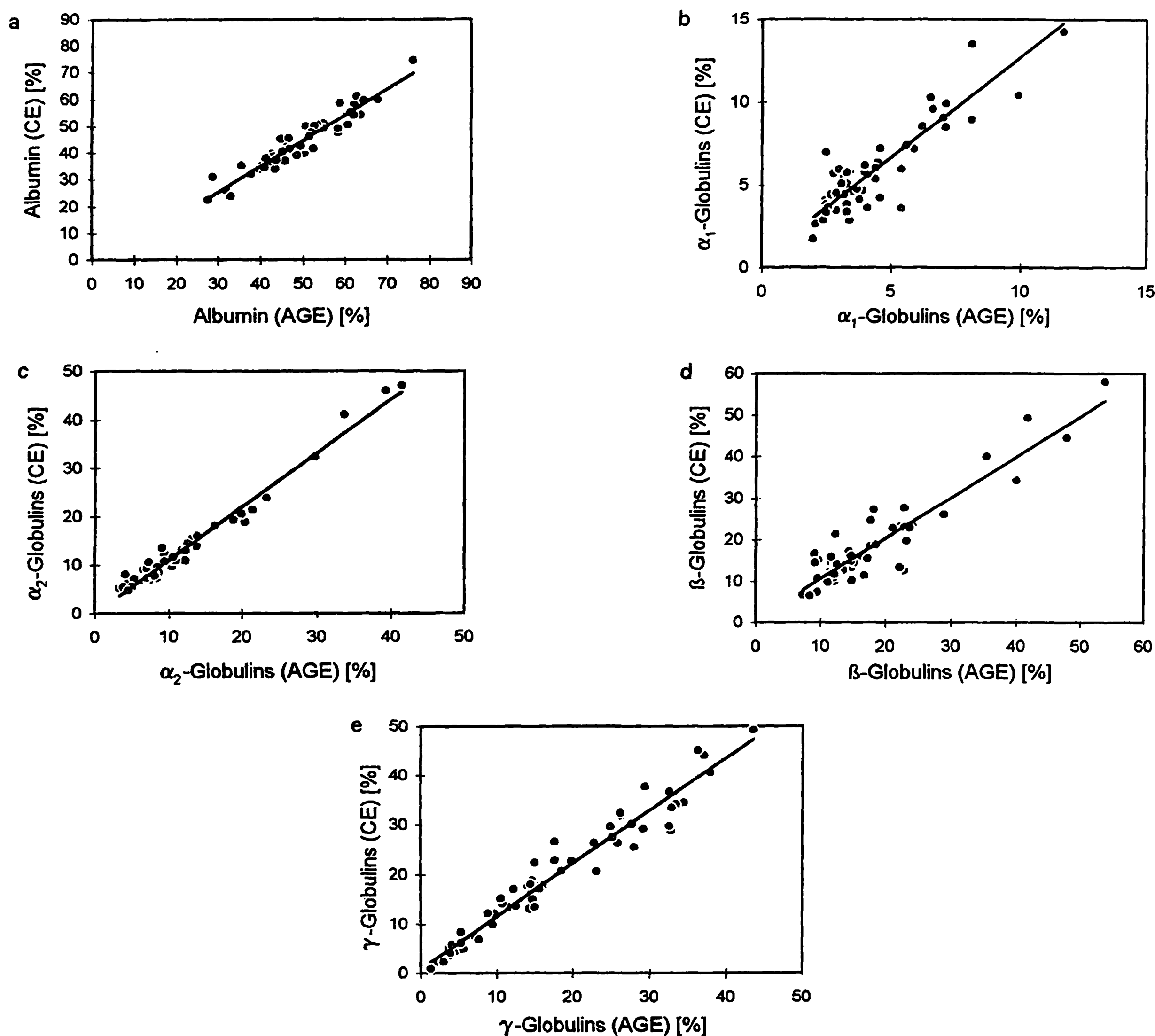


Fig. 4a–e Correlation between capillary electrophoresis data and agarose gel electrophoresis data for the five currently accepted fractions, albumin, α_1-, α_2-, β- and γ-globulin (n = 61). Fractions are indicated as fractions of the total protein concentration.
Regression lines: a. $y = 0.96x - 3.34$, $r = 0.95$; b. $y = 1.21x + 0.62$, $r = 0.90$; c. $y = 1.10x - 0.02$, $r = 0.99$; d. $y = 0.98x + 0.76$, $r = 0.93$; e. $y = 1.07 + 0.84$, $r = 0.97$

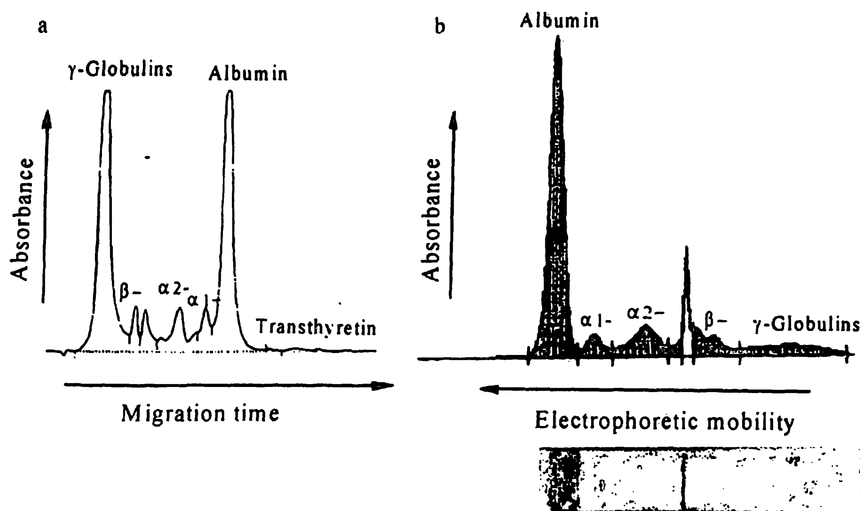


Fig. 5 Capillary electrophoresis (a) and agarose electrophoresis (b) of a sample containing large proteins that remain at the application slot after agarose gel electrophoresis separation (both electropherogram and gel are shown) (b) and not after separation with capillary electrophoresis (a).

diameter was 50 µm. At 10 kV we obtained a separation time of five instead of six minutes. We obtained slightly better results with this procedure. The most important factors affecting the final electropherogram shapes are discussed below.

Capillary length and diameter

Shorter separation times can be obtained with small and thin capillaries. Higher detection limits can be obtained with larger bore capillaries due to an increase in the absorbing path length. Our analysis time could be shortened by using a 20 µm thick capillary, but this can give more technical problems like obstruction of the capillary. A 50 µm thick capillary gave reliable results within 10 minutes and therefore we did not change to a smaller capillary diameter.

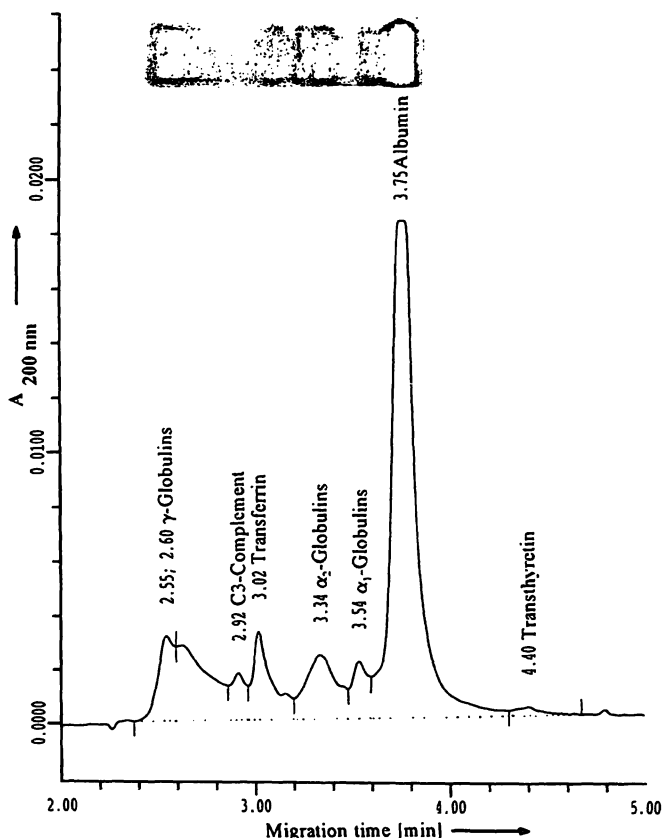


Fig. 6 Identification of gammopathies is possible both by capillary electrophoresis and by agarose gel electrophoresis. The figure shows two weak M-components that can be detected with both techniques, capillary electropherogram and agarose gel.

Voltage effects

The applied field seems to affect only the migration times up to a point of adverse *Joule* heating. High voltage gives short separation times, but has repercussions for the quality of the separation (7).

Sample injection

Sample injection can be performed electrokinetically or hydrodynamically. For charged particles like proteins, hydrodynamic injection is preferred to obtain optimal peak resolution (8, 10).

Capillary temperature control

Adequate temperature control is of utmost importance in quantitative analysis of serum proteins by capillary electrophoresis. This is primarily due to thermal change of the pH of the buffers, small changes in pH can have noticeable effect on pattern shape (8). In some of the papers published until now, temperature control was inadequate (1, 6, 13). Liquid cooling of the capillary in the cartridge gives excellent cooling results, which is of importance for reproducibility of migration times.

Detection

Detection of proteins by absorbance ranges from 190 to 280 nm. If possible, UV detection should be performed

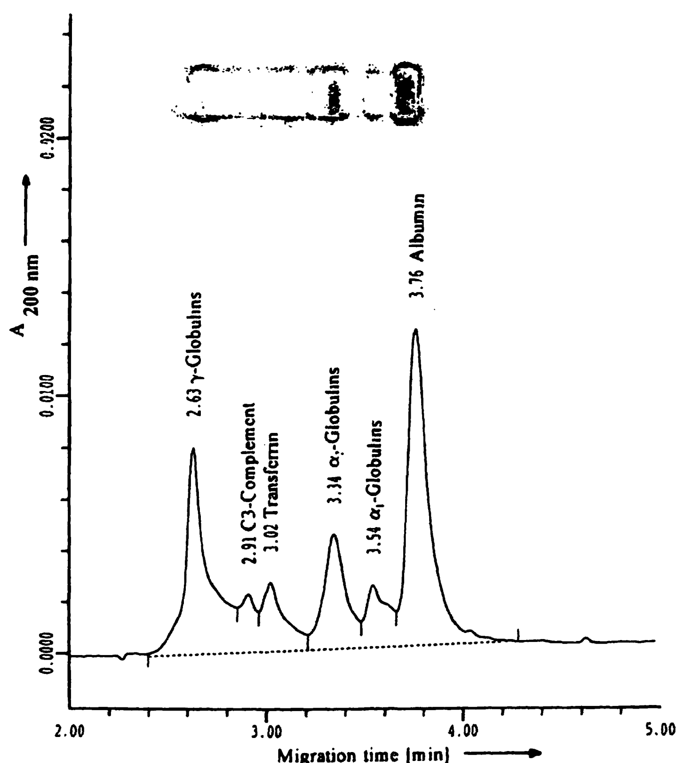


Fig. 7 Capillary electropherogram and agarose gel of a serum sample with a clear M-component.

at low wavelengths where sensitivity is much higher (10).

Buffer ionic strength effects and sample dilution

A borate buffer is used because of its transparency, especially in the far-ultraviolet (UV) spectral regions. An increase of the ionic strength of the separation buffer gives a decrease of the mobility. The mobility of the proteins is retarded by the surrounding buffer ions. With respect to analysis time and resolution, we obtained the best results with a buffer with an ionic strength of 100 mmol/l. At 75 mmol/l, analysis time was within four minutes, but peak resolution was insufficient. At 150 mmol/l analysis time was six minutes, but differentiation between γ-globulins and C3 was no longer possible.

Serum samples were diluted in phosphate buffered saline with half the ionic strength, resulting in a lower conductivity and proportionally higher field strength in the sample plug as compared to the separation buffer. A higher field strength in the diluted sample plug causes sample stacking (sample concentration) at the border between the sample and separation buffers. However, dilution in distilled water resulted in a higher signal to noise ratio. Therefore, phosphate buffered saline with half the ionic strength was used. Moreover, the 1 : 19 dilution in phosphate buffered saline gave a higher background, baseline, compared to the 1 : 39 dilution.

Effect of pH

A higher pH of the buffer gives an increase in the electro-osmotic flow. At high pH (approximately 10) the pH of the protein is typically less than that of the buffer. Therefore, both the proteins and the capillary wall are negatively charged and the adsorption process is minimized as a result of a charge repulsion effect. However, at too high pH a relatively high electro-osmotic flow is generated, which has negative consequences for the peak resolution.

Wall-solute interactions

Even though the pH of the running buffer was selected to induce a strong negative charge on all proteins, regions of the protein with net positive charges remained. Experiments involving successive runs without the NaOH rinse showed progressive lengthening of retention times and a loss of resolution. After the NaOH rinse, the capillary returned to normal (8). In our case a pH of 10.2 gave optimal results.

Determination of electro-osmotic flow

A higher pH of the running buffer results in an increase of the electro-osmotic flow and, as a consequence, of migration times. To ensure that the system is properly controlled it is necessary to measure the electro-osmotic flow. In contrast to *Chen* (2), we did not add a solution of dimethylformamide (0.1 ml/l) to the sample diluent as a neutral marker of electroendoosmotic flow, because the dimethylformamide-peak appeared in the γ-globulin peak. We diluted the serum samples in phosphate buffered saline of half ionic strength. Therefore, the electro-osmotic flow could be determined from the negative peak occurring at the transition of the sample to running buffer.

Migration time

Migration time of a protein is the result of its electrophoretic mobility plus the buffer's electro-osmotic flow. Changes in viscosity, temperature, pH, ion depletion etc. can influence the mobility and thus the migration time. Changes in mobility can lead to false identification of peaks and improper quantification. In the abnormal control, a higher variation is observed, especially for the β-globulin fraction (tabs. 3 and 4).

Variation in peak areas

Reif et al. (9, 10) reported standard deviations of peak areas of less than 6% (migration times less than 1%). No further information is given on how this value was obtained. *Stob* et al. (7) reported variations for the peak areas between 1.2 and 6.2% for the different fractions. Variation was obtained by measuring one sample six times. Variation in migration times was

less than 0.4% for all fractions. In the present study variation in peak areas was obtained from at least 200 measurements and gave slightly larger variation in peak areas for capillary electrophoresis than for agarose gel electrophoresis. The high γ-globulin fraction makes quantification of the C3 less reliable, but the localization of the peak is precise (fig. 3). In agarose gel electrophoresis, C3 cannot be detected as a separate peak in samples with a high γ-globulin fraction, for instance in patients with polyclonal gammopathy. If in capillary electrophoresis the C3 is counted with the γ-globulin fraction, the CV for the β-globulin fraction becomes 5% instead of 18%, which is comparable to agarose gel electrophoresis.

Colour correction

Quantification of stained agarose gels is subject to a number of factors that can influence stain binding. In contrast, the capillary electrophoresis profiles represent the direct measurement of protein via the peptide bonds, a more accurate method for quantifying their relative concentrations.

Peak resolution

From the standpoint of resolution, the smaller the capillary internal diameter and the shorter the separation time, the better the separation. However, a smaller diameter results in increased heat production. Therefore, a sufficient cooling system is required.

Capillary electrophoresis is generally characterized by a higher peak resolution as compared to agarose electrophoresis. In general, agarose electrophoresis is not suitable for identification of a separate transthyretin (prealbumin) fraction. However, in capillary electrophoresis it is possible to quantify transthyretin because of its clear resolution. Transthyretin occurs in a concentration range that allows quantification (12). However, we found an inter-individual variation of 31%, making capillary electrophoresis not suitable for reliable quantitative pre-albumin determinations.

In addition to transthyretin, a clearer separation is also possible for the β-globulin fraction, giving separate C3 and transferrin peaks. Precise localization of C3 and transferrin in capillary electrophoresis results in a more accurate detection of the β-globulin fraction. When C3 appears in the γ-globulin fraction it is not detected as a separate peak in the agarose electropherogram, whereas it is in capillary electrophoresis. Generally the β-globulin peak is overestimated in agarose gel electrophoresis because it overlaps with the γ-globulin fraction. *Chen* et al. (2) showed a separate β-lipoprotein peak and differentiation between α_2-macroglobulin and haptoglobin. In later studies a comparable resolution was not shown.

Agarose electrophoresis sometimes shows the phenomenon that large proteins remain at the application slot. In capillary electrophoresis this problem is fully solved.

In capillary electrophoresis reliable quantification of transferrin is possible, inter-individual variation about 10%, this is not the case for C3, where an inter-individual variation of 41% is found.

Correlation with agarose gel electrophoresis

We found a good correlation of capillary electrophoresis with agarose gel electrophoresis and even better compared to the correlation found by *Kim* et al. (6).

We conclude that capillary electrophoresis is a very useful technique, suitable for reliable quantification and separation of serum proteins. Total variation for capillary electrophoresis seems slightly higher compared to agarose electrophoresis. For capillary electrophoresis of the β-globulin fraction, a clear separation of the complement C3 and transferrin fraction is possible. The same holds for the separation of transthyretin. Generally, a better peak resolution is obtained with capillary electrophoresis. Some artifacts of gel electrophoresis are eliminated when using capillary electrophoresis.

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