

4. Grossman PD, Colburn JC (eds.). *Capillary Electrophoresis: Theory and Practice*, Academic Press, San Diego, California, USA, 1992.
5. Vindevogel J, Sandra P. *Introduction to Micellar Electrokinetic Chromatography*. Hüthig Buch Verlag GmbH, Heidelberg, 1992.
6. Li SFH. *Capillary Electrophoresis, Principle, Practice and Applications*. Elsevier, Amsterdam, 1992.
7. Kuhn R, Hoffstetter-Kuhn S. *Capillary Electrophoresis: Principles and Practice*, Springer Verlag, Berlin-Heidelberg, 1993.
8. Foret F, Krivankova L, Bocek P. *Capillary Zone Electrophoresis*. VCH Verlagsgesellschaft mbH, Weinheim, 1993.
9. Altria KD. *Quantitative Analysis of Pharmaceuticals by Capillary Electrophoresis*. Vieweg Press, Weisbaden, 1998.
10. CE and CEC Reviews. Ziad El Rassi (ed.). *Electrophoresis* 1999; 20: No. 15-16.
11. Atkins PW. *Physical Chemistry*. Oxford University Press, Oxford, 1982.
12. Ackermans MT, Everaerts FM, Beckers JL. *J Chromatogr* 1991; 549: 345-355.
13. Speciale editie over "Isotachoforese en stacking technieken" van *Electrophoresis*.
14. Thormann W et al. *Electrophoresis* 1994; 15: 3-12.
15. Jellum EJ. *Cap Elec* 1994; 1: 97-105.
16. Chen F-TA et al. *Clin Chem* 1991; 37: 14-19.
17. Landers JP. *Clin Chem* 1995; 41: 495-509.
18. Kohlrausch F. *Ann Phys (Leipzig)* 1897; 62: 209-239.

Ned Tijdschr Klin Chem 2000; 25: 219-229

Capillary zone electrophoresis as a tool to detect proteins in body fluids: reproducibility, comparison with conventional methods and a review of the literature

Y.M.C. HENSKENS¹ and M.P. van DIEIJEN-VISSER²

Capillary zone electrophoresis involves the separation of charged molecules, e.g. proteins, in a buffer-filled capillary tube by application of high voltage. CZE is a sensitive and rapid alternative for conventional gel electrophoresis techniques to separate proteins in body fluids. Serum, urine, cerebrospinal fluid, synovial fluid and saliva analysis by CZE have been investigated in the past 10 years. CZE is a promising tool for routine clinical laboratories to automate serum protein analysis. At present the separation and identification of serum proteins in order to detect monoclonal gammopathies has been extensively studied. For this purpose, a complete automated system was evaluated (Paragon CZE 2000) and already introduced into routine clinical chemistry laboratories. In general, CZE shows comparable results to conventional methods for serum protein screening and monoclonal component detection and identification. Applications for routine CZE analysis of urine or cerebrospinal fluid analysis are still in development and might be suitable to replace the conventional methods in the near future.

Medical Laboratories, Reinier de Graaf Group, Delft¹; Department of Clinical Chemistry, Academic Hospital, Maastricht²

Correspondence to: Dr. ir. Y.M.C. Henskens, Laboratorium voor Algemene Klinische Chemie (LAKC), Academisch Medisch Centrum, F1-213, Postbus 22660, 1100 DD Amsterdam.
E-mail: y.m.henskens@amc.uva.nl

Key-words: capillary electrophoresis (CE); capillary zone electrophoresis (CZE); agarose gel electrophoresis (AGE); immunosubtraction capillary zone electrophoresis (CZE/IS); immunofixation electrophoresis (IFE); monoclonal component (MC)

Capillary electrophoresis (CE) is an analytical tool for separating molecules based on molecular size, electric charge and hydrophobicity. Capillaries can be filled with a replaceable or fixed solid gel (capillary gel electrophoresis) or with a replaceable running buffer (capillary zone electrophoresis). Capillary zone electrophoresis (CZE) has been suggested as a new tool for separation and quantification of serum proteins (1-12). In addition, proteins in other body fluids such as urine (13-19), cerebrospinal fluid (15), synovial fluid (20, 21) and saliva (22) can also be detected or quantified by CZE. CZE combines the separation principles of conventional electrophoresis with the advanced instrumental design of high-performance liquid chromatography and capillary technology. The 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. Figure 1 presents the specific requirements for (serum) protein analysis as used by Wijnen and van Dieijen-Visser (11). Changing the injection time can regulate the amount of sample applied. 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.

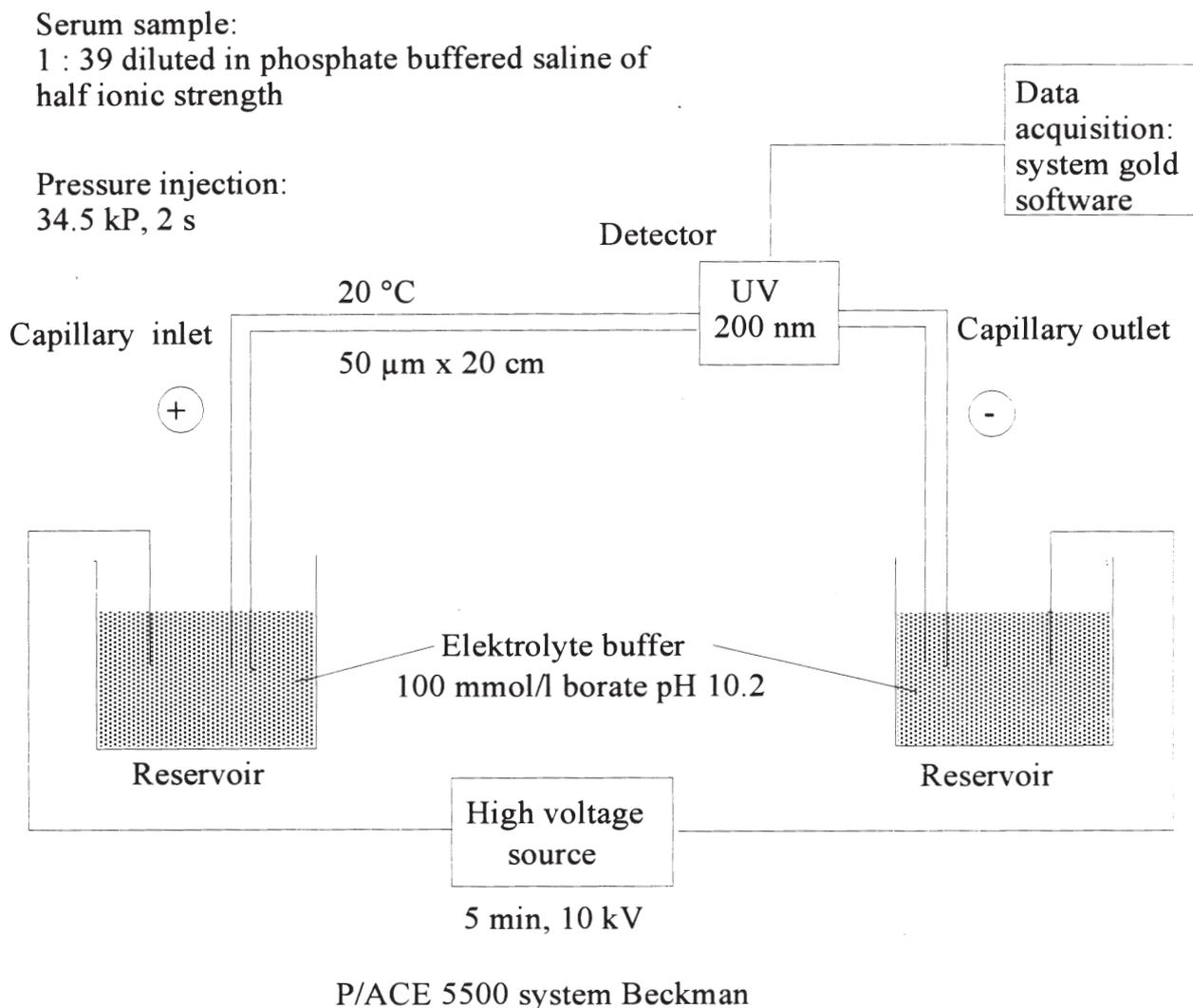


Figure 1. Capillary electrophoresis system with configuration used in the text.

The applied voltage causes the analytes to migrate through the capillary and pass the detector window. In this system the velocities of electroosmotic flow (EOF) exceeds the electrophoretic mobilities of the protein fractions leading to a cathodal migration of the molecules which are then detected (UV, 214 nm). The obtained data 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 ionisation of surface silanol groups ($pI=1.5$). The negatively charged silica surface attracts positively charged ions, 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 resulting EOF 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 electroosmotic flow, solute concentration effects, wall-solute interactions and applied field. The present review summarises CZE studies of proteins in those body fluids that are most analysed in routine clinical laboratories: serum, urine and cerebrospinal fluid.

Detection of serum proteins by CZE

CZE has been suggested as an alternative for the conventional agarose gel electrophoresis (AGE) in separating human serum proteins since it allows fast protein separation with good resolution, using only small amounts of sample. AGE is widely used for the screening and monitoring of several pathological processes by separating human serum proteins into five fractions: the albumin, alpha-1, alpha-2, beta and gamma region. Similar to the procedure of AGE, the CZE electropherogram shows the conventional separation into five regions (Fig. 3). CZE can be used to screen serum samples for the presence of monoclonal components (MCs), since these proteins produce sharp spikes in the beta or gamma region. Further identification of MCs can also be performed on capillary zone electrophoresis by the technique of

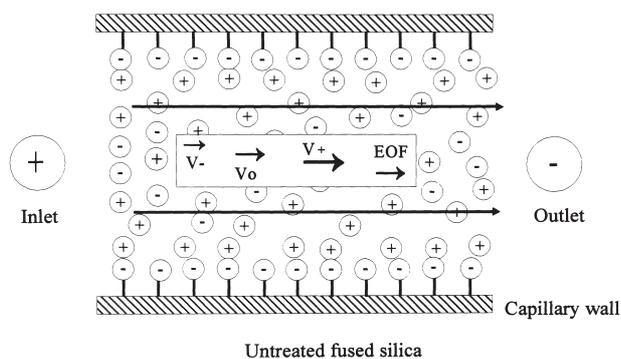


Figure 2. Untreated fused silica capillary. The negatively charged silica surface attracts cations from the buffer, creating an electrical double layer. The result is a net flow of buffer in the direction of the negative electrode, electroosmotic flow (EOF). V is the migration velocity of the different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

immunosubtraction (CZE/IS). Immunosubtraction as an alternative method for IFE was first described by Aguzzi and Poggi in 1977 (23). Classes or types of immunoglobulins are removed from a serum sample using an antibody specific for the immunoglobulin, coupled to beads. The serum sample is exposed to five different coupled antibodies against the heavy chains of IgG, IgA, and IgM and the kappa and lambda light chains. Before and after exposure to each of the beads, the treated sera and an untreated serum are run by CZE. The treated samples will show subtraction only if the coupled antibody removes the specific protein. Otherwise, the electrophoresis pattern will remain unchanged. Since 1995 several method comparison studies were published concerning the separation of serum proteins using CZE. Most studies were performed on different single capillary CZE instruments using running buffers which varied in molarity and pH. In order to obtain reproducible results, standardising the running conditions in serum protein CZE is of utmost importance (12, 24-27). Recently, a dedicated automated multicapillary CZE system has become available with standardised running conditions and reagents (Paragon CZE 2000, Beckman Instruments). On this multicapillary instrument, 7 serum samples can be analysed at the same time for serum protein screening. Furthermore, immunosubtraction capillary electrophoresis is completely automated on the Paragon CZE 2000. This section reviews the results achieved on different CZE systems and focuses on the separation of serum proteins in five fractions and the detection and identification of monoclonal components.

Separation of serum proteins into five fractions

Peak resolution

Fig. 3 shows the CZE electropherogram of a normal serum sample. In general agarose electrophoresis is not suitable for identification of a separate prealbumin (transthyretin) fraction. However, in capillary electrophoresis it is possible to quantify prealbumin, because of its clear resolution. However, an inter-individual variation of 31%, makes CZE not suitable for

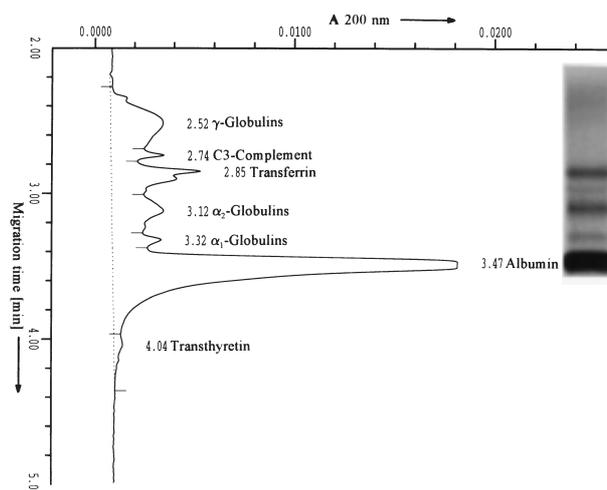


Figure 3. Capillary zone electropherogram of a normal serum sample.

reliable quantification of prealbumin (11). Apart from prealbumin a more clear separation is also possible for the beta-fraction giving a separate C3 and transferrin peaks. Precise localisation of C3 and transferrin using CZE results in more accurate detection of the beta-fraction. When C3 appears in the gamma-fraction it is not detected as a separate peak in the agarose electropherogram, whereas it is in CZE. Generally the beta peak is overestimated in AGE because it overlaps with the gamma-fraction. Fig. 4 shows a serum sample with a clearly separated monoclonal band in the gamma region. Fig. 5 shows an example of a serum sample, where AGE showed a band on the application slot. This occurs when large molecules are kept in the agarose layer and cannot be separated. In capillary zone electrophoresis this artefact disappears and the band appears in the gamma-fraction.

Reproducibility and accuracy

In most clinical laboratories, protein electrophoresis is used as a screening method for detection of major abnormalities of the main proteins in biological fluids

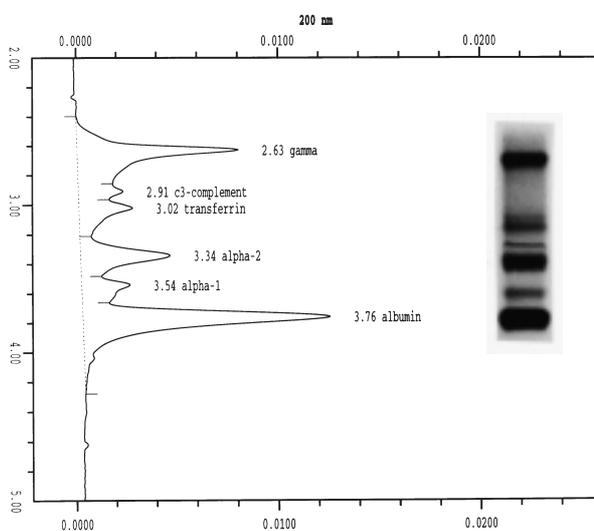


Figure 4. Capillary zone electropherogram of a serum sample containing a monoclonal component typed IgM lambda.

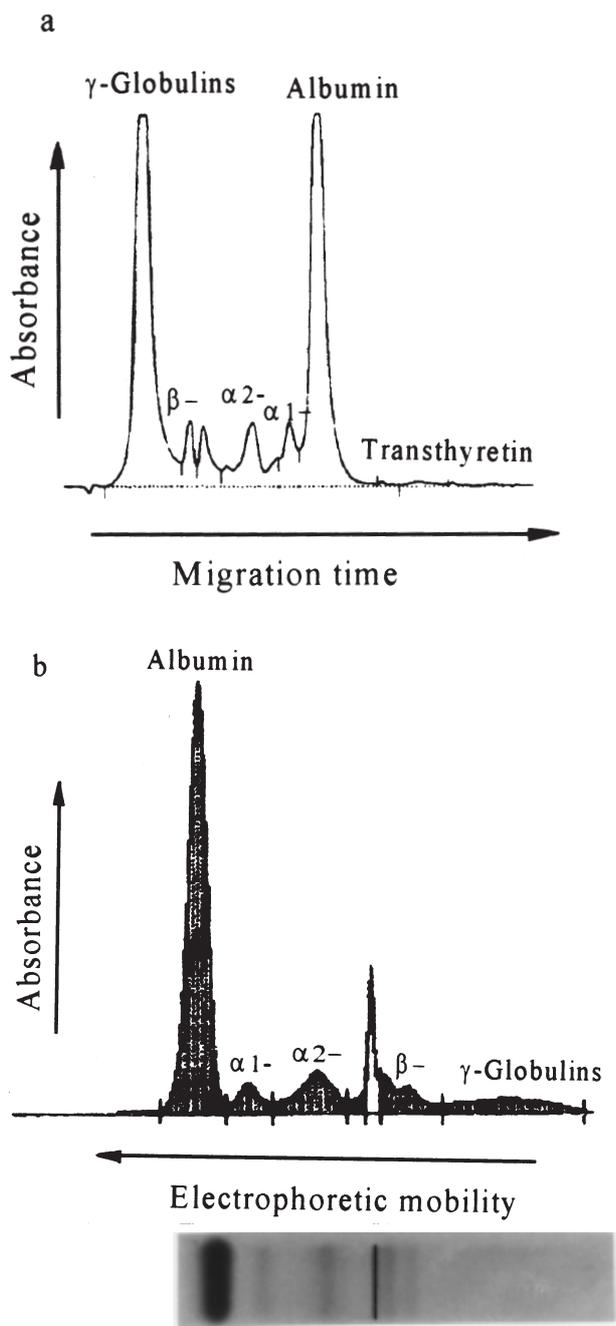


Figure 5. Capillary zone electrophoresis (a) and agarose electrophoresis (b) of a sample containing large proteins that remain at the application slot after agarose electrophoresis separation and not after separation with capillary zone electrophoresis.

like serum, urine and cerebrospinal fluid and not for quantitative purposes. From an analytical point of view it is interesting to compare the reproducibility of capillary electrophoresis (migration times and protein quantification) with existing methods. Variation in migration times on different days, using different capillaries and with different control sera, on a single capillary CZE instrument, appears to be less than 3.5% for all fractions, except for prealbumin, where a variation of 4.7% was found. The variations in relative peak areas are sometimes higher with capillary electrophoresis, compared to agarose electrophoresis (11). For the dedicated automated multicapillary system (Paragon CZE 2000), reproducibility data were

found (28, 29) which were comparable to the study with the single capillary CZE instrument (11). The recovery of artificially prepared protein mixtures (albumin and gamma-globulins) was studied using the CZE technique and resulted, when compared to the theoretical values, in similar values as the AGE technique (11).

Method comparison: percentages of the five fractions
Using the single capillary CZE system Wijnen et al. (11) found a good correlation of CZE with AGE and even better compared to the correlation reported by Kim et al. (7). For the five fractions correlation coefficients were found ranging from 0.90 (alfa1-globulins) to 0.99 (alfa2-globulins) (11). With the automated Paragon CZE 2000 system a better correlation was found with cellulose acetate, correlations varied from 0.85 to 0.97, compared to agarose gel electrophoresis, where the correlations were between 0.52 and 0.67 (28).

Reference values

Table 1 presents the inter-individual variations (reference values) obtained by measuring 140 serum samples of normal healthy controls (11). Bossuyt et al compared reference values of agarose electrophoresis and cellulose acetate electrophoresis with capillary zone electrophoresis. Compared with agarose electrophoresis substantially higher values for the alpha1-fraction and lower values for the gamma-fraction were found using capillary electrophoresis (28). Apart from the differences found by Bossuyt et al, Wijnen et al. found a substantially lower value for the albumin fraction using capillary zone electrophoresis.

Detection and identification of monoclonal components in serum by CZE

Studies on CZE using a single capillary instrument

Between 1995 and 1998 a number of studies have been published on the method comparison of the detection of monoclonal components (MCs) using single capillary CZE. Since 1998 most method comparison studies on CZE and serum protein screening were performed on the multicapillary Paragon CZE 2000 system (see the next section). Jenkins (4) published one of the first method comparison studies on CZE and monoclonal band detection and included 1000 specimens, containing 362 MCs with protein concentrations ranging from 1 to 71 g/l. CZE detected 4 MCs that were not found by AGE (three IgA and one IgG). On the other hand, CZE failed to detect two IgM MCs that were not visible on the electropherogram but caused a change of retention times for the five major protein fractions. Furthermore 8 small MCs were missed by CZE. A comparable study was published by Clark et al (30). They concluded that the results obtained by CZE were comparable or better than those obtained with AGE. Doelman et al (31) investigated 250 serum samples for the presence of MCs and found only minor differences between CZE and AGE. Both methods appeared to miss a few small MCs that could be detected by conventional IFE.

Table 1. Reference values for CZE and AGE (n=140)

Fractions	<i>Capillary zone electrophoresis</i>				
	Mean		CV	Median	Reference
	%	g/l	range %	%	%
albumin	55.9	37.1	5.98	55.6	49.2 - 62.6
α_1	5.62	3.73	21.2	5.60	3.24 - 8.01
α_2	8.79	5.84	18.5	8.74	5.53 - 12.1
β	15.3	10.2	13.7	15.3	11.1 - 19.5
γ	14.4	9.58	18.4	14.4	9.08 - 19.7
Fractions	<i>Agarose electrophoresis</i>				
	Mean		CV	Median	Reference
	%	g/l	range %	%	%
albumin	63.3	42.1	5.19	63.4	56.7 - 69.8
α_1	3.39	2.25	19.9	3.3	2.04 - 4.74
α_2	8.48	5.64	17.1	8.4	6.58 - 10.4
β	13.5	8.98	18.1	13.5	8.57 - 18.3
γ	11.4	7.58	21.8	11.5	6.43 - 16.4

Albumin fraction: prealbumin + albumin; β -fraction: C_3 + transferrin. Total protein 66.5 ± 3.5 g/l (mean \pm SD), range 56.5 - 75.6 g/l. Albumin 39.7 ± 2.7 g/l (mean \pm SD), range 32.4-45.3 g/l

Most of the studies concerning the use of CZE in the clinical laboratory focused on the detection of monoclonal bands on the electropherogram. Therefore, Henskens et al (12) investigated not only detection of MCs by CZE but also their identification by immunosubtraction capillary electrophoresis (CZE/IS). Electropherograms containing suspected MCs were identified by CZE/IS. In total, 74 MCs were detected by both methods. The routine method (AGE) demonstrated 73 MCs out of 468 serum samples. Using CZE, 70 MCs were demonstrated. Four MCs were not detected by CZE of which three were IgG of 0.6, 1.1 and 2.2 g/l, respectively. One was an IgM MC of 20.3 g/l according to routine method. Although the running conditions were optimised for MC detection, an IgM band of 20.3 g/l could not be detected by CZE. Changing the dilution of this sample did not reveal a peak in the gamma region. On the other hand, changing the ionic strength (from 150 to 130 mmol/l) or the pH (from 9.9 to 10.0) of the running buffer did result in a spike in the gamma region. Using densitometric scanning 58 MCs could be quantified from the agarose gel electropherogram, having protein concentrations ranging from 0.6 to 50.9 g/l. One large MC (IgG, 53.9 g/l) could not be quantified by routine densitometric scanning since the colouring of the band was too intense and could not be quantified by CZE.

Furthermore, one small monoclonal band, not detected by AGE, was demonstrated by CZE and subsequently identified by CZE/IS (IgA lambda). The routine IFE could confirm this MC. Except for one MC (IgM kappa) all MCs could be typed by CZE/IS. Furthermore, it was sometimes difficult to type small MCs in a polyclonal raised background using CZE/IS. Correlation between monoclonal protein concentration as determined by the two methods was 0.95. In contrast to Jenkins et al (4), for all MCs types, the CZE quantification's resulted in higher mean protein values compared to AGE. These differences were statistically significant. The results of Henskens et al (12) showed that the composition of the running buffer appears to be of major importance. Ionic strength and pH of the running buffer have in all cases a large influence on the capillary electrophoresis pattern. Just how crucial the buffer pH is, was demonstrated by a 20.3 g/l IgM MC, not detected at pH 9.9, that reappeared in the pre-gamma region at pH 10.0. This phenomenon has been described before by Jenkins and Guerin (26). They reported eight MCs (4) that did not separate correctly on CE using their standard buffer conditions. These MCs could only be demonstrated on the electropherogram by minor changes in buffer molarity and pH (26).

Studies on CZE using a multicapillary CZE instrument: Paragon CZE 2000

The Paragon CZE 2000 system (Beckman Instruments) is a multicapillary instrument designed for the automation of both the detection and identification of monoclonal components in serum. The last two years this dedicated CZE instrument has been extensively studied on reproducibility (29, 32), carry-over (29, 32) and compliance with the conventional methods for MC detection and typing (29, 33-36). Litwin (34) found excellent results for MC detection by CZE, but concluded that CZE/IS was less accurate than IFE in determining the immunotype of the monoclonal gammopathy. Only 60 to 75 % of the MCs were correctly immunotyped with CZE/IS by 4 reviewers. In this respect they comment that it is difficult to immunotype (CZE/IS) small MCs in a background of polyclonally raised total immunoglobulins. On the other hand, Joliff (35) demonstrated 100 % agreement in MC classification using CZE/IS compared with IFE. They also studied the detection limits for monoclonal gammopathies, providing that the MCs were not comigrating with other proteins: IgG 0.5 g/l, IgM 0.75 g/l and IgA 0.75 g/l. Comparable detection limits were reported by Bienvenue (29). Henskens et al evaluated the performance of the Paragon CZE 2000 system in two Dutch hospital laboratories (36). The conventional methods that were used in this comparison were AGE and IFE. In total, 578 serum samples were first analysed in both hospitals using CZE, AGE and IFE. CZE/IS was only performed on samples that were positive for a MC based on the CZE pattern. Fig. 6 shows an example of the CZE immunosubtraction method that was used to immunotype MCs in a serum sample. The disappearance of the large spike in the gamma region indicates the type

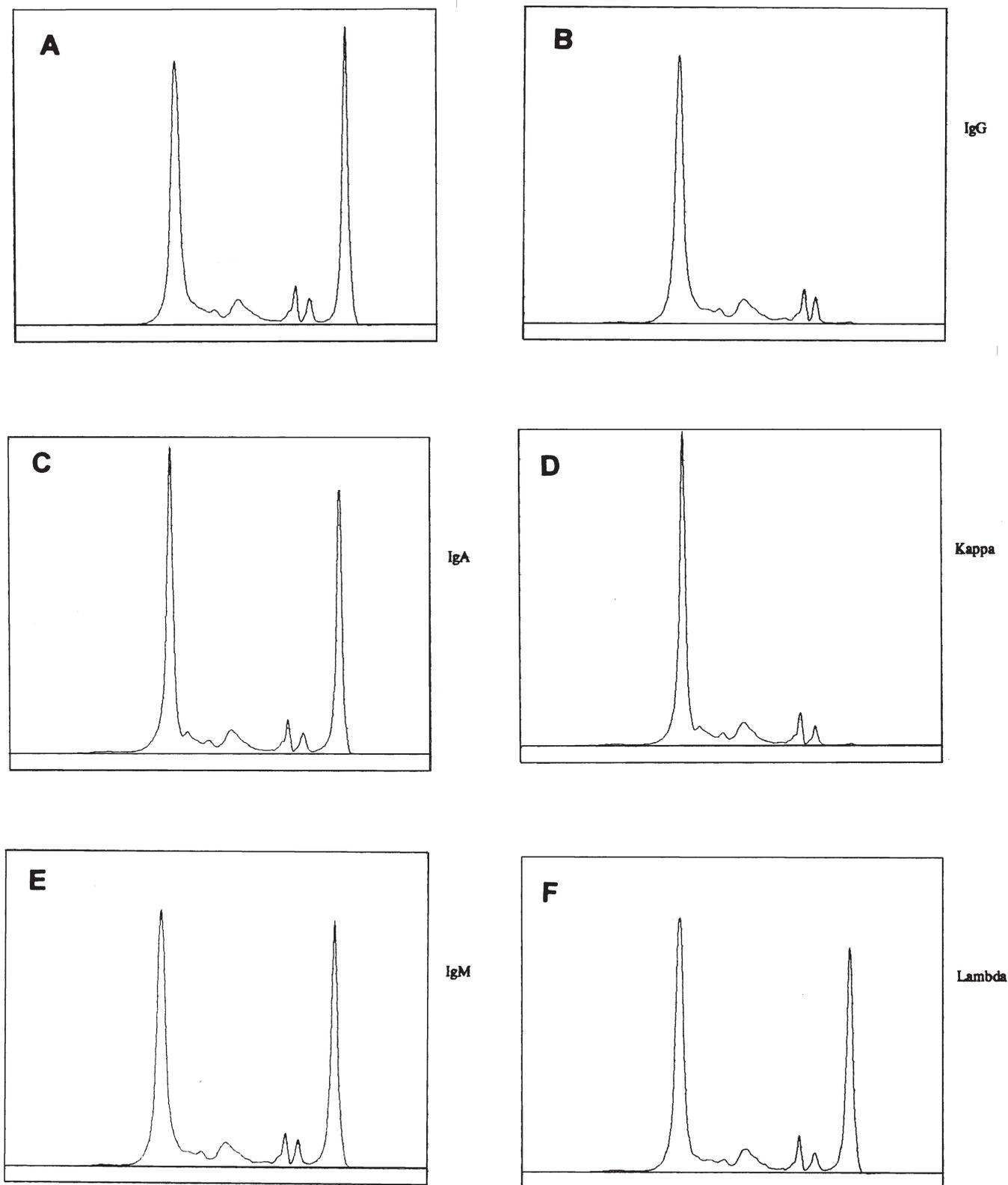


Figure 6. Example of immunosubtraction electropherograms (CZE/IS) of a serum samples with a MC (IgG kappa, 24.4 g/l, migrating in the gamma region). Classes or types of immunoglobulin are removed from a serum sample using a binder specific for the immunoglobulin, coupled to a solid support. The serum sample is exposed to five antibodies (coupled to beads) against the heavy chains of IgG, IgA, and IgM and the kappa and lambda light chains. Before (A) and after (B: IgG, C: IgA, D: kappa, E: IgM, F: lambda) exposure to each of the antibodies, the treated sera and an untreated control serum are separated by CZE. The disappearance (immunosubtraction) of the large spike in the gamma region indicates the type of the monoclonal component.

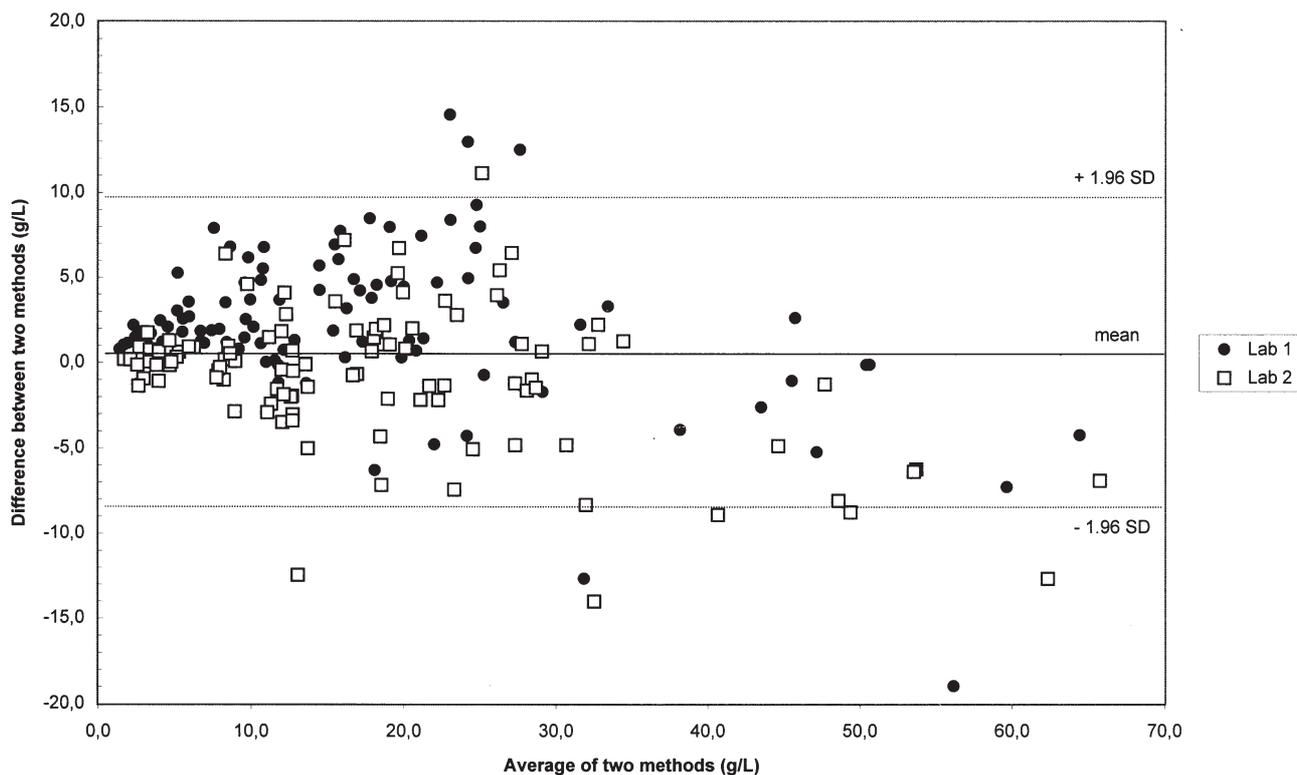


Figure 7. Bland and Altman analysis shows the differences between methods in the protein concentration of monoclonal components plotted against the average between the methods for two laboratories.

of the monoclonal component. Similar results (between methods and hospitals) were found on 558 serum samples that contained 124 monoclonal components. Differences in the detection or identification of MCs, which were found in 20 serum samples, concerned very small MCs. Fig. 7 shows the Bland and Altman analysis of the comparison of protein quantification of the MCs ($r = 0.96$). Overall, the authors conclude that all methods, even IFE, miss small MCs and that CZE/IS gives almost 100 % comparable results with IFE in immunotyping MCs. The Paragon CZE 2000 multicapillary system gives reliable results and can be used to automate both AGE and IFE in a routine clinical laboratory.

Summary of advantages and disadvantages of CZE in the separation of serum proteins

Automation

An important advantage of CZE for routine clinical laboratories is the full automation of MC detection and identification. Sample loading, separation and detection are all computer programmed and therefore standardized and patient results (electropherograms) can be directly stored into the laboratory computer. This in contrast to the traditional methods such as agarose gel electrophoresis and immunofixation electrophoresis which are labour intensive, time-consuming and lack the possibility of hard copy of patient data.

Resolution

Several authors demonstrated a major advantage of CZE, due to its better resolution, in the detection of a

gammopathy of the IgA type. Using AGE the IgA monoclonal band appears as a slightly elevated beta fraction that can be easily misinterpreted as an elevated transferrin. In the CZE electropherogram even a small IgA peak is distinctly separated from the transferrin band. Other MCs that co-migrate in the beta region can be missed by AGE. These MCs are much better detectable by CZE because of the better resolution in this area. Most investigators of CZE studies have reported this advantage of CZE (4, 5, 12, 33, 34, 37).

Monoclonal light chains often are difficult to detect with AGE. Although CZE is more sensitive for detecting light chains compared to AGE or CAE (30, 33) there is also a report of CZE missing them in clinically important cases (37). Another advantage of CZE is that point-of-application artefacts don't occur using CZE (see also Fig. 5 (11)).

Immunosubtraction technique

The Paragon CZE 2000 is a capillary electrophoresis system in which monoclonal component detection (alternative for traditional AGE) and identification (alternative for IFE) are combined and automated in one instrument. This is an advantage for use in a routine clinical laboratory. The interpretation of CZE immunosubtraction electropherograms is simple and easy (Fig. 6). On the other hand, the interpretation of the CZE/IS pattern of small IgG bands on a polyclonal background is more difficult compared to the routine IFE pattern since the polyclonal IgG in the gamma region will also be subtracted by the anti-IgG antibody using IS-CE. In contrast, Clark et al (38) conclude that small monoclonal bands in a polyclonal

raised background are sometimes easier to detect and identify with CZE and CZE/IS compared by AGE and IFE.

Detection and quantification

In comparison to AGE and IFE, CZE sometimes appears to miss small MCs (1-4 g/l). This is not relevant for monoclonal component detection in the diagnosis of multiple myeloma but might be a problem for detecting small amounts of a monoclonal components which are seen by e.g. polyneuropathy. Furthermore, studies performed on the standardized multicapillary Paragon CZE 2000 system appear to give better results in comparison with AGE than the single capillary instruments. Quantification of large bands is more accurate on CZE compared to AGE. CZE has on-line protein detection at about 200 nm, which makes time-consuming staining of agarose gels and densitometric scanning unnecessary. In practice, protein staining of large bands on the agarose gel is often incomplete and different bands display different dye uptake. Quantification of MCs by CZE depends on the height of the spike, the polyclonal background and the integrating software of the CZE system. For example, Henskens et al (12) reported eight small MCs that were more difficult to quantify on CZE, because of the fact that the computer software failed to recognise small bands on the CZE electropherogram as separate peaks. So, these bands were visible on the CE electropherograms whereas the relative peak areas could not be calculated. The latest CZE instruments have overcome these problems.

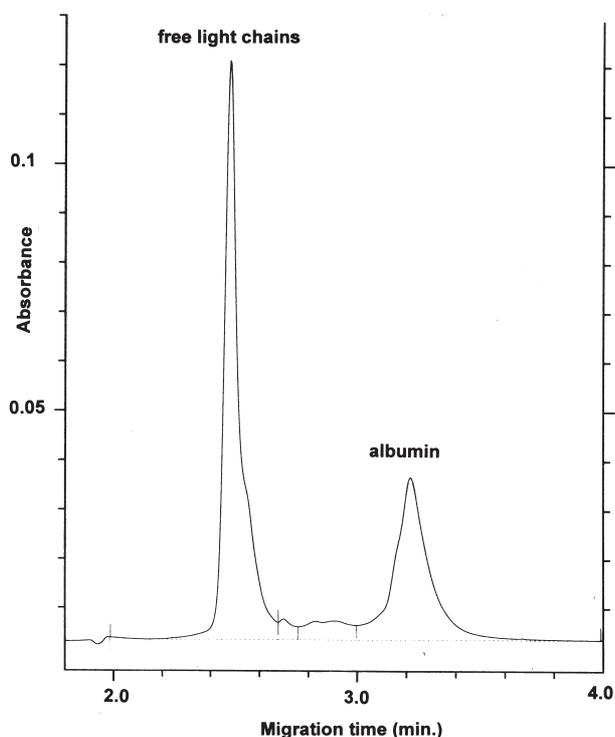


Figure 8. Agarose gel electrophoresis (AGE) and capillary zone electrophoresis (CZE) of urine. A urine sample containing albumin and Bence Jones proteins by CZE.

IgD or IgE immunotyping

At this moment it is not possible to obtain reagents for the detection of IgD or IgE monoclonal components in serum by CZE/IS. In practice this means that the traditional methods such as AGE and IFE have to be maintained in the laboratory for immunotyping IgD or IgE.

Detection of urinary proteins by CZE

Urine protein analysis is important for the diagnosis of disorders such as Bence Jones (BJ) proteinuria and nephrotic syndrome. Hypogammopathy should always be followed by a method to exclude light chain disease. Applications of capillary electrophoresis in nephrology have been reviewed by Stocking et al (18) and Oda et al (15). The analysis of urine proteins that originate from serum by CZE can be more difficult than CZE analysis of serum because urine samples contain components such as electrolytes, organic acids and other metabolites that can interfere with the CZE technique. Other problems are urine components that absorb at the same wavelength that is used for protein detection or urine samples that vary widely between individuals in protein concentration. To prevent these problems urine samples normally have to be pre-treated before CZE analysis by filtration (13, 17) dialysis or precipitation (17). On the other hand, Jenkins (19) published a study in which they applied urine on CZE without pre-treatment (see next section). The current configuration of the automated and dedicated multicapillary Paragon CZE 2000 system is not yet suitable for the analysis of urine specimens since the fixed software settings and reagents are not optimised for urine analysis.

Bence Jones proteins

Henskens et al (13) developed a CZE method to detect Bence Jones proteins in urine using a single CZE system. In a pilot study they compared CZE analysis of 10 normal urine samples and 10 samples containing Bence Jones proteins with the routine analysis (AGE). Before analysis (CZE and AGE) urine samples were concentrated 200 times. For CZE of urine the same running conditions were used as serum CZE analysis (12). Reproducible electropherograms of normal urine samples could only be obtained after a desalting step. Normal urine samples (n=10) showed no spikes in the electropherogram whereas all samples containing Bence Jones proteins showed a peak in the gamma region. Fig. 8 shows an example of the CZE electropherogram of a urine sample with free light chains (Bence Jones proteins) and albumin. Using this method a selective urinary protein loss can also be detected. Jenkins (19) demonstrated a CZE method in which Bence Jones proteins were detected in centrifuged unconcentrated urine samples which were directly diluted in running buffer and injected into the capillary and which had protein concentrations ranging from 0.004 to 9.7 g/l. Summarising, using single channel CZE instruments several authors have shown that Bence Jones proteins can be detected using the CZE method. On the other hand, the dedicated Paragon CZE 2000 system for the serum protein investigation has no application for urine analysis yet.

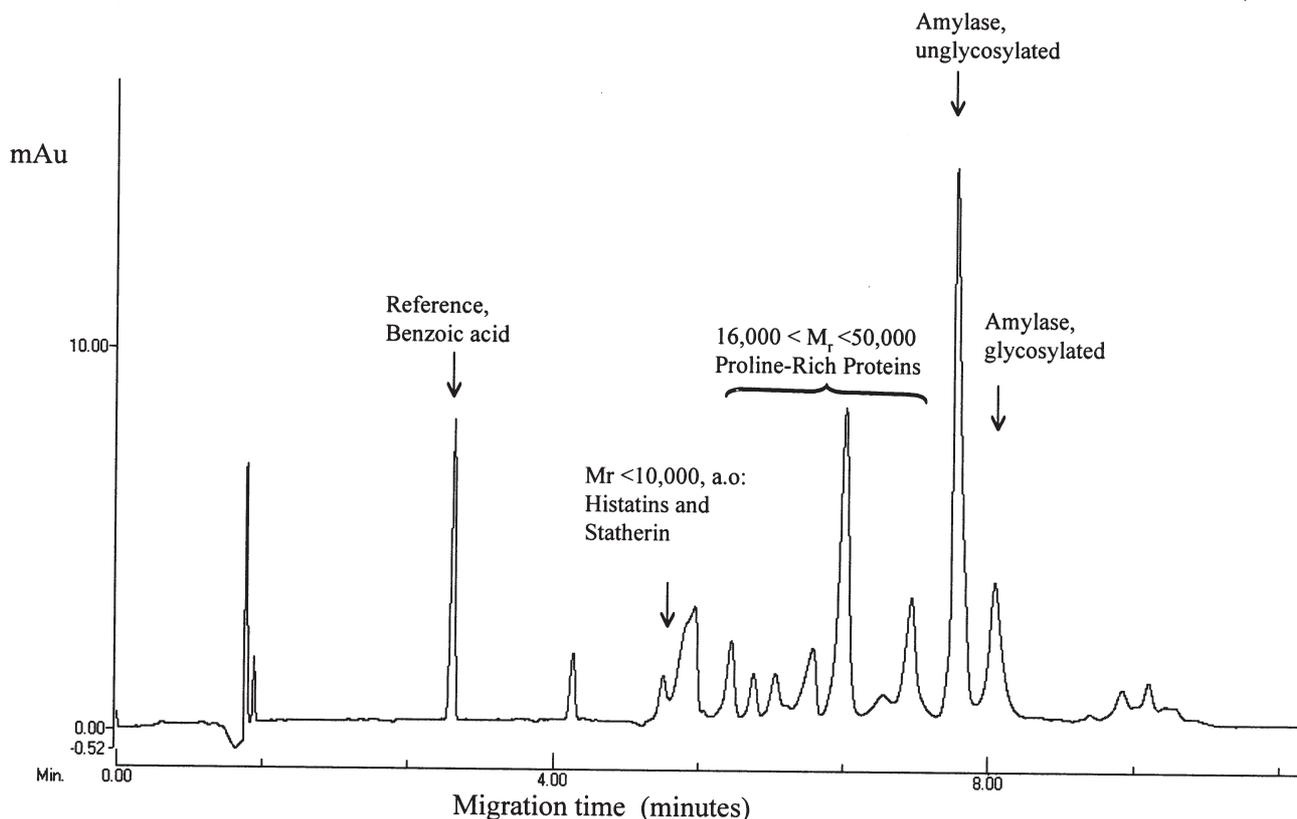


Figure 9. Separation of typical proteins of human parotid saliva (amylase, proline-rich-proteins, histatins, statherins) by SDS capillary electrophoresis on the BioFocus 2000 (Biorad) Running conditions: CE-SDS-protein running buffer, 24 cm uncoated capillary, 50 μ m ID, voltage 15 kV, temperature 20 $^{\circ}$ C, pressure injection 100 psi/sec, detection wavelength 220 nm.

Detection of proteins in cerebrospinal fluid by CZE

Capillary zone electrophoresis has been used by a small number of investigators to analyse cerebrospinal fluid (CSF) for several purposes: general screening of CSF (39), oligoclonal banding (40) or detection of beta-trace protein in CSF of patients with central nervous system diseases (41, 42). CSF analysis in a routine clinical laboratory is often performed to investigate oligoclonal bands (gamma globulins) as a diagnostic marker for multiple sclerosis. Sanders et al developed a CZE method for detection of oligoclonal bands and compared CZE analysis of CSF of 54 patients to their conventional electrophoresis methods (40). To improve the resolution of the CZE electropherograms they added polyethylene glycol and zwitterionic additives to their running buffer. Although Sanders et al found promising results (90% concordance with the conventional method) this field is still in development and further research is needed to recommend CZE as a suitable alternative on CSF analysis.

Detection of proteins in saliva by CZE

Salivary proteins are mainly studied for research purposes or for future diagnostic possibilities in relation to dental caries or oral inflammatory diseases. Some investigators use capillary electrophoresis to replace conventional SDS-polyacrylamide gelelectrophoresis (22, 43). An example of a diagnostic purpose of saliva is the analysis of the protein composition of parotid saliva that can be used to monitor the effects of

pathological conditions, including Sjögrens syndrome and radiotherapy, on the functioning of the parotid glands. Conventional electrophoretic analysis of parotid saliva by SDS-PAGE is hampered by the fact that a number of (proline-rich) proteins are only poorly stained by Coomassie Brilliant Blue. Capillary electrophoresis using a SDS running buffer of parotid saliva permits quantitation of the main protein components in these samples, including amylase and a number of proline rich proteins. An example of a capillary electropherogram of a parotid saliva sample is given in Figure 9. (personal communication and gift from dr. E. Veerman, Department of Oral Biology, Academic Centre for Dentistry, Amsterdam).

Conclusion

Capillary zone electrophoresis (CZE) is a very useful technique, suitable for reliable quantification and separation of serum proteins. Total variation of capillary zone electrophoretic detection is slightly higher compared to agarose electrophoresis. Using capillary zone electrophoresis a clear separation of the complement C3 and transferrin fraction in the beta-region of the electropherogram is possible. The same holds for the separation of prealbumin. Generally, a better peak resolution is obtained with capillary zone electrophoresis. Capillary zone electrophoresis is also a suitable method to automate serum analysis in the detection and immunotyping of monoclonal components. Sometimes interpretation of the CZE electropherogram is difficult, but this can also be the case

with the classical serum protein electrophoresis methods like agarose gel electrophoresis or cellulose acetate electrophoresis. A detection limit of 0.5-0.75 g/l has been found for CZE, which apart from light chain disease and polyneuropathy, is far beyond the clinically relevant concentration of monoclonal component detection in the diagnosis of multiple myeloma. Applications for routine CZE analysis of urine or cerebrospinal fluid analysis are still in development and might be suitable to replace the conventional methods in the near future.

Literature

- Chen FT, Liu CM, Hsieh YZ, Sternberg JC. Capillary electrophoresis - a new clinical tool. *Clin Chem* 1991; 37 (1): 14-9.
- Chen FT, Sternberg JC. Characterization of proteins by capillary electrophoresis in fused-silica columns: review on serum protein analysis and application to immunoassays. *Electrophoresis* 1994; 15 (1): 13-21.
- Hiraoka A, Miura I, Hattori M, Tominaga I, Machida S. Capillary-zone electrophoretic analyses of the proteins and amino acid components in cerebrospinal fluid of central nervous system diseases. *Biol Pharm Bull* 1993; 16 (10): 949-52.
- Jenkins MA, Kulinskaya E, Martin HD, Guerin MD. Evaluation of serum protein separation by capillary electrophoresis: prospective analysis of 1000 specimens. *J Chromatogr B Biomed Appl* 1995; 672 (2): 241-51.
- Jenkins MA, Guerin MD. Quantification of serum proteins using capillary electrophoresis. *Ann Clin Biochem* 1995; 32 (Pt 5): 493-7.
- Jenkins MA, Guerin MD. Capillary electrophoresis as a clinical tool. *J Chromatogr B Biomed Appl* 1996; 682 (1): 23-34.
- Kim JW, Park JH, Park JW, Doh HJ, Heo GS, Lee KJ. Quantitative analysis of serum proteins separated by capillary electrophoresis. *Clin Chem* 1993; 39 (4): 689-92.
- Jenkins MA, Guerin MD. Capillary electrophoresis procedures for serum protein analysis: comparison with established techniques. *J Chromatogr B Biomed Sci Appl* 1997; 699 (1-2): 257-68.
- Landers JP. Clinical capillary electrophoresis. *Clin Chem* 1995; 41 (4): 495-509.
- Stob S, Lauer HH, Swart A. Capillaire zone elektroforese in de klinische chemie. *Tijdschr Ned Ver Klin Chem* 1993; 18: 299-305.
- Wijnen PA, van Dieijen-Visser MP. Capillary electrophoresis of serum proteins. Reproducibility, comparison with agarose gel electrophoresis and a review of the literature. *Eur J Clin Chem Clin Biochem* 1996; 34 (7): 535-45.
- Henskens Y, de Winter J, Pekelharing M, Ponjee G. Detection and identification of monoclonal gammopathies by capillary electrophoresis. *Clin Chem* 1998; 44: 1184-90.
- Henskens YMC, Ponjee GAE. Method for the detection of Bence Jones proteins by capillary electrophoresis (abstract). *Ned Tijdschr Klin Chem* 1998; 23: 68.
- Jenkins MA, O'Leary TD, Guerin MD. Identification and quantitation of human urine proteins by capillary electrophoresis. *J Chromatogr B Biomed Appl* 1994; 662 (1): 108-12.
- Oda RP, Clark R, Katzmann JA, Landers JP. Capillary electrophoresis as a clinical tool for the analysis of protein in serum and other body fluids. *Electrophoresis* 1997; 18 (10): 1715-23.
- Shihabi ZK. Myoglobinuria detection by capillary electrophoresis. *J Chromatography B* 1995; 669: 53-58.
- Friedberg MA, Shihabi ZK. Urine protein analysis by capillary electrophoresis. *Electrophoresis* 1997; 18 (10): 1836-41.
- Stocking CJ, Slater JM, Simpson CF. Applications of capillary electrophoresis in nephrology. *Exp nephrol* 1998; 6: 415-420.
- Jenkins MA. Clinical application of capillary electrophoresis to unconcentrated human urine proteins. *Electrophoresis* 1997; 18: 1842-1842.
- Grimshaw J, Trocha-Grimshaw J, Fisher W, Rice A, Smith S, Spedding P, et al. Quantitative analysis of hyaluronan in human synovial fluid using capillary electrophoresis. *Electrophoresis* 1996; 17(2): 396-400.
- Sharif M, Osborne DJ, Meadows K, Woodhouse SM, Colvin EM, Shepstone L, et al. The relevance of chondroitin and keratan sulphate markers in normal and arthritic synovial fluid. *Br J Rheumatol* 1996; 35 (10): 951-7.
- Lal K, Xu L, Colburn J, Hong AL, Pollock JJ. The use of capillary electrophoresis to identify cationic proteins in human parotid saliva. *Arch Oral Biol* 1992; 37 (1): 7-13.
- Aguzzi F, Poggi N. "Immunosubtraction" electrophoresis: a simple method for identifying specific proteins producing the cellulose acetate electrophoretogram. *Boll Ist Sieroter Milan* 1977; 56 (3): 212-6.
- Shihabi ZK, Hinsdale ME. Some variables affecting reproducibility in capillary electrophoresis. *Electrophoresis* 1995; 16 (11): 2159-63.
- Klein GL, Joliff CR. Capillary electrophoresis for the routine clinical laboratory. CRC press Inc. CRC handbook of capillary electrophoresis 1994; 419-457.
- Jenkins MA, Guerin MD. Optimization of serum protein separation by capillary electrophoresis [letter]. *Clin Chem* 1996; 42 (11): 1886.
- Keren DF. Detection and characterization of monoclonal components in serum and urine (editorial). *Clin Chem* 1998; 44: 1143-1144.
- Bossuyt X, Schiettekatte G, Bogaerts A, Blanckaert N. Serum protein electrophoresis by CZE 2000 clinical capillary electrophoresis system. *Clin Chem* 1998; 44 (4): 749-59.
- Bienvenu J, Graziani MS, Arpin F, Bernon H, Blessum C, Marchetti C, et al. Multicenter evaluation of the Paragon CZE 2000 capillary zone electrophoresis system for serum protein electrophoresis and monoclonal component typing. *Clin Chem* 1998; 44 (3): 599-605.
- Clark R, Katzmann JA, Wiegert E, Namyst-Goldberg C, Sanders L, Oda RP, et al. Rapid capillary electrophoretic analysis of human serum proteins: qualitative comparison with high-throughput agarose gel electrophoresis. *J Chromatogr A* 1996; 744 (1-2): 205-13.
- Doelman CJA, Siebelder CWM, Penders TJ. Routine serum protein analysis using capillary electrophoresis. (letter). *Eur J Clin Chem Clin Biochem* 1997; 35: 393-394.
- Bossuyt X, Bogaerts A, Schiettekatte G, Blanckaert N. Serum protein electrophoresis and immunofixation by a semiautomated electrophoresis system. *Clin Chem* 1998; 44 (5): 944-9.
- Bossuyt X, Bogaerts A, Schiettekatte G, Blanckaert N. Detection and classification of paraproteins by capillary immunofixation/subtraction. *Clin Chem* 1998; 44 (4): 760-4.
- Litwin CM, Anderson MT, Philipps G, Martins TB, Jaskowski TD, Hill HR. Comparison of capillary zone and immunosubtraction with agarose gel and immunofixation electrophoresis and identifying monoclonal gammopathies. *Am J Clin Pathol* 1999; 112: 411-417.
- Jolliff CR, Blessum CR. Comparison of serum protein electrophoresis by agarose gel and capillary zone electrophoresis in a clinical setting. *Electrophoresis* 1997; 18 (10): 1781-4.

36. Henskens YMC, van de Water C, Lanser A, van der Meijden A, van der Kraaij A, de Winter J, H. Bekenes, W. van Gelder. Evaluation of the Paragon 2000 CZE system for the detection and identification of monoclonal components. (abstract) Tijdschr Ned Ver Klin Chem 2000; 25: 75.
37. Keren DF. Capillary zone electrophoresis in the evaluation of serum protein abnormalities. Am J Clin Pathol 1998; 110 (2): 248-52.
38. Clark R, Katzmann JA, Kyle RA, Fleisher M, Landers JP. Differential diagnosis of gammopathies by capillary electrophoresis and immunosubtraction: analysis of serum samples problematic by agarose gel electrophoresis. Electrophoresis 1998; 19(14): 2479-84.
39. Cowdrey G, Firth M, Firth G. Separation of cerebrospinal fluid proteins using capillary electrophoresis: a potential method for the diagnosis of neurological disorders. Electrophoresis 1995; 16(10): 1922-6.
40. Sanders E, Katzmann JA, Clark R, Oda RP, Shihabi Z, Landers JP. Development of capillary electrophoresis as an alternative to high resolution agarose electrophoresis for the diagnosis of multiple sclerosis. Clin Chem Lab Med 1999; 37(1): 37-45.
41. Hiroaka A. Detection by capillary electrophoresis of changes in the beta-trace protein levels in cerebrospinal fluid from patients with central nervous system diseases. Prostaglandins 1996; 51: 290.
42. Hiraoka A, Arato T, Tominaga I, Anjyo A. Capillary electrophoretic analyses of beta-trace protein and other low molecular weight proteins in cerebrospinal fluid from patients with central nervous system diseases. J Pharm Biomed Anal 1997; 15(9-10): 1257-63.
43. Liang H, Wang Y, Wang Q, Ruan MS. Hydrophobic interaction chromatography and capillary zone electrophoresis to explore the correlation between the isoenzymes of salivary alpha amylase and dental caries. J Chromatogr B Biomed Sci Appl 1999; 724: 381-8.

Ned Tijdschr Klin Chem 2000; 25: 229-232

Capillaire zone elektroforese en hemoglobinevarianten en -derivaten: de stand van zaken

C.J.A. DOELMAN en C.W. WEYKAMP

In dit artikel wordt de capillaire zone elektroforese met dynamische coating beschreven. Deze methode wordt op dit moment toegepast bij de analyse van hemoglobinederivaten en -varianten, zoals HbA1c, HbF, HbS. Deze methode, welke goede correlatie vertoont met andere methodieken, is zeer eenvoudig uitvoerbaar en heeft een korte analysetijd. Daarnaast biedt de automatisering en de digitale opslag een groot voordeel. Een overzicht van de literatuur wordt gegeven.

Verschillende elektroforetische technieken, zoals elektroforese in agargel, celluloseacetaat, isoelektrische focussing in polyacrylamide worden gebruikt voor de analyse van hemoglobinederivaten en hemoglobinevarianten. Ook HPLC-technieken worden toegepast (1). Voortbordurend op deze scheidingstechniek zijn er publicaties verschenen waarbij capillaire elektroforese wordt toegepast voor de analyse van hemoglobinevarianten en -derivaten. De capillaire elektroforetische scheidingstechniek is snel. Er wordt een geringe hoeveelheid reagens gebruikt en bovendien is de methode eenvoudig automatiseerbaar. De mogelijkheden voor scheiding van hemoglobinederivaten en -varianten zijn beperkt door de absorptie van eiwitten aan de wand van het capillair. Dit betekent,

naast de variabele snelheid van de elektroosmotische flow, dat de reproduceerbaarheid van de bepaling dan te wensen overlaat. Sommige onderzoekers hebben getracht dit probleem te verhelpen door de elektroforese uit te voeren bij een relatief hoge pH waardoor een sterke en constante electro-osmotische flow verkregen werd. Dit leidde tot een zeer sterke vermindering van de resolutie waardoor lange capillairen gebruikt dienden te worden wat weer een lange analysetijd tot gevolg heeft (2,3,4). Castagnola c.s. beschrijven methoden om hemoglobine tetrameren, globineketens en/of aminozuren te scheiden met behulp van capillaire zone elektroforese (CZE) of micellaire elektrokinetische capillaire chromatografie (MECC) (2). Ook wordt capillaire isoelektrische focussing met gecoate capillairen toegepast, waarmee getracht werd om hemoglobinevarianten te scheiden. Alhoewel analysetijden van rond de twintig minuten zijn beschreven, wordt de CIEF-techniek toch gezien als een techniek voor routineonderzoek. Ook werd aangegeven dat de mogelijkheid bestond om geglyceerd hemoglobine te scheiden van de andere hemoglobinederivaten, echter de data ontbreken daarvoor. Gezien de eenvoud van de techniek verdient het gebruik van capillaire zone elektroforese de voorkeur boven de capillaire isoelektrische focussing. De onder andere door onszelf geïntroduceerde techniek van capillaire zone elektroforese met een dynamische coating brengt voor het scheiden van hemoglobinevarianten en -derivaten een duidelijk voordeel. In dit artikel zullen wij deze techniek voor het voetlicht brengen.

Streekziekenhuis Koningin Beatrix, Winterswijk

Correspondentie: Dr. C.J.A. Doelman, Beatrixpark 1, Postbus 9005, 7100 GG Winterswijk
E-mail: c.j.a.doelman@skbwinterswijk.nl