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Review

Capillary electrophoresis as a clinical tool

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Abstract

Clinical laboratories must produce accurate results for patients with a minimum turn-around time. Automated commercial capillary electrophoresis instrumentation has been available to the clinical laboratory for the past five years. Our laboratory has utilised capillary electrophoresis (CE) to automate serum protein electrophoresis. We have used the technique of CE to produce clinical results for nearly two years. CE methods are also available for the quantitation of haemoglobin variants, by both isoelectric focusing and free solution techniques. Micellar electrokinetic separations by CE have been developed for some specialised drug assays and for B-group vitamin analysis, while gel-filled capillaries have the capability to separate DNA fragments, such as PCR products. Isoenzyme analysis has shown possibilities by CE, but quantitative results are needed to be clinically useful. Analysis of amino acids for newborn screening programs and as an arterial clotting indicator are being developed. The next five years should see a proliferation of clinical laboratory methods using automated CE.

Keywords: Reviews; Proteins

Contents

1. Introduction 24
2. Serum protein separations 24
3. Haemoglobin variants 28
4. Drug analysis 29
5. Vitamin analysis 30
6. Isoenzyme analysis 30
7. DNA separations 31
8. Metabolic disorders 31
9. Porphyria 32
10. Discussion 33
References 33

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1. Introduction

In the modern medical environment, patient diagnosis and treatment relies heavily on investigation procedures. With the introduction of productivity-based funding of laboratories, they are now required to provide rapid, cost-effective tests. This is often best achieved by the use of automated methods.

Capillary electrophoresis (CE) involves a separation of charged molecules in a buffer-filled capillary by the application of a very high voltage. The outstanding characteristic of CE is the high separation efficiency which can be achieved: 100 000 theoretical plates are able to be attained, at an applied voltage of 30 kV [1]. This allows an analysis time in the order of min [2], although some assays may take over 1 h for all components to be detected [3].

Areas of instrument design, injection methods and detection systems have been previously well described by a number of authors [4–6]. With commercially available CE instrumentation, automation of the assays is now available. Some instruments provide up to 50 position sample carousels, thus allowing sufficient space for a full overnight run.

The reproducibility of CE for serum protein assays in the authors' laboratory were of the order of 2.5% for intra-assay and 5.5% for inter-assay [7]. Other authors have obtained similar or slightly better precision [8].

As CE uses only nanolitres of buffer (often made in-house), the main consumable costs relate to the capillaries and sample cups. The automation of the system allows for considerably lower labour costs compared with other electrophoretic methods, including agarose gels.

The analytical capabilities of CE allow clinical laboratorians to consider automation of tests previously performed only on a manual basis. These include serum and urine protein electrophoresis, haemoglobin electrophoresis and vitamin studies. Assays for several newer drugs by CE could have significant advantages over the sometimes difficult HPLC techniques currently used.

2. Serum protein separations

The primary use of protein analysis by electrophoresis in the clinical laboratory is for the diagnosis of myeloma, a malignant disorder found in greater than 3% of the population, aged over 70 years. Myeloma is usually characterised by the finding of a monoclonal protein in the serum or the presence of monoclonal free light chains in the urine. For over twenty years, the presence of these monoclonal bands in serum and urine have been diagnosed by high resolution agarose gel electrophoresis, a procedure which is technically demanding and costly, due to it being labour intensive. Whilst there are automated instruments dedicated to gel electrophoresis, these are relatively expensive both in capital costs and consumables.

Tiselius [9], in his original "moving boundary" electrophoresis experiments in 1937, separated algae proteins and serum proteins. From his description, three peaks were obtained, one representing albumin and the other two representing globulin peaks. Jorgenson and Lukacs [1], in 1983, separated serum proteins by CE into six peaks, using a surface-modified capillary and UV detection at 230 nm. The comment was made that the classic serum bands, including albumin and globulin were apparent in the electropherogram.

Hjertén [10], in 1990, described a high resolution method for the separation of human serum in a multi-pH buffer system. Five protein peaks were detected at 215 mm using a 0.75 *M* Tris–HCl buffer system, the pH of the leading buffer being 9.7, while that of the stacking buffer was pH 6.8.

Through this early work with proteins, it was recognised that zone broadening of peaks could, in part, be related to adsorption of proteins to the walls of fused silica capillaries due to endosmotic effects related to exposed silanol groups. Two alternative approaches emerged as a possibility for minimising this adsorption: firstly, to use a coated capillary which in effect covered the silanol group and secondly, a possibility was to use fused silica capillaries, but to optimise the conditions so that adsorption is minimised.

The use of "coated" capillaries (where the silanol groups are reacted with various chemical reagents) provided a relatively simple solution to the problem of endosmosis. However, coated capillaries also introduced their own problems; it was difficult to find a compound to coat the capillary which was stable over a wide pH range. Also, reproducibility within batch and between batches of capillaries was

difficult to guarantee. In addition, certain washing procedures, such as the use of sodium or potassium hydroxide, tended to strip parts of the coating off the capillary.

Hjertén and Kubo [11] have described a high stability coating for capillaries which is attached to the silica surface via Si-O-Si-C bonds. This coating permits electrophoresis experiments to be carried out at pH extremes (such as at pH 12 or pH 0.3). The solution used for coating was a bifunctional silane (such as γ-methacryloxypropyltrimethoxysilane) mixed with acetic acid. Subsequently, the free methacryl groups were coupled to molecules containing allyl or acryl groups. Model proteins separated at pH 9.8 in these coated capillaries showed no adsorption in 50 runs performed over sixteen days.

Marked progress over the past four years has been made with serum protein separations in fused silica capillaries. In 1991, Chen et al. [12], in an industry-oriented paper, described serum protein separation into five bands, absorbance measurements being made at 214 nm. Whilst the composition of the proprietary buffer was not stated, the use of pH 10.0 ensured repulsion of the proteins from the silanol wall of the capillary.

At about the same time, Gordon et al. [13] described an approach for the minimisation of wall adsorption of proteins, by adding ethylene glycol to the protein sample which was in boric acid buffer at an acid pH, and using sodium borate decahydrate at pH 9.6 or above as the running buffer. A mixture of five individual proteins with a p*I* range of 4.6 to 6.2, as well as serum proteins from a patient with IgG myeloma, were separated using the system. Ethylene glycol multipeaks were identified either prior to the protein peaks or within the serum protein peaks. The authors felt that the resolving power of capillary zone electrophoresis (CZE) was superior to standard agarose gel electrophoresis, and also concluded that, without ethylene glycol, the electropherograms were not reproducible. Perhaps the stringent capillary washing that the authors followed also impeded the protein adsorbing to the capillaries walls. In addition, the molarity of borate and the higher pH also probably enhanced resolution. However, the electropherograms displayed were not similar to those produced by Chen et al. [12].

Later, Kim et al. [14] using a "home-made" CE apparatus similar to that described by Jorgenson and Lucas [1], assayed 37 samples, comparing the results to agarose gel electrophoresis and SDS–PAGE under non-reducing conditions. The 37 samples included normal samples as well as those from patients with various disease states, such as polyclonal gammopathy, liver cirrhosis, arthritis and chronic renal failure. All exhibited almost identical patterns in both CE and agarose gel electrophoresis. No serum from paraproteinaemic patients was reported. Linearity of response at 200 nm was established, the correlation between CE and agarose gels suggesting that CE was a reliable and reproducible technique for the clinical diagnosis of serum protein abnormalities.

Kim et al. [14] examined linearity plots of relative peak areas *versus* amount of serum proteins, and concluded that albumin and gamma fractions gave reasonable agreement compared to agarose electrophoresis. The comment was also made that the better resolution by CE compared to agarose gel electrophoresis would make it possible for more accurate quantitative analysis of serum proteins.

In another paper, Chen used a higher strength proprietary buffer at pH 10.0 to obtain up to ten peaks or inflections which related to various serum protein components [8]. This was obtained using an applied voltage of 10 kV in less than 4.5 min, absorbance measurements being made at 200 nm.

Our laboratory looked at the aspect of quantitation of serum proteins, particularly in regard to paraproteins, when we commenced investigations of serum proteins by CE [7]. By using a suitable dilution and injection time, we were able to calibrate the fused silica capillary, and quantitate the paraproteins by peak area, thus dispensing with the need for densitometry of high resolution agarose gel electrophoresis. For these measurements, 50 m*M* boric acid buffer, pH 9.7, was used, with 1 m*M* calcium lactate additive improving separation of the beta components into transferrin and C3. Monoclonal IgAs were often easier to identify, as they were separated from transferrin and C3 (see Fig. 1). Overall, the separation obtained resembled that of commercial high resolution agarose gel electrophoresis.

We then embarked on a prospective correlation of 1000 samples from patients, comparing CE and high resolution agarose gel electrophoresis (HRAGE) [15]. These specimens included 362 monoclonal paraproteins with concentrations ranging from 1 to 71 g/l. HRAGE gave slightly higher results for the

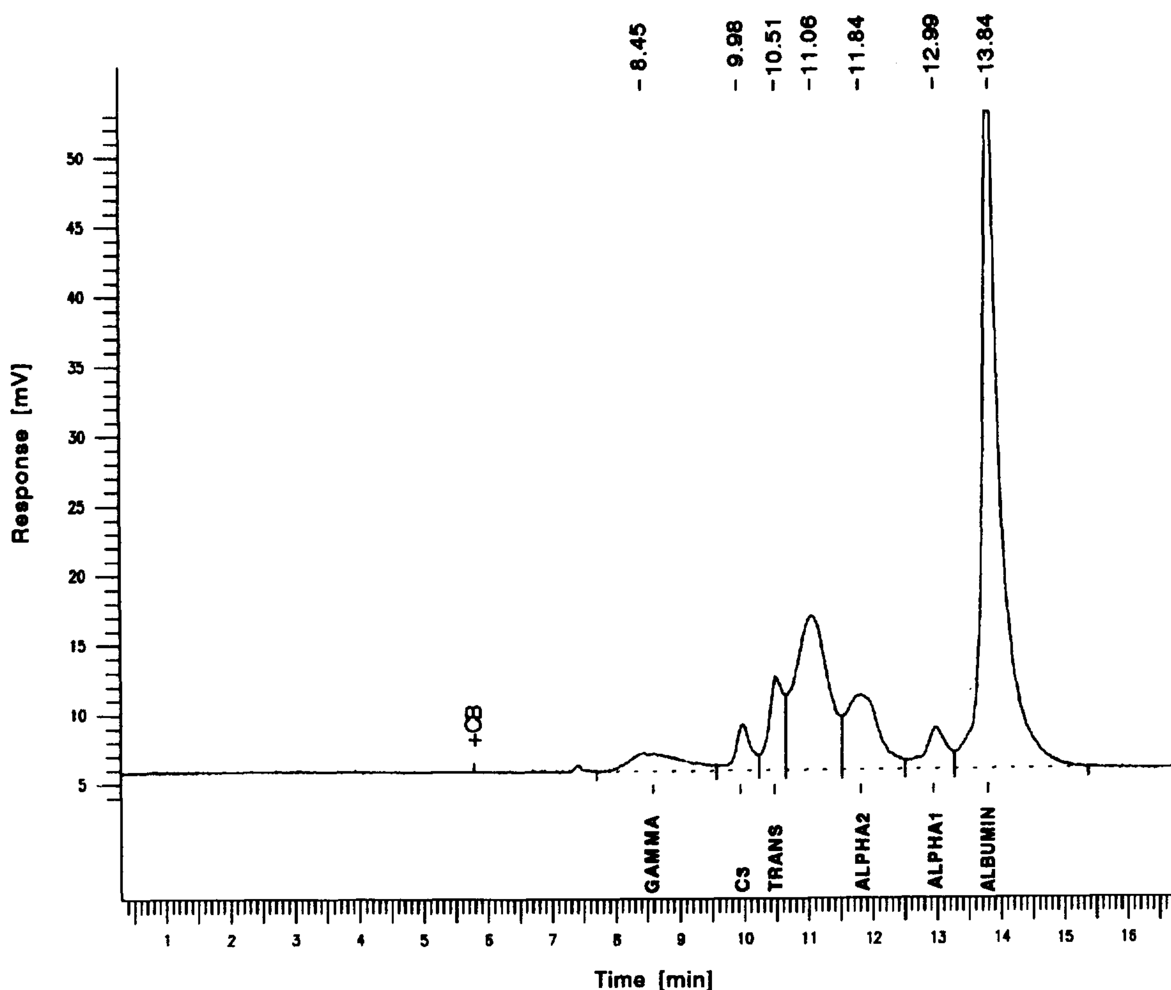

Fig. 1. Electropherogram of a monoclonal IgA detected by capillary electrophoresis showing separation from α2 and transferrin components. Conditions: Fused-silica capillary, 72 cm × 50 μm I.D.; detection, 200 nm; applied voltage, 18 kV; buffer, 50 mM boric acid, pH 9.7, containing 1 mM calcium lactate.

monoclonal bands, the quantitation of IgG and IgA paraproteins showing a statistically significant difference between the methods. Our conclusions indicated that automation of serum protein electrophoresis by CE was a cost-effective reality, and has been used routinely by our laboratory for nearly two years.

Identification of paraproteins after detection of a monoclonal band on agarose gel has routinely been achieved by immunofixation of either electrophoretic gels or isoelectric focusing [16–18]. Using CE, the identification of paraprotein can be based on immunosubtraction, an original concept proposed by Aguzzi and Poggi [19], in 1977. For CE identification of the paraprotein, the serum is first incubated with immunoglobulin-specific antibodies bound to solid supports, such as beads. When the specific protein is removed from the serum, a change is apparent when the treated serum is rerun [20]. This method is suitable for identification of IgG, IgA, IgM, κ and λ light chains. However, a number of complexities with the methodology lead one to question the cost-effectiveness of the procedure.

Analysis of proteins in human urine was alluded to

by Jorgenson and Lucas [21] when they separated dansyl amino acids in human urine. They were unable to identify the resulting peaks, as was Chen [12] when he dialysed urine through a membrane with a molecular mass cut-off of 14 000 [12]. Guzman et al. [22] demonstrated the co-elution of urea and creatinine. Our laboratory later identified albumin, Bence Jones Protein as well as a number of other proteins and quantitated the albumin and Bence Jones Protein by peak areas [23] (Fig. 2). This led to the correlation of albumin and Bence Jones Proteins in 71 patients by CE and high resolution agarose gels with correlation coefficients of 0.93 and 0.95, respectively. CE is now used routinely for the analysis of human urine proteins on a quantitative basis in our laboratory.

3. Haemoglobin variants

The diagnosis of haemoglobinopathies, by identification of haemoglobin variants, has relied on a variety of techniques. These include electrophoresis on cellulose acetate at alkaline pH and citrate agar at acid pH for common normal and abnormal bands. Ion exchange or affinity chromatography may be

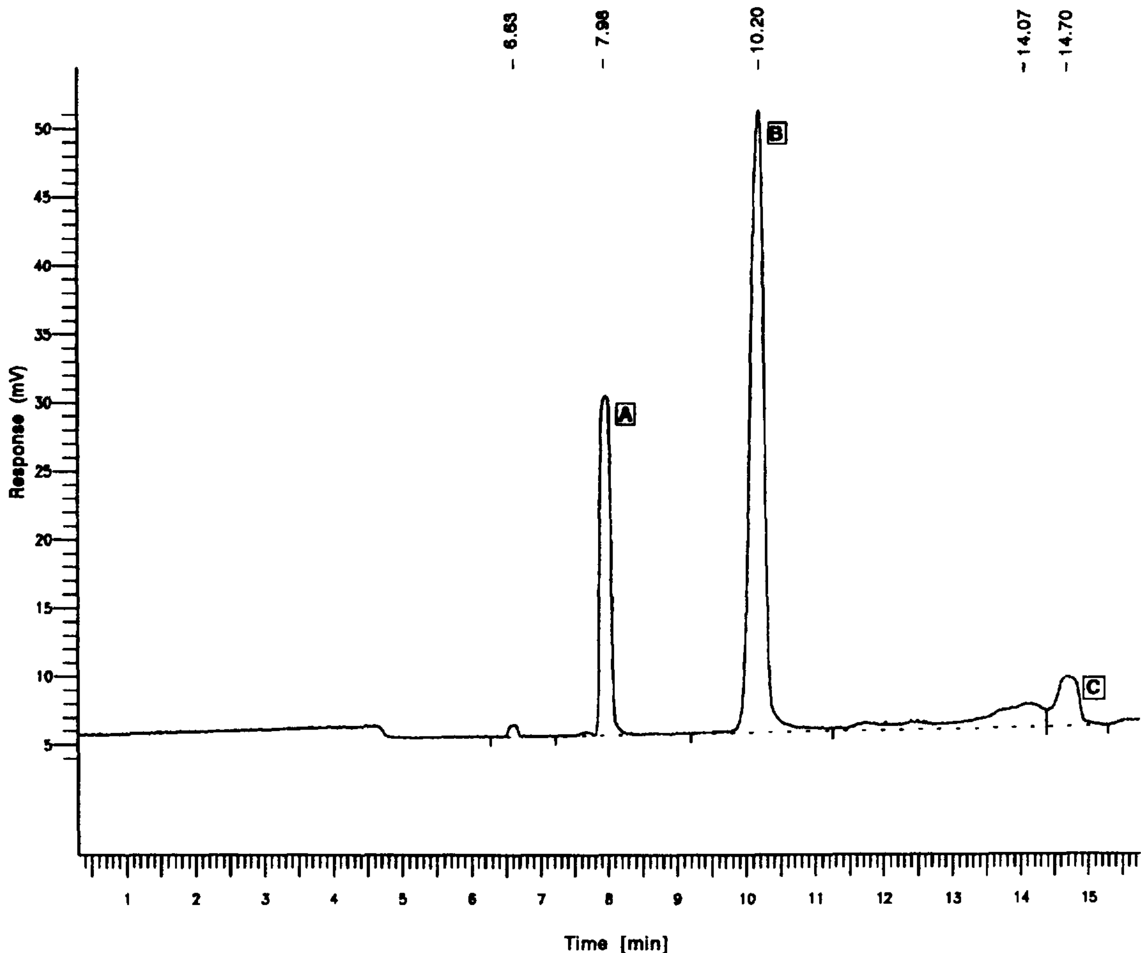

Fig. 2. Electropherogram of human urine proteins showing the components: A = Urea/creatinine; B = Bence Jones protein and C = albumin. Conditions: fused-silica capillary, 72 cm × 50 μm I.D.; detection, 200 nm; applied voltage, 18 kV; running buffer, 150 mM boric acid–1 mM calcium lactate, pH 9.7.

used for minor constituents such as HbA_2 or $HbA1_C$. HbF is characteristically resistant to alkali denaturation. Microscopic techniques have been employed for the detection of sickle haemoglobins, whilst supra-vital stain, to visualise "golf ball" cells, has been diagnostic for HbH. Isoelectric focusing and HPLC have been used to detect a wide range of variants. Using several techniques is costly due to the labour involved, and may also introduce unnecessary errors.

Two approaches have been made to the separation of haemoglobin variants by CE, these using either conventional electrophoresis or isoelectric focusing. An electrophoretic approach was used by Chen et al. [12], who chose a fused-silica capillary and a proprietary buffer at pH 8.6. Measurement of HbA, S and C was made at 415 nm, separation of HbF being obtained by slightly modified (unstated) conditions. Unfortunately, because of a lack of detail on the buffer composition, reproduction of this method would be difficult.

Ong et al. [24] have also used a fused-silica separation of haemoglobin components in normal individuals and thalassemia patients. Using a high pH phosphate buffer, the analysis time for haemoglobin variants was less than 8 min.

Classical gel IEF is labour-intensive and time-consuming. However, IEF by commercial CE instruments can be automated by computer-controlled sample injection, on-line detection and data acquisition. Using IEF, Hempe and Craver [25] have been able to use CE to separate HbA, $A1_C$, F, D, S and A_2 in less than 15 min, with a rapid screening method to separate components in 4 min. The described separations appeared excellent and should be able to be reproduced on different instrumentation with only minor modification.

One problem we have encountered with coated capillaries is the short lifetime, approximately one tenth the number of runs as compared to fused-silica capillaries. This repeated problem encouraged us to use fused-silica capillaries for the separation of haemoglobin variants. Following the work of Ishioka et al. [26], we have separated commercial haemoglobin variant control material as well as samples from patients, using a 72 cm fused-silica capillary in 12 min (see Fig. 3).

Other recent studies have demonstrated the separation of Hb variants by CE [27]. Molteni et al. [28] used a CIEF approach in fused-silica capillaries, choosing a mixture of ampholytes that had over twice the concentration used by Hempe and Craver [25]. Results published appear to be of similar quality by the two methods.

A micro chromatograph approach to the separation of haemoglobins has been made by Hjertén et al. [29]. These authors used a 0.32 nm continuous bed column prepared from piperazine diacrylamide and methacrylamide, eluting haemoglobins C, S, A and F by use of a NaCl gradient in Tris–HCl buffer. The other novel aspect of this paper is the description of a desalting and concentrating technique for small volume biological samples, using polyacrylamide gels with pores so small that only low-molecular-mass compounds could penetrate. The authors have applied this concentrating technique to a number of biological samples, including human urine, amniotic fluid and cerebrospinal fluid.

A side benefit of the haemoglobin variants work mentioned earlier was that $HbA1_C$ could also be identified. This constituent of haemoglobin reflects long-term control in diabetic patients. There is no reason why the technique for identifying haemoglobin variants could not be used for quantitating $HbA1_C$. In Hempe and Craver's pH 6–8 gradient system [25], separation of HbA and $HbA1_C$ only differ in pI by 0.038 pH units and were separated by 0.35 min. Methods for quantitation of $HbA1_C$ at the moment involve ion-exchange chromatography on disposable columns which are expensive, or use of monoclonal antibody haemoglobin $A1_C$ reagent cartridges, which are even more expensive. The third alternative available at present is the use of the Diamat, an instrument dedicated to analysing only $HbA1_C$, the drawback here being the initial cost of the instrument. Recently, however, a further modification has allowed expanded use of the instrumentation for haemoglobin variants.

4. Drug analysis

Routine drug analyses in the clinical laboratory are usually performed by immunoassay methods using four groups of labels, radio-isotopes, enzyme, fluorescent and chemiluminescent. A number of

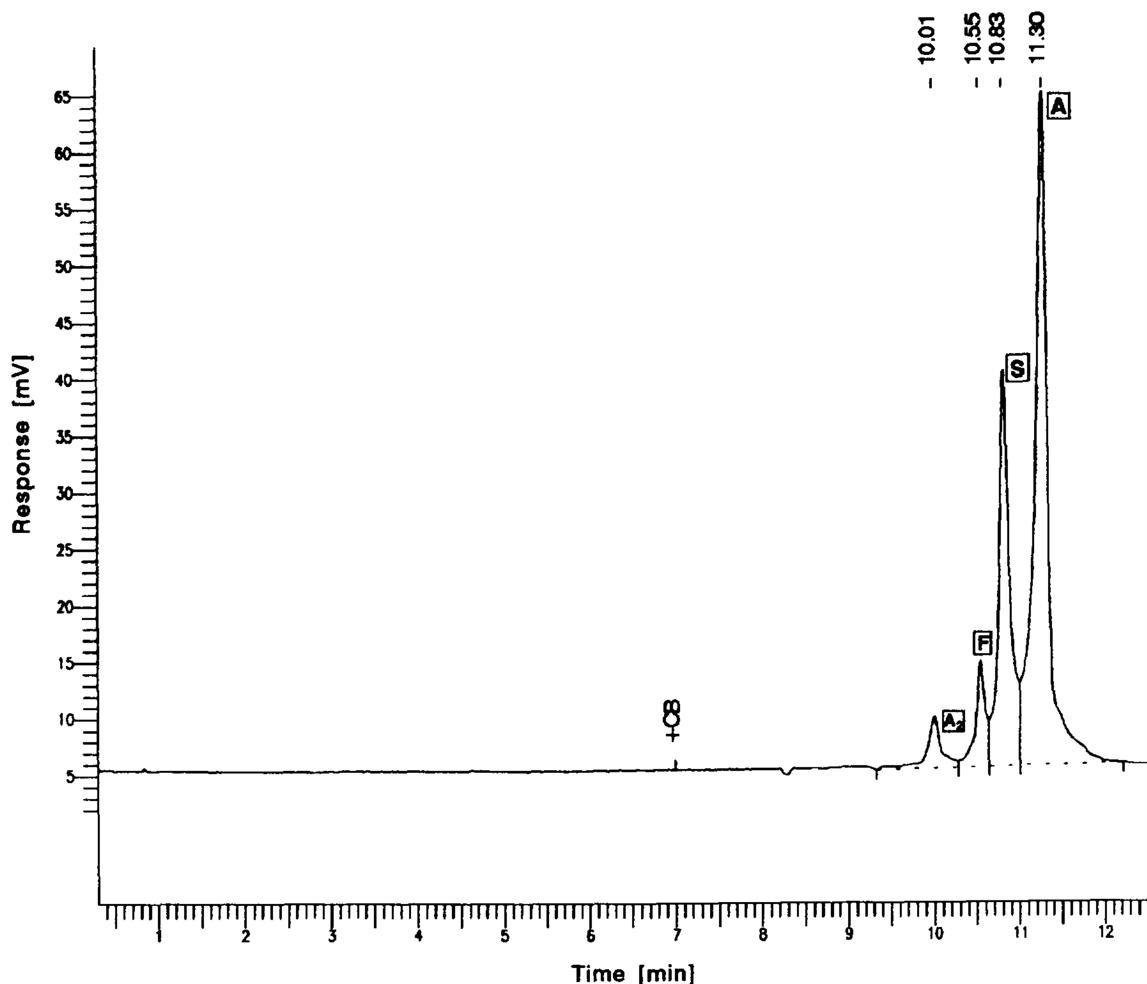

Fig. 3. Electropherogram of a Biorad haemoglobin A_2 control, showing haemoglobin A, S, F and A_2 as marked. Conditions: Applied Biosystem fused-silica capillary, 72 cm × 50 μm I.D.; detection, 200 nm; applied voltage, 15 kV; injection, 1 s; running buffer, 0.1 *M* boric acid, pH 9.98.

these assays are now available on random access instrumentation and data-reduction is performed with automated curve fitting procedures on computers.

Newer drugs, however, are still generally analysed by HPLC. It is in this particular area, where cost is a priority, that CE may play a significant role. Following the original work of Terabe et al. [30], on micellar electrokinetic capillary chromatography (MECC) separations for neutral compounds, a substantial amount of developmental work has already been done for various drug separations by CE.

Evenson and Wiktorowicz [31] described separations of barbiturates, cardio-active drugs, benzodiazepines, anticonvulsants, diuretics and xanthines. Nishi and Terabe [32] described the analysis of penicillin and cephalosporin antibiotics. McLaughlin et al. developed practical guidelines for pharmaceutical drug separation by CE [33].

Other authors have developed methods for the separation of a single drug such as felbamate, a new anticonvulsant drug that was recently approved for the treatment of partial seizures [2], or cefpiramide, a third generation cephalosporin [34]. Shihabi [35] developed a serum pentobarbital analysis on acetonitrile deproteinized serum using CE. 3-Isobutyl-1-methyl xanthine was used as an internal standard, the

separation being monitored at 254 nm. Details of the commercial buffer were not given, which makes reproduction of the work almost impossible. In the same paper, Shihabi also demonstrated serum iohexol analysis by CE. The same internal standard was used as for the pentobarbital separation, the pH of the unstated buffer changing from 8.5, with serum pentobarbital separation, to pH 8.8, for serum iohexol.

One of the major drawbacks in analysing drugs by CE is related to serum protein interference. Nakagawa et al. [34] first reported the determination of antibiotics in human plasma by micellar separations using a direct injection method, similar to micellar HPLC. Protein interference is partly eliminated by MECC separations, as the migration time of plasma proteins is increased with an increase in surfactant concentration. Addition of SDS also eliminates the adsorption of plasma proteins to the capillary wall, thus enabling determination of drugs in plasma by the direct injection method.

An alternative approach for protein elimination of serum samples is by solvent extraction acetonitrile deproteinisation. The use of a fluorimetric detector could be beneficial for direct drug analysis from serum samples.

5. Vitamin analysis

Vitamin analyses by CE originally used MECC separations similar to the SDS preparations which Terabe et al. [30] had used to separate phenol derivatives.

Fujiwara et al. [36] reported separation of B group vitamins and vitamin C by CE, and optimised their SDS–phosphate MECC separation at pH 9.0. Although the absorption maximum of the vitamins varied from 210 nm to 270 nm, they selected 254 nm for measurements and went on to investigate quantification of vitamins using an internal standard in preference to peak-area measurements.

Nishi and Terabe [37] successfully separated eleven water-soluble vitamins in 20 min, the absorbance being measured at 210 nm.

Later, Ong et al. [3], using SDS modified with γ-cyclodextrin, separated five B group vitamins plus vitamins C+H and fat-soluble vitamins A and E. The disappointing aspect of this paper was that the separation took over 70 min per sample.

Jegle separated water-soluble vitamins in phosphate buffer at pH 7.0, using a CZE separation without assistance from any micelles [38]. He went on to analyse an over-the-counter vitamin preparation, comparing the tabled analysis to his reference sample containing eight vitamins.

Koh et al. [39] advocated a fused-silica separation for vitamin C, using 100 m*M* tricine buffer, pH 8.8, with absorbance measurements at 254 nm. A stereoisomer of ascorbic acid, isoascorbic acid, was used as the internal standard. The technique was demonstrated to be suitable for clinical evaluation of human vitamin C status.

6. Isoenzyme analysis

Isoenzymes are multiple forms of an enzyme that all possess the ability to catalyze the enzyme's characteristic reaction, but differ in structure because they are encoded by distinct structural genes. Isoforms are non-genetically determined multiple forms of isoenzymes, which result from post-translational modification of the molecule as a result of changes to glycosylation, sialation, deamidation, etc.

Isoenzyme components of lactic dehydrogenase (LDH) are known to have a pH of between 8.3 and 8.55. Wiktorowicz and Colburn [40] have shown, by CE, increased separation of these isoenzymes of LDH, using the principle of surface charge reversal, which achieves separations of basic proteins by reversing the charge on the capillary surface. Results described in their paper would indicate that this is superior to methods using fused-silica capillaries.

Trace amounts of three LDH isoenzymes have also been separated by Xue and Young [41], using laser-induced fluorescence detection and monitoring the product of the enzyme-catalysed reaction between lactate and NAD.

A third method has been reported by Klein and Jolliff [20]. These authors added substrate for the particular isoenzyme to the running buffer which was used at the pH required for optimal enzyme activity. After application of the electric field for a short time to separate the isoenzymes, the applied voltage was interrupted to allow the resolved isoenzymes to

catalyse the conversion of the substrate in the running buffer. When the electric field was re-established, the isoenzymes formed were quantitated at the detector window, by measuring absorbance at the wavelength optimum for the product.

The ability of CIEF to resolve proteins which may differ by as little as 0.005 of a p*I* unit has been demonstrated. Since isoenzymes are composed of a combination of subunits [liver and heart LDH isoenzymes, and M and B protein subunits for creatine kinase (CK)], perhaps the logical approach for the isoenzymes analysis by CE should be by IEF. As stated previously (Section 3), IEF measurement by commercial CE instruments can be automated by computer-controlled sample injection, on-line detection and data acquisition.

7. DNA separations

The usual mechanism for separation and analysis of DNA fragments has been by submarine or slab gel electrophoresis. The basis of separation is related to the size of the DNA fragments, since the charge density on large individual fragments is similar. Slab gel electrophoresis can be tedious for the application of samples and often non-quantitative and cumbersome in the isolation of individual fragments.

While submarine gels have been routinely used in the past for delineation of PCR products or DNA endonuclease digests, the possibility now exists for using gel-filled capillaries for processing these samples. An example where gel-filled capillaries could be used in the clinical laboratory is in the determination of deletions in cystic fibrosis.

Hjertén [42] introduced gel filled capillaries in 1983. Polyacrylamide gel-filled columns have been used for the separation of oligonucleotides differing by a single base. Cohen et al. [43] have separated restriction fragments using open-tube CE filled with Tris–borate–EDTA–urea–1% SDS buffer. The authors separated nineteen fragments ranging in size from 72 to 23 130 base pairs.

Karger et al. [44], using a similar Tris–borate–EDTA–urea–0.1% SDS buffer, observed that injection of heated samples proved most effective in the high resolution of deoxyoligonucleotides. Another approach for CE separation of restriction fragments is to add a cationic surfactant to the buffer [45]. This is an example of electrokinetic chromatography in which ion pairing and micelles are employed for oligonucleotide separation.

A further possible application of gel-filled capillaries is in automated DNA sequencing. Luckey et al. [46] have developed a CE instrument using a fluorescence detector capable of measuring fluorescence at four wavelengths. Detection sensitivity is the key factor in this paper, the optimum sample size being 0.4 pmol or a femtomole of DNA per band. DNA sequencing is currently a research tool but, with the introduction of molecular biology techniques to the routine clinical laboratory, could, by its nature, see the introduction of CE as well.

8. Metabolic disorders

These disorders are suspected in children with clinical indicators such as failure to thrive, unexplained metabolic acidosis, failure of neurological development or significant family history. Usually, urine or serum samples are analysed by thin-layer chromatography and paper chromatography to determine mucopolysaccharides and carbohydrates. Previously, HPLC or an automated amino acid analyser have been used for quantitative amino acid analysis and to diagnose amino acidopathies.

Amino acid analysis involving separation of phenylalanine and tyrosine in newborn screening programs is another clinical application of CE. Patients undergoing dietary treatment for phenylketonuria (PKU) may be monitored by CE [47]. CE analysis of sulphur-containing amino compounds such as cysteine, homocysteine and glutathione derivatized with monobromobimane, and detected fluorimetrically is a simple alternative to classical amino acid analysis [48]. The association between plasma homocysteine concentrations and extracranial carotid artery stenosis has recently been recognised [49].

Methods have been developed by Araki and Sako [50] for the determination of free and total homocysteine in human plasma by HPLC with fluorescence detection. Stamler and Loscalzo [51] have demonstrated a simple, rapid and reproducible method for separating cysteine and homocysteine by CE, using a phosphate buffer at pH 2.5. With detection at

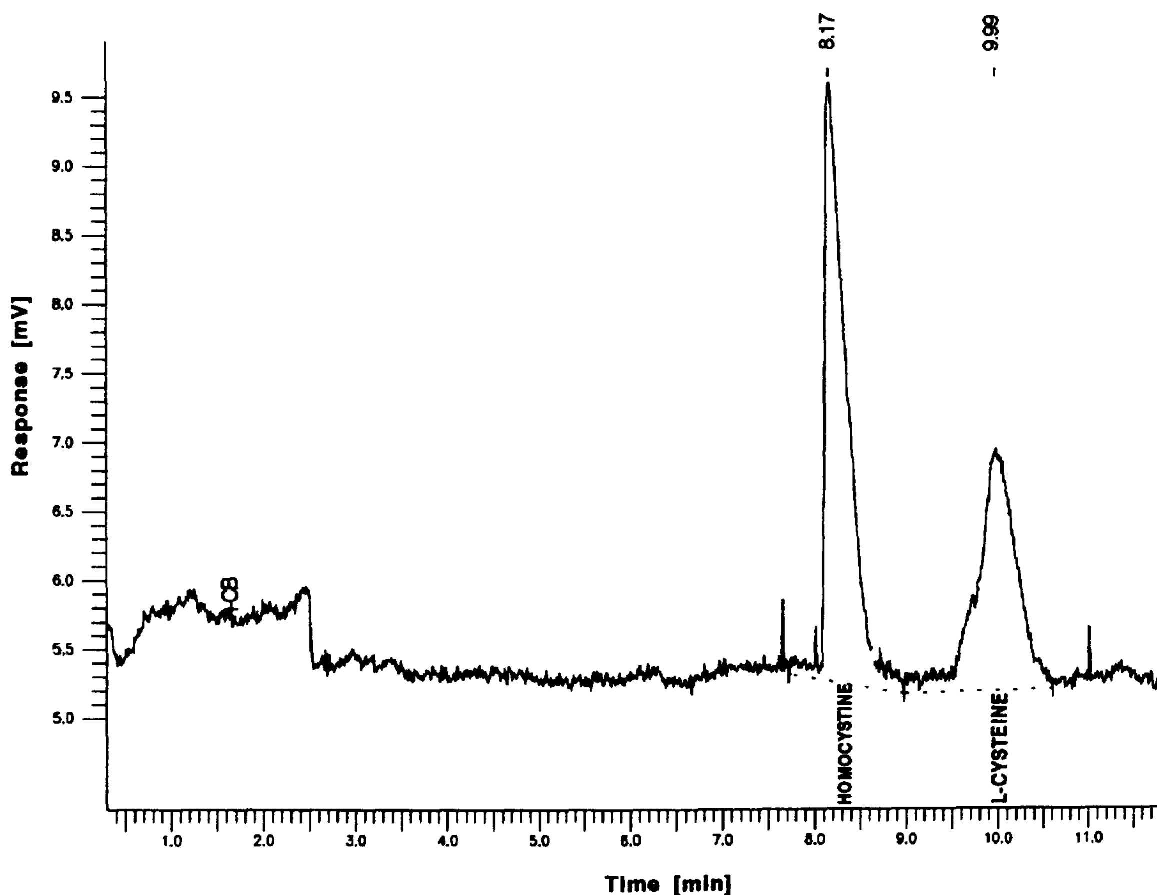

Fig. 4. Electropherogram showing the separation of homocystine (100 μM) and L-cysteine (100 μM). Conditions: fused-silica capillary, 62 cm × 100 μm I.D.; detection, 200 nm; applied voltage, 15 kV; buffer, 10 mM phosphate buffer, pH 2.5.

200 nm, the authors were able to show electropherograms of 250 μM homocysteine and 500 μM cysteine. We have been able to reproduce the separation of biological thiols on different equipment with a slight improvement in sensitivity (see Fig. 4). Hence, large-scale testing for plasma homocysteine may become important in the near future. A fast, sensitive and direct method has also been developed for the determination of glutathione peroxidase activity (both selenium and non-selenium dependent) [52].

9. Porphyria

The porphyrias are due to a deficiency, usually inherited, of one of the enzymes of the haem pathway, resulting in impaired haem production.

The symptoms of porphyria correlate well with the biochemical abnormalities; skin lesions varying from mild photosensitivity to severe blistering, whilst neurological disturbances such as peripheral neuritis and abdominal pain may be found. The porphyrins are classified according to whether the main site of porphyrin accumulation is in the liver or in the erythropoietic system. Diagnosis has been made previously by examination of urine, faecal or blood specimens. CE has been used by Weinberger et al. [53] for analysis of urinary porphyrins by MECC using either absorbance at 400 nm or by their natural fluorescence (excitation 400 nm; emission at 550 nm). Fluorescence was found to be about ten fold more sensitive than absorbance for the six test porphyrins studied.

10. Discussion

Automated CE instrumentation will be commonplace in the routine clinical laboratory within three to five years. Depending on the size of the hospital, it may be cost-effective solely for serum and urine protein electrophoresis. For smaller workloads, the initial cost of the instrumentation could probably be justified if haemoglobin variants and/or vitamin assays were run overnight, one or two nights per week.

We have not attempted in this review to assess available instrumentation. Prior to 1989, when a number of the current CE commercial instruments were developed, CE was a research tool, not a routine tool in the clinical laboratory. However, since 1989, clinical applications have been developed for CE. Perhaps it is time for the commercial CE companies to consider the development of a third-generation CE analyser.

Clinical laboratories would welcome any modification of instrumentation that allowed two capillaries to be inserted into the instrument, each with its own detector system. This would allow the throughput to be double. Cost analysis with electrophoretic and manual techniques would then be overwhelmingly favourable to CE.

The other area for CE instrument development must be in the area of modular instruments, particularly in the use of various detectors. If the UV–VIS detector system was able to be removed and a fluorescence detector inserted in its place, the number of analytes considered for CE in a clinical laboratory could be considerably increased.

In conclusion, there is a place for CE in the clinical laboratory. Significantly more developmental work is necessary for a number of analytes.

References

[1] J.W. Jorgenson and K.D. Lukacs, Science, 222 (1983) 266.
[2] Z.K. Shihabi and K.S. Oles, Clin. Chem., 40 (1994) 1904.
[3] C.P. Ong, C.L. Ng, H.K. Lee and S.F.Y. Li, J. Chromatogr., 547 (1991) 419.
[4] Z. Deyl and R. Struzinsky, J. Chromatogr., 569 (1991) 63.
[5] M.J. Gordon, X. Huang, S.L. Pentoney, Jr. and R.N. Zare, Science, 242 (1988) 224.
[6] W.G. Kuhr, Anal. Chem., 62 (1990) 403R.
[7] M.A. Jenkins and M.D. Guerin, Ann. Clin. Biochem., 32 (1995) 493.
[8] F.-T.A. Chen, J. Chromatogr., 559 (1991) 445.
[9] A.Tiselius, Trans. Faraday Soc., 33 (1937) 524.
[10] S. Hjertén, Electrophoresis, 11 (1990) 665.
[11] S. Hjertén and K. Kubo, Electrophoresis, 14 (1993) 390
[12] F.-T.A. Chen, C.-M. Liu, Y.-Z. Hsieh and J.C. Sternberg, Clin. Chem., 37 (1991) 14.
[13] M.J. Gordon, K.-J. Lee, A.A. Arias and R.N. Zare, Anal. Chem., 63 (1991) 69.
[14] J.W. Kim, J.H. Park, J.W. Park, H.J. Doh, G.S. Heo and K.-J. Lee, Clin. Chem., 39 (1993) 689.
[15] M.A. Jenkins, E. Kulinskaya, H.D. Martin and M.D. Guerin, J. Chromatogr. B, 672 (1995) 241.
[16] R.F. Richie and R. Smith, Clin. Chem., 22 (1976) 1982.
[17] R.A. Kyle and J.P. Garton, Seminars in Oncology, 13 (1986) 310.
[18] R. McLachlan, The Clinical Biochemist Monograph, The Australian Association of Clinical Biochemists, Perth, 1985, p. 38.
[19] F. Aguzzi and N. Poggi, Bull. Inst. Sierater Milan, 56 (1977) 212.
[20] G. Klein and K. Jolliff, in J.P. Landers (Editor), Handbook of Capillary Electrophoresis, CRC Press, Boca Raton, FL, 1993, p. 419.
[21] J.W. Jorgenson and K.D. Lukacs, Clin. Chem., 27 (1981) 1551.
[22] N.A. Guzman, C.M. Berck, L. Hernandez and J.P. Advis, J. Liq. Chromatogr., 13 (1990) 3833.
[23] M.A. Jenkins, T.D. O'Leary and M.D. Guerin, J. Chromatogr. B, 662 (1994) 108.
[24] C.-N. Ong, L.S. Liau and H.Y Ong, J. Chromatogr., 576 (1992) 346.
[25] J.M. Hempe and R.D. Craver, Clin. Chem., 40 (1994) 2288.
[26] N. Ishioka, N. Iyori, J. Noji and S. Kurioka, Biomed. Chromatogr., 6 (1992) 224.
[27] M. Zhu, T. Wehr, V. Levi, R. Rodriguez, K. Shiffer and Z.A. Cao, J. Chromatogr. A, 652 (1993) 119.
[28] S. Molteni, H. Frischknecht and W. Thormann, Electrophoresis, 15 (1994) 22.
[29] S. Hjertén, L. Valtcheva and Y.-M. Li, J. Capillary Electrophoresis, 1 (1994) 83.
[30] S. Terabe, K. Otsuka, K. Ichikawa, A. Tsuchiya and T. Ando, Anal. Chem., 56 (1984) 111.
[31] M.A. Evenson and J.E. Wiktorowicz, Clin. Chem., 38 (1992) 1847.
[32] H. Nishi and S. Terabe, Electrophoresis, 11 (1990) 691.
[33] G.M. McLaughlin, J.A. Nolan, J.L. Lindahl, R.H. Palmieri, K.W. Anderson, S.C. Morris, J.A. Morrison and T.J. Bronzert, J. Liq. Chromatogr., 15 (1992) 961.
[34] T. Nakagawa, Y. Oda, A. Shibukawa, H. Fukuda and H. Tanaka, Chem. Pharm. Bull., 37 (1989) 707.
[35] Z.K. Shihabi, Ann. Clin. Lab. Sci., 22 (1992) 398.
[36] S. Fujiwara, S. Iwase and S. Honda, J. Chromatogr., 447 (1988) 133.
[37] H. Nishi and S. Terabe, Electrophoresis, 11 (1990) 691.
[38] U. Jegle, J. Chromatogr. A, 652 (1993) 495.

[39] E.V. Koh, M.G. Bissell and R.K. Ito, J. Chromatogr. A, 633 (1993) 245.
[40] J.E. Wiktorowicz and J.C. Colburn, Electrophoresis, 11 (1990) 769.
[41] Q. Xue and E.S. Yeung, Anal. Chem., 66 (1994) 1175.
[42] S. Hjertén, in H. Hirai (Editor), Electrophoresis '83, Walter de Gruyter, Berlin, New York., 1984, p. 71.
[43] A.S. Cohen, D. Najarian, J.A. Smith and B.L. Karger, J. Chromatogr., 458 (1988) 323.
[44] B.L. Karger, A.S. Cohen and A. Guttman, J. Chromatogr., 492 (1989) 585.
[45] T.J. Kasder, M. Melera, P. Gozel and R.G. Brownlee, J. Chromatogr., 458 (1988) 303.
[46] J.A. Luckey, H. Drossman, A.J. Kostichka, D.A. Mead, J. D'Cunha, T.B. Norris, L.M. Smith, Nucl. Acids Res., 18 (1990) 4417.
[47] F. Tagliaro, S. Moretto, R. Valentini, G. Gambaro, C. Antonioli, M. Moffa and L. Tato, Electrophoresis, 15 (1994) 94.
[48] E. Jellum, A.K. Thorsrud and E. Time, J. Chromatogr., 559 (1991) 455.
[49] J. Selhub, P.F. Jacques, A.G. Bostom, M.D. Ralph, B. D'Agostino, P.W.F. Wilson, A.J. Belanger, M.A. Daniel, H. O'Leary, P.A. Wolf, E.J. Schaefer and I.H. Rosenberg, New Engl. J. Med., 332 (1995) 286.
[50] A. Araki and Y. Sako, J. Chromatogr., 422 (1987) 43.
[51] J.S. Stamler and J. Loscalzo, Anal. Chem., 64 (1992) 779.
[52] P. Pascual, E. Martinez-Lara, J.A. Bárcena, J. López-Barea and F. Toribio, J. Chromatogr., 581 (1992) 49.
[53] R. Weinberger, E. Sapp and S. Moring, J. Chromatogr., 516 (1991) 271.