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Review

Capillary electrophoresis procedures for serum protein analysis: comparison with established techniques

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Abstract

Methods using automated capillary electrophoresis (CE) instrumentation are available for serum protein electrophoresis with monoclonal band quantitation, isoelectric focusing and sodium dodecyl sulphate-polyacrylamide gel electrophoresis separations. The advantages of CE over previous gel methods relate to the time and labour saved by the automated instrumentation. High p*I* monoclonal bands and cryoglobulin specimens can be successfully analysed by CE. However, if the CE application uses a standard company supplied kit, then the cost savings are often negated by the high cost of the kit. Improvements such as the inclusion of both a UV–Vis as well as a fluorescence detector as standard within the one commercial instrument, the production of coated IEF capillaries with a useful life of at least 100 samples, and the introduction of a capillary array into all commercial instrumentation would ensure greater use of CE within both the clinical and other protein laboratories. © 1997 Elsevier Science B.V.

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1. Introduction

The first electrophoretic technique used to study serum proteins was the free solution or moving boundary technique described by Tiselius in 1937 [1]. The resolution obtained by electrophoresis was further improved by the introduction of solid support media such as paper, cellulose acetate [2] and agarose [3]. For the past twenty years, however, the reference method for serum protein electrophoresis in the routine clinical laboratory has been high resolution agarose gel electrophoresis (HRAGE) [4]. Several other methods of protein separation are available to compliment serum protein electrophoresis. These include isoelectric focusing, immunofixation, gel exclusion techniques and capillary electrophoresis.

Isoelectric focusing (IEF) is a technique incorporating ampholytes into gels and hence separates proteins according to their isoelectric point. Since the early work of Kolin [5,6] on artificial pH gradients and the subsequent work of Svensson [7] the technique of IEF has developed into one of the most useful tools for protein analysis. The high resolving power of IEF should have ensured greater acceptance than it enjoys today. However, problems such as cathodic drift, pH gradient instability and low sample load capacity have lead to limited application, even though the technique may be used in either polyacrylamide or agarose gels [8,9]. Even so, the technique is relatively simple.

Afonso [10] first described immunofixation, a more practical procedure later published by Alper and Johnson [11]. Identification of a particular protein using immunofixation of agarose gels following electrophoresis was reported by Ritchie and Smith [12,13] and other workers [14,15]. Later immunofixation was combined with IEF to give increased resolution and sensitivity of the particular protein under investigation [16].

For molecular mass identification of proteins the standard gel technique has been sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) in either continuous or discontinuous buffer systems. Since the original work by Shapiro et al. [17] and that of Weber and Osborn [18], the technique has developed to the stage where good separation of protein subunits within a specified molecular mass range can be obtained. Laemmli [19] added SDS to the Tris–glycine/Tris–HCl system developed by Ornstein [20] and Davis [21] creating the 'Laemmli system' for discontinuous SDS electrophoresis which gave further improvement in resolution.

Capillary electrophoresis (CE) involves the separation of charged or uncharged species in a fine capillary under the influence of a very high voltage. Whilst the pattern of separations obtained by CE resembles that obtained by the original technique of Tiselius, the use of capillaries with reduced diameter has vastly improved the resolution obtained. In 1983 Jorgenson and Lukacs [22] used a surface modified capillary to separate serum proteins into six peaks, whilst Hjerten [23] in 1990 used a multibuffer system to detect five protein peaks. Since the introduction of automated CE instrumentation, in the late 1980s, the technique has allowed rapid identification of proteins with minimal technical input.

Capillary IEF was first developed by Hjerten [24] and modified by Zhu et al. [25]. Mazzeo et al. [26] and Thorman et al. [27] independently reported using an uncoated capillary for IEF. Hjerten [28] introduced gel-filled capillaries for molecular mass separations in 1983. Subsequently his work was developed by Cohen et al. [29] for fragment analysis and Karger et al. [30] who observed that injection of heated samples improved separation of deoxyoligonucleotides. Kasder et al. [31] used ion pairing agents with micellar separation in oligonucletide analysis whilst Lucky et al. [32] utilised gel-filled capillaries for automated DNA sequencing.

2. High resolution agarose gel electrophoresis

The primary objective of protein electrophoretic analysis in clinical laboratories is for the identification and quantification of paraprotein in patients' sera. The aim in handling a particular patient is to monitor changes in paraprotein quantitation, irrespective of changes in operators, batches of stain, variation in staining times etc. The current reference method for serum protein electrophoresis is high resolution agarose gel electrophoresis (HRAGE).

The reference method for electrophoresis on agarose gel uses 75 m*M* barbiturate–sodium barbiturate

(pH 8.6) with 2 m*M* calcium lactate. The separation of plasma proteins achieved depends on the buffer composition and the gel temperature. Increasing the concentration of buffer from 0.04 to 0.10 m*M* increases the resolution in the albumin–alpha 2 zone but decreases the resolution in the beta–gamma zone [3]. Calcium ions e.g. calcium lactate added to the buffer will increase resolution of the beta components (transferrin, β-lipoprotein, C3 and IgA). Heat produced during a run may be diminished by the use of a cooling system. Following electrophoresis the separated proteins are stained, and any paraprotein quantitated by densitometric analysis.

Manual techniques such as HRAGE, are prone to large operator dependent variations. This is because the technique involves many manual steps starting with the application of a small volume of each specimen. Factors such as the separation obtained being dependent on the reproducibility of run and staining times and the difficulty in getting even uptake of stain into very large monoclonal bands increase the variation in results obtained. The precision of results obtained by HRAGE must be less than for any automated method. Although there are numerous papers on HRAGE, the precision and accuracy of the method is not frequently reported. Jeppsson's proposed selected method [3] only reports subjective evaluation of precision which is determined by different observers to be excellent: however, no limits of acceptability are reported. In a paper by Dennis et al. [33] the coefficient of variation (C.V.) values for cellulose acetate were stated as ranging from 5.8–15.7% compared to those of 2.6–10.6% for agarose.

In a correlation study between HRAGE and CE which our laboratory published in 1995 we found that the C.V. values for HRAGE ranged from 5.2–8.1 for monoclonal bands of 16 and 33 g l^{-1} [34].

Commercial automated protein electrophoresis and isoenzyme analysis systems have been available since the late 1980s. Whilst the initial cost of the automated system was approximately $100 000, the manufacturers claimed that subsequent labour costs could be reduced by 90%. With the use of Peltier cooling, total run time for electrophoresis was cut to less than 15 min. C.V. values for protein separations were not available, although those for enzyme separations were quoted at 2–3%. Because of high capital cost these dedicated electrophoretic instruments only found a small niche market in extremely high throughput laboratories.

3. Capillary electrophoresis

Since 1991 significant progress has been made in the separation of serum proteins by CE using fused-silica capillaries. Gordon et al. [35] added ethylene glycol to sodium borate decahydrate buffer (pH 9.6) to separate serum proteins from a patient with IgG myeloma. Chen et al. [36] described serum protein separation into five bands, detection being at 214 nm. The use of pH 10.0 buffer ensured repulsion of the proteins from the silica wall of the capillary. However, the composition of the buffer was not disclosed. In a further paper Chen [37] increased the ionic strength of the buffer to give ten peaks or inflections which related to various protein components. Measurements in this case were made at 200 nm. In a later paper Chen et al. [38] discussed protein separations in untreated fused-silica capillaries. The electropherograms showed five bands only for the serum protein specimens.

Dolnik [39] recognised that the standard agarose buffer system (5,5″-diethylbarbiturate pH 8.6) was unsuitable for CE because of its high UV absorbance and investigated different operational electrolytes. His choice of 10 m*M* methylglucamine–5 m*M* lauric acid permitted the use of 30 kV to separate serum proteins into 5 fractions in about 3 min.

Kim et al. [40] using a laboratory-made CE apparatus, similar to that described by Jorgenson and Lukacs, compared 37 serum samples by CE to agarose gel electrophoresis and SDS-PAGE under non-reducing conditions. No results from serum of paraproteinaemic patients were reported. Linearity of quantitation response at 200 nm was established, the correlation suggesting that CE was a reliable and reproducible technique for clinical diagnosis of serum protein abnormalities.

Lehmann et al. [41] favourably compared CE separations using 30 m*M* borate (pH 10.0) to cellulose acetate membrane electrophoresis. The electropherograms showed separation into six bands. Lehmann et al. [41] stated that CE profiles showed higher resolution yielding more detailed diagnostic

information compared with conventional agarose gel or cellulose acetate membrane electrophoresis.

Our laboratory looked at quantitation of serum proteins, particularly in regard to paraproteins [42]. We controlled the amount of protein introduced to the capillary by adjustment of the serum sample dilution and injection time so that we could calibrate the fused-silica capillary and quantitate the paraproteins by peak area. This obviated the need for densitometric analysis of HRAGE. The automated nature of the CE apparatus, and the data capture program, allowed considerable savings in technical time.

There were two alternatives available for serum protein separations by capillary electrophoresis, these being the use of coated capillaries versus fused-silica capillaries.

Coated capillaries appear to provide a relatively simple solution to the problem of endosmosis. However, they also introduce an alternative set of problems. Reproducibility within batch and between batches of capillaries has not been satisfactory. Also, certain washing procedures, such as the use of sodium and potassium hydroxide, tended to strip the coating off the capillary. The quality of published electropherograms for serum protein separations by CE using a coated capillary by both Jorgenson and Lukacs [22] and also by Hjerten [23] are not recognisable as mirror-images of densitometer tracings compared to the electropherograms obtained using fused-silica separations. In addition, we have been unable to find any reference to quantitation of monoclonal bands using coated capillaries.

Huang et al. [43] have recently produced a two-layer coated capillary, the polyalkylsiloxane-bonded phase being attached to the capillary surface with a second hydrophilic polymer or polymer surfactant layer then attached to the first layer. These modified capillaries have been tested for separation of a variety of proteins over a wide range of buffer pH values. However, the separation of human serum proteins using these columns with phosphate buffer took 50 min.

Fused-silica capillaries have been used by our laboratory for routine serum protein electrophoresis for the past three years. The calibration of the capillary using albumin samples is done on installation of the capillary and checked weekly. Each day a serum which contains a 20 $g\,l^{-1}$ paraprotein band is analysed as a quality control sample, the C.V. value of this sample being recently shown to be 6.3%. Our experience is that we can obtain at least 300 protein separations per capillary, using a standard wash procedure comprising 2 min of 0.1 *M* sodium hydroxide, 2 min distilled water and 3 min running buffer between samples. We have obtained up to 650 separations from the one 50-μm fused-silica capillary, which is only guaranteed for 100 assays.

One of the major advantages of CE over HRAGE is that monoclonal IgAs were often easier to identify as they separated from other beta components such as transferrin and C3.

Using our routine buffer of 75 m*M* boric acid plus 0.25 m*M* calcium lactate we have included a CE electropherogram of a normal serum, a serum containing oligoclonal IgG, serum containing increased acute phase reactants, and a serum demonstrating bisalbuminaemia. (Fig. 1)

Moore et al. [44] in a recent paper examined the combination of microcolumn size-exclusion chromatography with capillary zone electrophoresis for the separation of serum proteins. Signal intensity was presented using logarithmic scale to accentuate low-intensity peaks. The author stated that the combined use of column techniques had greater resolving power than the independent use of either technique. However, the illustration included showed only 5 or 6 distinct protein areas indicating a qualitative separation.

4. Low-end sensitivity by CE

By CE small monoclonal bands i.e. 0.5–1.0 $g\,l^{-1}$ usually show as an irregularity in the gamma region. Other clinical situations such as the appearance of oligoclonal IgG due to acute antigenic stimulation, the finding of free κ- or λ-light chains in a serum specimen indicating the presence of a Bence Jones proteinuria or a grossly elevated C reactive protein may also cause irregularities in the gamma area. The sensitivity of detection of monoclonal bands in our hands is 0.5–1.0 $g\,l^{-1}$. This detection limit is influenced by adequate sensitivity of detection of the instrument, which, unfortunately, we have seen vary

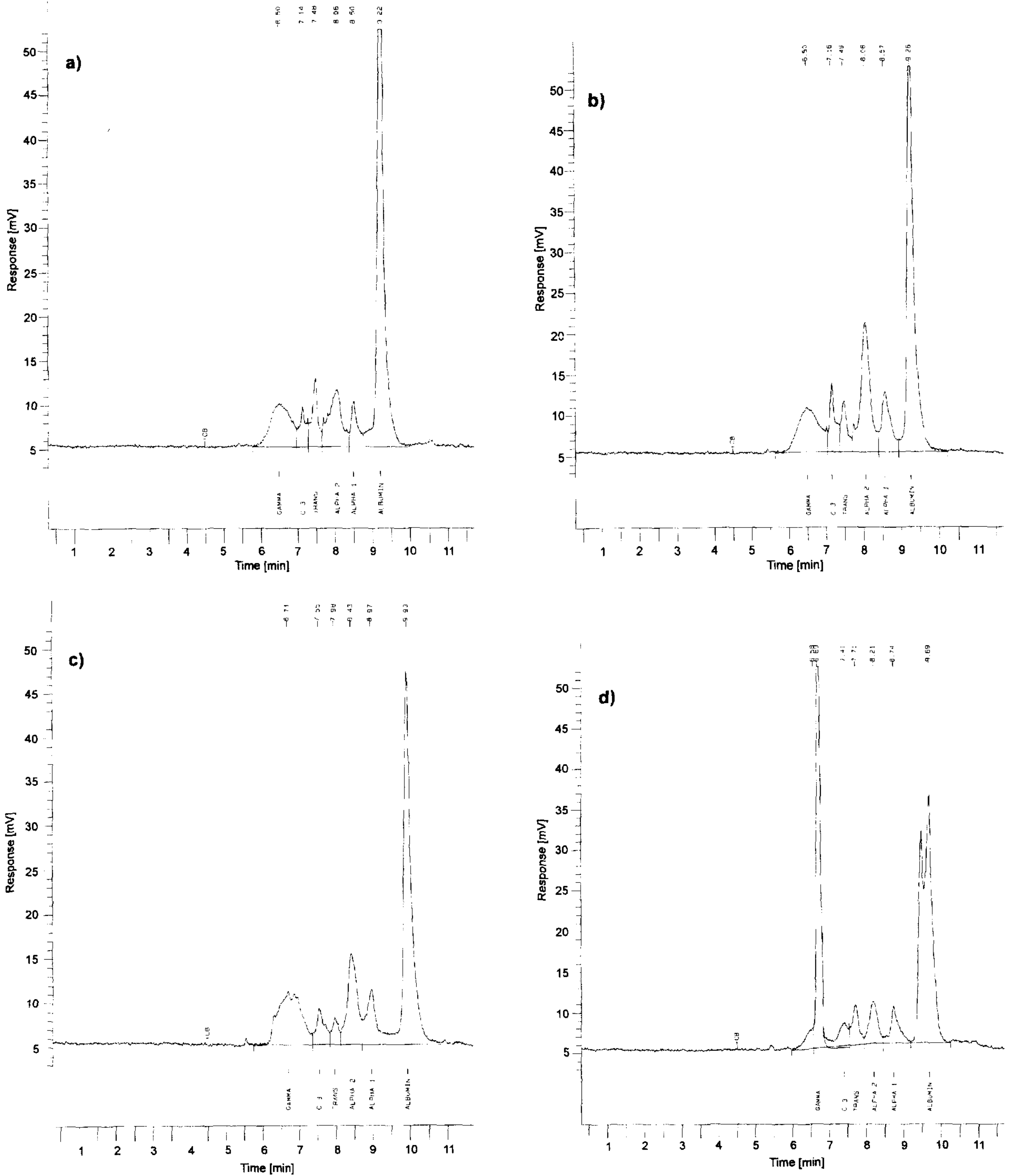

Fig. 1. Capillary electropherograms of (a) normal laboratory control, (b) increased acute phase reactants, (c) oligoclonal IgG and (d) bisalbuminaemia with IgG paraprotein of 22 $g\,l^{-1}$. Albumin, alpha 1, alpha 2, transferrin, C3 and gamma components are marked on each tracing.

six fold between the same model of instrument from the one manufacturer.

For small abnormal bands identification by HRAGE is usually by visual inspection of the electropherogram. Non-specific background staining can cause gross errors in the estimation of small monoclonal bands [45]. Further specialised testing such as immunofixation of isoelectric focusing gels is needed to distinguish these small monoclonal bands from free light chains or an oligoclonal picture.

5. High p*I* monoclonal bands

During the past three years we have processed over 6000 specimens in our laboratory for serum protein electrophoresis using CE. During that time we have discovered 8 sera where CE did not properly quantify the paraprotein. Originally we noted that the migration time for albumin in these specimens were markedly increased. Removal of the calcium lactate from the buffer allowed quantification. However, the electropherogram became grossly shortened.

Five of these sera contained an IgM paraprotein, whilst three contained an IgG paraprotein which migrated in the slow gamma region. By increasing the pH of the buffer to pH 10.3 i.e. approximately 1 pH unit above the highest p*I* of any of the monoclonal paraproteins and simultaneously increasing the ionic strength to 75 m*M* boric acid, we were able to quantify correctly all of these monoclonal bands [46].

6. Cryoglobulin specimens

Cryoglobulins are proteins which show temperature dependent precipitation or gel formation at temperatures below 37°C. The importance of proper specimen collection of samples for cryoglobulin detection cannot be over emphasised.

Quantitation of cryoglobulins can be achieved by careful washing of a measured aliquot transferred to 4°C for 72 h. Identification of the type of cryoglobulin is by immunofixation of the electrophoretic strip or by isoelectric focusing of the washed cryoglobulin precipitate [47,48]. Type 1 cryoglobulins refer to monoclonal cryoimmunoglobulins which are most commonly associated with IgM found in lymphoid malignancies such as Waldenstrom's macroglobulinemia and multiple myeloma. Type 2 cryoglobulins occur in association with autoimmune diseases, the monoclonal immunoglobulin usually being IgM with a polyclonal IgG background. Type 3 cryoglobulins are the most common cryoglobulins comprising polyclonal IgM plus polyclonal IgG [49]. An example of immunofixation of a Type 2 cryoglobulin is shown in Fig. 2.

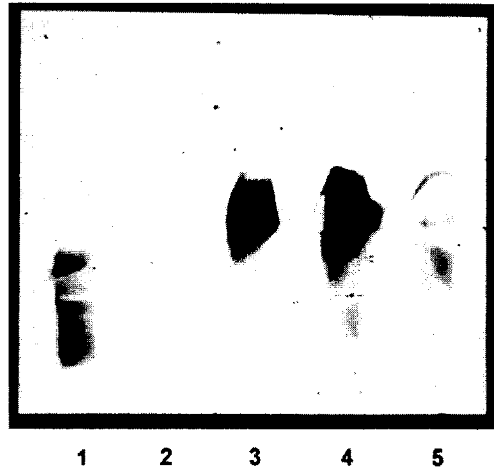

Fig. 2. Isoelectric focusing with immune fixation of a Type 2 cryoglobulin showing monoclonal IgM (κ) plus small amount of polyclonal IgG. Lane 1: Immunofixed with anti-IgG antisera; lane 2: anti-IgA; lane 3: anti-IgM; lane 4: anti-κ light chains; lane 5: anti-λ light chains.

Cryoglobulin determination by CE is by identification of a monoclonal band on an electropherogram, and then comparison of the electropherogram with that of a cooled specimen on the same patient. Any specimen with a cryoglobulin will show a marked reduction in the quantitation of the monoclonal protein after the specimen is held at 4°C. (See Fig. 3)

7. Isoelectric focusing

Using ampholytes incorporated into gels, proteins may be focused in a pH gradient so that they will migrate to their electrically neutral or isoelectric point. The main benefit of IEF is the excellent

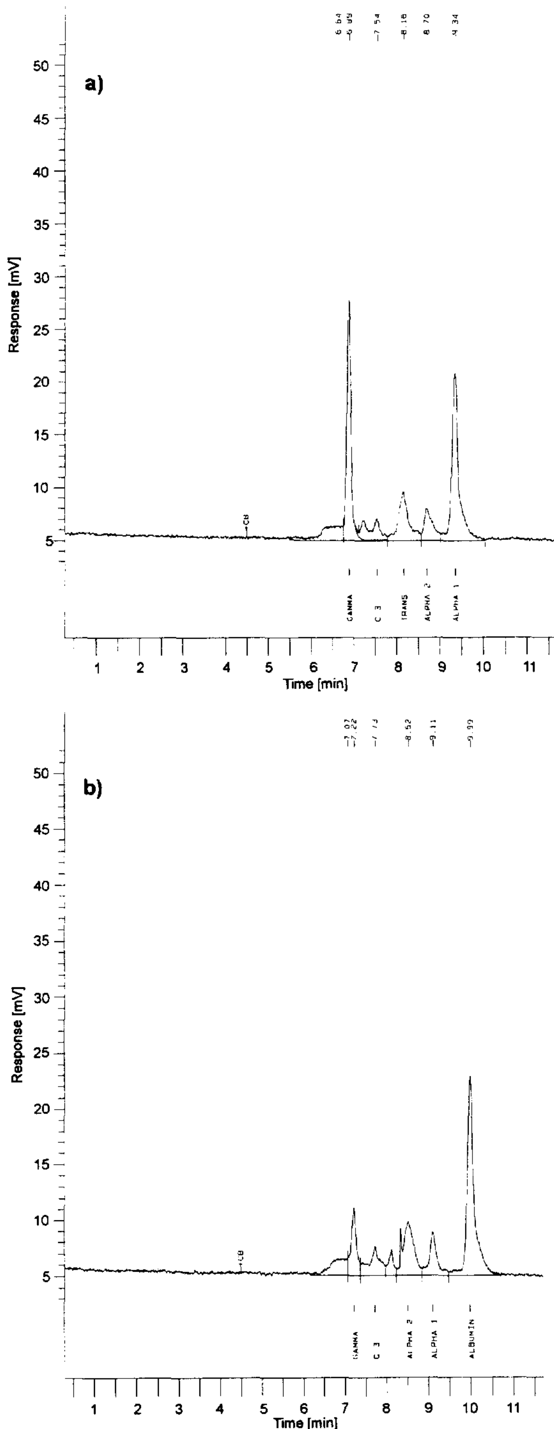

Fig. 3. Capillary electropherograms of Type 2 cryoglobulin before and after refrigeration overnight. Size of monoclonal band decreased from 24 to 8 g l^{-1}. Albumin, alpha 1, alpha 2, transferrin, C3 and gamma components are marked on each tracing.

resolution which can be achieved – proteins which differ by an little as 0.001 pH unit can be successfully resolved [50]. The use of varying ampholyte ranges may alter the final position of the protein of interest – hence the correct selection of the ampholyte range incorporated into the gels is vital. The quality of IEF separation which can be achieved is a direct reflection of the quality of the gels produced. Gels which have not had complete dissolution of the agarose will not produce good focusing results.

The technique of IEF can be used to distinguish irregularities in the gamma region detected by HRAGE or CE. 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. Capillary IEF can be performed either in the absence of or in the presence of electroosmotic flow. Electroosmotic flow can be reduced or even eliminated by using a capillary coated with either methylcellulose or non-crosslinked acrylamide [24,51].

Following the establishment of a pH gradient by the application of an electric current, mobilization of peaks may either be achieved by three alternative methods. The first is electrophoretic mobilization which involves adding salt to one of the electrolytes, an example being the addition of 80 m*M* NaCl to 20 m*M* NaOH [52]. Alternatively, mobilization of focused peaks may be achieved by the application of a vacuum to the capillary as well as the maintenance of the high voltage [53,54]. In this approach methylcellulose is utilized in the ampholyte mixture to support the stability of the coating which is usually dimethylpolysiloxane (DB-1). Whilst commercial CE has provided reliable instrumentation, we have encountered a major problem with these coated capillaries. Using capillaries from several different sources, we have found the lifetime of the capillaries to be severely limited thereby making IEF by CE uneconomic. An example of the resolution which can be achieved using DB-1 capillaries is shown in Fig. 4. For comparison we have included gel IEF on the same patient.

A third alternative is the recording of the pH gradient without mobilization [55]. Detection of components in each capillary was achieved by imaging the whole length of the short glass capillary.

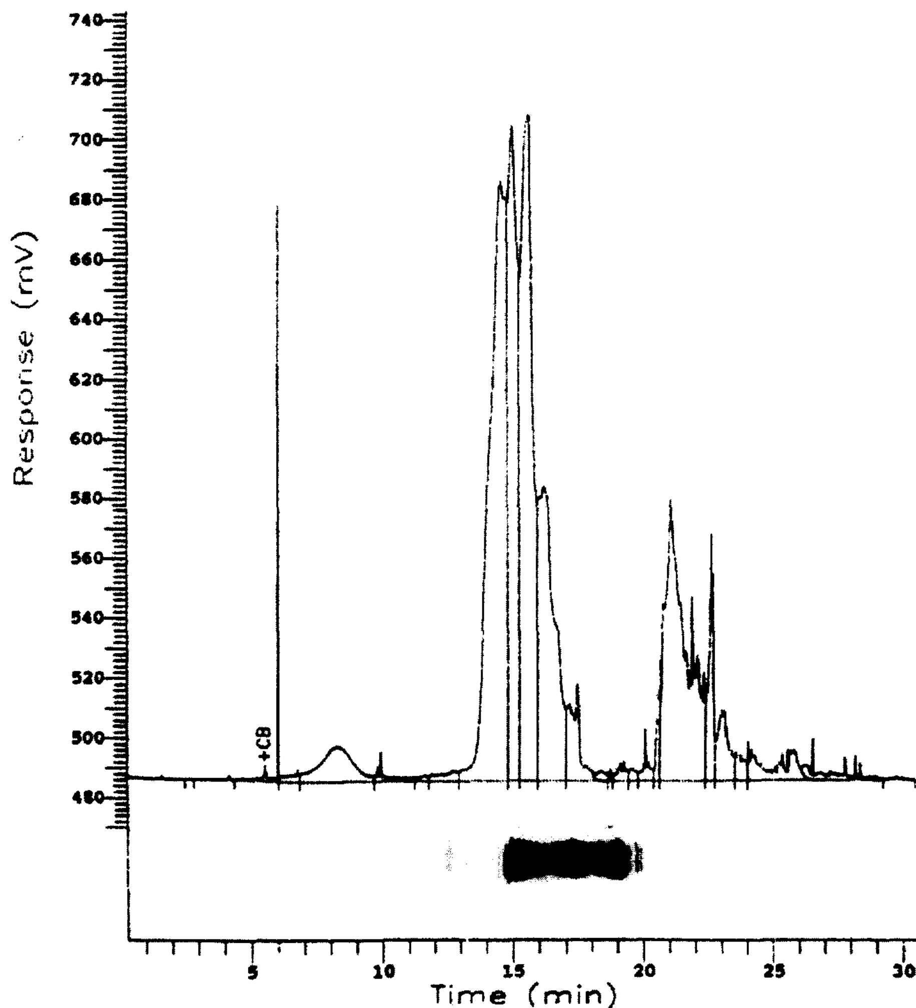

Fig. 4. Capillary isoelectric focusing of an IgG paraproteinaemic serum. Insert near time axis shows gel isoelectric focusing of same serum.

In a later paper Wu and Pawlisyn used the capillary IEF–adsorption imaging detector with a two capillary instrument to quantitate human haemoglobin variants in a single run of less than 3 min [56]. The percentage of HbA_2 could be determined accurately for control material by peak area for concentrations up to 200 $\mu g\ ml^{-1}$. Human haemoglobin variant S was also quantitatively determined using the instrument.

As a further alternative, if the electroosmotic flow is sufficiently reduced to allow focusing to occur, IEF can be carried out in fused-silica capillaries. It is important that a suitable additive such as hydroxypropylmethylcellulose or methylcellulose is added to the buffer to reduce interaction between the proteins and the capillary wall.

8. Immunoelectrophoresis

Immunoelectrophoresis (IEP) which combines the techniques of electrophoresis and immunodiffusion is a qualitative technique rather than a quantitative technique. The serum proteins are electrophoresed and then allowed to diffuse against antiserum placed in adjacent wells. When concentrations of immuno-

Table 1
Comparison of techniques of isoelectric focusing by gels and capillary electrophoresis with immunoelectrophoresis

	Gel IEF	CE IEF	IEP
Resolution	++++	+++	±
Time per sample (min)	8	50	135
Ruggedness of technique	++++	+	+
Technical skill	++	+	++
Interpretation skill	+	+	+++
Consumable cost	+	+++	+

globulins are normal, regularly shaped arcs are formed. However, when a monoclonal protein is present, the arc produced will show a widening or distortion from the normal pattern [57]. Experience is required in the interpretation of various patterns.

Commercially prepared IEP slides are available. When a monoclonal IgM is present, the aggregates of IgM which occur with some specimens may need to be depolymerised by treating the sample with a reducing agent such as mercaptoethanol before applying the sample for IEP. Urine samples may be used for IEP, the optimal concentration being 6–8 $g\,l^{-1}$. CSF samples should be concentrated before being applied to the well. As IEP is a qualitative technique, it is unsuitable for testing for oligoclonal bands in CSF.

A comparison of the resolution, technical skill required and consumable costs involved in the techniques of IEP and IEF both by gels and CE is shown in Table 1.

9. Immunofixation

Identification of paraproteins after detection of a monoclonal band on agarose gel has routinely been achieved by immunofixation of either electrophoretic or IEF gels [10–16]. In immunofixation the proteins of interest are precipitated by overlaying the gel with cellulose acetate strips soaked in specific antisera. The unfixed proteins are removed by washing and the immune fixed proteins are stained and examined. Fig. 5 shows a biclonal gammopathy from a serum specimen immunofixed using both the electrophoretic HRAGE and IEF gel.

Using CE, the identification of paraprotein can be based on immunosubtraction, a concept proposed by Aguzzi and Poggi [58], in 1977. With this procedure, the serum is first incubated with immunoglobulin-specific antibodies bound to solid supports, such as beads. Comparison of treated and untreated specimen provides a decrease in the immunoglobulin component when the appropriate antisera are present [59]. This method is suitable for identification of IgG, IgA, IgM, κ- and λ-light chains. However, producing the immunoglobulin-specific antibodies bound to solid supports is a time consuming method

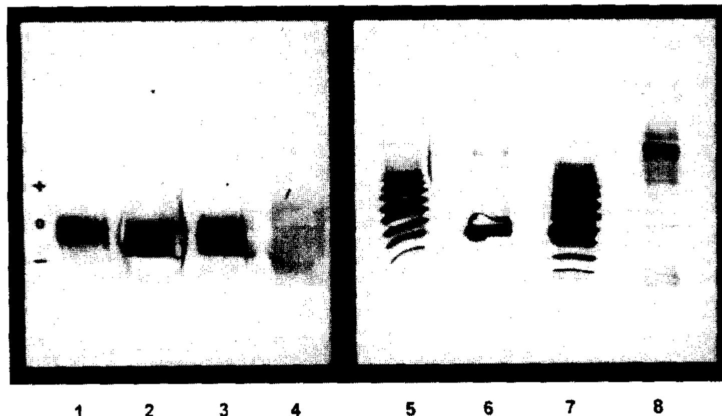

Fig. 5. Immune fixation of biclonal gammopathy by electrophoresis (on LHS) and isoelectric focusing (on RHS) illustrating superior resolution of isoelectric focusing. Lanes 1 and 5: Immunofixed with anti-IgG; lanes 2 and 6: anti-IgM; lanes 3 and 7: anti-κ light chains; lanes 4 and 8: anti-λ light chains.

and consumes large amounts of expensive antibody. Hence the CE method for immunosubtraction currently would appear either too time consuming or too costly. A commercial system using the procedure is available.

10. SDS-PAGE

Under normal electrophoretic conditions the movement of proteins is determined by the net charge of the protein and its mass. In SDS-PAGE the effective pore size of a gel is determined by the concentrations of acrylamide and bisacrylamide in the polymerization mixture. By adjusting the concentration of acrylamide and the crosslinking agent it is possible to make a gel which gives optimal separation for a particular protein. A high concentration of acrylamide (40%) plus N,N-methylene-bisacrylamide combined with a high concentration of crosslinking (12.5%) will restrict the passage of proteins of M_r less than $20 \cdot 10^3$ u [60].

The inclusion of urea in SDS gels allows the protein to remain dissociated in the sample buffer. High concentrations of urea produces increased sieving in the case of small proteins and peptides whereas low concentrations of urea helps maintain SDS solubility at low temperatures [61].

Buffer systems used for SDS-PAGE may be continuous or discontinuous throughout the vertical gel apparatus used. Continuous buffer systems using sodium phosphate are often chosen for use with small pore size gels. In contrast discontinuous buffer systems utilise different buffer ions at different pH values in the electrode reservoirs, Tris–glycine/Tris–HCl being originally developed by Ornstein and Davis in the mid 1960s [20,21]. The differing buffer pH values permit stacking or sample concentration and provide superior resolution. In discontinuous buffer systems the stacking gel is at a slightly acid pH whereas the separating gel is at a slightly alkaline pH. The running reservoir is typically at a slightly alkaline pH.

Alternatively, using linear polyacrylamide in which molecules are free to entwine themselves rather than being held in a fixed mesh of rigid pore size and a coated capillary, proteins may be separated in order of molecular mass by capillary electrophoresis. Native proteins ranging in size from 14 to $669 \cdot 10^3$ u mol mass may be separated in approximately 20 min. Using marker proteins of known molecular mass, the time of elution for standard M_r proteins can be plotted as a function of gel concentration creating a Ferguson plot. Subsequently the molecular weight of the unknown protein can be calculated from this plot.

Alternative conditions include the use of fused-silica capillaries and Tris–borate buffer plus 8 *M* urea which will separate proteins of smaller M_r i.e. those up to $69 \cdot 10^3$ u mol. mass units.

Karger et al. [62] have examined DNA and protein separation using polymer matrices. This excellent paper outlined their experience with covalent coatings which were found to be highly reproducible as well as listing the commercial kits available for CE polymer separations. The preparation of linear polyacrylamide-coated capillaries were detailed and their use for nucleic acid separations, oligonucleotide analysis and DNA sequencing was discussed.

Manabe used cross linked polyacrylamide gel in short fused-silica capillaries to separate standard proteins from 17 to $116 \cdot 10^3$ u in 30 min [63]. The effective length of these capillaries was less than 10 cm with polyacrylamide gels ranging from 2.4–4.8%T (5%C). UV detection at 280 nm on these gel capillaries of 200 or 75 μm I.D. indicated higher sensitivity was achieved for proteins by CE than by Coomassie staining of slab gels.

Lehman et al. [64] have compared the analysis of CE with SDS-PAGE and cellulose acetate membrane electrophoresis for human serum proteins and apolipoproteins A-1 and A-11 [64]. They concluded that CE analysis of serum proteins and apolipoproteins appeared superior with respect to resolution, separation time and sample requirement to conventional methods such as SDS-PAGE or electrophoresis on cellulose acetate membranes. They measured CE peak area versus concentration for apolipoprotein A-1 up to 100 mg dl^{-1} and concluded that quantitative determinations were possible.

A comparison of the techniques of SDS-PAGE by gels and CE is contained in Table 2.

11. Conclusion

The outstanding characteristic of CE is the sensitivity of the technique compared to other chromato-

Table 2
Comparison of SDS-PAGE separations by gel technology and capillary electrophoresis

	Gel SDS-PAGE	CE SDS-PAGE
Resolution	++++	++++
Time per sample (min)	50	12
Reproducibility	+++	++
Technical skill required	+++	+
Interpretation skills	+	+
Consumable costs	+	++++

graphic methods. This sensitivity combined with the automated nature and speed of the technique provided by commercial instrumentation delivers an analytical tool of enormous potential.

Although the technique of automated CE has only been available for approximately 6 years, the number of applications have been increasing almost exponentially. The use of the technique for serum protein separation has gained increasing popularity, despite the fact that the low-end sensitivity of CE for the technique of electrophoresis is not as great as gel IEF. By optimisation of the buffer high p*I* monoclonal bands and 'precipitating' IgM specimens can be successfully dealt with by CE. Cryoglobulins can be routinely quantitated by CE – however cold precipitation of the specimen diminishes the band to the same size that would occur if the cryoglobulin was being assayed by gel techniques.

We have evaluated a number of different buffers for serum protein separation by CE and have shown several to be suitable. These include borate, phosphate and Tris buffer as well as combinations such as Tris–borate and phosphate–borate.

The advantage of CE as an alternative to other protein separation techniques depends on two important factors: labour and consumable costs. At the moment CIEF relies on the use of a kit produced by the instrument manufacturer. Using this kit for the technique of IEF, CE takes six times longer (due to specimens being analysed sequentially) and costs twice as much as conventional gel IEF. As mentioned earlier there is also the problem of the short life of some coated capillaries used for CIEF. Until coated capillaries are available which will give at least 100 runs per capillary, our conclusion must be that CIEF is unlikely to gain widespread acceptance as a routine tool. This is unfortunate considering the sensitivity of the technique.

With the technique of immunofixation, our conclusion is that CE is less advantageous than immunofixation of gel techniques such as IEF and electrophoresis. In the case of SDS-PAGE, gel techniques are likely to remain the preferred option due to consumable cost and time considerations.

Another aspect that needs to be explored for all commercial instrumentation is the introduction of multi capillaries so that two or more samples can be assayed for the same analyte at the same time. The recently released Paragon CZE 2000 Clinical Chemistry Electrophoretic System (Beckman Instruments Brea, CA, USA) has 7 removable fused-silica capillaries whilst Molecular Dynamics (Sunnyvale, CA, USA) have developed a 48-capillary apparatus [65]. Other commercial companies need to give consideration to these modifications so that the time per test could be decreased. Then perhaps the potential of CE as an analytical tool would be fully realised.

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