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REVIEW

HIGH-PERFORMANCE CAPILLARY ELECTROPHORESIS IN THE BIOLOGICAL SCIENCES

BARRY L KARGER*, AHARON S COHEN and ANDRAS GUTTMAN

Barnett Institute, Northeastern University, Boston, MA 02115 (U S A)

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1 INTRODUCTION

High-performance capillary electrophoresis (HPCE), the instrumental approach to electrophoresis, is a method with great potential for the high-resolution separation and purification of biological substances. There is much publicity and significant activity in this field An international symposium on this topic (1st International Symposium on High Performance Capillary Electrophoresis) was held in Boston, MA, U S A , on April 10–12, 1989, in which over

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80 papers have been submitted Review articles have appeared [1–3] as well as news features [4–6] Commercial instruments have been or will shortly be introduced for whole systems

This paper will overview basic principles of separation and instrumentation of HPCE and illustrate some of the typical applications The goal of this review is to provide the reader with a sense of the direction of the methodology (a) what is the power of the approach? (b) what are the significant areas of instrumentation and applications that are on the horizon?

Historically, rods were utilized prior to slab gel operation [7], while capillary columns are often employed today for isoelectric focusing, as the first dimension in two-dimensional electrophoresis [8] What is new in HPCE is on-column or on-line microcapillary detection in an analogous fashion to on-line detection in high-performance liquid chromatography (HPLC) A small section of the polyamide layer is thermally removed to expose the quartz silica tubing for the on-column detection Fused-silica capillary tubing (typically 25–100 cm length and 50–100 μm I D) is employed The narrow diameters are used to dissipate the Joule heat generated from the high fields necessary for rapid analysis and high efficiency

On-column detection in electrophoresis was successfully developed by Catsimpoolas [9] and especially Hjerten [10] using a rotating tube In these early examples, the total tube was scanned for detection purposes Then in the 1970s,

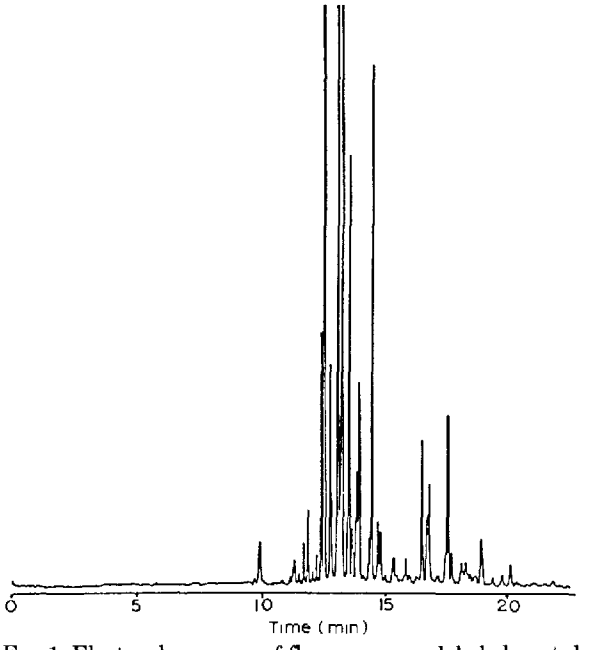


Fig 1 Electropherogram of fluorescamine-labeled peptides from a tryptic digest of egg white lysozyme Column 100 cm × 75 μm I D , 0 05 *M* phosphate buffer at pH 7 (see ref 1)

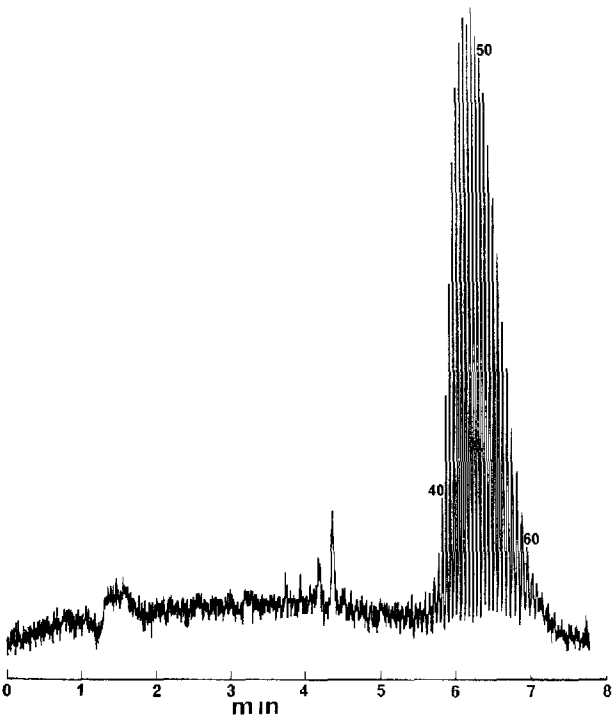


Fig 2 HPCE separation of polydeoxyadenylic acid mixture, $(dA)_{40-60}$ Column 27 cm×75 μm I D , effective length 13 cm, running buffer was 0 1 *M* Tris–0 25 *M* borate–7 *M* urea, pH 8 3, and the gel contained 7 5% T and 3 3% C The applied field was 400 V/cm (see ref 26)

TABLE 1

MODES OF OPERATION OF HIGH-PERFORMANCE CAPILLARY ELECTROPHORESIS

Capillary mode		Separation principle	Typical refs
1A	Zone electrophoresis	Electrophoretic mobility - charge, charge density	22,27
1B	Zone electrophoresis with electroosmotic flow		14,15,18,28,29
2	Micellar electrokinetic chromatography	Hydrophobicity, complexation, charge	20,30,31
3	Gel electrophoresis	Size, charge	21,26,32
4	Isoelectric focusing	p*I*	16,22,32
5	Isotachophoresis	See Zone electrophoresis, displacement mode	11,33,34
6	Packed column electro-osmotic chromatography	HPLC modes	35

Everaerts et al [11] developed capillary displacement electrophoresis or capillary isotachophoresis Interestingly, commercial instrumentation of capillary isotachophoresis was introduced in the mid 1970s, incorporating many of the features of current equipment (albeit at a less developed level) However, capillary isotachophoresis was not a commercial success for several reasons One of the most significant factors was that the needs of the biological sciences of the 1970s were different from those of today Today, separation and purification methods which handle low sample amounts are required for analysis and for subsequent characterization, e g microsequencing of peptides and mass spectrometry HPCE, a method well suited to low sample quantities, matches the needs of the times

Two papers on HPCE introduced the potential of this approach [12,13] Then, in the early to mid 1980s, several laboratories played a significant role in the advancement of HPCE Jorgenson and Lukacs [14] demonstrated the very high resolution possible by this approach and Hjerten and Zhu contributed significantly to open-tube electrophoresis [15] and capillary isoelectric focusing [16] Tsuda et al [17], Lauer and McManigill [18] and our group [19] have also shown the high efficiency and selectivity possible Terabe et al [20] introduced micellar electrokinetic chromatography. Our group [21], as well as Hjerten's [22], introduced gel-filled capillary columns Zare's laboratory [23], as well as Sepaniak's [24] among others, initially showed the power of laser-induced fluorescence detection, and Olivares et al [25] were the first to demonstrate the potential of coupling HPCE to mass spectrometry (MS)

The power of HPCE can be illustrated by Fig 1 which shows an open-tube separation of fluorescamine-labeled peptides obtained from the tryptic digest of lysozyme [1] and Fig 2 shows the separation of polyadenylic acids (40–60 bases) in a polyacrylamide gel column [26] Along with the great resolving power, an HPCE instrument can be utilized in a variety of selectivity modes of separation as listed in Table 1 Thus, a great deal of analytical information on complex mixtures can be obtained by the use of different principles of electrophoresis HPLC separation deals, on the other hand, with the interaction of solutes with chromatographic surfaces, and therefore different selectivities and elution orders often exist in HPLC relative to HPCE Hence, the two methods, HPLC and HPCE, may be viewed as complementary tools Indeed, the integration of HPLC and HPCE may represent one of the most powerful approaches to separation and analysis in the 1990s

In this review we will first explore basic principles that allow utilization of the full power of HPCE This will be followed by a discussion of current and future trends in instrumentation We will then focus on the various modes of HPCE to illustrate current and future applications

2 PRINCIPLES

In zone electrophoresis, the velocity of migration of an ion (v) is proportional to the electrophoretic mobility (μ_e) and the field, (E)

$$v = \mu_e E = \mu_e \frac{V}{L} \tag{1}$$

where V = the applied voltage and L = the length of capillary column between two reservoirs The electrophoretic mobility depends on the charge density of the substance and, thus, the overall valence and size, as well as the viscosity and dielectric constant of the medium The mobility also varies strongly with temperature, approximately 2% per degree [36] The time (t) necessary to migrate the distance between the injection and the detection points (i e the effective length l) can be determined from the velocity in eqn. 1 as·

$$t = \frac{l}{v} = \frac{l}{\mu_e E} = \frac{lL}{\mu_e V} \tag{2}$$

It should be emphasized that l and L are usually not the same value.

Band broadening in capillary electrophoresis can result from a variety of factors When conditions are properly designed, the major effect controlling band width can be axial diffusion of the solute in the capillary tube. This will represent the condition of maximum efficiency HETP (height equivalent to the theoretical plate) then follows the standard molecular diffusion term in chromatography and the theoretical plates N can be expressed as

$$N = \frac{\mu_e E l}{2D} = \frac{\mu_e V}{2D}\left[\frac{l}{L}\right] \tag{3}$$

where D is the diffusion coefficient of the substance in the buffer system It has been emphasized by a number of workers that the higher the field E, the lower the time the molecule spends in the capillary tube (eqn 2) and therefore the higher will be the number of theoretical plates Thus, all things being equal, higher fields yield faster separations with higher efficiencies

One of the limits to ever increasing fields is the Joule heating generated from the applied power This heat will cause the center of the tube to be at a higher temperature than the walls. leading to sample diffusion and solvent density differences and resultant band broadening Many workers have discussed the heat effects on column efficiency and concluded that under normal operating conditions with capillaries the effect is small [30,37–39] However, because of the sensitivity of the electrophoretic mobility with temperature as well as the temperature dependence of sample species to complexation with buffers, micelles, etc , good temperature control is important for migration reproducibility

The Joule heating problem has recently been addressed in detail [37–39] The temperature difference ΔT from the center to the wall (parabolic temper-

ature profile) can be expressed as

$$\Delta T \sim E^2 \lambda C d_c^2 \tag{4}$$

where C is the concentration of the electrolyte, λ is the molar conductivity of the solution and d_c is the tube diameter This relationship clearly shows that the higher the field the narrower the tube diameter must be Thus, capillaries are employed in order to reduce the temperature difference across the tube (as well as the overall column temperature increase) Small temperature effects have been estimated for 25- or 50-μm capillaries if proper thermostatting is employed [39] Typical tube diameters today are 50–100 μm; however, 25 μm is commercially available as well

Eqn 4 further shows that for a given field ΔT depends on the concentration of the buffer selected (C) and the conductivity of that buffer The use of organic univalent buffers is recommended for their relatively lower ionic conductivity, e g Tris borate In addition, zwitterionic buffers can be useful However, the buffer and its concentration need to be chosen with column loadability in mind (see later)

The temperature rise also depends on removal of heat from the outside of the capillary wall Forced air convection is a significant improvement over static air, and flowing liquid baths are an even better means of heat dissipation Solid state Peltier devices also provide a very efficient means of heat removal [40] Care must be exercised to control the temperature of the whole column, including the detector region, in order to minimize hot zones along the capillary

With respect to resolution, we can write that [41]

$$R_s = \tfrac{1}{4}\left[\frac{\Delta\mu_e}{\bar{\mu}_e}\right] N^{1/2} \tag{5}$$

where $\Delta\mu_e$ is the difference in electrophoretic mobility between two substances and $\bar{\mu}_e$ is the mean mobility for those species Substitution for N (eqn 3) into eqn 5 yields a final expression for the resolution

$$R_s = 0\ 18\Delta\mu_e\left[\frac{El}{D\bar{\mu}_e}\right]^{1/2} = 0\ 18\Delta\mu_e\left[\frac{Vl}{LD\bar{\mu}_e}\right]^{1/2} \tag{6}$$

Eqn 6 demonstrates that higher fields, as expected, produce higher resolution Moreover, the lower the diffusion coefficient of the solute, the higher will be the resolution, as well This latter effect can be highly significant in that high-molecular-weight species can be efficiently separated in HPCE if molecular diffusion is the limiting factor in band broadening For example, in the case of DNA restriction fragments, over 600 000 theoretical plates have been attained for species with molecular masses in the several millions [42] Frequently, when species are adsorbed to surfaces, as in HPLC, the larger the size of the molecule the lower will be the rate of desorption and, as a consequence, the lower the efficiency and resolution However, in HPCE, since no surfaces are in principle involved, very high efficiencies can be obtained and high mass recoveries should be possible Indeed, a future advantage of HPCE with respect to HPLC may be the higher recoveries possible for HPCE, particularly for

hydrophobic species

The above equations are directly applicable to zone electrophoresis in which there is no bulk flow An important area of open-tube HPCE today is zone electrophoresis with electroosmotic flow, the bulk flow being derived from the zeta potential on the walls of the capillary Thus, in the case of fused silica where a negative charge exists on the walls, bulk flow will occur in the direction of the negative electrode because of the excess of positive ions in the double layer at the wall

The electroosmotic flow creates an apparent mobility (μ_e^*) which can be directly determined from eqn 1 by measuring the velocity of an uncharged species The apparent mobility of the solute (μ_e^a) now becomes

$$\mu_e^a = \mu_e + \mu_e^* \tag{7}$$

where the mobilities are added vectorially Eqns 2, 3 and 6 are then appropriately modified using μ_e^a instead of μ_e

There are several important conclusions that can be derived from eqn 7 First, with negatively charged fused-silica walls, positive species will migrate faster and negative species slower than a neutral molecule The more electrophoretically active a negatively charged species, the later it will elute in the electropherogram Ultimately, if the absolute value of μ_e is greater than μ_e^*, then the negatively charged species will not be detected as it will have a net migration towards the positive electrode

In principle, both positively and negatively charged species can be detected at one detector site using electroosmotic flow It must, however, be mentioned that oppositely charged species often may adsorb electrostatically to the walls of the fused silica Even the slightest adsorption, e g k' less than 0 1, could drastically lower efficiencies achievable by HPCE due to slow desorption kinetics In order to minimize adsorption, low-pH buffers are often selected, particularly for peptides and proteins [28,29], in order to reduce the charge on the surface of the walls Phosphate buffer is particularly recommended [28] Since all the oligopeptides will be positively charged at the low pH, a further reduction in adsorption can be achieved by coating the wall with a positively charged polymer [28] High pH (e g ~8–9) can also be utilized for reduction of adsorption, as both the capillary walls and the protein (generally) will be negatively charged [18] One of the important problems to be solved in the years ahead is to find stable coating procedures that minimize adsorption of species to the walls

Returning to the issue of band broadening, there are several other potential factors that need to be minimized in order to achieve high performance Consider first extra column effects arising from the injector and detector Minimization of these effects can be of critical importance as the total volume of the capillary tubing is often 1 μl or less It can be calculated that nanoliter or lower injection volumes are necessary to eliminate the injection volume influencing peak width [43] Indeed, a paper recently was published where 9 μm diameter capillary tubing has been used for HPCE and low picoliter injection volumes

were required [44] For the injection of larger volumes of dilute samples in a sharp plug, a preconcentration or focusing step is necessary Examples of focused injection are disc electrophoresis [22], and injection of low-conductivity sample solutions into gel columns [26] The injecon solvent as well as the buffers selected both downstream and upstream from the sample can thus markedly affect performance in HPCE

With respect to the detector, as in HPLC, the on-column cell volume is a critical factor As the injection volume, the cell volume should be in the low nanoliter range Thus, the opening of the exposed capillary through which light is allowed to penetrate must be small [45] For example, for a 100-μm capillary, a 1 5-nl volume would require an opening of only 0 2 mm in order to have no major effect on efficiency (assuming a 600 000 theoretical plate column) Counter-balancing the need to minimize the opening of the capillary is the requirement to have sufficient light intensity for solute absorption and low noise levels One of the important issues in detector design is therefore to obtain high amounts of light through the cell without substantially decreasing column performance

Another factor which can result in band broadening is sample overloading of the column As has been pointed out [46], if the sample component contributes significantly to the conductivity of the band, then the resultant band shape may be either tailed or fronted Two ways of overcoming this overloading effect are (a) reduce sample sizes such that the conductivity of the band is made up predominately from the buffer or (b) match closely the mobility of the buffer and the solute species. In general, it has been found that the use of low sample sizes helps substantially to improve column efficiency [1,18] When appropriate care is exercised, columns which generate several hundred thousand or even greater than one million theoretical plates are possible [18] Such high resolution can be utilized for the separation of complex mixtures or in the assessment of purity for similar species

3 INSTRUMENTATION

A basic instrument of HPCE has many similarities to high-performance liquid chromatography, as seen in Fig 3 The power supply often is in the 30–60 kV range In addition, a device will be found on all commercial equipment which will release the power before the high-voltage electrode can be handled Buffers are contained in reservoirs that are large (several milliliters) relative to the column volume (~ 1 μl) in order to minimize the effects of electrolysis (low currents also minimize electrolysis) It is important to degas the buffers prior to use, and frequent change of buffers will yield best results

Injection is achieved by dipping the end of the capillary into the sample and either pressurizing the sample in the capillary (e g siphoning) or using electrophoresis [47,48] In the latter case, sample discrimination can occur as a

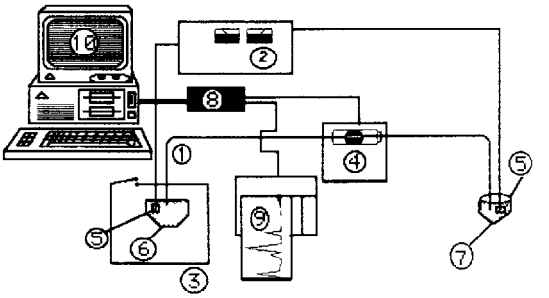


Fig 3 Diagram of high-performance capillary electrophoresis instrument (1) Fused-silica tubing, (2) 30-kV power supply, (3) plexiglas interlock system, (4) on-column UV detector, (5) platinum electrodes, (6) buffer reservoirs, (7) microcentrifuge vial for collection, (8) analog–digital interface, (9) recorder, (10) IBM PC control unit (see ref 26)

consequence of the different mobilities of the sample species [49], however, appropriate corrections can be made if the mobilities are known Both procedures depend on the viscosity of the sample solution and, therefore, care should be exercised with respect to the temperature control of the sample solution for good quantitative reproducibility

3 1 Detection

Detection is one of the most critical aspects of the instrumentation Most commercial instruments will initially use a UV or UV–VIS detector, although in one case simultaneous UV and fluorescence in a diode array configuration is offered [50] For UV detection, the conflicting demands of low cell volume for high performance and high cell volume for light passage and low noise levels have already been noted Beyond this, light scattering off the capillary walls, the location of the photomultiplier tube relative to the capillary and the intensity of the light source must be considered In an improperly designed detector, one can be tricked into believing signals result from samples when in fact they represent refractive index changes due to components in the injection sample Careful evaluation is thus necessary with regard to *true* signal-to-noise levels

Fluorescence will undoubtedly become an important HPCE detector in the immediate future Fluorescence can be useful in reducing the limit of detection and in aiding in specificity. In the case of peptides or proteins, both free and post-column derivatization can be an important feature for HPCE analysis Post-column derivatization is particularly appealing in the case of proteins, since a controlled reaction occurs and quantitation is readily achievable Post-column derivatization has been successfully developed with minimal band broadening in very small volumes [51,52] The utilization of either pre- or post-column derivatization for fluorescence labeling will become particularly

important in areas such as amino acid analysis and sequencing. In the case of oligonucleotides, fluorescence dyes such as ethidium bromide can be utilized for selected detection of DNA molecules [53]

The use of lasers or laser-induced fluorescence opens up the possibility of extremely low detection limits Whereas in HPLC, laser detection has not yet been commercially introduced, there is a real possibility that such detection may occur for HPCE, due to the low concentration detection limit If we assume a detector volume of 2 nl and a mass detection limit of 2 ng, then the lower concentration detection limit in the detector cell is 1 mg/ml The cell volume in an HPLC system is roughly 2 μl, hence, for the same mass detection limit (2 ng), the concentration limit in the HPLC detector would be 1 μg/ml or 1000-fold less than that in HPCE In order to handle conveniently dilute concentrations of sample, it is necessary to reduce the detection limit in HPCE by using more sensitive detectors A 2-pg mass detection limit in HPCE would yield 1 μg/ml concentration limit, corresponding to that in HPLC, and a 2-fg level would represent 1 ng/ml Laser-induced fluorescence, which can detect in the femtogram range or even lower, thus offers the possibility of detection of dilute solutions of sample components as well as a wide dynamic range Recently, Dovichi's laboratory [54] has shown impressive separations of fluorescein isothiocyanate amino acid derivatives to the attomole and subattomole levels (see Fig 4) The molecules are detected in a flowing sheath after the capillary in order to minimize light scattering

A useful spectroscopic approach for universal detection at low levels is to employ indirect fluorescence Here, as developed by Kuhr and Yeung [55], a fluorescently active component is either added to the buffer or is a buffer component itself, e g salicylate, and individual molecules are detected as vacancy peaks from the high fluorescent background Using indirect laser-induced fluorescence, Kuhr and Yeung [55] have been able to detect attomole levels of

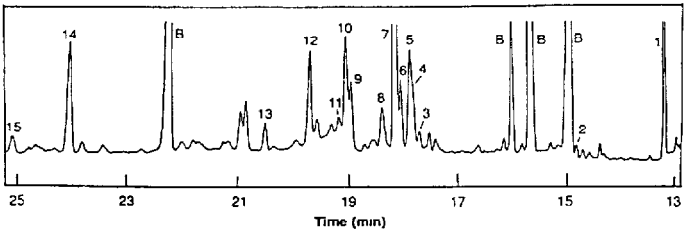


Fig 4 Separation of between 2 and 7 amol of eighteen fluorescein isothiocyanate derivatives of amino acids The separation is driven by a 25-kV potential, and a pH 10 buffer is used for both the separation and the sheath stream Injection was for 10 s at 2 kV Amino acids identified 1 = Arg, 2 = Lys, 3 = Leu, 4 = Ile, 5 = Trp, 6 = Met, 7 = Phe, Val, His and Pro, 8 = Thr, 9 = Ser, 10 = Cys, 11 = Ala, 12 = Gly, 13 = Tyr, 14 = Glu, 15 = Asp, peaks marked B are associated with the reagent blank (see ref 54)

nucleotides This approach holds much promise as a universal detection scheme of high sensitivity

Besides the spectroscopic approaches, other detection methods have also been developed Conductivity has been successfully utilized by Huang et al [56] in the detection of lithium This detector can prove useful for small ions which are highly conducting Electrochemical detectors can also be employed, using an approach developed by Wallingford and Ewing [57] Here, the electrochemical detector is isolated from the high electric field of the capillary by connecting a separate short piece of capillary to the column using porous glass The electrode that completes the circuit for HPCE is positioned on the outside of the porous glass and the electrochemical detector is downstream from the capillary joint Ions that exit the electrophoretic capillary move by laminar flow towards the electrochemical detector where they are subsequently detected The flow through the capillary need not cause excessive broadening [58] A third detector currently under development is a radioactivity detector [3] This approach promises to be useful in the transfer to HPCE of those slab gel electrophoretic applications where radiolabelling plays a significant role

A final detector under rapid development is the direct coupling of HPCE to MS This is an obvious marriage, given either zero flow or low liquid flows exiting the capillary In the first attempt at coupling, the capillary exit has been maintained at atmospheric pressure, employing either electrospray by Smith's group [59] or, more recently, atmospheric pressure ionization MS by Henions group [60] Both of these approaches can be successfully used and with further development, including higher vaporization and ionization efficiencies, can lead to low-level detection, e g picogram detection limits of peptides The electrospray method represents a new approach to MS in which the species fragment into a series of multiply charged ions Using this approach, Smith [61] has been able to detect ions of over 100 000 MW using a standard low-molecular-mass mass spectrometer Most recently, Ewing's [62] and Caprioli's [63] laboratories have adopted the porous-glass connection approach cited above to flowing fast atom bombardment Successful results have been obtained in the analysis of peptides from tryptic digests separated by open-tube HPCE [63] There is no question that there will be much activity in the MS coupling area in the immediate future, and there is a great likelihood that HPCE–MS will take its rightful place beside gas chromatography (GC)–MS and HPLC–MS

3 2 Collection

Initially, HPCE was viewed simply as an analytical tool, however, without the ability to collect and to characterize and utilize the purified materials, HPCE would be severely limited It is essential that HPCE have micropreparative capabilities in order to identify and confirm individual peaks With current methodology, low picomole amounts of material are sufficient for microse-

quencing, e g 150 ng for 100 pmol of MW 1500 and 1 5 μg for 100 pmol of MW 10 000 Such amounts are well within the micropreparative capabilities of HPCE

The original commercial capillary isotachophoretic instrument had collection capabilities in which eluted bands were transported to a collection device by a cross flow of solvent [11] Hjerten and Zhu [64] have advocated a sweep liquid after the capillary to carry the samples to a standard HPLC detector and fraction collector A simpler approach is to collect samples directly in a fraction collector With electroosmotic bulk flow, one can automatically collect the liquid as it exits the capillary [65] If there is no bulk flow as in the case of gel columns or coated tubes, the capillary and electrode can be dipped into a fraction vial containing several microliters of a conductive medium of buffer or even water [26]

Small peptides have been successfully collected from sodium dodecyl sulfate (SDS) polyacrylamide gel columns and microsequenced at the picomole level [66] Indeed, the above collection in water partially decoupled the buffer system from the fraction collection step In addition, a 20-mer primer was purified from a polyacrylamide gel column and identified by a standard dot blot assay [26] In both examples, reinjection of fractions revealed high recoveries of species

Relative to slab gel electrophoresis, micropreparative HPCE offers the advantages of speed, recovery, automation and resolution capability As an example of speed, standard procedures for purification of oligonucleotides require two days [26], whereas the HPCE experiment can be completed in less than 1 h The ability to collect trace amounts of samples without lyophilization can be advantageous relative to HPLC with respect to recovery In addition, biologically active species can be collected from HPCE columns in open-tube or non-denaturing gel systems

Studies are required on optimized conditions for sample capacity The compromise with respect to tube diameter (for capacity) and speed (i e Joule heating) needs to be addressed Bundles of capillaries have been suggested as another approach to increase capacity [48] It should also be noted that the rapid separations possible on 100-μm capillaries may favor a multiple injection approach on such columns for increased capacity Depending on the resolution available, 500 ng–1 μg should be conveniently collected in one run In addition, commercial fraction collectors that are computer-controlled are necessary for routine operation In the future, both HPCE–MS and micropreparative HPCE will be utilized in the identification and confirmation of species

4 OPEN-TUBE HIGH-PERFORMANCE CAPILLARY ELECTROPHORESIS

At present, the greatest activity in HPCE involves open-tube operation using fused-silica capillaries Most work utilizes fused silica with charged surfaces in which electroosmotic flow takes place, however, some separations are

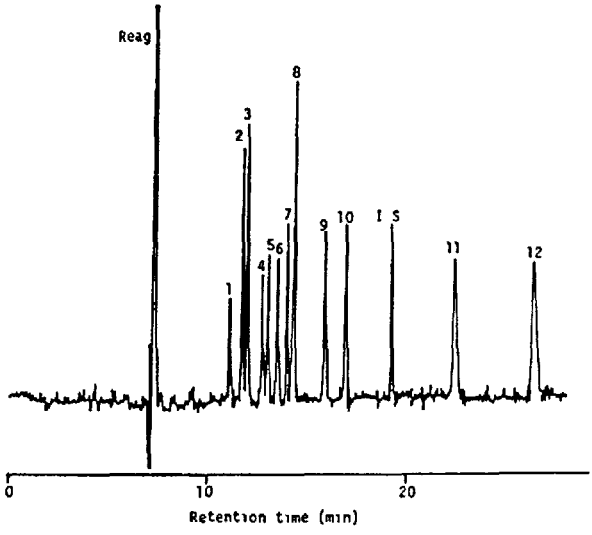


Fig 5 Separation of N-2-pyridylglycamines derived from various reducing monosaccharides Carrier, 200 m*M* borate buffer, pH 10 5, applied potential, 15 0 kV, detection, UV absorption at 240 nm Peak assignment of parent saccharides Reag = reagent, 1 = N-acetylgalactosamine, 2 = lyxose, 3 = rhamnose, 4 = xylose, 5 = ribose, 6 = N-acetylglucosamine, 7 = glucose, 8 = arabinose, 9 = fucose, 10 = galactose, I S = cinnamic acid (internal standard), 11 = glucuronic acid, 12 = galacturonic acid (see ref 72)

being conducted with neutral coated surfaces in which electroosmotic flow is zero or very low [27] We can anticipate a wide variety of applications in the next few years dealing with diverse organic and biochemical mixtures

One area of potential importance is amino acid analysis While amino acid analysis has become well developed and automated using HPLC [67], the need for the determination of amino acid content at low mass levels remains As already cited, Fig 4 shows the separation of derivatized amino acids using an ultratrace laser detector, in which very low levels of mass detection have been achieved For example, in the best case, this cited paper reports a detection limit of 0 05 amol for arginine corresponding to only 5700 molecules As rightly noted by the authors [54], real-world detection at such minute levels may not be feasible since extreme care would be required with respect to sample manipulation to minimize losses due to adsorption, etc However, the application of HPCE separation and detection procedures to sample amounts below that possible today is clearly going to occur in the future One can also anticipate a decrease in sample size in Edman peptide microsequencing using HPCE with laser detection and fluorescent-active phenylthiohydantoin-type derivatives

As another example with respect to amino acids, there is often a need to determine the optical purity of specific amino acids. For example, the racemization of aspartic acid is sometimes used as a basis for dating the organic con-

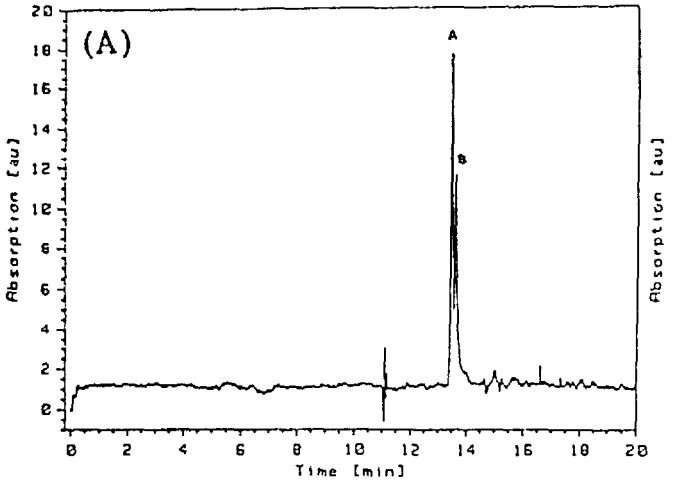
tent of archeological objects [68] Two approaches for chiral analysis can be used in this case, either the formation and separation of a diastereomeric derivative such as with Marfey's reagent [69] or the direct separation of individual amino acids using chiral additives or buffers [2,23,70] Chiral separation at the low sample amount level is clearly an area that will be developed in the next several years using as a foundation the important information already available from GC and HPLC [71]

Recently, an intriguing paper on the separation of monosaccharides via derivatization to N-2-pyridylglycamines has appeared [72] In this example, anionic borate complexes of *cis*-diols created the charge for electrophoretic separation, as shown in Fig 5 The same type of borate complexation has been utilized in the selective separation of catecholamines [73] It is clear from these results, as well as the chiral separation work, that there is a wealth of chemistry that can be manipulated to affect separation in HPCE One of the goals of the next few years will be to examine a variety of complexation schemes with respect to the separation of ionic species

4 1 Peptides and proteins

Another area of great interest for open-tube HPCE is peptides and proteins, and we can anticipate a great deal of activity on this topic over the next several years It has been possible to resolve peptides and proteins by HPCE on the basis of charge differences at a given pH [18,28,29,74] As a consequence, manipulation of pH can be an important selectivity tool If adsorption on the walls takes place, a small amine (e g putrescine [18]) can be added to cover the adsorption sites Alternatively, as already mentioned, low-pH buffers can be utilized [28,29] One must, however, be mindful that proteins may not be stable at low pH For example, McCormick [28] has shown that subunits of hemoglobin could be dissociated in the column at pH 2 1, leading to multiple peaks for a single pure species (Increased temperature caused by Joule heating could also denature proteins) For both strategies to reduce adsorption, i e addition of base or low pH, the electroosmotic velocity will be lowered as the zeta potential on the walls is decreased This change in μ_e^* can improve resolution [1] albeit at the price of longer analysis times

Closely related peptides and proteins have also been resolved by open-tube zone electrophoresis For example, seven amino acid peptides differing only in one neutral amino acid have been successfully separated (i.e silent mutants) [29] In another example [75], Fig 6A shows the separation of a 70 amino acid peptide IGF I (peak B) and a by-product (peak A) in which the disulfides have been scrambled (see Fig 6B) At pH 11 in this figure, a high electroosmotic velocity occurs, reducing resolution Perhaps addition of a base, as noted above, would prove useful Nevertheless, the separation does demonstrate the power of the method to biotechnology problems



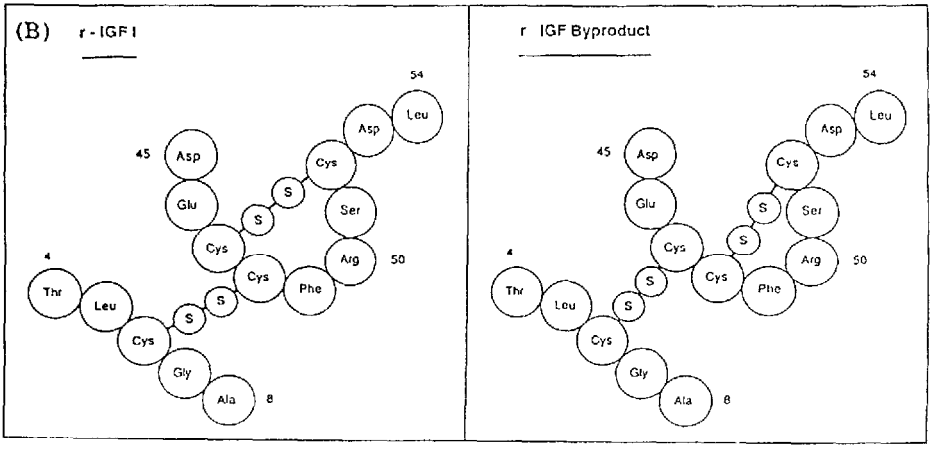


Fig 6 (A) Electropherogram peak A = r-IGF by-product (0 1%), peak B = r-IGF I (0 1%) Electrolyte buffer, pH 11 1 10 m*M* CAPS (3-cyclohexylamino-1-propanesulfonic acid)-5 m*M* sodium borate-1 m*M* EDTA Injection, 10 kV for 10 s, length of capillary, 120 cm (105 cm to the detector), voltage for electrophoresis, 250 V cm^{-1} at 25 μA, detection, 215 nm (B) Schematic view of the structures of r-IGF I and r-IgF by-product (see ref 75)

Other impurities of degradative products of recombinant materials can be determined as well. For example, deamidation will yield an additional carboxyl group on a peptide, and the deamidated product can be resolved from the amidated one by taking advantage of the charge difference [76]. Sulfoxides of methionine are also resolvable We can also anticipate important applications in the glycoprotein area where size (carbohydrate) and charge (sialic acid) will play a role on migration time

TABLE 2

COMPARISON OF PEPTIDE RETENTION ORDER ON HPCE AND REVERSED-PHASE HPLC [78]

Peptide	Residue number	HPCE, pH 8 5[a]	Reversed-phase HPLC, pH 2 1[b]	Reversed-phase HPLC, pH 7 4[b]
Oxytocin	9	4	1	2
Bradykinin	9	3	2	5
Angiotensin II	8	1	3	1
Neurotensin	13	2	4	4
Angiotensin I	10	2	5	3

[a]Buffer 0 1 *M* Tris borate, 0 1 *M* SDS
[b]See J L Meek, Proc Natl Acad Sci , 77 (1980) 1632

Since the above degradative analyses are also possible by HPLC [77], HPCE should be viewed as a complementary tool to HPLC for assessment of purity of recombinant materials As we have noted, HPCE provides different information than HPLC as a result of an alternative mechanism of separation This can clearly be seen in Table 2 in the elution order variations of some simple peptides by HPCE and reversed-phase HPLC at pH 2 1 and 7 4 Interestingly, SDS micelles have been utilized for the low-pH HPCE separation Retention of the peptides is affected both by ion pairing and by partition within the interior of the micelle (see later)

Researchers are exploring a variety of parameters, such as pH, SDS, etc that affect retention of peptides in open-tube HPCE Recently, Nyberg et al [79], based on earlier work [80], have shown that the retention of small peptides can be predicted by the relationship of $M^{2/3}/Z$, where M is the molecular weight and Z is the valence of the molecule More study is necessary in order to examine the range of validity of this relationship and to understand the role of buffer–peptide interactions on retention in HPCE In addition, as the molecules become larger, the overall conformation of the species may affect electrophoretic mobility

Another important potential open-tube HPCE application is enzymatic (e g tryptic) digest analysis (see Fig 1) Early results are promising for peptide mapping, but work needs to continue, as in peptide separation by HPCE itself, on minimizing adsorption and opening up the time window of separation Because of rapid separation, it should also be possible to use HPCE to optimize the digest conditions themselves Ultimately digest analysis may be routinely conducted by HPCE, with direct coupling to MS [25,60,62,63]

4 2 Electrokinetic chromatography

We have already pointed out the power of manipulation of separation by the addition of complexing species to the buffer phase An important advance in

this area was the introduction of micelles in the zone electrophoretic experiment [20,30] A surfactant, such as SDS, above its critical micelle concentration (~ 10 mM, depending on conditions) will form colloidal micelles in which the interior of the colloid is hydrophobic and the exterior is negatively charged While the micelles break and form on the millisecond time scale, there is sufficient time to solubilize hydrophobic species within the interior Because of its high charge density, the micelle will migrate at a slow fixed rate toward the negative electrode under electroosmotic flow caused by the negatively charged fused-silica capillaries. The colloidal micelle acts in a manner similar to a stationary phase in chromatography, and separation is based on partition within the micelle

Neutral species were first separated by micellar electrokinetic chromatography. When no micelles are present, neutral solutes will migrate with the electroosmotic flow, and no separation will result. However, with the addition of SDS, separation is possible as shown in Fig. 7 [81], where cold medication sample components elute in order of increasing hydrophobicity Note the high efficiency possible with this approach. The capacity factor $\tilde{k}'$ for partition can be written as

$$\tilde{k}' = \frac{t_R - t_0}{t_0(1 - t_R/t_m)} = K\frac{V_S}{V_M} \tag{8}$$

where t_R = retention time of solute, t_0 = retention time of solute moving at the rate of bulk flow, t_m = micelle retention time, K = partition coefficient, V_S and

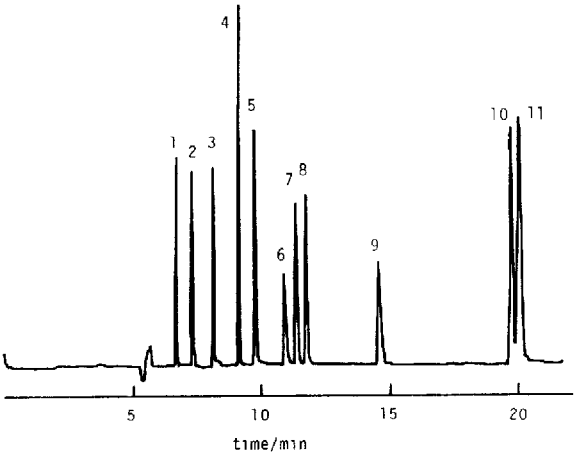


Fig 7 Micellar electrokinetic chromatographic analysis of cold medicine Peaks 1 = acetaminophen, 2 = caffeine, 3 = sulpyrin, 4 = naproxen, 5 = guaiphenesin, 6 = impurity, 7 = phenacetin, 8 = ethenzamide, 9 = 4-isopropylantipyrine, 10 = noscapine, 11 = chlorpheniramine and tipepidine Applied voltage, 20 kV, capillary, 650 mm × 0 05 mm I D , 500 mm to the detector, detection wavelength, 220 nm (see ref 81)

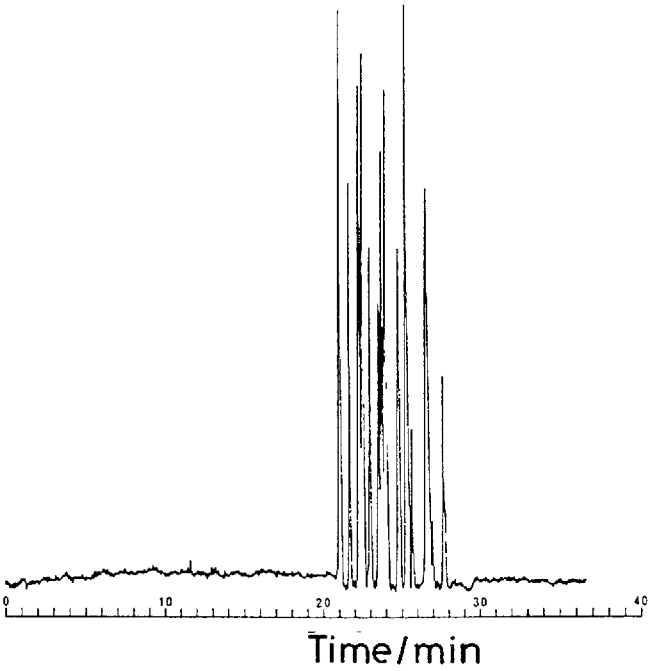


Fig 8 Separation of a mixture of eighteen deoxyoligonucleotides, each with eight bases Buffer 7 *M* urea, 20 m*M* Tris, 5 m*M* Na_2HPO_4, 50 m*M* SDS, 3 m*M* Zn^{2+} (see ref 19)

V_M = volume of stationary (micellar) and mobile phases, respectively Differences in $\tilde{k}'$ (or K) for different substances are the cause of separation It should also be mentioned that besides allowing resolution of neutral species, the time window for possible separation is expanded up to the elution time of the micelle (i e K very large) Interestingly, as in HPLC, gradient elution is possible by addition of either organic solvent or non-ionic surfactants [82]

Recently, workers have recognized that the micelle system can create added selectivity when species of different charges are to be resolved, for example, the separation of neutral from negatively charged nucleic acid constituents [83] In addition, positively charged species can ion pair to the negatively charged SDS micelles with significant retention changes [73,84]. It should be noted that the negatively charged micelle will also be a competitor with the negatively charged wall, thus reducing electrostatic adsorption of positive species with the wall and enhancing efficiency

Besides ion pairing, one can manipulate the chemistry on the surface of the micelles for separation. For example, metal ions will be electrostatically attracted to the SDS micelle surface and be available for complexation with solute species [19]. Using zinc–SDS micelles, Fig. 8 shows the separation of fourteen out of eighteen 8-mers of deoxyoligonucleotides that differ in their base

sequence Differential metal complexation of the deoxyoligonucleotides plays a significant role in the separation mechanism As another example, optically active micelle surfaces can be utilized to separate chiral species For example, an optically active chelate–detergent complex with metals can be used to separate D,L-dansylated amino acids [2]

Micelles represent only one type of complexing additive for electrokinetic chromatography Cyclodextrins, that have been chemically altered to create charge on the complexing agent, e g 2-O-carboxymethyl-β-cyclodextrin, can be used for selective separations of inclusion compounds [85] Ionic polymers, e g polybrene, can also be added to the buffer to create a type of ion-exchange chromatography [86] These and other additive approaches follow a strategy already developed in affinity electrophoresis or affinophoresis [87] As is the case in HPLC [88], we can anticipate the introduction of many chemical selectivities in the next few years to manipulate separation in open-tube HPCE

4 3 Deoxyoligonucleotide restriction mapping

Restriction fragment separation and mapping of DNA molecules is today one of the most important applications of slab gel electrophoresis. In this application, using an appropriate restriction enzyme [89], double-stranded DNA is specifically cut (depending on sequence) into fragments of different base number or molecular mass As presently conducted on slab gels [90], the separation is typically achieved using different gel compositions, since the molecular masses of the fragments can vary from a few thousand to several million Open-tube zone electrophoresis has the capability of resolving a very wide molecular weight range of species in one run, however, it has generally been believed that oligonucleotides would possess the same electrophoretic mobilities independent of base number in free solution [91].

Recently, several reports have appeared that lend encouragement that open-tube HPCE can be used for restriction mapping Fig 9A shows the separation with electroosmotic flow of a λDNA-Hind III–ϕX-174DNA-Hae III mixture, containing at least 18 deoxyoligonucleotides from 72 base pairs (46 000 MW) up to 23 000 base pairs (15 000 000 MW) [42] Fig 9B presents the same mixture which has been spiked by slab gel-purified fragments The buffer system consisted of 0 1 *M* Tris borate (pH 8 1), 7 *M* urea, 2 5 m*M* EDTA and 0 1% SDS Injection of heated samples also proved most effective in high resolution The average plate count for individual peaks was 600 000, which means that roughly a 0 5% difference in mobility will yield baseline separation Since the free zone electrophoretic mobility of deoxyoligonucleotides at room temperature is roughly 10^{-4} cm^2/V s [90], the high plate count means that species with mobility differences as small as $5 \cdot 10^{-7}$ cm^2/V s can be resolved The high resolving power is undoubtedly one of the reasons for the result in Fig 9

A second approach for open-tube HPCE separation of restriction fragments

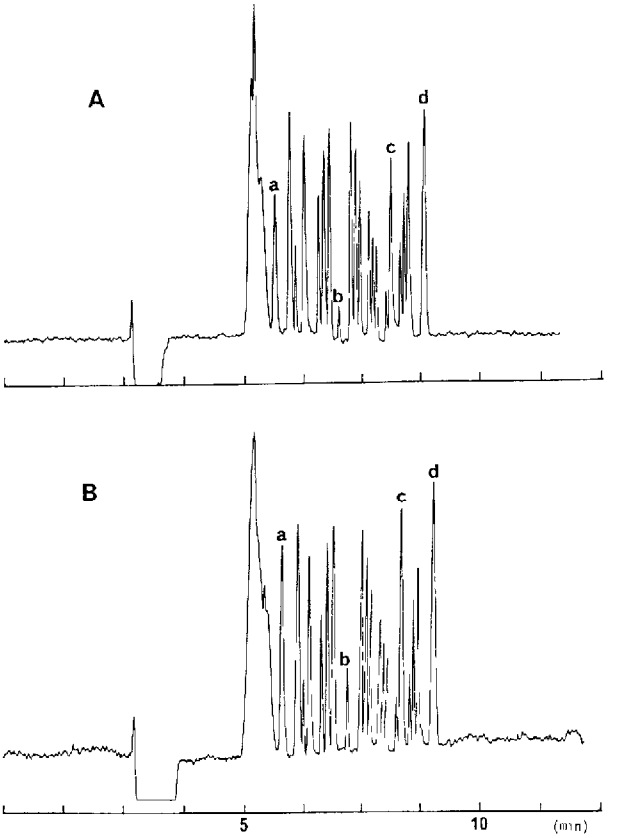


Fig 9 (A) Separation of DRIgest™ III (λDNA-Hind III-φX-174DNA-Hae III) Sample heated for 20 min at 60°C, injected hot by siphoning (3–4 nl) (B) Separation of DRIgest III sample spiked with four slab gel electrophoretically purified fragments 72 (a), 564 (b), 4362 (c) and 23 130 (d) bp Note the increased peak areas for the spiked peaks relative to those in (A) Conditions (A and B) buffer, 0 1 *M* Tris borate, pH 8 1, 2 5 m*M* EDTA, 0 1% SDS, 7 *M* urea, column, 300 mm × 0 075 mm I D , effective length 150 mm, applied voltage, 15 kV, 30 μA, detection, UV, 260 nm, temperature, 27 ± 0 5°C (see ref 42)

is to add a cationic surfactant to the buffer [53] This is another example of electrokinetic chromatography in which micelles and ion pairing are utilized for separation There are probably a variety of other chemistries that can be incorporated into the buffer for the achievement of oligonucleotide separation HPCE clearly has great potential in restriction fragment mapping for both molecular biologic and forensic science applications (DNA fingerprinting)

5 ISOELECTRIC FOCUSING

Another approach which can be incorporated within the capillary electrophoresis instrument is isoelectric focusing in which substances are separated

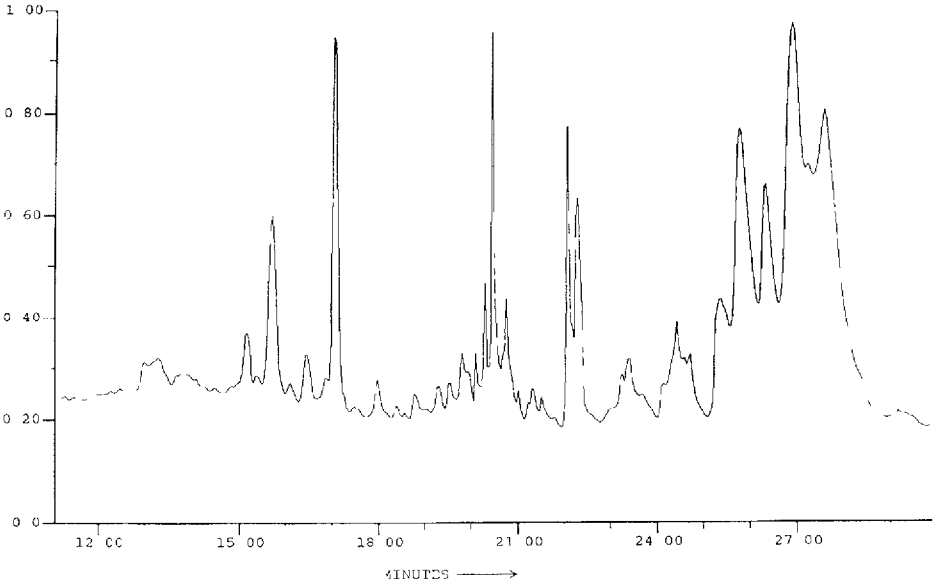


Fig 10 Capillary isoelectric focusing of proteins Sample commercial standard protein mixture containing nine proteins with pI range of 3 5–10 6, 2 mg/ml with 0 05% methylcellulose in 3% ampholyte mixture with pH range of 2–11 Conditions 20 min focusing at 6 kV, 10 μA followed by 40 min mobilization at 10 kV Detection at 280 nm (see ref 32)

on the basis of their isoelectric points or pI values [92] This method can be highly resolving, and it can be anticipated that rapid development will occur in the years ahead As shown by Hjerten and Zhu [16], the open-tube capillary is initially coated with a neutral hydrophilic polymer The sample components are then typically mixed with the carrier ampholytes which span the desired pH range, e g. 3–10, and the mixture is injected into the capillary Upon the application of an electric field, the sample ions concurrently focus with the carrier ampholytes, and this focusing can be followed by the drop in current The focused material can then be eluted from the capillary by a pressurized flow, e g simply lifting the height of one capillary and permitting the sample to flow by the detector cell Alternatively, after focusing, salt (e g sodium chloride) can be added to the anolyte (acid reservoir) or catholyte By the principle of electroneutrality, sodium ions can exchange for protons in the tube, generating a pH imbalance gradient which causes the elution of the components [93] Sharp peaks are obtained with good resolution, and a large peak capacity is observed An example of isoelectric focusing is shown in Fig 10 which demonstrates the separation of nine standard proteins [32] Interestingly, as in this case, one often observes extra peaks in an electropherogram for seemingly 'pure' proteins

The resolving power in isoelectric focusing can be expressed in terms of Δ(pI), the difference in pI of two species for separation [92]

$$\Delta(\mathrm{p}I) = 3\left(\frac{D[\mathrm{d(pH/d}x)]}{E[-\mathrm{d}\mu/\mathrm{d(pH)}]}\right)^{1/2} \quad (9)$$

Good resolution results from species with low diffusion coefficients and a high mobility slope at the isoelectric point, a shallow rate of change of pH with tube distance and a high electric field High fields will also speed focusing The use of well cooled columns can be of value for resolution and separation speed in capillary isoelectric focusing [42]

In principle, it is not necessary that the electroosmotic flow be negligible during the focusing step, only that it be less than the time necessary for focusing However, reproducibility from column to column could be problematic In addition, the coating on the walls must be sufficiently stable to allow good reproducibility from run to run with the same column Clearly, coating chemistry will play a significant role in this method

Since the whole tube is simultaneously used, larger sample amounts can be injected than normally in free zone electrophoresis [22] Nevertheless, one must be cognizant of the possibility of precipitation at the p*I* of the protein Such an effect will show up on the electropherogram as a spiked signal and a non-linear calibration curve Further study is necessary to fully utilize capillary isoelectric focusing, but one can envision this method becoming a powerful tool in HPCE, particularly in peptide mapping In addition, it is likely that when fully developed, the approach may be used as the first dimension in two-dimensional electrophoresis [8]

6 HIGH-PERFORMANCE CAPILLARY GEL COLUMNS

Gels were originally introduced on flat plates as a means of reducing convection (i e an anticonvective medium) In the case of capillaries, the wall can serve to some extent as an anticonvective medium, particularly at the high surface area-to-volume ratios possible in narrow-bore capillaries [13] Gel columns, however, provide unique modes of selectivity, and it is for this reason that they represent an important approach in HPCE The fibrous nature of polyacrylamide and agarose causes a sieving effect in which small molecules migrate faster than larger-sized species In the case of SDS polyacrylamide gel electrophoresis (SDS-PAGE) of peptides and proteins, each SDS species has the same charge density and shape, and separation is based solely on size or molecular mass For oligonucleotides as well, gel columns also separate by size (base number), since mobility in free solution is roughly independent of size In the case of non-denaturing gels for peptides and proteins, both size and charge will contribute to separation

It is possible to conduct SDS-PAGE using narrow-bore capillaries [21] Previously, it was shown that cyanogen bromide fragments of myoglobin could be

separated on such columns A linear relationship existed between log MW of the species and electrophoretic mobility. A similar relationship was found for proteins and by variation in monomer composition (% T), and use of Ferguson plots, it was demonstrated that pure size separation was achieved An SDS-PAGE separation of six molecular mass marker proteins is shown in Fig 11. As already mentioned, peptides have been collected from SDS-PAGE gel columns and successfully sequenced [67]. In addition, non-denaturing polyacrylamide gels can be employed [2]. Further work is needed to extend the molecular mass range and to maximize the resolving power of PAGE columns.

In a second example, Fig. 2 has shown the polyacrylamide gel separation of polydeoxyadenylic acids. High-performance separation has been achieved with single base resolution in short time. Such results suggest that capillary gel electrophoresis has real potential as the separation method for DNA sequencing Interestingly, the original work on an automated sequencing instrument involved a gel tube [94], before slab gels were commercially incorporated Capillary gel columns will need to be further examined with a variety of oligonucleotide mixtures to optimize separation for DNA sequencing In addition, for

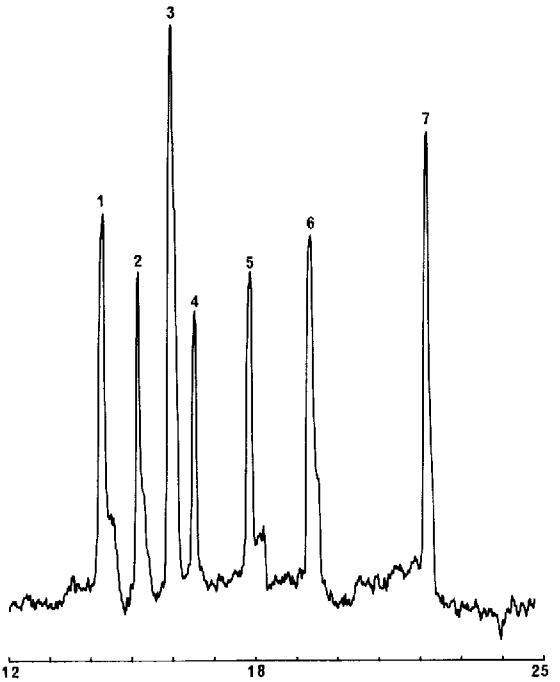


Fig 11 SDS-PAGE separation of molecular mass markers Conditions 300 V/cm, 12 μA, 25°C migration, distance, 15 cm, fused-silica capillary, 75 μm (1), T=7 5%, C=5% Buffer 0 1 *M* Tris borate, pH 7 3, 0 1% SDS, 8 *M* urea Samples 1=tracking dye, 2=lysozyme, 3=β-lactoglobulin A, 4=trypsinogen, 5=pepsin, 6=egg albumin, 7=bovine albumin Monitoring at 220 nm

the required low-level detection, laser-induced fluorescence or some other comparably sensitive detection principle will be required.

Besides the potential for sequencing, the gel columns provide a rapid means of purity assessment of synthesized oligonucleotides and micropreparative isolation of such species [26] It is often found by HPCE that oligonucleotides (or proteins) purified by slab gel electrophoresis still contain trace (or higher) amounts of impurities migrating close to the major peak Since fractions can easily be collected from capillary gel columns, micropreparative applications of such columns may prove to be significant in biotechnology

The gel columns can also be used with complexing agents to achieve unique selectivities As practiced in affinity electrophoresis [95], one can either chemically attach complexing agents to the gel matrix or incorporate the agent into the polymeric fiber gel matrix Our laboratory has used the latter approach of entrapment by polymerizing polyacrylamide in the presence of the neutral inclusion compound, β-cyclodextrin By this incorporation into the gel matrix, the complexing agent is immobile when uncomplexed and migrates only slowly, if at all, when complexed with ionic species The column can be considered to be analogous to an HPLC column in which the complexing agent is fixed to the solid support Fig 12 shows the separation of D,L-dansylated amino acids using a β-cyclodextrin gel column These inclusion compounds are well known to resolve chiral species [97] Over 100 000 plates have been obtained with an effective length of 15 cm at a field of 1000 V/cm As with open-tube operation, there is a wealth of chemistry that can be utilized in gel column operation

There are several general features of gel columns that are worth noting First, the diffusion coefficient of any solute will be lower than in open-tube electrophoresis because of the high viscosity of the gel Large plate counts are thus achievable (eqn 3), for example, in Fig 2 the average plate count is 600 000

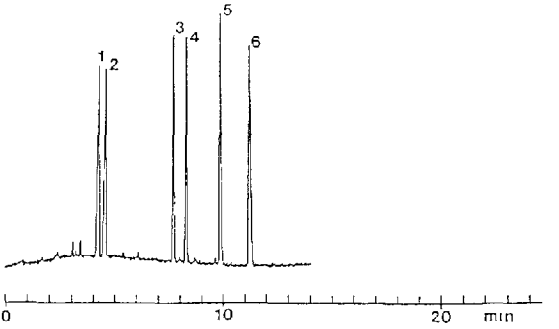


Fig 12 High-efficiency separation of D,L-dansylated amino acids 1 = Dns-L-Glu, 2 = Dns-D-Glu, 3 = Dns-L-Ser, 4 = Dns-D-Ser, 5 = Dns-L-Leu, 6 = Dns-D-Leu Buffer 0 1 *M* Tris, 0 25 *M* boric acid (pH 8 3), 7 *M* urea Gel T = 5%, C = 3 3% Capillary 150 mm × 0 075 mm I D , *E* = 1000 V/cm (see ref 96)

for an effective column length of 13 cm Secondly, when the field is switched off as in collection [26], minimal diffusional band broadening will occur This result means that computer-controlled heart cutting of peaks for high purification should be achievable, even for high-performance columns. Thirdly, electric fields can be readily programmed without significant band broadening, both for manipulating the rate at which substances enter a mass spectrometer or for enhancing separation [98] Finally, focusing of samples upon injection in the gel column is possible, either by manipulating the conductivity of the sample solution or by disc electrophoresis

Further work is necessary for full utilization of the gel columns Care must be exercised not to introduce bubbles into the column upon injection Secondly, UV detection of proteins at 210 nm or lower is difficult because of the interference of the gel An answer around this issue is to couple an open tube to the gel column and to detect in the open-tube region However, while successful, this approach needs further optimization Successful operation at 220 or 280 nm is also possible Nevertheless, the gel columns offer unique selectivity possibilities under high resolution, and they will be utilized along with open-tube capillaries for a wide variety of biochemical applications

7 CONCLUSION

As this review has demonstrated, HPCE is a rapidly advancing field It needs to be emphasized that almost none of the cited work has been performed on commercial instruments which are only now in the early stages of introduction Within the next several years, we can anticipate a wealth of applications on commercial systems

The immediate future should see the validation of HPCE as an important analytical and micropreparative tool in peptide or protein chemistry Oligonucleotide sequencing, restriction fragment mapping and macro DNA separations will also be intensely investigated As already shown [22], open-tube capillary electrophoresis is a viable tool for migration of bacteria and viruses Separation of very closely related species will also be demonstrated, taking advantage of the high resolving power A recent good example of the high resolution possible is the isotopic separation ($^{16}O/^{18}O$) of carboxylic acids [99] HPCE–MS will also become a widely utilized tool for analysis of biological substances The next few years should see enormous advances

Finally, it should be again noted that HPCE is not a tool to replace HPLC or slab gel electrophoresis Rather it is complementary Each method has its advantages and disadvantages The proper perspective is to consider adding HPCE rather than replacing current methodology

8 SUMMARY

High-performance capillary electrophoresis (HPCE), the instrumental approach to electrophoresis, is a method undergoing rapid development at the present time There is a high expectation that HPCE will become a widely applicable tool within the biochemical community This review presents principles and instrumentation followed by typical applications of HPCE in the biological area In the instrumentation area, particular attention is paid to current and future detectors, including laser-induced fluorescence and HPCE–mass spectrometry In the applications section, both peptide or protein and oligonucleotide high-resolution separations are described for open-tube and polyacrylamide gel capillary columns The use of isoelectric focusing and electrokinetic chromatography to manipulate separation is also presented Future directions of methodology are suggested, and it is predicted that in the next few years HPCE will become a complementary tool to liquid chromatography and slab gel electrophoresis

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ADDENDUM

Since the time of the completion of the manuscript, January 1989, HPCE has advanced at a rapid pace with a high interest level The 1st International Symposium on High Performance Capillary Electrophoresis (HPCE '89) was held with roughly 450 participants. Originally, plans were made to schedule a second meeting in February, 1991, however, because of the accelerating pace of the field, the scientific committee voted to hold a meeting in 1990 HPCE '90 will be held in San Francisco, January 29–31, 1990

At present six companies have introduced full systems, Applied Biosystems, Beckman, Bio-Rad, Dionex, Shimadzu and Spectrovision Two companies have introduced HPCE–UV detectors (modified from HPLC), ISCO and Linear Instruments, and one company recently introduced an HPCE–MS interface to their atmospheric pressure ionization mass spectrometer, SCIEX Several new review articles have also recently appeared [1A,2A]

Further advances in the theory of band broadening in electrokinetic micellar chromatography have been published [3A] and presented at HPCE '89 [4A] The need for efficient Joule heat removal from the capillary in terms of overall column temperature control has been emphasized [5A] With respect to equipment, an on-line radioisotope detector for HPCE has been introduced [6A], and detection limits below 1000 molecules have been achieved by laser induced fluorescence [7A] HPCE–MS interfacing continues its very rapid advance using electrospray ionization, where a bovine serum albumin dimer (MW 133,000) was determined on a normal quadrupole instrument [8A] Peptide determination by electrospray MS is clearly becoming established [8A,9A] and a coaxial continuous flow fast atom bombardment HPCE system using 10 μm diameter capillaries seems promising [10A]

In the peptide–protein area, an interesting paper illustrating the use of HPCE isoelectric focusing for resolution of isoforms of transferrin has appeared [11A] A further validation of the power of open-tube HPCE to peptide and protein analysis has been shown [2A] Especially interesting is the lack of coherence between elution order in HPLC and HPCE of peptide maps, emphasizing the complementarity of the two methods The separation of degradated products of the recombinant materials has been further advanced [2A,12A–14A] as well as peptide mapping [15A] A new relationship between free mobility, μ, and charge/size of peptides has been developed [16A]

$$\mu \propto \frac{\ln(q+1)}{n^{0.43}} \tag{1A}$$

where q = charge and n = number of amino acids Electrophoretic mobility should turn out to be important in peptide identification [17A]

In electrokinetic micellar chromatography, a broader understanding of the parameters that can be manipulated for separation is beginning to appear [18A–20A] Ion pairing can be very important when solutes oppositely charged to

the micelles are employed [21A] The role of pH has been shown to be particularly complex but highly useful [22A]

In the gel column area, plate numbers as high as $15 \cdot 10^6$ for lengths of 50 cm have been achieved for deoxyoligonucleotides [23A] These very high resolving powers hold major promise for DNA sequencing New approaches to collection using field programming have also been introduced [23A] Here, a high field is employed until just before migration of the band from the column The field is then decreased to a low value (e g 30 V/cm) for collection By this approach, high resolution (narrow time band widths) can be accomplished while simplifying collection with wide time band widths

The pace of advances is expected to continue over the next several years It is clear that HPCE will become an important quantitative and micropreparative tool in the analytical and biological sciences

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