resis toward the use of thinner gels; these gels, for example, give better retention of nucleotides for DNA sequencing and, because of more efficient cooling, allow higher voltages to be used for faster separations. In the past, gels have typically been 2 mm thick; today, they are often less than 0.5 mm thick. This has been made possible primarily by refinements in preparing the gels. Many more investigators are also now using agarose gels, which are less toxic than polyacrylamide. And finally, it has become possible within the past year to prepare gels with immobilized *p*H gradients.

Detection and analysis of the samples have also improved markedly. Laser densitometry and computer analysis of specimens, in particular, have made analysis of product distributions a more exact science. New electroblotting techniques have also helped to preserve specimens and make analysis easier. It is often difficult to derivatize samples left in the gel; moreover, with time they diffuse through the gel, diluting the sample and blurring resolution.

In 1978 E. O. Southern reported that the distribution pattern of DNA in the final gel can be preserved by placing nitrocellulose paper on the surface and "blotting" up the nucleotides. At first, the DNA was covalently bound to the paper, but subsequent investigators have concluded that simple adsorption is suitable for most purposes. The sample can then readily be derivatized for precise identification, and the distribution pattern preserved for comparison with future gels. The eponymous Southern blot was followed soon after by the Northern blot for RNA and, more recently, by the Western blot for proteins.

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Capillary Zone Electrophoresis

James W. Jorgenson and Krynn DeArman Lukacs

Electrophoresis has developed into a powerful technique for the separation and analysis of charged substances, especially biopolymers. In large part, the success of modern electrophoresis rests on the effective utilization of stabilizing media such as polymer gels. These gels stabilize the separation medium against convection and flow, which would otherwise disrupt separations. A large part of the science of modern electrophoresis is devoted to understanding and controlling the formation of these gels (*1*, *2*). Still, electrophoresis as commonly practiced would not be considered an instrumental method of analysis. True instrumental versions of electrophoresis analogous to modern column chromatography are rather rare. In part, this can be traced to the essential role of stabilizing gels in electrophoresis. Because of the presence of gels, the method has not been easily adapted to on-line sample application, detection, quantification, or automated operation. Instead, modern electrophoresis is a powerful and yet manual-intensive methodology.

Instrumental versions of electrophoresis have been developed. Among these the "rotating tube" system of Hjerten (*3*) and the "transient-state isoelectric focusing" technique of Catsimpoolas (*4*) are particularly notable. These techniques, although novel and quite powerful, have failed to come into routine usage due to their complexity. Capillary isotachophoresis is probably the only instrumental version of electrophoresis to see extensive application, although here, too, acceptance has been slow. The unconventional format of data output in isotachophoresis, coupled with the fact that it appears better suited for separations of relatively small molecules, is the probable reason for its slow acceptance (*5*).

In the course of considerations of causes for zone broadening in zone electrophoresis, it occurred to us that an "open" capillary tube—that is, one containing buffer without stabilizer—offered a unique and simple situation in which to study electrophoresis. In such a system electrophoresis could be studied with minimal interferences, and at the same time causes of zone broadening could be

J. W. Jorgenson is an assistant professor of chemistry and K. D. Lukacs is a graduate student in the Department of Chemistry, University of North Carolina, Chapel Hill 27514.

minimized. It now appears that such systems also offer a practical way to perform rapid and highly effective electrophoretic separations in an instrumental format.

Zone Broadening in Zone Electrophoresis

There are many possible causes of zone broadening in electrophoresis. Unstable density gradients within the separation medium, caused by samples with a different density than the buffer or by Joule heating, can lead to disruptive convective flows. This is usually minimized by inclusion of a stabilizer in the separation medium, as previously mentioned. The microporous nature of stabilizers permits relatively free electromigration of molecules through the stabilizer, while, owing to its viscosity, the separation medium itself does not undergo gross hydraulic flow. Unfortunately, stabilizers can introduce new causes of zone broadening. One of these is called eddy migration, in analogy with eddy diffusion in chromatography. Eddy migration refers to the fact that not all possible routes of migration through and around the stabilizer will yield the same net migration velocity. More tortuous routes will be slower. The very high surface areas of these microporous stabilizers also create extensive possibilities for adsorptive interactions between solutes and stabilizer. Because of slow desorption kinetics, this may result in extensive zone broadening (*6*).

In zone electrophoresis with or without stabilizers, there are a few more potential causes of zone broadening. The first of these arises from the Joule heat generated by the passage of electricity through the buffer medium. Since heat is generated uniformly throughout the medium, but is removed only at the edges of the separation compartment or gel, a temperature gradient results. The temperature in the center of the system will be higher than at the edges, with two undesirable consequences. First, fluid in the warmer central regions will be less dense than fluid in the cooler periphery, resulting in convection currents. Stabilizers can be used to correct this. However, the warmer fluid is also less viscous, and therefore electrophoretic mobilities are greater in the central regions. As a rule, electrophoretic mobilities increase by 2 percent for each 1°C increase in temperature (*6*). Thus an electrophoretic zone can develop a bowed shape, with the zone center migrating faster than the edges.

Sample concentration can also play a role in zone broadening. The presence of a sample in the buffer medium can alter the medium's electrical conductivity. It may increase the conductivity by acting as an additional conducting electrolyte. But it may also decrease the overall conductivity by adsorbing small ions. As the concentration of a sample increases, the sample can cause significant changes in the local conductivity of the medium, and this, in turn, will distort the electric field in the sample's vicinity. This distorted electric field ultimately leads to asymmetric and broadened zones (*6*). This effect was described in quantitative terms by Mikkers *et al.* (*7*) and is discussed below. The solution to this problem is to keep sample concentration low compared to the buffer concentration.

The remaining cause of zone broadening is simple molecular diffusion. In ordinary practice, this cause of zone broadening is often insignificant compared to the causes previously mentioned.

Summary. Zone electrophoresis in open tubular capillaries is a useful approach to high-resolution separations of charged substances. Efficient heat transfer from small diameter capillaries permits application of unusually high voltages, which promote more effective separations and increase the speed of analyses. A sample injection technique and on-line zone detection create an instrumental format for zone electrophoresis. The basic theory, system parameters, and preliminary results are described.

Zone Broadening in Capillaries

Capillary tubes offer an interesting alternative to gels. First, the capillary tube acts like the microporous "capillaries" in a conventional gel or paper, counteracting convective flow. This anticonvective effect has been called the wall effect by Mikkers *et al.* (*7*). The stabilizing influence of the capillary increases as the capillary diameter is decreased because of the increasing surface-to-volume ratio. With the omission of porous stabilizer, eddy migration is eliminated. However, adsorptive interactions between solutes and the capillary wall can still present a problem. One advantage of the capillary is that its total surface area is quite small compared to that of a conventional stabilizer in an equivalent volume of buffer.

Temperature inequalities are also present in a capillary in the form of a parabolic lateral temperature profile (*6, 8, 9*). The magnitude of the temperature difference between the center and wall of the tube is roughly proportional to the square of the tube diameter (*6*). Decreasing the capillary diameter serves to increase the surface-to-volume ratio, increasing the efficiency of heat dissipation. Thus, in a capillary, temperature gradients can be kept rather small. However, some finite temperature differences will exist even in very small diameter capillaries, and this may lead to measurable zone broadening. Here, a very subtle additional advantage of small diameter capillaries comes into play. In zone broadening caused by a temperature profile, each solute molecule is thought of as remaining in essentially the same radial position during its entire migration down the tube. Thus a molecule starting at the wall will remain near the wall and will migrate more slowly than one in the warmer and less viscous central region of the tube. While this is essentially true for large diameter tubes, in capillaries the lateral distances may be diminished enough that solute molecules easily diffuse many times across the entire cross section of the capillary while migrating down the tube length. Thus, there is significant diffusional randomization of a solute's radial position. The greater the extent of this randomizing or averaging process, the greater the tendency is for all solute molecules of a particular species to migrate with the same net velocity, minimizing this thermal source of zone broadening (*10*).

Because of the combined advantages of more efficient heat transfer and increased effectiveness of lateral diffusion, capillaries of very small diameter seem advantageous. Introduction of excessive sample into a capillary will still cause serious distortion of the electric field and zone shape, as it does in gel-stabilized media. Thus a low ratio of sample to buffer concentration must be maintained. Under the proper circumstances in an open-tubular capillary of small diameter, the dominant cause of zone broadening can be molecular diffusion.

Basic Theory of Capillary Zone Electrophoresis

Jorgenson and Lukacs (*10*) developed an approach to carrying out zone electro-

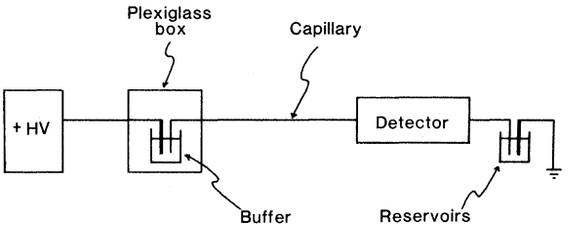


Fig. 1. Schematic of a capillary electrophoresis system.

phoresis in open tubular capillaries. A buffer-filled capillary is suspended between two reservoirs filled with buffer. Samples are introduced at one end and, under the influence of an applied electric field, migrate toward the other end of the capillary. Just before leaving the capillary, sample zones migrate through a detector, which senses their passage, yielding a recording of detector response versus time which is analogous to a chromatogram but is called an electropherogram. Because of the parallels between this system and a chromatographic system, it is natural to borrow such concepts as migration time (retention time), theoretical plates, and resolution from chromatography.

In capillary zone electrophoresis, the migration time for a solute is given by

$$t = \frac{L^2}{\mu V} \tag{1}$$

where t is a solute's migration time, L is the tube length, μ is the solute's electrophoretic mobility (electrophoretic velocity in a unit electric field), and V is the applied voltage. The separation efficiency, in terms of the total number of theoretical plates, N, is

$$N = \frac{\mu V}{2D} \tag{2}$$

where D is the solute's diffusion coefficient. These two equations are the basis of some interesting predictions. First, high separation efficiencies are best achieved through the use of high voltages. Electrophoretic mobilities and diffusion coefficients are dictated by the solute and are not easily manipulated in some overall useful way. Second, column length plays no role in separation efficiency, but has a profound influence on migration times and hence analysis times. It appears that the ideal situation is to apply as high a voltage as is available to capillaries as short as possible, yielding the highest separation efficiency in the shortest possible time. There are practical limits to this approach. As the capillary is made shorter the amount of Joule heat that must be dissipated increases because of the decreasing electrical resistance of the capillary. At the same time, surface area available for heat dissipation is decreasing. At some point significant thermal effects will ultimately appear, placing a practical limit on how short a tube can be used with a particular applied voltage. This conclusion assumes that the capillary diameter, voltage, and buffer composition have remained constant.

In the previous equations of migration time and separation efficiency an important phenomenon, electroosmosis, was neglected. Electroosmosis is the flow of liquid that occurs when an electrical potential is applied to a liquid-filled porous medium. In an unobstructed capillary the shape of the electroosmotic flow profile is piston-like. The flow velocity is constant over most of the tube cross section and drops to zero only near the tube walls (*11*). This is fortunate as the flat flow profile of electroosmosis will add the same velocity component to all solutes, regardless of their radial position, and will thus not cause any significant dispersion of the zone. The more familiar parabolic laminar flow profile, such as occurs in capillaries in ordinary hydraulic flow, would lead to serious zone spreading. Electroosmotic flow does, however, modify the equations for migration time and separation efficiency. The migration time becomes

$$t = \frac{L^2}{(\mu + \mu_{osm})V} \tag{3}$$

and the separation efficiency is now

$$N = \frac{(\mu + \mu_{osm})V}{2D} \tag{4}$$

where μ_{osm} is the electroosmotic flow coefficient, or the linear flow velocity achieved in a unit electric field.

Equation 4 leads to the same general conclusions regarding separation efficiency and voltage. However, it suggests a misleading approach for using electroosmotic flow to enhance separation. This is to promote large values of electroosmotic flow in the same direction as electrophoretic migration. Although this will yield high separation efficiencies in terms of theoretical plates, it is actually detrimental to resolution of zones (*10*). Electroosmotic flow does not act directly to accomplish any separation but instead affects all substances in the same way. The only effect of rapid electroosmotic flow is to sweep all solutes quickly through the capillary, leaving little time for zones to separate. The resulting zones will be sharper (increased theoretical plates) but more poorly resolved. It is resolution of zones that we ultimately wish to accomplish. Following the approach of Giddings (*12*), we have derived an equation to predict the resolution of two zones in capillary zone electrophoresis.

$$R_s = 0.177\,(\mu_1 - \mu_2)\left[\frac{V}{D(\bar{\mu} + \mu_{osm})}\right]^{1/2} \tag{5}$$

where R_s is the resolution, μ_1 and μ_2 are the electrophoretic mobilities of the two solutes, and $\bar{\mu}$ is their average mobility (*10*). From this equation it is clear that the resolution of two zones will be poorer if there is a large component of electroosmotic flow in the same direction as electrophoretic migration. In fact, good resolution of substances having very similar mobilities can be achieved by balancing electroosmotic flow against electrophoretic migration ($\mu_{osm} = -\bar{\mu}$). The cost of this approach to higher resolution is long analysis time. This is readily apparent by referring to Eq. 3 and assuming that the electroosmotic flow coefficient and the electrophoretic mobility are equal in magnitude but opposite in sign (direction).

Description of System

A schematic diagram of the system we use to perform electrophoresis in capillaries is shown in Fig. 1. In almost all cases the strong electroosmotic flow carries solutes, regardless of charge, toward the negative (grounded) electrode. For this reason samples are usually introduced at the positive (high-voltage) end, and a detector capable of sensing zones within the capillary (on-column detector) is located just before the grounded outlet end. Samples are introduced into the capillary in the following manner. With the high voltage turned off, the buffer reservoir is removed and replaced with a container of sample (we have made sample "containers" with volumes as small as a few microliters). Voltage is briefly applied, causing electromigration of a small "slug" of sample into the capillary. Next, the buffer reservoir is returned, voltage is again applied, and electrophoresis proceeds. This simple method of "injection" is quite effective, accomplishing sample introduction with a minimum of zone broadening. The operator is protected from accidental con-

tact with high voltage during sample introduction by an interlock system. The high voltage end of the system is enclosed in a plexiglass box. Opening the box automatically cuts off the high voltage in the event that the operator has neglected to turn off the voltage manually (*10, 13–15*).

All our attempts to physically inject samples have resulted in extremely broad zones. This is probably due to the broadening effect of the parabolic flow profile that occurs during the injection. The quantity of a particular sample component introduced by our electromigratory injection technique depends on many factors. The quantity injected should be given by

$$Q = \frac{(\mu + \mu_{osm})VAC\, t_{inj}}{L} \qquad (6)$$

where Q is the quantity injected, A the cross-sectional area of the capillary, C the sample concentration, and t_{inj} the duration of injection. Although many factors control the quantity injected, all factors are easily measured. We have verified experimentally that the quantity injected is proportional to the concentration of the solute present in the sample (*16*).

Zone detection has been accomplished with fluorescence and ultraviolet absorption detectors, both in an on-column mode. Mikkers *et al.* (*7*) demonstrated the use of conductance detection, but this mode does not appear sensitive enough to permit full realization of the separation power of capillary zone electrophoresis. As discussed earlier, high separation efficiency dictates use of a very small ratio of sample to supporting electrolyte concentration, conditions unfavorable to conductimetric detection.

We have investigated capillaries of a variety of diameters and materials. When used with a potential of 30,000 V, borosilicate glass capillaries 80 μm or less in diameter and 1 m long provided good results. Larger diameters or shorter lengths yielded inferior separations because of excessive thermal overloading. Pyrex borosilicate glass was completely suitable for samples containing smaller molecules, and where detection at longer ultraviolet wavelengths was appropriate. Borosilicate could not be used at wavelengths shorter than approximately 280 nm. For these shorter wavelengths, capillaries of fused silica were a better alternative. Larger molecules such as proteins suffered serious adsorption to the surface of both fused silica and glass. In a search for a more inert surface, Teflon capillaries were investigated. Teflon proved transparent to short-wavelength ultraviolet light, but, surprisingly, also exhibited significant adsorptivity toward proteins. Furthermore, the poorer thermal conductivity of Teflon than of fused silica and glass dictated use of lower voltages to prevent significant thermal effects.

After many attempts to deactivate the surface of fused silica with modifying groups such as trimethylsilane, octadecylsilane, aminopropylsilane, and crosslinked methylcellulose, an effective method was found. We applied the technique of Chang *et al.* (*17*) for bonding glycol-containing groups to the surface. This particular surface-modified silica exhibits markedly lower adsorptivity toward proteins and is the most satisfactory capillary material we have used for protein separations so far. Untreated fused silica appears perfectly acceptable for molecules with molecular weights roughly below 1000.

Preliminary Results

In the first publication on zone electrophoresis in capillaries, Mikkers *et al.* (*7*) showed a good separation of a mixture of 16 relatively small anions, ranging from chloride to benzylaspartate. Their separation was carried out in a Teflon capillary 200 μm in diameter, using potential gradient (conductimetric) detection. This mode of detection is relatively insensi-

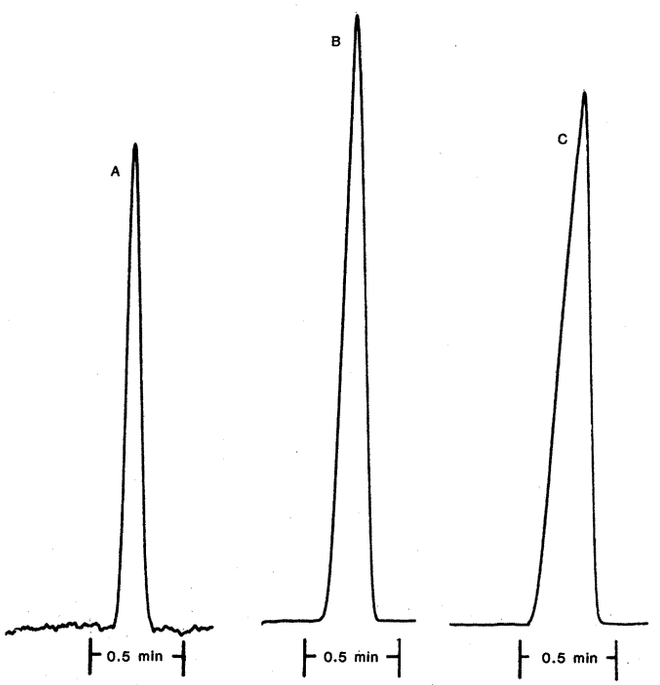


Fig. 2 (left). Effect of sample concentration on zone profile. Glass capillary, 75 μm (inner diameter) by 100 cm long, filled with 0.05*M* phosphate buffer at *p*H 7; potential, 30 kV. Dansyl leucine at peak A, 0.4 m*M*; peak B, 5 m*M*; and peak C, 10 m*M*. [From (*14*)]

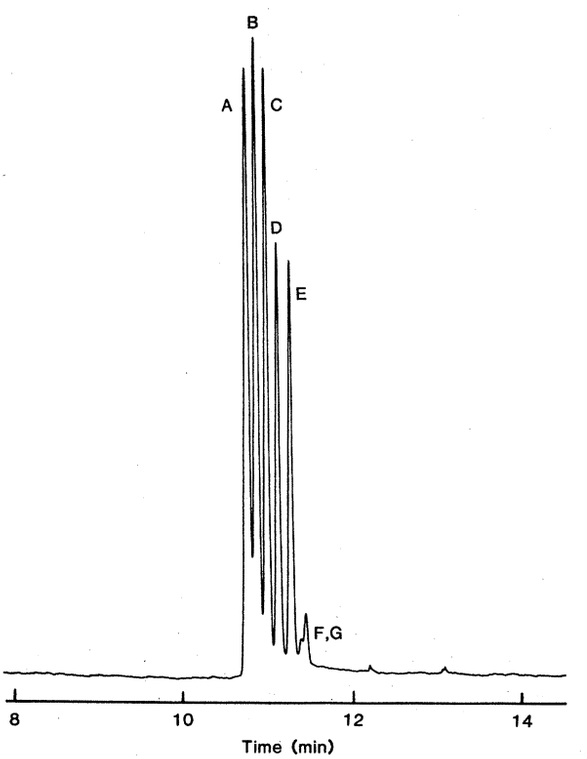
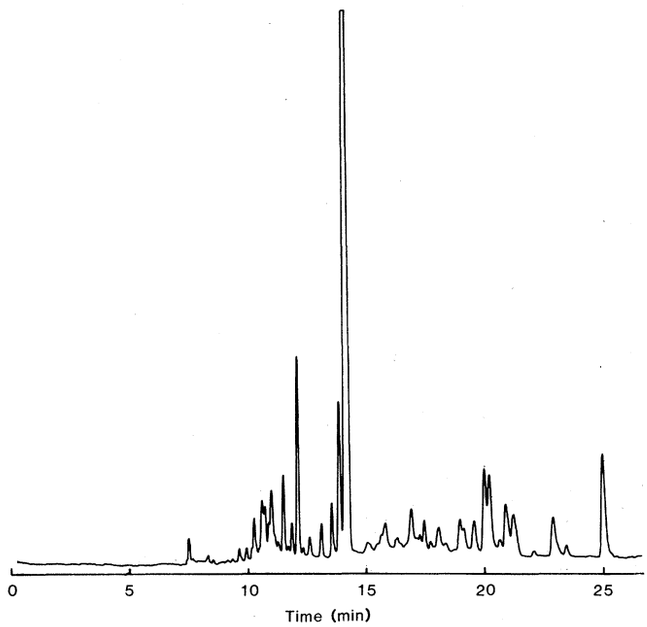


Fig. 3 (right). Electropherogram of *n*-alkylamines as fluorescamine derivatives (conditions as in Fig. 2): peak A, octyl; peak B, heptyl; peak C, hexyl; peak D, pentyl; peak E, butyl; peak F, unknown impurity; and peak G, propyl. [From (*15*)]

tive, requiring overloading of sample and resulting in peak broadening. Still, it does have the advantage of being completely general, responding to any ionic material.

In our initial work we made use of on-column fluorescence detection, a sensitive and selective detection mode (*10*). The solutes studied were dansyl and fluorescamine derivatives of amine-containing compounds. With these solutes and fluorescence detection we were able to work at low enough sample concentrations that solute overloading effects were minimal, and very sharp electrophoretic zones were observed. The effect of solute concentration on zone shape is shown in Fig. 2 (*14*). Clearly, high solute



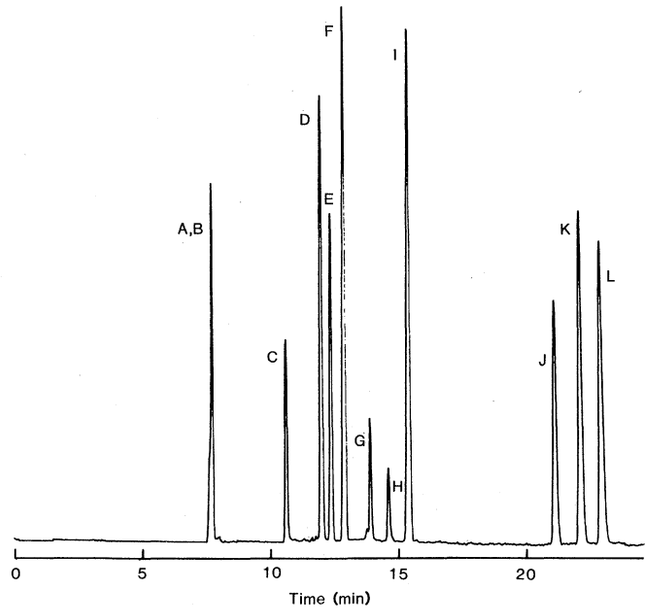
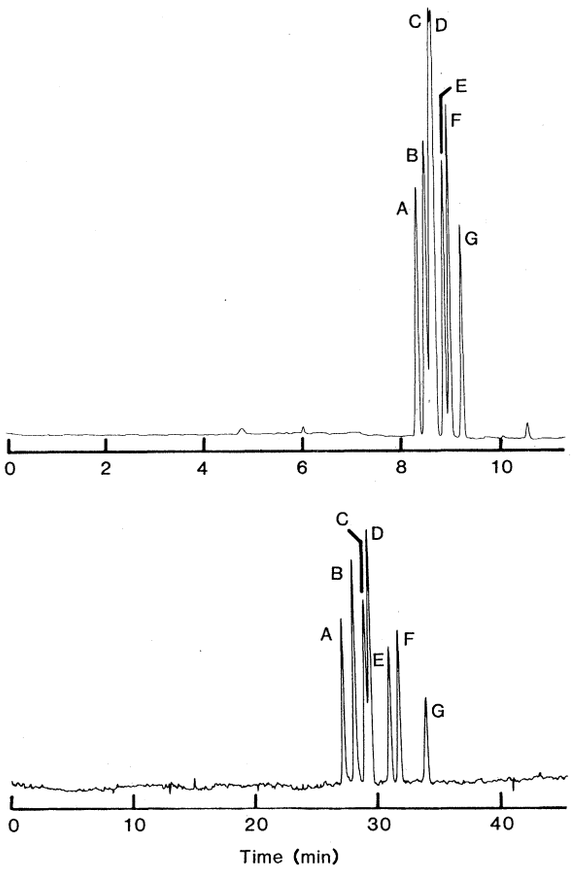


Fig. 4 (left). Electropherogram of fluorescamine-labeled human urine. Conditions as in Fig. 2. [From (*14*)] Fig. 5 (right). Electropherogram of dansyl amino acids (conditions as in Fig. 2): peak A, unknown impurity; peak B, ϵ-labeled lysine; peak C, dilabeled lysine; peak D, leucine; peak E, serine; peak F, glycine; peaks G and H, unknown impurities; peak I, dilabeled cystine; peak J, glutamic acid; peak K, aspartic acid; and peak L, cysteic acid. [From (*14*)]



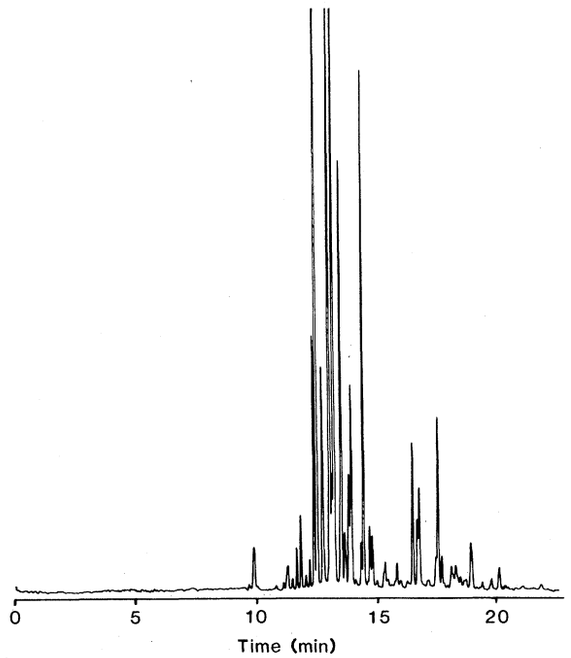


Fig. 6 (left). Effect of electroosmosis on resolution and analysis time. (Top) Untreated capillary; (bottom) capillary treated with trimethylchlorosilane. Peak A, asparagine; peak B, isoleucine; peak C, threonine; peak D, methionine; peak E, serine; peak F, alanine; and peak G, glycine. Conditions as in Fig. 2. [From (*10*)] Fig. 7 (right). Separation of fluorescamine-labeled peptides from a tryptic digest of egg white lysozyme. Conditions as in Fig. 2. [From (*13*)]

concentrations have undesirable effects on zone shape and width. Using low sample concentrations, we were able to verify that separation efficiency is proportional to the applied electrical potential and that migration time is inversely proportional to applied potential (*10*). Quite high efficiencies were easily achieved. As an example, the dansyl derivative of the amino acid isoleucine had a migration time of approximately 13 minutes, and a separation efficiency of approximately 250,000 theoretical plates was achieved with an applied potential of 30,000 V (*10*).

Figure 3 shows the separation of a homologous series of normal alkyl amines as their fluorescamine derivatives (*15*). Each successive derivative differs from its neighbor by only 4 percent in molar mass or roughly 1 percent in diameter, demonstrating the potential separating power of this system. The sensitivty of fluorescence detection is also well illustrated in this figure, as each amine derivative except for propylamine is present at roughly 7 picomoles.

To demonstrate the ability of the system to handle a real sample, human urine was diluted tenfold with buffer and allowed to react with fluorescamine to yield fluorescent labeled amines. The labeled urine was then run in the capillary, with the results shown in Fig. 4. Many amine-containing substances are evident but their identity remains unknown (*14*).

Amino acids as dansyl derivatives were also investigated. A typical electropherogram is shown in Fig. 5. Separation efficiencies here are approximately 100,000 theoretical plates (*14*). It is of interest to note the migration order of the solutes; basic amino acids migrate fastest, neutrals at an intermediate rate, and acids slowest. This is as expected since they are migrating toward the negative electrode. The magnitude of the electroosmotic flow is demonstrated by the fact that it carries doubly charged anions such as dansyl aspartic acid toward the negative electrode. Even small triply charged anions have been observed to migrate toward the negative electrode, carried by the strong electroosmotic flow (*10*).

The effects of electroosmotic flow on zone resolution and analysis time are demonstrated in Fig. 6. Shown is the separation of several dansylated amino acids, first run in an untreated borosilicate glass capillary and then run in the same capillary after treatment of the glass surface with trimethylchlorosilane (*10*). The silane eliminates much of the negative surface charge on the glass, thereby reducing electroosmotic flow. As predicted by Eq. 3, the separation in the second case is slower. Also evident is the increase in resolution between adjacent zones as predicted in Eq. 5. In both electropherograms the electroosmotic flow is in a direction opposite to that of electrophoretic migration. However, in the second case the electroosmotic flow velocity is more comparable to the electrophoretic migration velocities, leaving the zones more time in the capillary to separate by differential electromigration.

Larger solutes such as peptides and proteins are of special interest, not only because of the important separation applications they present, but also because their larger size results in lower diffusion coefficients, which should yield still sharper zones. Figure 7 shows an electropherogram of the fluorescamine-labeled peptides from a tryptic digest of egg white lysozyme (*13*). This sample contains rather small peptides, the largest being 12 amino acid residues long. An obvious increase in zone sharpness and separation efficiency is observed when the electropherograms of these peptides are compared to those of amino acids. This seems to confirm the theory that as diffusion coefficients become smaller, separation efficiency increases. The separation efficiency approaches 1 million theoretical plates in the case of the tryptic peptides of lysozyme.

These results are promising and suggest that separation of proteins will be highly efficient because of their still

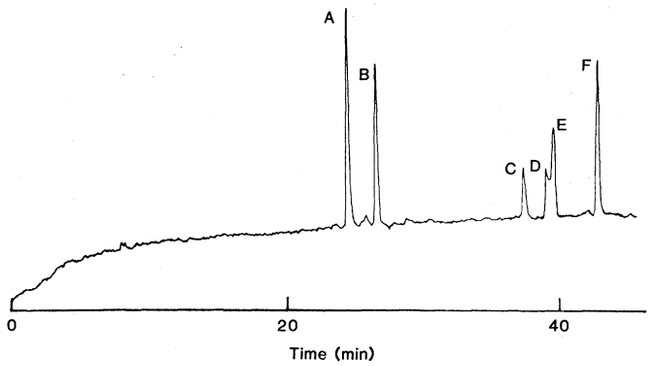
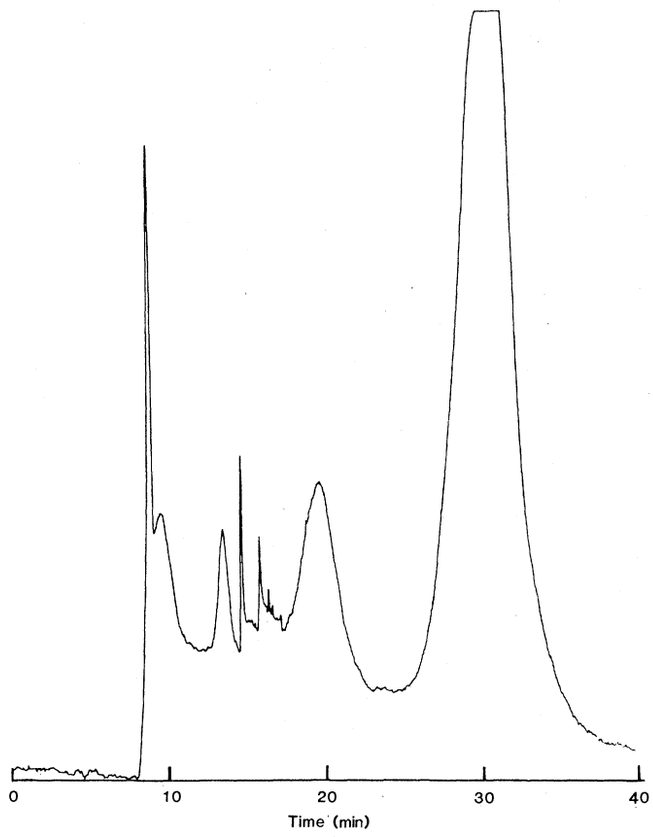


Fig. 8 (left). Electropherogram of protein standards. Surface-modified fused silica capillary, 75 μm (inner diameter) by 100 cm long, filled with 0.05*M* phosphate buffer at *p*H 7; potential, 20 kV; ultraviolet absorption detection at 230 nm. Peak A, egg white lysozyme [isoelectric point (pI), 11; molecular weight (MW), 14,000]; peak B, horse heart cytochrome C (pI, 10.25; MW, 12,500); peak C, bovine pancreatic ribonuclease A (pI, 9.45; MW, 13,700); peak D, impurity; peak E, bovine pancreatic α-chymotrypsinogen A (pI, 9.1; MW, 25,000); and peak F, equine myoglobin (pI, 7; MW, 17,000). Fig. 9 (right). Electropherogram of human blood serum. Surface-modified fused silica capillary, 75 μm (inner diameter) by 50 cm long, filled with 0.1*M* tris-HCl at *p*H 8.5; potential, 10 kV; ultraviolet absorption detection at 230 nm.



smaller diffusivities. However, new problems emerge with proteins. First, we found that we could not label proteins in a useful manner with fluorescent tags. Because of the large number of sites available for labeling in most proteins, these reactions produce a complex mixture of products which are of little value for detection purposes. Further work on fluorescent labeling techniques, or use of the intrinsic fluorescence exhibited by most proteins, may eventually result in an effective detection technique. At present we are forced to use a less sensitive mode, on-column ultraviolet absorption at a wavelength of 230 nm. The lower sensitivity of this detector forced us to operate with overloaded samples, preventing realization of the potentially high separation efficiencies. Second, most proteins exhibit significant adsorption to the surface of both fused silica and borosilicate glass capillaries. Adsorption affects electropherograms in two undesirable ways. First, it leads to broad asymmetric "tailed" zones. Second, adsorbed protein modifies the capillary surface, usually decreasing electroosmotic flow significantly. This leads to completely unpredictable migration for all sample zones. As previously described, modification of the surface of fused silica with glycol-containing groups markedly decreases the adsorption of proteins (*17*). These surface-modified capillaries have been the most satisfactory solution to protein adsorption so far. Figure 8 is an electropherogram of five different proteins run in a surface-modified fused silica capillary. It is of interest to note that solutes elute in order of their isoelectric points, as might be expected for a group of proteins of similar size. Most of the adsorption problems associated with proteins appear to have been solved, and zone broadening is dominated by the sample overloading necessitated by insufficient detector sensitivity. The peak for cytochrome c in Fig. 8 exhibits approximately 70,000 theoretical plates, far short of the approximately 2 million plates that can be predicted from Eq. 4.

An electropherogram of a human blood serum sample run in a surface-modified fused silica capillary is shown in Fig. 9. Several peaks, both broad and narrow, are seen. Judging from the symmetric shape of the broad peaks, we feel that adsorption is not a likely cause for their broadening. Microheterogeneity—the fact that each zone is composed of extremely similar and yet unique proteins—is one likely cause. Also, kinetically slow conformational changes in the structure of proteins is a possibility. A review of these factors and their contribution to zone broadening can be found in the discussion by Wieme (*6*). However, the classic serum bands, including albumin and the globulins, are apparent in this electropherogram.

Conclusions

Zone electrophoresis in capillaries is a technique complementary to electrophoresis in supporting media, and each approach has its own particular advantages. Efficient heat transfer from small-diameter capillaries permits use of unusually high voltages, resulting in both high resolution and rapid analyses. Capillaries also seem well suited for automation. Our present electromigration injection technique is relatively straightforward and should be simple to automate. Capillaries are reusable, which is an advantage over gels. On-line electronic detection permits good quantification, further enhancing possibilities for fully automatic operation. The greatest obstacle to further development and utilization of capillaries is the requirement of extremely sensitive detectors, and more types of detectors with higher sensitivity are greatly needed. A better understanding of capillary surface modification will also be important, both for improved capillary surface deactivation and for better control over electroosmotic flow.

There are many interesting areas for future application of this technique. Automated versions would be useful in routine analyses such as separation of serum proteins in a clinical laboratory. Capillary systems offer higher resolution, greater speed, and better accuracy than conventional methods. Capillary zone electrophoresis can also be useful in the generation and application of fundamental physicochemical data. The technique should yield accurate values for electrophoretic mobilities, while at the same time diffusion coefficients may be obtainable from zone width data. Omission of gel also makes this approach attractive for separation of particulate-containing samples, especially viruses, cells, and organelles. Compared to other electrophoretic systems designed to separate particles, capillaries are quite simple.

Capillaries should provide an ideal system in which to explore nonaqueous separation media. Electrophoresis is rarely carried out in nonaqueous media for two principal reasons. First, it is generally considered only in terms of separation of water-soluble biomaterials. Second, formation and use of suitable stabilizing gels in nonaqueous solvents is a largely unexplored problem. In reality, nonaqueous solvents allow manipulation of acidity and basicity over a much wider range than water, permitting ionization of substances that are not easily ionized in water. Also, nonaqueous media permit dissolution of hydrophobic solutes, further expanding the range of applicability of electrophoresis. The prospects for nonaqueous media in electrophoresis are similar to those in electrochemistry, and capillaries should prove an excellent system in which to begin their study.

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