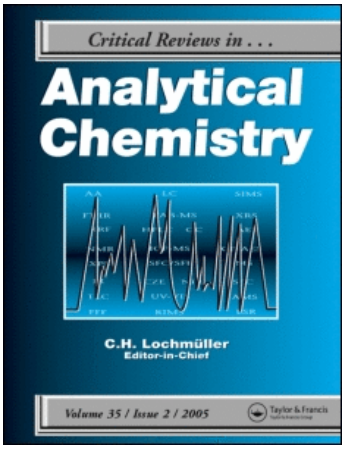

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Electrochromatography

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Electrochromatography

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ABSTRACT: Fundamentals of electrochromatography are reviewed in this work. Theory of solute migration and band broadening is included. The types of columns used (packed capillary and open tubular columns) and their influence on efficiency are shown. The most important applications of this technique are described.

KEY WORDS: electrochromatography, electroosmotic-driven flow, pressure-driven flow, open tubular column, packed capillary column.

I. INTRODUCTION

The search for greater resolving power in high-performance liquid chromatography (HPLC)[1] is one of the major fields of activity in contemporary chromatographic research. HPLC enables the separation and analysis of highly complex samples with species of both neutral and ionic nature. Packed columns (PCs), conventional and microbore, are excellent for achieving moderate separation powers, characterized by column efficiency below 1.0×10^5 plates per meter approximately (about 3.0×10^4 plates in a real column). Increasing the efficiency without accepting a decrease in the theoretical plate productivity (i.e., at a constant number of plates per unit time of analysis) implies the reclamation of columns with a particle diameter smaller than 3 μm. However, two major problems exist: the proper packing and coupling of large pressures, in excess of 1 kbar. Barring the appearance of a major breakthrough in PC technology, it seems that the prospects of solving these problems are slim.

Open tubular columns (OTCs) were first proposed for gas chromatography (GC) by Golay[2] in 1958. Only the capillary inner wall is coated with the stationary phase in OTCs, whereas in PCs the stationary phase is on the surface of the packing particles. The excellent efficiencies and resolving power that can be obtained with OTCs, which have replaced PCs in most analytical applications in GC, enabled OTCs in the early 1980s[3–5] to be considered an alternate solution to PCs in improving significantly the resolving power in liquid chromatography (LC). Since that time, the use of OTCs in LC has been extensively and experimentally investigated.[6–24] Efficiencies above 5.0×10^5 plates per meter (about 10^6 plates in real column) and plate generation velocities around 1.0×10^3 plates per second were obtained using such columns with a 5-μm inner diameter (i.d.).[21] Despite their advantages, OTCs are not as popular in LC as their counterparts in GC and, at present, OTCs are used only at university laboratories and research institutes. This may be due to practical problems in their preparation, and the demanding fea-

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tures of the injection and detection systems associated with these columns. It has been found that to obtain a theoretical plate productivity higher than that of PCs, the diameter of the open tube should be about twice the size of that of the smallest particles used in PCs (1.5 to 3 μm), i.e., it should be around or smaller than 3 to 5 μm.[3,21,25] However, a most difficult problem remains; i.e., the preparation of a thick layer[14] of a stable stationary phase with an adequate high-phase ratio in capillaries of a 5-μm i.d. approximately. The phase ratio should be high, first to enable manipulation of the retention and second (and possibly more important) to allow sufficient loadability. In addition, it was found that detection and injection volumes must be kept in the range 1 to 10 pl to achieve a very small efficiency loss.[3,21,26,27] Although it has been shown that these stringent requirements can be met in practice when applying on-column detection and split-injection devices, the insufficient sensitivity and low mass loadability of OTCs still inhibit the interest of instrument manufacturers in developing an OTC–LC instrument.

An alternate solution to the use of OTCs in LC has been considered. As first shown in 1974 by Pretorius et al.,[28] there are two ways to drive a liquid through a capillary column, i.e., either by application of a hydrostatic pressure difference (pressure-driven flow [PDF] as in the case of LC) or of a potential difference (electroosmotic-driven flow [EDF]) across the length of the column. The possibility of making an electroosmotic flow (EDF) to work as a pumped system gave rise to the so-called electroseparation techniques. In contrast to OTC-LC's lack of commercial development, interest for these techniques is growing rapidly, as demonstrated by the enormous increase in the number of publications and the availability of several commercial instruments since the introduction in 1980 of the first electroseparation technique named capillary zone electrophoresis (CZE).[29,30] This technique, in which the capillary column is an open tube of silica and the capillary walls serve to establish an EOF, has enabled the separation and analysis of charged species via their different electrophoretic mobilities, using ultra-low sample volumes with high resolution and efficiency. Although this analytical technique[31] is particularly interesting for separating ionic species, the separation of neutral species through an electroseparation technique remains uncertain.

In 1984, Terabe et al.[32] demonstrated that the introduction of micelles into the separation electrolyte results in the separation of some uncharged species. In this electroseparation technique, called micellar electrokinetic chromatography (MEKC), the separation is based on the partitioning of the uncharged analytes between the electrolyte and the micelles that serve as a pseudo-stationary phase. Although easy to implement, MEKC does not offer the choice of stationary phases available in HPLC. Consequently, MEKC is not used routinely in the laboratory.

Another possible solution to the separation of neutral species by means of electroseparation techniques was reported by Jorgenson and Lukacs in 1981.[30] They showed EOF can act as a pump for chromatographic separations applying a potential difference across a PC with microparticles such as those used in LC. This electroseparation technique, known as electrochromatography (EC), was considered a variant of HPLC where the eluent is driven via EOF. As opposed to LC, EC may only be performed in capillary columns with tube bore limited to no more than 200 μm by self-heating.[33–35] This is why Jorgenson and Lukacs[30] used columns of 170 μm i.d. packed with particles of 10 μm. Some time later, Tsuda et al.[36] demonstrated that EC can also be performed in OTCs.

Unfortunately, since the introduction of EC in 1981, several names have been coined for this type of electroseparation technique,

and confusing terminology may be found in the literature. The term *electrochromatography* is used to indicate all capillary separation procedures in which the flow results from electroosmosis.[34,37] Recently, Knox[38] has used this term for electroseparation techniques such as CZE and MEKC. However, in our opinion, the difference between electrophoresis (CZE and MEKC) and EC is essential. In electrophoresis, the separation process is based on the differences in the electrophoretic mobility of the solutes (CZE) or between two phases, the mobile phase of an electrophoresis buffer and the pseudo-stationary phase of a dispersed micellar phase (MEKC). These processes occur throughout the interior of the column, being the main source of in-column zone broadening, at least in theory, axial diffusion[30] (Figure 1). It has been demonstrated[39] that intermicellar diffusion is not very important, and therefore, it can be considered as a chromatographic modification of electrophoresis, rather than as a form of EC. The column diameter, although not relevant for the separation process itself, is limited by another phenomenon, i.e., in-column heating.[40] In EC, the separation mechanism essentially originates at the interface between the mobile and stationary phases, and exchange kinetics between two phases are important. In this case, there are two sources of zone broadening, axial and radial diffusion (Figure 1). Especially when it comes to performing fast analyses, radial diffusion determines the column diameters that can be used. Finally, in chromatography, the separation mechanism is essentially the same, the only difference is in

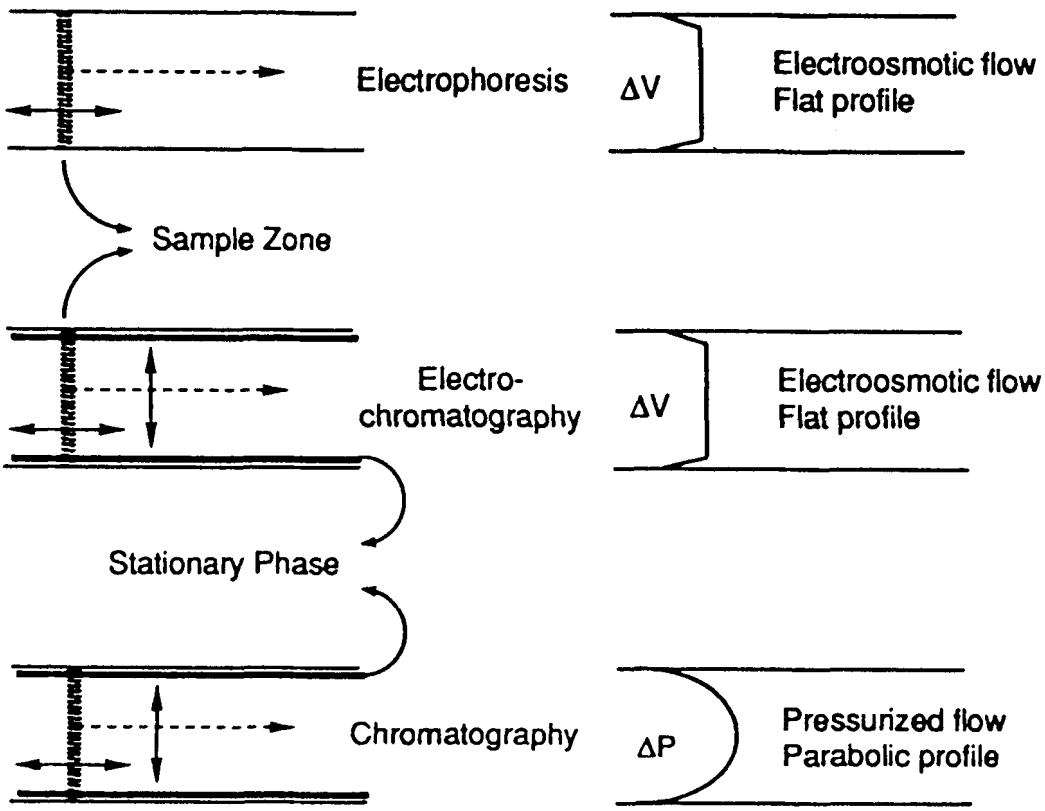

FIGURE 1. Difference between electrophoresis, electrochromatography, and chromatography. Moving of mobile phase (– – →); Zone broadening due to axial diffusion (↔); Zone broadening due to radial diffusion (↕). (From Vindevogel, J. and Sandra, P.; *J. Chromatogr.* 541, 483, 1991. With permission.)

the profile flow; parabolic in chromatography and flat in EC (Figure 1). Therefore, it is our contention that the term *electrochromatography* should be applied when dealing with conventional stationary phases in conjunction with electroosmosis, as opposed to CZE and MEKC. The use of capillary columns has made the name *capillary electrochromatography* (CEC) very popular. However, since the use of EC already entails a capillary format, we will only refer to that term in this article.

The separation of uncharged analytes in EC is based on partitioning, but the separation of charged analytes is also possible based both on partitioning and electrophoretic mobility. The power of EC as an analytical technique lies in the combination of CZE's high efficiency and resolution with the high selectivity, versatility, and universality of HPLC. An example of EC's potential can be observed in Figure 2, where the different separations obtained[41] with CZE, OTC–LC, and OTC–EC are shown. CZE and OTC–LC do not provide effective separation of naphthalene sulfonic acid isomers due to the small difference in the electrophoretic mobilities and partition coefficients of these isomers. However, OTC–EC can solve this problem by providing a complete separation.

Finally, it should be noted that in EC, a voltage of 10 to 50 kV is applied across a capillary column that is 0.5 to 1.5 m long and between 10 and 100 μm i.d. containing an electrolyte the concentration of which is in the range 0.001 to 0.1 *M*. With the appropriate operating parameters, electroosmotic velocities of around 1 mm/s can be achieved. In OTC–EC systems, the sample introduction is performed by means of electromigration[42,43] or by the hydrodynamic method (vacuum or pressure).[44] Both techniques lead to automation and are easier to use than those used in OTC–LC systems, in which sample introduction is usually done by split injection[45] (which requires a relatively large sample volume) or by pressure-pulse-driven stopped-flow injection[46] (excellent injection system for extremely small sample volumes,[27] but is very sophisticated and expensive). However, as in OTC–LC, detection is one of the major problems in OTC–EC, but considering the great interest in capillary electroseparation techniques, new research efforts in microdetection will certainly lead, in the near future, to commercially available ultramicrodetectors having better characteristics with respect to sensitivity.

The characteristics of EC associated with EOF are reviewed and studied in this work. Its possibilities as opposed to those of HPLC are compared both with PCs and with OTCs. Particular attention is paid to the efficiency of this technique, and its most important applications are also presented.

II. TYPES OF COLUMNS USED IN EC

As stated above, EC may only be performed in capillary columns with tube bore limited to no more than 200 μm by self-heating.[33–35,47,48] The capillary columns used in EC can be grouped into two types of columns, packed capillary columns (PCCs)[30,33–35,49–63] and OTCs.[23,24,36,64–67] PCCs consist of a capillary tube of 40 to 200 μm i.d. and about 50 cm of length, which is totally packed with particles between 3 and 10 μm in size. Generally, the particles used are of porous silice (particles of 1.5 μm of nonporous silica, with the stationary phase located in their outer surface, have been also used but with bad results). OTCs also consist of a capillary tube, but it is narrower and longer than before, between 10 and 50 μm i.d. and 100 cm of length, with the stationary phase located in its inner wall like a very thin film. With regard to the stationary phase, chemically modified silica (octadecylsilica, ODS) is used in both types of columns. Therefore, the form or geometry to place the stationary phase is the main difference between both columns.

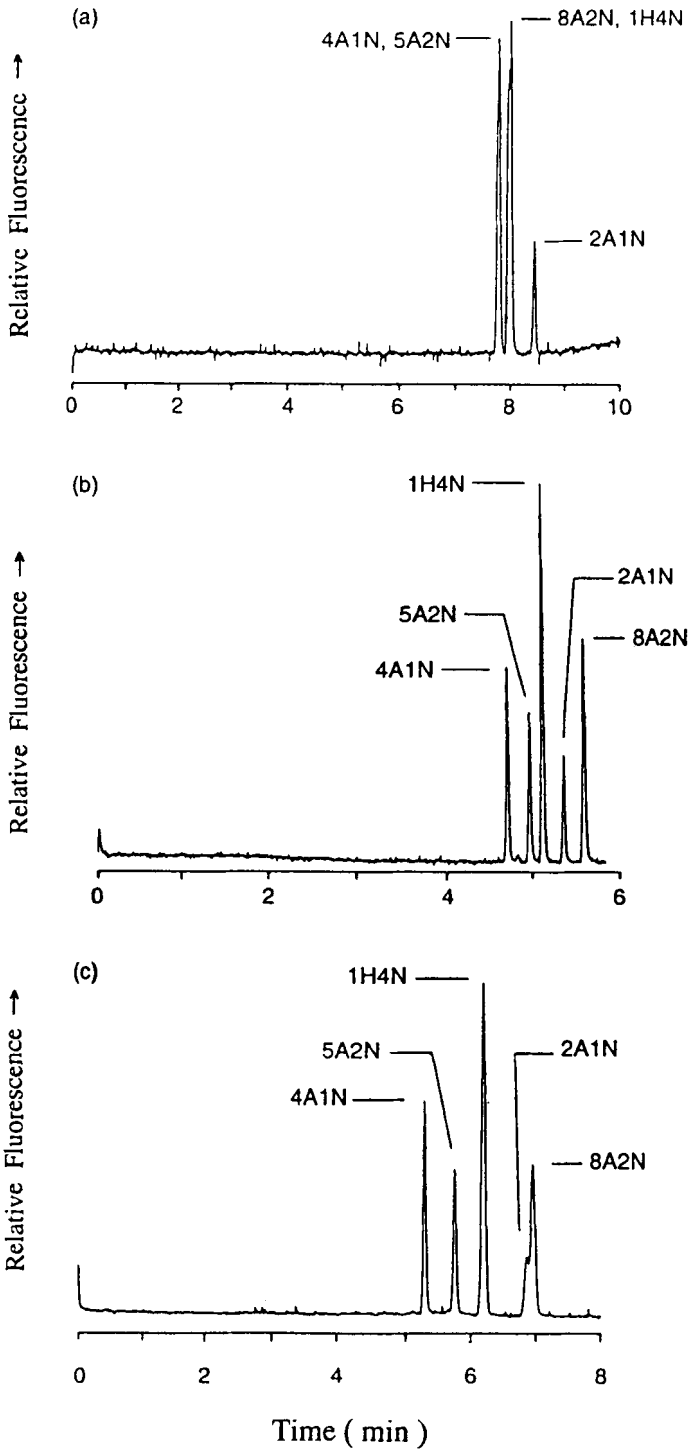

FIGURE 2. Comparison of elution profiles obtained for the five anions using: (a) CZE, (b) OTC–EC, and (c) OTC–LC. Conditions for (a): column: 50 cm × 10 μm, uncoated with 40 cm separation distance; eluent: 10 m*M* phosphate buffer at pH 7.0, containing 1.25 m*M* tetrabutylammonium hydroxide; applied voltage: +21 kV; hydrodynamic injection; and on-column laser-induced fluorescence detection. Conditions for (b): column 50 cm × 10 μm, coated with a polymer, PS-264; applied voltage: –21 kV; the other conditions as for (a). Conditions for (c); column: same as for (b); applied voltage: 0 V; and the other conditions as for (a). Peaks: 4-amino-1-naphthalenesulfonic acid (4A1N), 2-amino-1-naphthalene-sulfonic acid (2A1N), 5-amino-2-naphthalenesulfonic acid (5A2N), 8-amino-2-naphthalenesulfonic acid (8A2N), and 1-naphthol-4-sulfonic acid (1H4N). (From Pfeffer, W. D. and Yeung, E. S., *J. Chromatogr.*, 557, 125, 1991. With permission.)

A. PCCs

Pretorius et al.[28] were the first to apply high voltages over columns packed with particles of 75 to 125 μm and 1 mm i.d. They demonstrated that adequate EOF could be developed using typical reversed-phase eluents (MeOH to H_2O ratio) on C_8 chemically modified silica. In this first work, EC potential could not be tested based on the great size of the particle and the i.d. chosen due to some instrumental problems. In 1981, Jorgenson and Lukacs[30] described the potential of PCs in EC, using columns of 170 μm i.d. packed with 10 μm ODS packing. In 1992, Yamamoto et al.[53] discussed the potential uses and limitations of PCCs in EC. They reported the use of a 50-μm i.d. column with 3 μm ODS packing and 1.6 μm ODS packing (nonporous particles), although a significant decrease in plate numbers for retained compounds was observed. This might be due to the small capacity of the nonporous packing and the limitations of mass transfer.

Some practical problems, such as the difficulty in preparing and operating PCCs (e.g., frit making, column packing, bubble formation, and Joule heating), have reduced the number of successful applications reported in EC. The formation of bubbles in the packing during a run on a PC is the most important practical problem precluding the extensive use of EC. It has been observed that the formation of a bubble invariably begins at a border between a packed and unpacked section of the capillary, hindering stable flow conditions in purely electroosmotic-driven systems. Behnke et al.[61] have investigated several parameters determining EC performance (e.g., eluent, frits, PCs, and optional supplementary pressure), and their results have shown that with thoroughly degassed eluent, frits made of sintered silica gel, and with the use of supplementary pressure, stable and reproducible conditions may be readily obtained. However, column packing is crucial for improving EC performance with PCCs, and for the future commercialization of this technique.

The accepted method for producing these capillaries[50,56,58] was previously that widely documented in the HPLC slurry method[68–70] (the slurry stationary phase in a suitable solvent is placed into a slurry reservoir and introduced in the capillary applying a high-pressure drop). The first problem was to produce a retaining plug at the column outlet. Columns were not normally plugged at the end opposite to the retaining frit. Surprisingly, when used in the EC mode, the sintered plug had to be at the inlet end of the column (i.e., in the anode compartment) because the silica gel particles moved against the EOF. Flow directions under pressure and electrodrive were therefore opposite. However, the production of efficient working capillaries depended on several important factors when the separation column had an i.d. of 50 to 100 μm.[71–73] Therefore, alternative ways had to be found to pack microparticles in stable and uniform beds in order to optimize EC. Boughtflower et al.[62] used a method obtaining typical efficiencies averaged to approximately 2.0×10^5 plates per meter for almost every capillary packed. This procedure introduced a novel and extremely easy method of preparing packed capillaries for EC. The practical contribution to the packing procedure was reduced considerably over the existing methods of packing, and setting up the procedure itself was very simple and fast. The lifetime of these PCCs varies from a few days to several months and the failure of a capillary seems inevitably to be caused by the formation of a void, leading to severe baseline noise and performance loss.

Recently, Yan et al.[63] demonstrated that PCCs with different i.d. (50 to 150 μm) and 3 μm ODS particles can be routinely produced via a novel electrokinetic packing method.[59,74] In addition, Dulay et al.[75] demonstrated that EC can be used routinely in

the laboratory to achieve reproducible and reliable separations, using a fully automated commercial CZE instrument. Hundreds of consecutive runs over a period of several weeks were performed.

Finally, it should be noted that Knox and Grant[51] have highlighted that EC with PCCs may enable efficiencies comparable to those obtained with OTCs if the capillary can be packed with submicrometer-sized particles. However, this has not been achieved yet.

B. OTCs

The preparation of the stationary phase is paramount to achieve the high performance rendered by these columns in EC. However, it is very difficult to prepare OTCs with very narrow capillaries (<15 μm i.d.) and with appropriate coating materials of sufficient retentive and mass loadability characteristics. Thus, although in the past 16 years many efforts have been made, there are still some problems associated with the methods developed so far. This is one of the reasons why OTC–LC, for example, is being used only in a few research laboratories, despite its many advantages[76] over the more conventional PCs used in LC. The packing materials used in LC have a large surface and small particle size, resulting in a high phase ratio and thus a suitable capacity factor for the solutes. In contrast, the smooth inner wall of glass or fused silica capillaries gives rise to phase ratio values around 350 times smaller than those of commercial LC packings.[3] In such conditions, the capacity factor for most of the solutes becomes so small that OTCs cannot be used for analytical purposes. Therefore, the surface area of the inner capillary wall has to be increased and the yield of the subsequent chemically modified reaction (silanization) optimized in order to improve phase ratios for OTCs.

The first procedures used to increase the surface area and reactivity of the inner wall of glass capillaries consisted of etching their internal surface, and then the stationary phase was chemically bonded directly onto the treated inner wall of the capillary.[7,77–79] Such columns provide very low phase ratios, which lead to low retention and low sample capacity. Another approach to increase the phase ratio in OTCs was cross-linked to polymeric stationary phases such as polysiloxanes[13,14,16,18,19,22,80,81] and polyacrylates[82,83] applied to fused silica capillaries by different procedures. These columns usually give good column stability, high phase ratio, and sufficient retention. Initially, these columns should not be suitable for EC because the EOF is suppressed (the inner surface of capillary has no charge). Nevertheless, a cationic surfactant can be added to the mobile phase to generate the EOF in these columns, and thus separate neutral compounds in the reversed-phase mode.[65] These columns also have been used to separate anions in a dynamic ion exchange mode,[66,67] and with a chiral polymeric stationary phase (Chirasil-Dex) even to separate enantiomers.[84] However, their column efficiency is poor, due to the low diffusion coefficient in the cross-linked phase[14] ($D_s \approx 10^{-12}$ m^2 s^{-1}). Film thickness should be small to increase efficiency, but this fact limits mass loadability, and consequently some detection problems may occur.

The diffusion coefficient in porous materials is much larger ($D_s \approx 10^{-9}$ m^2 s^{-1}) and therefore the preparation of porous adsorbent layers in capillaries has better prospects. Silica was chosen as the porous material because of (1) its chemistry, (2) its use as a support or adsorbent in LC, and (3) the possibilities of modifying its surface are well documented. The third approach to increase surface area and phase ratio involves two major steps: (1) laying on a thin porous silica layer of large surface on the inner wall of the capillary and (2) attaching functional groups onto the prepared layer through chemical bonding. Tock et al.[15] described the prepara-

tion of a porous layer in 10 to 25 μm fused silica capillaries by precipitation of porous silica from a dynamically coated film of polyethoxysiloxane (PES) with gaseous ammonia. However, the thickness of the layer obtained by dynamic coating was too small and its porosity was so low that it was unable to bond a substantial amount of alkylsilane, and therefore, the columns could not be used in reversed-phase mode. Tock et al.[85] modified the previous method by using a static procedure in which a volatile solvent was used to introduce the PES into the capillary and the solvent was evaporated under vacuum. The PES layer was subsequently treated with ammonia solution to change it into silica. They achieved a layer with sufficient surface and activity to bond an important amount of ODS moieties. However, the method reproducibility was poor and it was unsuitable for preparing columns with i.d. smaller than 10 μm. Crego et al.[21] also developed a procedure for the preparation of a thin layer of silica gel with chemically bonded ODS on the internal wall of fused silica capillaries. The preparation of the silica layer was based on the hydrolytic polycondensation and gelling of tetraethoxysilane (TEOS) in a pH-fixed water–ethanol solution.[86] Although a similar reaction was already used in previous works, several modifications were carried out in this new work to achieve the preparation of OTCs with smaller inner diameters. In addition, the reproducibility of the method in terms of k' and reduced plate height from column to column was quite good.

Recently, Guo and Colón[23,24] explored sol-gel technology to produce porous silica glass films that can be cast onto the inner walls of OTCs for LC and EC applications. This procedure enables preparation of an organically modified silica glass coating material to be used as the stationary phase for reversed-phase OTCs. Generally, the sol-gel process consists of hydrolysis and polycondensation of metal alkoxide monomers, tetramethoxysilane (TMOS), or TEOS, which are commonly used in silica glasses.[87] During the process, a liquid-like colloidal suspension (*sol*) is transformed first into a gel through gelation (polycondensation of the *sol* to form a wet network of porous material); then, a xerogel (i.e., the dried gel) is formed after the loss of solvent through aging and drying. The initial hydrolysis can be either acid or base catalyzed,[87,88] and the rates of hydrolysis and polycondensation are highly pH dependent. Control of the processing parameters (e.g., drying temperature, pressure, pH, molar ratio of water, alcohol solvent, and the structure of alcoxy groups, among others) during different stages of the process (polymerization, gelation, drying) has enabled production of sol gel-derived materials with desired structural characteristics (e.g., pore size and surface area).[87,88] The surface area of the inner wall of fused silica capillaries is significantly increased with the porous glass coating. The material has been shown to be stable in acidic and basic conditions. Furthermore, the stationary phase (i.e., octyl groups) was incorporated into the porous glass matrix during the glass formation process. Preparation of the supporting porous glass matrix and the stationary phase into a single step eliminates chemical attachment procedures to fix the stationary phases; otherwise, phase attachment would be required after the porous layer is prepared.

III. THEORY

A. Migration Principles in EC

Any capillary electroseparation technique must involve one or both of the primary electrochemical phenomena, electrophoresis and electroosmosis, originated by the presence of the electrical double layer. Furthermore, EC also involves chromatographic partitioning phenomena between phases. See these phenomena.

1. Electrical Double Layer

The electrical double layer is a feature of all surfaces immersed in a liquid.[89-91] The typical surfaces of silica, hydrocarbon-bonded silica, and graphite possess fixed negative charges. With silica, these charges arise from partially ionized acidic silanol groups ($-SiO^-$) on the surface. When such a surface is in contact with a solution, there is a slight excess of opposite charge in the solution (electrolyte) to balance the fixed negative charges on the surface. The excess charge area in the solution is very thin, and it is generally accepted that this zone can be separated into two parts or layers.[92,93] Part of this excess charge is very close to the solid surface and includes ions effectively fixed to the surface, which do not enter into the electrokinetic phenomena. This is called the Helmholtz or Stern layer. The remainder of the excess charge is distributed among ions which freely exchange with those in the bulk of the solution, and are indistinguishable from them. This is often called the diffuse part of the double layer or Gouy–Chapman layer. The excess charge density associated with these ions decreases rapidly with distance from the surface, along with the associated electrical potential which is proportional to the charge density. The potential at the boundary between the Stern layer and the start of the Gouy–Chapman layer is called the zeta potential, denoted by ζ. The fall-off in electrical potential with the distance from the surface is roughly exponential, and the distance over which ζ falls by a factor e (=2.718) is called the thickness of the electrical double layer, δ, often denoted by $1/\kappa$ (where κ is the Debye length), and is given by

$$\delta = \left[(\varepsilon_0\varepsilon_r RT)/(2CF^2)\right]^{1/2} = \left[(\varepsilon RT)/(8\pi CF^2)\right]^{1/2} \quad (1)$$

where ε_0 is the permitivity of vacuum (8.85×10^{-12} C^2 N^{-1} m^{-2}); ε_r is the relative permitivity or dielectric constant of medium (±78 for water at 25°C); R is the universal gas constant (8.314 J K^{-1} mol^{-1}); T is the absolute temperature; C is the molar concentration of electrolyte; F is the Faraday constant (96,500 C mol^{-1}); and ε is the permitivity of buffer ($4\pi\varepsilon_0\varepsilon_r$). Addition of an electrolyte to the bulk solution compresses the double layer. For example, adding a monovalent cation-anion salt to water is reported[49,94] to result in the following estimated δ values: for 10^{-6} M, 0.3 μm; for 10^{-5} M, 0.1 μm; for 10^{-4} M, 0.03 μm; for 10^{-3} M, 0.01 μm; for 10^{-2} M, 0.003 μm; and for 0.1 M, 0.001 μm.

The zeta potential and the thickness of the diffusion double layer have the following relationship:[95,96]

$$\zeta = \frac{4\pi\delta\sigma}{\varepsilon} = \frac{\delta\sigma}{\varepsilon_0\varepsilon_r} \quad (2)$$

where σ is the charge density of the excess ions in the Gouy–Chapman layer. Zeta potentials depend on the nature of the solid surface and the ionic state of the liquid (pH and ionic strength). Polar solvents (e.g., water) give rise to zeta potentials between 10 and 100 mV in contact with either polar (e.g., silice or glass) or nonpolar (e.g., graphite) surfaces.[97] Nonpolar, nonconducting solvents (e.g., heptane) do not normally exhibit a zeta potential when in contact with either polar or nonpolar surfaces. However, by adding small concentrations (10^{-5} M) of substances such as calcium diisopropyl salicylate, similar zeta potentials to those quoted above can be obtained.[98]

2. Electroomosis

In EC, the eluent flows along the column (open or packed) as a result of a potential gradient parallel to the surface (inner surface of the wall in open tube or outer surface of the packing material in packed tube) of the electrical double layer. When a potential

gradient is applied across the length of the column (E = V/L), excess ions within the double layer, or at least those not firmly adsorbed in the Stern layer (diffuse part of the double layer), move toward the appropriately charged electrode carrying any liquid along with themselves. This is resisted by the viscosity of the liquid (η), and shearing of the solution occurs only within the diffuse part of the double layer resulting in the flat-flow profile already shown in Figure 1 when the tube diameter is much larger than δ. The process is called electroosmosis and the resulting flow of liquid is known as EOF. The EOF is a fundamental process in EC because it is the driving force for the separation. This has been covered in detail by Rice and Whitehead[99] and Van de Goor et al.[100] and its mean flow velocity (u_{eo}) can be given by the following expression:

$$u_\infty = (\varepsilon_0 \varepsilon_r \zeta/\eta) \cdot E = (\varepsilon\zeta/4\pi\eta) \cdot E = \mu_\infty \cdot E \quad (3)$$

where μ_{eo} is the electroosmotic mobility, E is the applied field, and η is the viscosity of the electrolyte and other parameters already described. From Equation 3 and taking typical values (ζ = 50 mV, $\eta = 10^{-3}$ N s m^{-2}, and a E = 50,000 V m^{-1}), the EOF velocity in water is calculated to be approximately 1.8 mm s^{-1}, a typical flow velocity in HPLC.

According to Equation 3, it is obvious that the electroosmotic mobility will depend directly on two factors, the zeta potential (ζ) and the viscosity (η), which are determined by other factors such as surface (charge density), electrolyte (concentration, ionic strength, and pH), temperature, cosolvents, or other additives. Electroosmotic mobilities have been related to buffer concentration[101–105] and ionic strength.[103–106] The studies confirm that, at a fixed pH, mobility decreases when increasing concentration or ionic strength, ionic strength being a more meaningful quantity than concentration[105] (when the ionic strength, I, is increased, the ζ will drop faster, $I = 1/2\Sigma C_i z_i^2$). The pH dependence of the μ_{eo} in fused silica capillaries has been determined,[107–111] and it has been observed that μ_{eo} is very low in strongly acidic buffers, rising above pH 3, and increasing strongly between pH values of 7 to 8. At higher pH values, mobility still increases, but more modestly.[106] However, these values are variable, influenced by the buffer system used, but also by the brand and history of the fused silica tubing.[112] On the other hand, the dependence of the EOF on the volume fraction of an organic modifier has been investigated for OTCs by Schwer and Kenndler.[113,114] They found a linear decrease of the electroosmotic migration velocity with an increasing volume fraction of the modifier. In contrast to their results, the dependence of the EOF on the volume fraction of the organic modifier in PCs is different, an increase of flow with an increasing volume of the modifier,[58] which is partly due to a decrease of the viscosity with increasing volume of the modifier.[114] The difference in μ_{eo} observed when methanol and acetonitrile are used as organic modifiers in the electrolytes can be explained by differences in the viscosity and dielectric constant of the electrolytes. Methanol did not show a significant reduced mean flow velocity and current with respect to water. However, the current observed with acetonitrile (aprotic solvent of low conductivity) was significantly reduced and μ_{eo} was duplicated.[36] The best eluent should develop a high ζ (i.e., have a high amount of counterions in solution beyond the plane of shear), have low electrical conductivity (low heat generation) and low viscosity. Finally, due to the influence of the temperature on the viscosity, mobilities show a temperature dependence of about 2 to 3%.[115,116]

The direction of flow was found to be determined by the type of background electrolyte used. With sodium phosphate as the background electrolyte, the EOF is directed toward the cathode (in packed capillaries or open tubular capillaries). The resulting di-

rection of EOF is beneficial to the EC separation of cationic analytes. However, anions will migrate against the EOF, resulting in an extremely low overall mobility and excessively long migration times. In such cases, a reversal of the EOF direction is desirable. When long-chain ammonium salts are added to the buffer (e.g., triethylammonium acetate [TEAA]), they can be adsorbed by the surface,[117–119] leaving an excess of counteranions in the buffer and, consequently, reversing the flow direction. The bulk solution is then moved toward the anode.

Several methods have been used to measure EOF: (1) directly observing the volume or level increase in the destination vessel;[49] (2) weighing the mass of buffer transferred;[100,101,120] (3) monitoring electrical current while a buffer with different conductivity is drawn into the capillary by electroosmosis;[121] (4) determining the zeta potential of the wall from streaming potential measurements;[100] and (5) monitoring the migration time of a neutral marker.[49,120,122,123] Among these methods, the latter is the most used, because it can be used to control EOF during analysis. With an ultraviolet (UV) detector, dimethylformamide, mesityloxide, or another UV-absorbing substance can be used. Even nonabsorbing species, like methanol, ethanol, or 1-propanol, cause baseline disturbances that can be used as a marker.[55]

3. Electrophoresis

Some substances in a buffer at certain pH levels acquire a net positive or negative charge and then becomes ionized substances. When these substances are in an EC system and an electric field is applied, they will move to its corresponding pole, anode (+) or cathode (–). The electrophoretic migration velocity (u_{ep}) of an ionized charge can be expressed by the following equation:

$$u_{ep} = \mu_{ep} \cdot E = \mu_{ep} \cdot (V/L) \qquad (4)$$

where μ_{ep} is the electrophoretic mobility, E is the field strength, V is the voltage applied across the capillary, and L is the capillary length. The μ_{ep} depends on ionic species through zeta potential (ζ) and the nature and concentration of buffer (η,ε):

$$\mu_{ep} = 2/3(\varepsilon_0 \varepsilon_r \cdot \zeta/\eta) \qquad (5)$$

The ζ potential of an ionic species depends on its charge ($q_{\pm}$), size (r_s), and the buffer (ε):

$$\zeta = q_{\pm}/r_s\varepsilon \qquad (6)$$

Combining Equations 4, 5, and 6 gives the general expression for the mean electrophoretic migration velocity:

$$u_{ep} = \frac{q_{\pm} \cdot E}{6\pi r_s \eta} \qquad (7)$$

In conclusion, the migration due to the electrophoretic phenomenon will only affect the ionic species ($q_{\pm} \neq 0$), and their electrophoretic velocity will only depend on the ion net charge and size, applied electric field, condition and concentration of the buffer, and temperature. Differences in solvation (strongest for the smallest ion), and the consequence for the effective ionic radius, must be taken into account.

Finally, for weak electrolytes the equilibrium between species with a different charge must be considered. When the buffer pH is close to the pK_a value (±2 units), the compound will occur in a partial state. When the exchange is fast, as is generally the case in acid–base equilibria, the fraction of the time in the charged state is equal to the dissociation constant α. Accordingly, the effective electrophoretic mobility (μ_{eff}) will only be a fraction of the absolute electrophoretic mobility (μ_{abs}), i.e., the mobility that would be observed for the fully charged ion:[124]

$$\mu_{eff} = \alpha\mu_{abs}$$

with, for proton acceptors (B/BH^+)

$$\alpha = \frac{10^{-pH}}{10^{-pH} - 10^{-pKa}} = \frac{1}{1+10^{pH-pKa}}$$

and, for proton donors (BH/B^-)

$$\alpha = \frac{10^{-pKa}}{10^{-pH} + 10^{-pKa}} = \frac{1}{1+10^{-pKa-pH}}$$

Therefore, by appropriately selecting the buffer pH, mobility differences can be induced between ions with similar absolute mobility.[125,126]

4. Chromatographic Partitioning

The retention of neutral molecules in EC is based on purely chromatographic partitioning between two phases, one stationary phase and one mobile phase. With a background electrolyte necessarily being the mobile phase, there are two possibilities for the stationary phase: (1) particles fixed in a packed bed as in HPLC and (2) a thin film in the inner wall of a capillary. In each instance, the extent of partitioning is better represented by the chromatographic capacity factor k′, which is defined as[1]

$$k' = \frac{\text{amount of analyte in the stationary phase}}{\text{amount of analyte in the mobile phase}} \quad (8)$$

The concept of k′ is related to the concentration distribution coefficient (D) through the phase ratio (ϕ):

$$k' = \frac{\text{concentration in the stationary phase}}{\text{concentration in the mobile phase}} \cdot \frac{\text{volume of the stationary phase}}{\text{volume of the mobile phase}} = D\phi \quad (9)$$

It is reasonable to assume that the phase ratio, ϕ, is very nearly independent of temperature and, therefore, that the temperature dependence of k′ is essentially the same as that of D.

5. Characterization of Elution Parameters in EC

a. Neutral Analytes

The migration of a neutral chromatographically retained compound in EC is characterized by the linear velocity of EOF (u_{eo}) and the extent of partitioning between the two phases (k′), according to the following equation

$$u_{mig} = u_{eo}/1+k' \quad (10)$$

The u_{eo} can be expressed as the electroosmotic mobility (μ_{eo}) of the electrolyte (eluent) using the potential drop (V) over the total length of the column (L):

$$u_{eo} = \mu_{eo}V/L \quad (11)$$

Combining Equations 10 and 11 gives

$$u_{mig} = \frac{\mu_{eo}}{1+k'}\frac{V}{L} \quad (12)$$

The retention time (t_r) of the neutral compound can be calculated introducing the injector-to-detector length of the column (L_{id}) and using Equation 12. So

$$t_r = \frac{L_{id}}{u_{mig}} = \frac{L_{id}\cdot L\cdot(1+k')}{\mu_{eo}\cdot V} \quad (13)$$

As the μ_{eo} is related to the time of unretained neutral marker (t_{eo}) by the equation

$$t_{eo} = \frac{L_{id}\cdot L}{\mu_{eo}\cdot V} \quad (14)$$

Combining Equations 13 and 14 gives

$$t_r = t_{eo}(1+k') \quad (15)$$

Being electropherograms sufficiently similar to chromatograms, it is tempting to think that the degree of retention or capacity factor (k′) for the different analytes can be obtained directly from the electrochromatogram as in HPLC by measuring retention times of the unretained neutral solute (t_{eo}), and of each analyte, t_r.

$$k' = (t_r - t_{eo})/t_{eo} \quad (16)$$

b. Ionized Analytes

The migration of ionized chromatographically retained compounds in EC has an added complication when compared to the migration of neutral compounds, because ions in the mobile phase will migrate at a different velocity than that of EOF. In these cases, the migration velocity of the compound can be described according to the following equation

$$u_{mig} = \frac{u_0}{(1+k')} \quad (17)$$

where u_0 is the mean linear velocity of an ionized chromatographically unretained compound, which can be expressed as the sum of the electroosmotic velocity of the electrolyte (u_{eo}) and the effective electrophoretic velocity of the unretained compound (u_{eff}):

$$u_0 = u_{eo} + u_{eff} \quad (18)$$

Combining Equations 17 and 18 results in

$$u_{mig} = \frac{u_{eo} + u_{eff}}{(1+k')} \quad (19)$$

where u_{eo} and u_{eff} can be expressed as the electroosmotic mobility (μ_{eo}) of the electrolyte (eluent) and electrophoretic mobility (μ_{eff}) of the ion using the potential drop (V) over the total length of the column (L):

$$u_{eo} + u_{eff} = (\mu_{eo} + \mu_{eff})(V/L) \quad (20)$$

Combining Equations 19 and 20 gives

$$u_{mig} = \frac{(\mu_{eof} + \mu_{eff})}{(1+k')}\frac{V}{L} \quad (21)$$

The retention time (t_r) of the ionized compound can be calculated introducing the injector-to-detector length of the column (L_{id}) and using Equation 21. So

$$t_r = \frac{L_{id}}{u_{mig}} = \frac{L_{id} \cdot L \cdot (1+k')}{(\mu_{eof} + \mu_{eff}) \cdot V} \quad (22)$$

Combining Equations 14 and 22, an expression can be drawn for the retention time of an ionized compound:

$$t_r = \frac{(1+k')}{\left[\frac{1}{t_{eo}} + \frac{\mu_{eff} V}{L_{id} L_{tot}}\right]} \quad (23)$$

This expression is more complicated than its equivalent for a neutral compound (Equation 15). Contributions to the elution time from electrophoresis and partitioning cannot be ascertained in a single experiment. In practice, separation of electrophoresis and partitioning contributions to the elution velocity will not be necessary, so that effective k′ values will most likely be quoted. The effective k′ values should be obtained from Equation 16 even when there are ionized analytes present having included an unretained neutral marker in the sample to establish t_{eo}.

B. Flow Profiles and Band Broadening in EC

The columns used in EC are restricted in general to the two types of columns described

before, i.e., PCCs and OTCs. With these types of columns, two forms of EC can be differentiated according to the columns used, and each one is subject to some limits under which the expected increase in separation efficiency can be obtained. In the case of PCCs, the EOF originates in the interparticular channels, and a lower limit on the channel diameter may exist as it will be shown later. In the case of OTCs with - coated wall (on which the remaining part of this discussion focuses), this lower limit is of no importance whatsoever. Even the smallest OTCs used, down to 2 μm, have a large diameter when compared with the channel diameter in PCCs. There is, however, a limit on the higher side established by the kinetics of the radial transport in the mobile phase, as already suggested in Figure 1.

There are important differences in the flow and dispersion properties of pressure-driven and electroosmotic-driven chromatography systems. The geometry of the two different types of columns in the flow profiles and solutes band dispersion will be discussed next.

1. Flow Profiles in EC

As stated before, there are two ways to drive a liquid through a column (open or packed) either by application of a hydrostatic pressure difference (PDF) or by a potential difference (EDF).[127] In PDF systems with OTCs or PCCs, there is a laminar (*Poiseuille*) flow, which has a parabolic velocity profile as a result of the distribution of shear stress in a viscous fluid. However, in EDF systems, the flow does not have a parabolic velocity profile. For an open tube filled with electrolyte, the double layer exists at the silica-coated wall of the tube. Because there is no imbalance of charge inside the sheath, in principle, there is no shear within the core region and the flow should have a *plug* profile, so that the velocity of the liquid is constant over more or less the entire tube section except for a very thin layer at the wall which is a few δ's thick. However, the theoretical work of Rice and Whitehead[99] on the subject of EOF in narrow capillaries concluded that the flow velocity predicted by Equation 3 is reduced as δ approaches the radius of the capillary, and the flow velocity begins to approach the typical parabolic shape of PDF. Therefore, the plug flow velocity profile diagram ascribed to electroosmosis in the LC literature could be inaccurate. Strictly, the EOF is a function of the distance from the wall and, with φ(x) representing the potential between wall and buffer, Equation 3 must be rewritten as[116]

$$u_{eo}(x) = \frac{\varepsilon}{4\pi\eta}(\zeta - \varphi(x))E \qquad (24)$$

Rice and Whitehead[99] described the transcolumn flow profiles to different ratios of the tube bore (d_c) to the double layer thickness (δ), and their results show that the flow does not vary over 90% of the tube bore when $d_c/\delta \geq 50$. Therefore, with typical values of δ (1 to 10 nm), the minimum d_c required should be between 0.050 and 0.5 μm, diameters at least 20 to 100 times narrower than typical d_c used in EC (10 to 50 μm). In addition, with the results described by Rice and Whitehead, Knox and Grant[34,51] demonstrated that the mean EOF velocity is independent of the tube bore in which it occurs, provided that the d_c is significantly greater than the thickness of the double layer. Both phenomena are very important features of the EOF and they are in sharp contrast with PDF where the profile is parabolic and the flow rate depends on the square of the tube bore.

The properties of the EOF in packed capillaries have been discussed[30,94] and are considered to be the same as open tubular capillaries, except that the electrical double layer exists on the surface of each silica-based particle in contact with the electrolyte

and not on the surface of the silica wall-coated tube. In addition, there are now numerous interconnected flow channels between particles of a packed bed. The mean channel diameter (d) is significantly smaller than the particle size (d_p), and it may be estimated from the ratio of the flow resistance parameter values ($\varnothing$) for an OTC and a PCC according to the equation:[34]

$$d/d_p = [\varnothing_{OTC}/\varnothing_{PCC}]^{1/2}$$

According to typical values obtained by Knox,[4] $\varnothing_{PCC} \approx 300$ and $\varnothing_{OTC} \approx 32$, the mean channel diameter is about one third that of the d_p. Hence, on the basis of the results by Rice and Whitehead,[99] the flow rate through a packed bed will begin to decrease noticeably only when the $d_P < 150\delta(d/\delta \geq 50)$. With the typical values of δ indicated before, this will occur when d_P is between 0.15 and 1.5 μm. Therefore, EOF behavior in capillaries packed with $d_P \geq 1.5$ μm is that of the flow not varying over 90% of the channel diameter, and the mean EOF velocity will be independent of the particle size. However, when the ratio of d to δ is unfavorable (a condition known as double layer overlap), the mean flow velocity is reduced and will vary along the radial position (the flow is supposed to be mixed with laminar and plug flow). In the worst case it approaches a parabolic-like profile, as with PDF.

These observations confirm the theoretical predictions of Knox and Grant,[34] proven experimentally,[33,51] indicating that the conclusions reached by Stevens and Cortes[49] regarding the double layer overlap when the d_p = 10 μm were erroneous. In addition, electroosmotic velocity is also independent of channel diameter and should therefore be more nearly the same in all channels across a packed bed.

It is important to note that although the electroosmotic velocity is not affected by particle size at least down to 1.5 μm, and for a given material (e.g., silica) it will exhibit the same zeta potential whether in the form of particles or an open tube, the EDF will be lower in packed capillaries than in open tubes. This is due[51] to the nonalignment of flow channels with the electric field and the packing porosity. First, channels in a packed bed are not, in general, aligned axially. The effective field in the direction of any channel will be E cos θ, where θ is the angle between the channel's and tube's axis. Second, HPLC packings are normally porous. EOF will occur only outside the particles, because the pores within the particles are normally so small that a complete double layer overlap occurs. The apparent mobile phase velocity traditionally used in chromatography is the true electroosmotic velocity multiplied by the factor (extraparticle porosity)/(total porosity). Accordingly, allowing for both effects, the electroosmotic velocities for beds packed with chromatographic packings are likely to be 40 to 60% of those for open tubes.

2. Band Broadening in EC

Band dispersion is characterized in electroseparation techniques in the same way as in chromatography, and the dispersive processes which can, in principle, occur are exactly the same. Thus, the theoretical plate number (N) for any analyte is obtained from the standard deviation of its peak profile or migration zone width in time units (σ_t) and its migration time (t) using the equation

$$N = (t/\sigma_t)^2 \qquad (25)$$

The height equivalent to a theoretical plate (H) is obtained from column length (L) and its theoretical plate number, according to the equation

$$H = L/N = L\,\sigma_t^2/t^2 \qquad (26)$$

If the peak profile or migration zone width (bandwidth) at the column outlet is expressed in length units, there are other expressions similar to those mentioned before, given by

$$N = \left(L/\sigma_L\right)^2 \quad (27)$$

$$H = \sigma_L^2/L \quad (28)$$

where σ_L^2 is the variance of the bandwidth in length units.

The observed theoretical plate height (H_{tot}) for any solute in an electropherogram can be expressed as:

$$H_{tot} = H_{col} + H_{ext-col} \quad (29)$$

where H_{col} is the plate height due to dispersion of the bandwidth across the column, and $H_{ext\text{-}col}$ is the plate height due to dispersion of the bandwidth out of the column (extracolumn contribution). In turn, the H_{col} and $H_{ext\text{-}col}$ in an EC system can be expressed as:

$$H_{col} = H_{mig} + H_{th} \quad (30)$$

$$H_{ext-col} = H_{inj} + H_{det} \quad (31)$$

where H_{mig} is the plate height due to migration of the solute across the column by EOF, H_{th} is the plate height caused by thermal effects across the column, and H_{inj} and H_{det} are the plate heights caused by extra band broadening as a result of the injection and detection, respectively.

a. Band Broadening in EC with OTCs due to Migration

The nature of the flow profile has some repercussions in the dispersion of the solute across the length of an open tube which has important implications in OTC separations. In PDF, with a parabolic-like profile, the flow variation across the tube disperses the solute (radial molecular diffusion), even when it is unretained. The resulting net dispersion is given by the Taylor equation,[128] from which the plate height (H) for an unretained solute in an open tube is determined by both axial and radial molecular diffusion according to the following expression (see Figure 1)

$$H = 2D_m/u + ud_c^2/96D_m \quad (32)$$

where u is the mean linear velocity, D_m the diffusion coefficient of the solute in the mobile phase, and d_c the inner diameter of the tube.

With EDF systems, the second term (radial molecular diffusion) in Equation 32 is absent[30] because the velocity profile is flat and there is no tendency of an unretained solute to be dispersed by the flow. Thus, the plate height for an unretained solute in an open tube is given

$$H = 2D_m/u \quad (33)$$

However, as soon as a solute is retained, transcolumn equilibration becomes necessary and the plate height is given by the following expression of Golay equation:[2,129,130]

$$H = \frac{2D_m}{u} + C_m\frac{d_c^2 u}{D_m} + C_s\frac{d_f^2 u}{D_s} \quad (34)$$

where d_f is the thickness of the stationary phase layer, D_s the diffusion coefficient of the solute in the stationary phase, and C_m and C_s are dimensionless coefficients for resistance to mass transfer in the mobile and stationary phase, respectively. In this case, there are three processes contributing to band broadening of component zones, i.e., the axial molecular diffusion term and slow equilibration in the mobile and stationary phase of the mass transfer terms. However, it is generally assumed that the resistance to mass

transfer in the stationary phase is negligible,[131] and Equation 34 can be adequately represented by the following equation:

$$H = \frac{2D_m}{u} + C_m \frac{d_c^2 u}{D_m} \qquad (35)$$

According to Equation 35, it is widely accepted that when OTCs are used in EC, the mass transfer term requires the use of very narrow tubes if high speeds and efficiencies are to be obtained. This requirement is not present in CZE[30] because with no retention at the walls of the tube and no variation in flow velocity across the tube, no contributions from C terms existed, and the plate height is given only by the axial diffusion term according to Equation 33. The same rationale applies to MEKC and capillary gel electrophoresis (CGE). With values of D_m around 10^{-9} m^2 s^{-1} (for small analytes in water, not valid for proteins or other polymers)[40] and u values of around 2 mm s^{-1}, plate heights can be of 1 μm and plate efficiencies of 10^6 plates per meter. In the case of CGE, the diffusion of the analytes (usually DNA fragments) is severely restricted by the presence of the gel, but being multicharged, they still experience a force large enough to provide adequate migration rates. The results are migration rates similar to those in CZE, but diffusion coefficients are 10 or possibly 100 times lower. The plate efficiencies than achieved, as shown in numerous examples, may be as high as 10^7 to 10^8 plates per meter. However, the situation in EC at first sight looks less encouraging, because additional plate height contribution from C-term (Equation 35) must be considered.

From the three contribution terms to the plate height (Equation 34), only resistance to mass transfer in the mobile phase is affected by the shape of the velocity profile of the eluent in the column. For the parabolic velocity profile from a PDF, C_m is derived[3,25] from the Golay equation,[130] and is given by

$$C_m^{PDF} = \frac{(1 + 6k' + 11k'^2)}{96(1 + k')^2} \qquad (36)$$

In general, for a given velocity profile, C_m may be obtained by evaluating the generalized dispersion theory of Aris.[132] According to this theory, the coefficient C_m for a flow profile perfectly flat is given by

$$C_m^{EOF} = \frac{k'^2}{16(1 + k')^2} \qquad (37)$$

However, as previously stated, the EOF profile is not completely flat. An accurate expression of the EOF profile can be obtained based on the theory of Aris,[132] but it is rather complex and cannot lead to analytical calculation of C_m. Therefore, Martin et al.[133,134] theoretically investigated the peak broadening with such flow in relation to the solute retention (k′) and the thickness of the double layer (δ) relative to the column radius (a), in order to propose an approximation of the profile generated in EOF. In their second,[134] they proposed an approximate expression more accurate and simpler than the first study for the evaluation of the peak broadening mobile parameter C_m, given by

$$C_m = \frac{4 + (4n + 16)k' + (n^2 + 10n + 20)k'^2}{4(n + 2)(n + 4)(1 + k')^2} \qquad (38)$$

For its application, a good estimate of the n parameter is necessary, which depends on the column radius (a) and the thickness of the double layer ($\delta \approx 1/\kappa$) according to the following expression:

$$n = \kappa a - \frac{3}{2} + \frac{3}{8\kappa a}\left(1 + \frac{1}{\kappa a} + \cdots\right) \qquad (39)$$

Martin et al.[134] compared C_m values obtained with Equation 38 with the C_m values for the true electroosmotic profile numeri-

cally computed according to the Aris dispersion theory.[132] In all cases studied ($10 \leq \kappa a \leq 50$),[134] the relative errors are less than 2% for $k' \geq 1$. In addition, relative errors decrease when increasing retention and, as expected, for a given k' value, they are smaller at larger κa.

C_m values obtained according to Equation 37 have been compared with those obtained by the expression of Martin et al.[134] (Equation 38). In all cases studied (different d_c [5 and 50 μm], δ [1 and 10 nm], and k' [1 and 2]), relative errors between the values obtained by two expressions are less than 4% (see Table 1), which is quite satisfactory. In addition, according to the above results, relative errors decrease as the retention increases and, again, as expected, for a given k' value they are smaller at a larger d_c to δ ratio. Therefore, the C_m coefficient is a function of the capacity factor (k') and of the flow profile. As shown by Equation 37, this is a very reasonable approximation for C_m values when there is an EOF process[133–135] which can be used to thoroughly estimate the EOF potential and establish a comparison with other techniques or types of flow.

In Figure 3, C_m^{EDF} and C_m^{PDF} are plotted vs. k'. It is clear that both functions increase when increasing retention, but C_m^{EDF} is always smaller than C_m^{PDF}. The improvement is significant (about 10) at k' values lower than 2, but C_m^{PDF} is typically twice as large as C_m^{EDF} at k' values higher than 2. In conclusion, solute dispersion due to the C_m-term is lower in an EDF than in a PDF system, which results in a lower plate height (see Equation 35) for an OTC–EC system compared with an OTC–LC system. Therefore, the difference between both C_m-terms is the reason for the different efficiency between EC and LC with OTCs.

On the other hand, the importance of column diameter in the plate height is also obvious in Equation 35. Figure 4 shows the influence of both column diameter and flow profile on plate height for a given k' according to Equation 35. Note that for a PDF system, the reduction of the d_c by a factor of 2 will have a more pronounced effect on efficiency than using an EDF system, which will reduce C_m by a factor of 3, especially at velocities higher than optimal. It is also interesting to note that to OTCs of 10 μm i.d. with EDF, H is practically constant at velocities higher than optimal.

In previous papers[136–138] it was shown that column performance may be most effectively assessed by logarithmic plotting of the plate height vs. velocity. In addition, in order to compare data obtained with otherwise similar chromatographic systems in which the column diameter and solute diffusivity may vary, Equation 35 should be written in dimensionless form using reduced parameters (the reduced velocity $v = u\, d_c/D_m$ and the reduced plate height, $h = H/d_c$).[138,139] Knox and Gilbert[3] have shown that a corresponding reduced plate height equation is given by

$$h = 2/v + C_m \cdot v \qquad (40)$$

This method of representation enables easy comparison of columns with different d_c, using different solutes and eluents and setting readily remembered standards of good performance.[140] Therefore, Figure 5, $\log h^{EDF}$ and $\log h^{PDF}$ are plotted vs. $\log v$, shows that the use of EC with OTCs is most advantageous at relatively low values of k', when it can provide a substantially improved column performance, although some improvement may be expected at any k'. This phenomenon opens up the possibility of using larger diameters in OTC–EC, fewer with existing practical problems with regard to instrumentation (injection and detection), loadability, and column preparation. If columns are operated at their optimum velocity and for a given capacity factor (between 0.2 and 2), EOF can be performed in a tube with diameters between 1.5 and 3.0 times larger than for pressure flow, which gives between two and ten times larger external volumetric requirements than for pressure flow (see Table 2). Therefore, instrumental require-

TABLE 1 Cm Values and Relative Error between the Values Obtained by Equations 37 and 38

					k′ = 1			k′ = 2		
Electrolyte	**δ (nm)**	**d_c (μm)**	**κa[a]**	**n[b]**	**C_m[c]**	**C_m[d]**	**Error (%)**	**C_m[c]**	**C_m[d]**	**Error (%)**
0.001	10	5	250	248.5		0.06450	3.2		0.11377	2.4
		50	2,500	2,499.5	0.06250	0.06252	0.32	0.11111	0.11138	0.24
0.1	1	5	2,5000.24	2,499.5						
		50	25,000	24,998.5		0.06270	0.03		0.11114	0.02

[a] $\kappa a \approx d_c/2\delta$.
[b] Equation 39, when $\kappa a \gg$ can be simplified by $n = \kappa a - 3/2$.
[c] According to Equation 37, but when in Equation 35 the inner diameter is substituted for the inner radius: $C_m = k'^2/4\ (1+k')^2$.
[d] According to Equation 38.

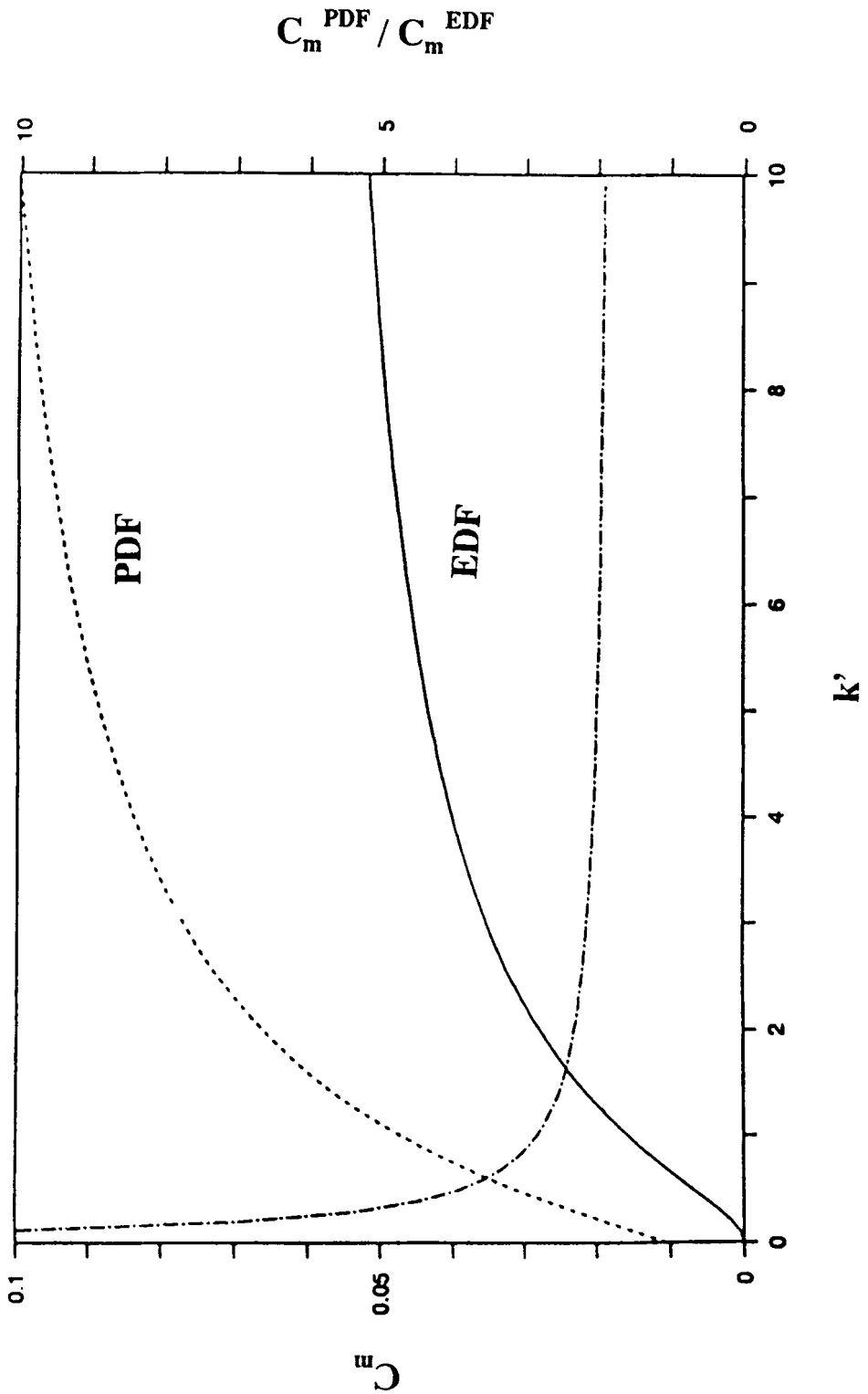

FIGURE 3. Functions of the C_m for a PDF, $C_m^{PD} = 0.0469$ (dashed line), and for an EDF, C_m^{EDF} (solid line). The dot-dashed line represents the ratio C_m^{PDF}/C_m^{EDF}. (From Bruin, G. J. M., T CK PPH, Kraak, J. C. and Poppe, H., *J. Chromatogr.*, 517, 557, 1990. With permission.)

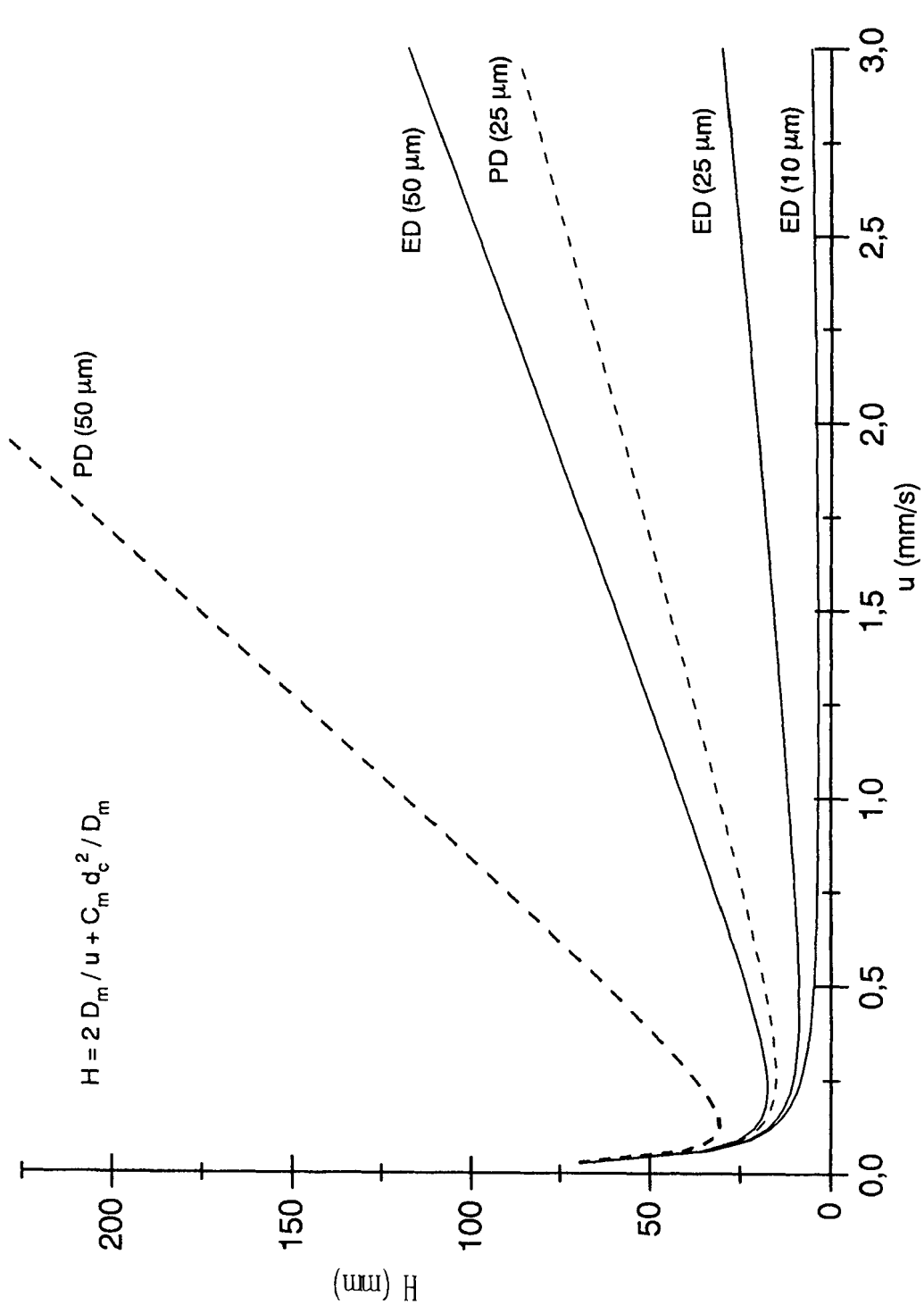

FIGURE 4. Theoretical plate height curves (according to Equation 35) for a pressure-driven (PD) system (dashed lines) and for an electroosmotic-driven (ED) system (solid lines) for different inner diameters of the OTCs. Other parameters: $k' = 1$; $D_m = 10^{-9}$ m^2 s^{-1}; $C_m^{PD} = 0.0469$ (according to Equation 36); and $C_m^{ED} = 0.0156$ (according to Equation 37).

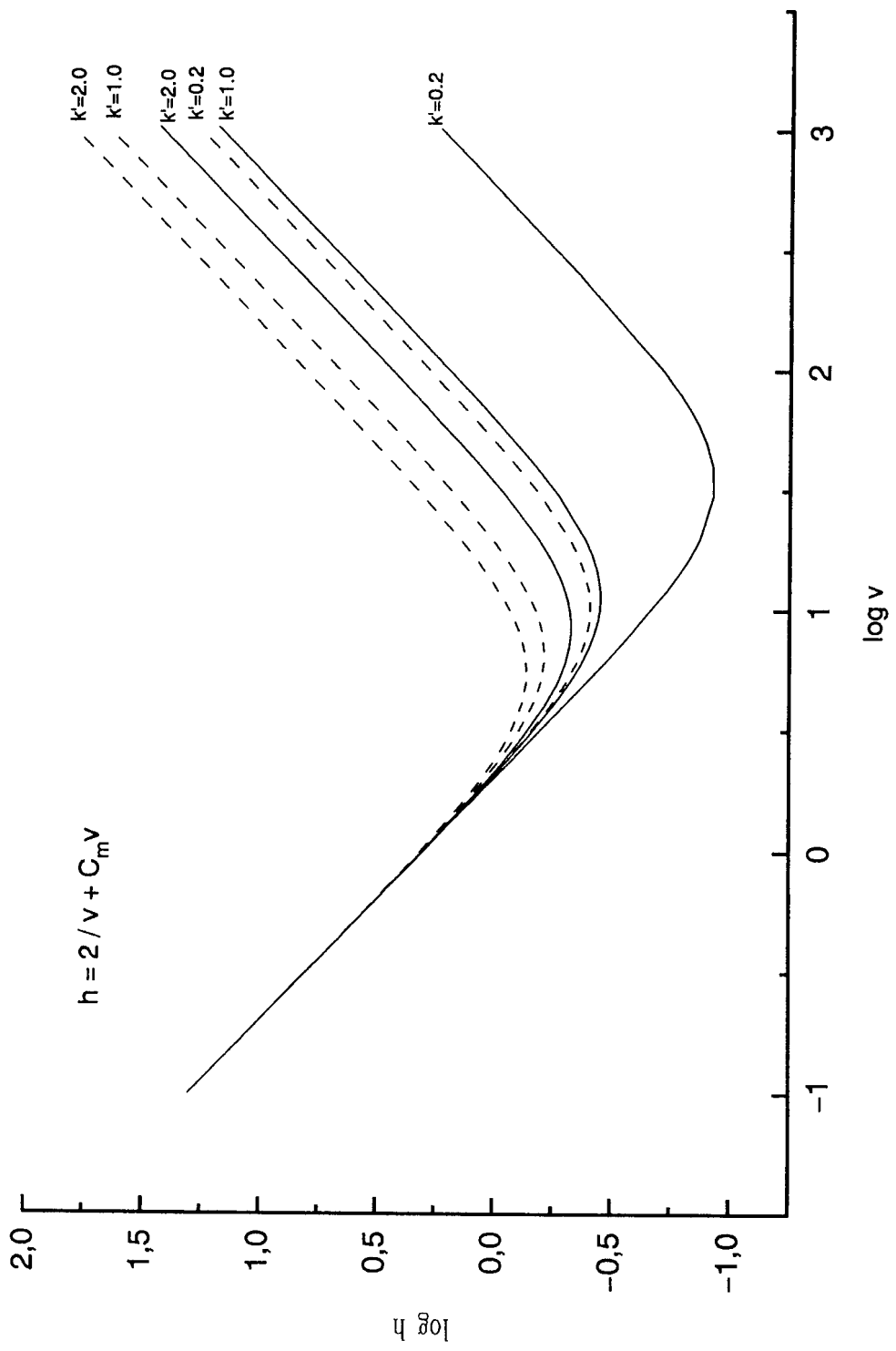

FIGURE 5. Logarithmic plots of reduced height (h) vs. reduced velocity (v) for electroosmotic-driven system (solid lines) and pressure-driven system (dashed lines) for three different k′ values (according to Equation 40).

TABLE 2 Relations between External Volumetric Requirements (V_{ext}) Demanding for EDF and PDF Systems

k′	h_{op}^{PDF} [a]	h_{op}^{EDF} [a]	d_c^{EDF}/d_c^{PDF} [b]	$V_{ext}^{EDF}/V_{ext}^{PDF}$ [c]
0.2	0.40	0.12	3.3	11
1.0	0.62	0.35	1.8	3.2
2.0	0.72	0.48	1.5	2.3

[a] See Figure 5.
[b] $h = H/d_c$, and if H in both systems is the same: $h_{op}^{PDF}/h_{op}^{EDF} = d_c^{EDF}/d_c^{PDF}$.
[c] $V_{ext} \propto d_c^2$, therefore: $V_{ext}^{EDF}/V_{ext}^{PDF} = (d_c^{EDF}/d_c^{PDF})^2$.

ments may be somewhat less demanding for EDF systems than for PDF systems, allowing injector or detector volumes at least three times larger for practical purposes ($k' < 2$).

b. Band Broadening in EC with PCCs due to Migration

In general chromatography with PCs, three main independent processes contribute to band broadening of solute zones when they migrate through the column, namely, eddy diffusion (the unevenness of flow through the packing), axial molecular diffusion, and solute resistance to mass transfer between the mobile and stationary phases. The plate height (H) is derived[141–143] from the Van Deemter equation,[144,145] and for practical purposes is adequately represented by the following semiempirical equation[146]

$$H = \frac{A \cdot d_p^{4/3}}{D_m^{1/3}} \cdot u^{1/3} + \frac{2\gamma D_m}{u} + C\frac{d_p^2}{D_m} \cdot u \qquad (41)$$

where u is the mean linear velocity, D_m is the solute diffusion coefficient in the mobile phase, d_p is the average particle diameter, γ is the tortuosity factor of the packing, and A and C are dimensionless coefficients for eddy diffusion and resistance to mass transfer, respectively.

In order to compare data obtained with similar chromatographic systems in which one or several of the following parameters may vary (e.g., the particle size of packing, the solutes, or eluents), Equation 41 should be written in dimensionless form. According to the concept of reduced parameters in PCs (the reduced plate height, $h = H/d_p$, and reduced velocity, $v = u\, d_p/D_m$) introduced by Giddings[147] and widely advocated by Knox et al.,[136,146,148] the reduced plate height equation is given by[149]

$$h = Av^{1/3} + B/v + Cv \qquad (42)$$

where A and C were described before, and B is a new dimensionless coefficient for axial molecular diffusion.

The dependence of h on k′ of the solute at low velocities is predicted theoretically.[149] In principle, B depends on k′ and C apart from k′ will depend on the D_m/D_s ratio, whereas the value of A is irrespective of these factors. However, diffusion coefficients in both phases are of the same order of magnitude in LC ($D_m/D_s = 1.7$),[3] and in practice, for well-packed columns, experimental data[150–152] suggest that it is adequate to take constant values for the three dimensionless coefficients. In general, B is equal to 2γ and usually close to 1.5 to 1.6, because γ has a value of about 0.8 for porous spherical particles normally used in HPLC packings (γ = 0.6 for nonporous spherical particles).[153] C generally ranges from 0.02 to 0.2. Finally, the A coefficient characterizes packing regularity and its value drastically determines column quality. Values of A significantly larger than unity indicate that the column is poorly packed, either because the packing technique used is inappropriate or because the batch of the stationary phase contains nonuniform particles (different size or irregular shapes).

Guiochon[154] estimated as normal for well-packed columns the following values: A = 0.6 to 1.0, B = 1.5, and C = 0.1, which can be used for theoretical studies. A logarithmic plot of Equation 42 using these pre-

vious values is shown in Figure 6. According to this Figure, the B-term is predominant at low reduced velocities and is responsible for the rising value of h as velocity decreases; at high velocities, the C-term is predominant and may account for the rise of h as the velocity rises; finally, in the intermediate region, where the A-term becomes predominant, h shows a well-defined minimum ($h \approx 2$ at $v \approx 3$).

On the other hand, Knox and Grant[34] affirmed that EC is significantly more efficient than conventional chromatography when carried out with the same PC, and predicted that the only difference between both techniques is the lower contribution of the A-term to the plate height. This is to be expected due to the nature of electroosmotic mobility being independent of the channel diameter. These predictions were corroborated some years later by the experimental results obtained by Knox and Grant.[51] They compared chromatography performance under both PDF and EDF using capillaries packed with both 3 and 5 μm silica gel particles for unretained solute. For both particles, it was notable that the minimum h values (h_{min}), dominated by the A-term and obtained with EDF ($h_{min} \approx 1.3$ at $v \approx 3$), were substantially lower than those obtained with PDF ($h_{min} \approx 2.0$ at $v \approx 3$). However, the HETP curves converge at high-flow velocities for both particles when the C-term is predominant. A study of these results has enabled estimation of an $A \approx 0.3$ value for an EDF and of an $A \approx 0.8$ value for a PDF, being $B \approx 1.5$ and $C \approx 0.1$ in both cases. Based on these results, efficiencies that could be obtained with PCCs using EDF and PDF systems are compared in Figure 7. According to this Figure, EC is more efficient than conventional LC when carried out with PCCs at velocities close to the optimum.

Knox[94] has suggested that, using submicron particles, all contributions from packing particles to the plate height other than axial diffusion should become insignificant. Thus the plate height will be given by

$$H = \frac{2\gamma D_m}{u}$$

This situation will be similar as in CZE, MEKC, and CGE, however, it has not been done yet. The smallest particles used so far are ≈1.5 μm. Knox and Grant[51] obtained excellent results (h = 1.3) for an unretained solute in a PC with 1.5 μm nonporous silica particles. However, Yamamoto et al.[53] obtained a significant decrease in plate numbers for retained compounds when 1.6 μm nonporous ODS-silica particles were used. This phenomenon was not observed when the particles were of 3 μm porous ODS-silica. This might be due to the small capacity of the nonporous packing and mass transfer limitations. Despite this fact, the real and feasible size limit of the particle used in PCCs is considered to be 1.5 μm.

Finally, the use of PCCs in EC enables a decrease in plate height and the possibility of using longer capillaries. Separations with PCC–LC can be carried out easily with 3 and 5 μm particles, but this is seldom possible with 1.5 μm particles unless very short columns are used. For example, to operate with approximately 60 cm and 1.5 μm at 2.0 mm/s (optimum velocity) with PDF would have required a pressure drop of about 6000 bar. The results with 1.5 μm establish that efficiencies of around 500,000 plates per meter are attainable in EC.[51] With a voltage limitation of 50 kV, it should be possible to attain 250,000 plates in a real column using EC with 2 mm/s. With LC limited to say 400 bar, the longest column, packed with 1.5 μm, that could be used would be about 6 cm long giving a maximum of 30,000 plates.

c. Band Broadening due to Thermal Effects

The above considerations suggest that an EDF system possesses some remarkable advantages over a PDF system. However, attainment of the best performance is limited

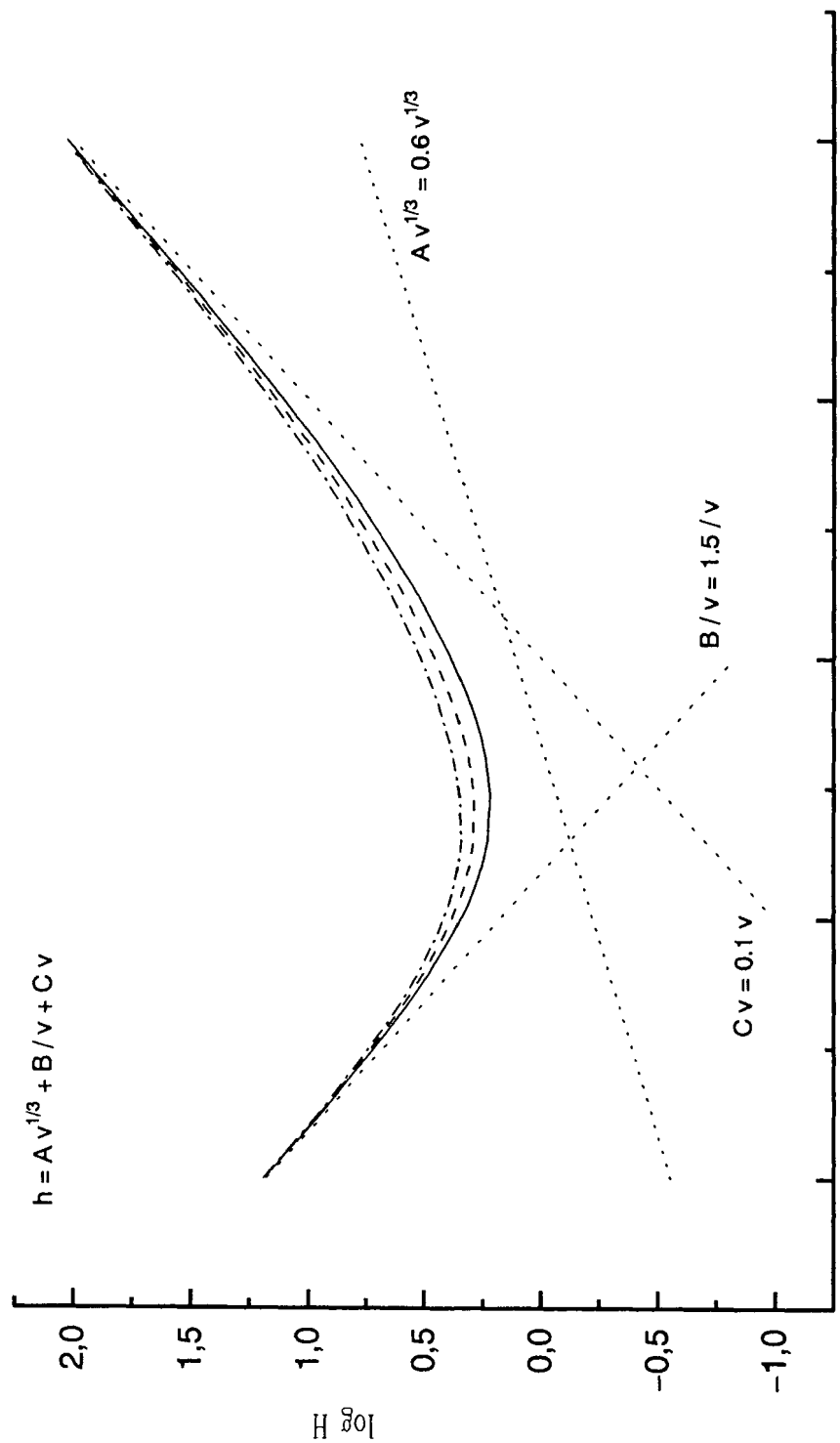

FIGURE 6. Logarithmic plots of reduced height (h) vs. reduced velocity (v) for PCs and the following values of the dimensionless coefficients: A = 0.6 (solid line) – 0.8 (dashed line) – 1.0 (dot-dashed line); B = 1.5; and C = 0.1 (according to Equation 42).

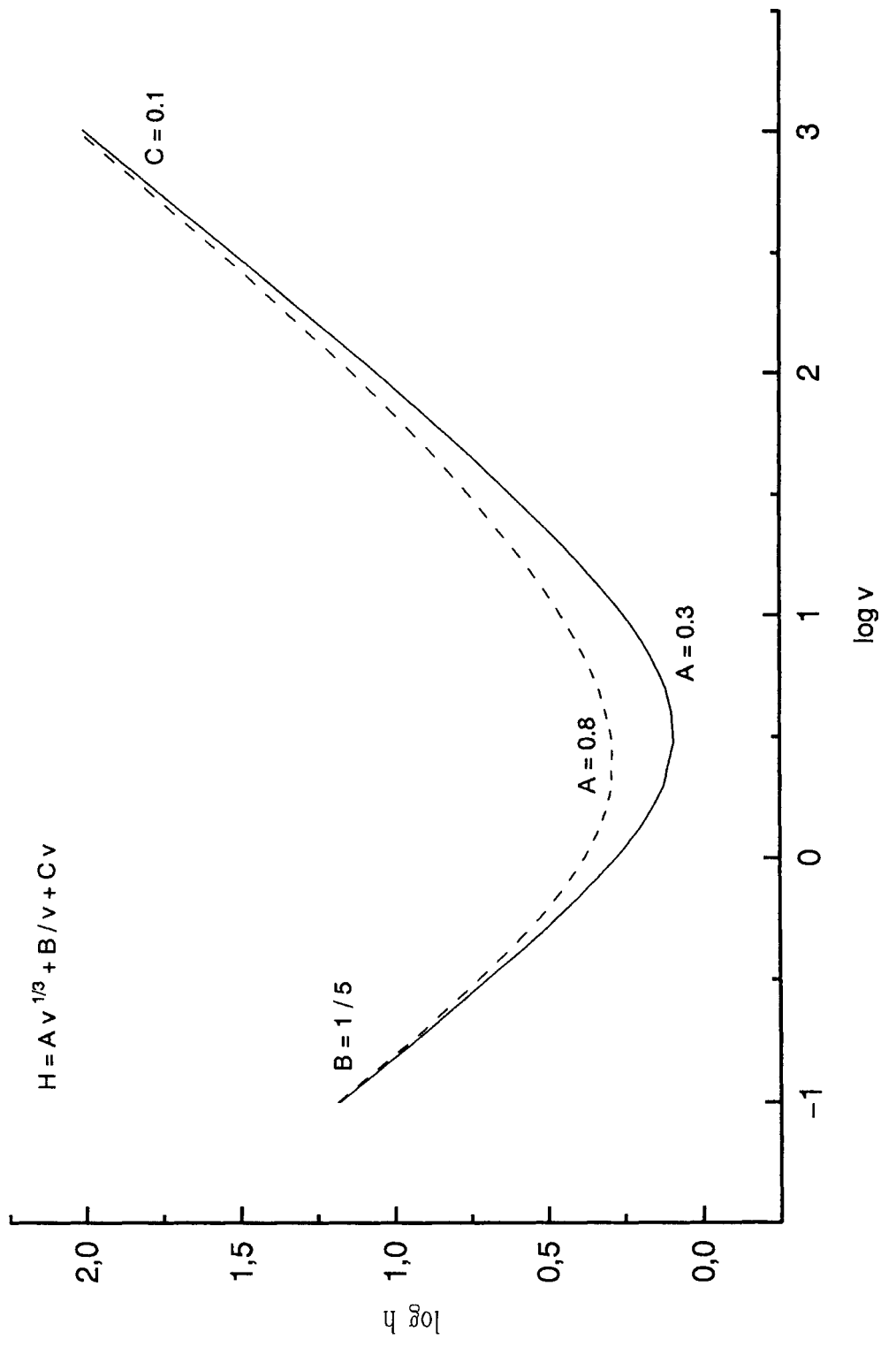

FIGURE 7. Logarithmic plots of reduced height (h) vs. reduced velocity (v) for PCs with EDF (solid line) and PDF (dashed line). The values for the dimensionless coefficients used are: B = 1.5 and C = 0.1 for both flows, and A = 0.3 for EDF and A = 0.8 for PDF (according to Equation 42).

by the much greater self-heating occurring in EDF systems. In order to attain high efficiencies, it is essential to ensure that an effective heat dissipation be accomplished in the system, because excess solution heating leads to a parabolic temperature gradient across the bore of the capillary, which can be a significant source of dispersion. Lauer and McManigill[155] discussed the importance of power dissipation for efficient separation in capillary electrophoresis (CE). They also provided theoretical and experimental evidence for separation efficiency in 125 to 500 μm i.d. capillaries. Poppe et al.[156,157] considered in detail the effects of homogeneous heat release arising from viscous drag in PCs. Their general and complex equations were used by Knox and Grant[34] to establish simpler equations which could be easily applied to a common situation such as that of a cylinder within which heat is generated, both in a PDF and in an EDF system.

The temperature difference between the center of a cylinder and its wall is given by

$$\Delta T_{core} = Qd_c^2/16\kappa \qquad (43)$$

where Q is the heat generation rate per unit volume within the cylinder, d_c is the inner diameter of the cylinder, and κ is the thermal conductivity of the medium (0.6 W m^{-1} K^{-1} for water, 0.2 W m^{-1} K^{-1} for methanol, and 0.4 W m^{-1} K^{-1} as typical values).

In a PDF system, Q is obtained from the frictional work dissipated in the column, and is given by[34]

$$Q = \left(\psi d^2/\varnothing \cdot \eta\right)(\Delta P/L)^2 \qquad (44)$$

where ψ is the total porosity of the medium, d is the inner diameter of the tube in the case of an open column and the particle diameter in the case of the PC, $\varnothing$ is the flow resistance parameter, η is the viscosity of the electrolyte, ΔP is the pressure drop across the column, and L is the column length. Combination of Equations 43 and 44 gives the temperature excess within the core region of the tube:

$$\Delta T_{core} = \left(\psi d^2 d_c^2/16\kappa\varnothing\eta\right)(\Delta P/L)^2 \qquad (45)$$

Note that although Equation 45 for the packed tube appears to show that ΔT_{core} will decrease as d_p is reduced, if one is to operate at a fixed reduced velocity in order to maintain peak performance, $\Delta P/L$ must be altered in proportion to d_p^{-3} and ΔT_{core} will be proportional to d_p^{-4}. Therefore, in order to maintain the ΔT_{core} constant under optimum operating conditions, it is necessary to reduce d_c in proportion to d_p^2 in a PDF system. According to Equations 44 and 45, Table 3 includes Q and ΔT_{core} values for different types of columns used in a PDF system (conventional packed, PC; packed capillary, PCC; and open tubular, OTC) under optimal conditions.[3] All values are very small and practically negligible, except for ΔT_{core} in a PC (0.25 K), which indicates that miniaturization is necessary in conventional LC with packed tubes only when the particle diameter becomes very small ($d_p < 3$ μm).

The situation is strikingly different with EDF systems. The tube containing the electrophoretic medium behaves similarly to a cylindrical ohmic conductor when a voltage is applied across the two ends. When a current is passed along the tube, Joule heating is generated and the medium heats up. Heat generation per unit volume in an electrolyte for an EDF system is given by[34]

$$Q = E^2\lambda C\psi \qquad (46)$$

where E is the electric field strength, λ is the molar conductivity of the electrolyte, and C its concentration. Combining Equations 43 and 46 then gives the temperature excess within the core region of the tube:

$$\Delta T_{core} = E^2\lambda C\psi d_c^2/16\kappa \qquad (47)$$

TABLE 3 Values of Q and ΔT_{core} for Different Types and Dimensions of Columns in Systems with PDF and EDF

Type of column	d_c	System	Q (W cm^{-3})	ΔT_{core} (K)
PC	4.6 mm	PDF	0.077	0.25
		EDF	300	992
	200 μm	PDF	0.045	$3 \cdot 10^{-4}$
		EDF	300	1.9
PCC	100 μm	PDF	0.045	$7 \cdot 10^{-5}$
		EDF	300	0.47
	50 μm	PDF	0.045	$2 \cdot 10^{-5}$
		EDF	300	0.12
	50 μm	PDF	$4 \cdot 10^{-6}$	$2 \cdot 10^{-9}$
OTC		EDF	375	0.15
	10 μm	PDF	$1 \cdot 10^{-4}$	$2 \cdot 10^{-9}$
		EDF	375	$6 \cdot 10^{-3}$

Note: Taking typical values: E = 50,000 V m^{-1}; κ = 0.4 W m^{-1} K^{-1}; η = 10^{-3} N s m^{-2}; λ = 0.015 S m^2 mol^{-1}; C = 10^{-2} M^3; ψ = 0.8 (PC or PCC) or 1.0 (OTC); d_p = 3 μm; $\varnothing$ = 500 (PC), or 300 (PCC), or 32 (OTC); L = 15 cm (PC), 30 cm (PCC), or 100 cm (OTC); ΔP (under optimal conditions) = 110 bar (PC), 130 bar (PCC), or 2 bar (OTC).

Q and ΔT_{core} values are included in Table 3 according to Equations 46 and 47 for different types of columns used in an EDF system (conventional packed, PC; packed capillary, PCC; and open tubular, OTC). According to the results obtained, both Q and ΔT_{core} are larger for EDF systems than for PDF systems under typical operating conditions. The high value of ΔT_{core} (992 K) obtained with a conventional PC of 4.6 mm i.d. needs to be highlighted, which proves the inability to use this type of column in an EDF system. Note also that self-heating is much greater in a wider-bore column, because it is proportional to the square of the column diameter (Equation 47). Therefore, miniaturization is required when an EDF system is used, with open and packed tubes, and the tube diameter used must be sufficiently smaller to avoid excessive temperature gradient, which can be a significant source of dispersion.

Knox and Grant[34] have developed an expression for the contributions of the temperature gradient to the plate height, H_{th}:

$$H_{th} = 7 \cdot 10^{-9} \frac{\varepsilon_0 \varepsilon_r \zeta E^5 d_c^6 \lambda^2 C^2 \psi^2}{D_m \eta \kappa^2} \qquad (48)$$

where ε_0 is the permitivity in vacuum and ε_r is the relative permitivity or dielectric constant.

Using some typical parameters (some of the values are rough estimates) shows that thermal band broadening caused by heating effects can be neglected for diameters smaller than 100 μm (see Table 4). Therefore, as a result of the self-heating in the EDF system, the inner diameter of the capillary used in EC must have a maximum value of 100 μm, top value for PCCs in EC but not for OTCs in EC, which will show an inferior inner diameter due to the dispersion factor caused by the migration referenced above. The results obtained concerning ΔT_{core} and H_{th} (Table 4) are in agreement with precedent studies. Grushka et al.[40] predicted significant loss of efficiency at high ionic strength and high field in columns larger than 75 μm i.d., due to temperature gradients across the column diameter; and Jones and Grushka[158]

TABLE 4 Values of Different Parameters Related to Thermal Effects for Different Types and Dimensions of Columns in EC Systems

Type of column	d_c (μm)	Q (W cm^{-3})	ΔT_{core} (K)	H_{mig} (μm)	H_{th} (μm)	ΔH (%)	ΔT_{wall} (K)
	200		1.9	3.9	0.44	11	128
PCC	100	300	0.47	3.9	$7 \cdot 10^{-3}$	0.2	32
	50		0.12	3.9	$1 \cdot 10^{-4}$	$2.5\ 10^{-3}$	8
OTC	50	375	0.15	17.5	$2 \cdot 10^{-4}$	$1.1\ 10^{-3}$	10
	10		$6 \cdot 10^{-3}$	3.5	$1 \cdot 10^{-8}$	$2.9\ 10^{-7}$	0.4

Note: Taking typical values: E = 50,000 V m^{-1}; $\varepsilon_0\varepsilon_r = 7.08 \cdot 10^{-10}$ C^2 N^{-1} m^{-2}; κ = 0.4 W m^{-1} K^{-1}; η = 10^{-3} N s m^{-2}; λ = 0.015 S m^2 mol^{-1}; C = 10^{-2} *M* = 10 mol m^{-3}; ψ = 0.8 (PCC) or 1.0 (OTC); $D_m = 10^{-9}$ m^2 s^{-1}; ξ = 50 mV; d_o = 375 μm; d_p = 3 μm; h_{min} = 1.3 (PCC) and h_{min} = 0.35 (OTC at k′ = 1).

assumed that a ΔT_{core} of less than 1 K across the bore of the capillary will not seriously decrease the efficiency. In addition, it may be observed that H_{th} is proportional to $E^5 d_c^6 C^2$, and thus quite small changes in potential gradient, column diameter, and electrolyte concentration can have dramatic effects on the contribution to H arising from self-heating. It is thus clear that careful consideration must be given to these interconnected parameters if adverse effects from self-heating are to be avoided.

Finally, the heat generated within the tube is conducted first through the walls of the tube and then through the surrounding medium, typically air or a cooling liquid. Under typical operating conditions, ΔT_{core} would be small compared with the temperature excess of the tube wall relative to the surrounding ambient air (ΔT_{wall}). Therefore, apart from the thermal nonuniformity of the liquid within the core of the tube, problems may also arise as a result of the mean temperature rise of the liquid, due to slow heat transfer through the tube material (of poor conductivity) and cooling bath medium. Knox[94] has studied the magnitude of this self-heating using data from Roberts,[159] and concluded that the temperature increase of the tube relative to the surrounding air (ΔT_{wall}), in an unstirred system where the tube is cooled only by convection, can be calculated approximately by the following expression

$$\log \Delta T_{wall} = 1.70 \log d_0(\mu m) + \log Q\left[\left(d_i/d_0\right)^2\right](W/cm^3) - 4.20 \quad (49)$$

where d_o and d_i are the outer and inner and outer diameter of the tube, respectively.

According to Table 4, the demands on temperature control become more serious when larger inner diameters are used. With 200 μm, the $\Delta T_{wall} \approx 130$ K and lack of performance is about 10%. But even with 100 μm, the capillary may run at a temperature as much as 30°C above ambient ($\Delta T_{wall} \approx 30$ K), although the lack of performance is <0.2%. Such temperature increases may affect the reproducibility and stability of components. This can be catastrophic, particularly in EC with PCCs, where particles and frits act as boiling chips, helping the formation of bubbles which generate baseline noises and dried-out sections in the column and eventually breaking the current and thus stopping the EOF.[51] In contrast to the internal thermal profiles, these effects can be smoothed by applying cooling, as shown by various workers.[94,160] If the tube is cooled by forced air at 10 m s^{-1}, the temperature excess

can be reduced to about one fifth of its instirred value.[94]

d. Band Broadening due to the Extracolumn Contributions

Basically, there are two possible contributions to the extracolumn band broadening in an EC system, one due to injection and another to detection. However, the extracolumn contribution of an on-column detection system can be neglected.[10,26] In addition, in light of the above paragraph, H_{th} can be neglected too. Therefore, the observed theoretical plate height in a system with on-column detection and without self-heating can be expressed as:

$$H_{tot} = H_{mig} + H_{inj}$$

Hence, according to Equation 28, the variance observed in an electropherogram is given by

$$\sigma^2_{L,tot} = \sigma^2_{L,mig} + \sigma^2_{L,inj} \qquad (50)$$

where $\sigma^2_{L,mig}$ is the variance due to migration of the solute across the column by EOF, and $\sigma^2_{L,inj}$ is the extra variance caused by the injection zone length. If the general viewpoint of a 5% increase in bandwidth by extracolumn contribution effects is accepted,[161] the following should hold:

$$\sigma^2_{L,tot} \leq \left(1.05\sigma_{L,col}\right)^2 \qquad (51)$$

Combining Equations 50 and 51, and considering that the variance caused by thermal effects ($\sigma^2_{L,th}$) is neglected, then the extra variance caused by the injection zone length must be

$$\sigma^2_{L,inj} \leq 0.103\ \sigma^2_{L,mig} \qquad (52)$$

On the other hand, the extra variance caused by the injection can be expressed as:[162]

$$\sigma^2_{L,inj} = \frac{L^2_{inj}}{K^2} \qquad (53)$$

where L_{inj} is the length of the injection and K^2 is the injection profile factor, for which a value of 12 can be assumed when injection is a rectangular plug. The restrictions on the injection zone length become even more critical when the injection profile approaches an exponential profile. Using the electromigration technique, the injection profile can be considered plug, but with a hydrodynamic technique the injection profile approaches an exponential profile. In this instance, one will find a lower K^2 value, thus a smaller maximum acceptable injection zone length. In addition, various phenomena may account for the deviation in K^2 value from the value for a block, 12. Martin and Guiochon[133] found that the rise and fall times in the voltages may have influenced the amount injected. Grushka et al.[40] found that hydrodynamic induced sample introduction spontaneous takes place. Finally, Bruin et al.[64] observed that the angle at which the capillary is cut is critical. Although a value of 8 can be considered satisfactory for an electromigration technique, with obliquely cut capillaries K^2 values as low as 3 were found.

Table 5 shows the injection volumes that should be used with different types and dimensions of columns, assuming a value of 8 for K^2 as satisfactory. It should be noted that although with an OTC of 10 μm i.d. sample volumes between 50 and 100 pl must be used, with PCCs it is possible to inject higher volumes, about nanoliters, which could be used in OTCs if these columns have at least a 25-μm i.d. However, in an EC system with OTCs of 25 μm i.d., the high efficiencies that could be expected from OTCs cannot be attained, obtaining instead similar efficiencies to those rendered by PCCs.

TABLE 5 Injection Volumes That Should Be Used with Different Types and Dimensions of Columns in EC Systems

Type of column	d (μm)[a]	k′	h_{min}	H_{min} (μm)	σ^2_{inj} (mm^2)[b]	L_{inj} (mm)[c]	V_{inj}[d]
		0.2	0.12	1.2	0.062	0.704	55 pl
	10	1.0	0.35	3.5	0.180	1.200	95 pl
		2.0	0.48	4.8	0.247	1.406	110 pl
OTC	25	0.2	0.12	3	0.154	1.110	0.55 nl
		2.0	0.48	12	0.617	2.222	1.1 nl
	50	0.2	0.12	6	0.309	1.572	3.1 nl
		2.0	0.48	24	1.234	3.142	6.2 nl
	1.5			2	0.100	0.894	1.8 nl
PCC	3	—	1.3	3.9	0.201	1.268	2.5 nl
	5			6.5	0.334	1.635	3.2 nl

[a] d is the tube inner diameter in the case of an OTC and the particle diameter in the case of the PCC.
[b] $\sigma^2_{L,inj} = 0.103\ H_{min} \cdot L_c$; considering a length of column, L_c = 50 cm for all columns.
[c] $L_{inj} = (\sigma^2_{L,inj} \cdot K^2)^{1/2}$; considering $K^2 = 8$.
[d] $V_{inj} = \pi/4 \cdot d_c^2 \cdot L_{inj}$; considering a d_c of 50 μm for all PCCs.

Finally, it is possible to inject suitably the very small sample volumes required for OTCs of 10 μm using the electromigration technique, described in detail by Rose and Jorgenson.[42] This technique would have discrimination between charged species of the sample, because charged compounds migrate at a different velocity into the column as a result of their different electrophoretic mobilities. In EC, however, preferably neutral compounds are separated, migrating at the same electroosmotic velocity into the capillary.

IV. APPLICATIONS

In principle, the quality of the sample introduction into the separation capillary depends only on the injection method used. Also, the separation parameters determine final quantitative results. Therefore, to ascertain the optimal quantitative repeatability for a specific instrument, both the parameters influencing the sample introduction and the separation parameters must be analyzed.

The repeatability of quantitative analyses on a home-made instrument for EC was studied by Coufal et al.[163] They used the peak area and the peak height as evaluation parameters for the quantitative repeatability, and found that the peak height generally yielded better repeatable quantitative results compared to the peak area. In addition, the effect of different injection and separation parameters on the repeatability of quantitative analyses was also studied. Dose and Guiochon[164] reported that immediately after a buffer-filled capillary is immersed into a sample solution, a rapid diffusion of sample components into the capillary will take place. Therefore, the duration of individual injection steps from injection to injection, when an injection method is carried out manually, can strongly impact the quantitative repeatability results. Degassing of the eluent is crucial in a test system. Insufficient degassing led to an increase of baseline drift, unstable current, and finally EOF breakdown as a result of bubble formation. These signs can be presented by thoroughly degassing the eluent. The best results were obtained by Behnke et al.[61] through a combination of purging with helium and applying vacuum under ultrasonication.

A. Separation of Complex Mixtures in EC

The potential of EC with OTCs in separations with high resolutions and high efficiencies of complex mixtures was first demonstrated by Bruin et al.[64] in 1990. They used OTCs of 10 μm i.d. coated ODS-silica layer. One example of a separation is shown in Figure 8, where eight polycyclic aromatic hydrocarbons (PAHs) are separated in less than 400 s, and all the peaks are baseline resolved in only 90 s (internal time). The efficiency and resolving power of an OTC–EC system using polymer-coated columns was shown by Pfeffer and Yeung.[65] The separation of several PAHs, geometrical and positional isomers, are all baseline resolved. Pfeffer and Yeung[66] also showed that five anions not separating due to mobility differences in CZE can be separated using OTC–EC (see Figure 2b). Anions can be separated based on affinity differences for a polymer-coated column via an ion-pairing agent added to the buffer. The efficiency achieved for such a separation in a 40-cm column (1.4×10^5 plates) is twice that required for a similar chromatographic separation and nearly equal to that of a similar but poorly resolved CZE separation. Separation of compounds with similar electrophoretic mobilities (as stated before) has also been shown by Garner and Yeung.[67] Finally, the use of OTCs has recently been shown by Guo and Colón.[23,24] Different test mixtures of PAHs were separated in OTCs coated with a silica layer and with C_8 groups under silica layer (reversed-phase mode). Efficiencies around 5.0×10^5 theoretical plates per meter were obtained for some test solutes under the following experimental conditions: capillary column, 13 μm i.d. × 50 cm; mobile phase, methanol/1 m*M* phosphate (67:33); separation voltage, 30 kV; electrokinetic injection, 1 s at 30 kV; detection, UV at 220 nm.

EC capabilities with PCCs were first demonstrated in 1991 by Knox and Grant,[51] who reported separations of a test mixture of PAHs. They showed that about 1.0×10^5 plate separations can be achieved in relatively short times (30 min) using drawn-packed capillaries with particles of 3 and 5 μm ODS-silica. Yamamoto et al.[53] showed high-resolution analysis of benzene derivatives with 50 μm i.d. capillaries packed with 3 μm ODS-silica particles, and reduced plate heights of 1.7 to 2.2 were obtained for test compounds (see Figure 9). In addition, using particles of 1.6 μm nonporous ODS-silica, an extremely high efficiency of up to 790 plates per second was obtained. Rebscher and Pyell[58] used PCCs of 100 μm i.d. with both 5 and 3 μm ODS packing materials to separate nitrotoluenes and biphenyls. Smith and Evans[56] used both 1.8 and 3 μm ODS packing materials to pack 50 μm i.d. capillaries of up to 1 m in length to analyze several drug compounds, obtaining efficiencies $>4.0 \times 10^5$ plates with reduced heights <1. In a different study, the same authors[60] used a strong cation exchanger in place of ODS-silica to resolve a series of highly polar compounds (tricyclic antidepressants), which are traditionally very difficult to analyze using conventional HPLC (see Figure 10), with very high efficiencies (N >8 million of plates per meter).

Recently, Yan and Rakestraw[165] separated a mixture of 16 PAHs (EPA priority pollutants) with a PCC of 75 μm i.d. and 3 μm ODS-silica particles (see Figure 11). The compounds were naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benz[*a*]anthracene, chrysene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, dibenz[*a,h*]anthracene, benzo[*ghi*]peylene, and indeno-[1, 2, 3-*cd*]pyrene. Efficiencies up to 4.0×10^5 theoretical plates per meter are obtained when detection is performed within the column packing, and up to 1.5×10^5 theoretical plates per meter when detection is performed following a frit.

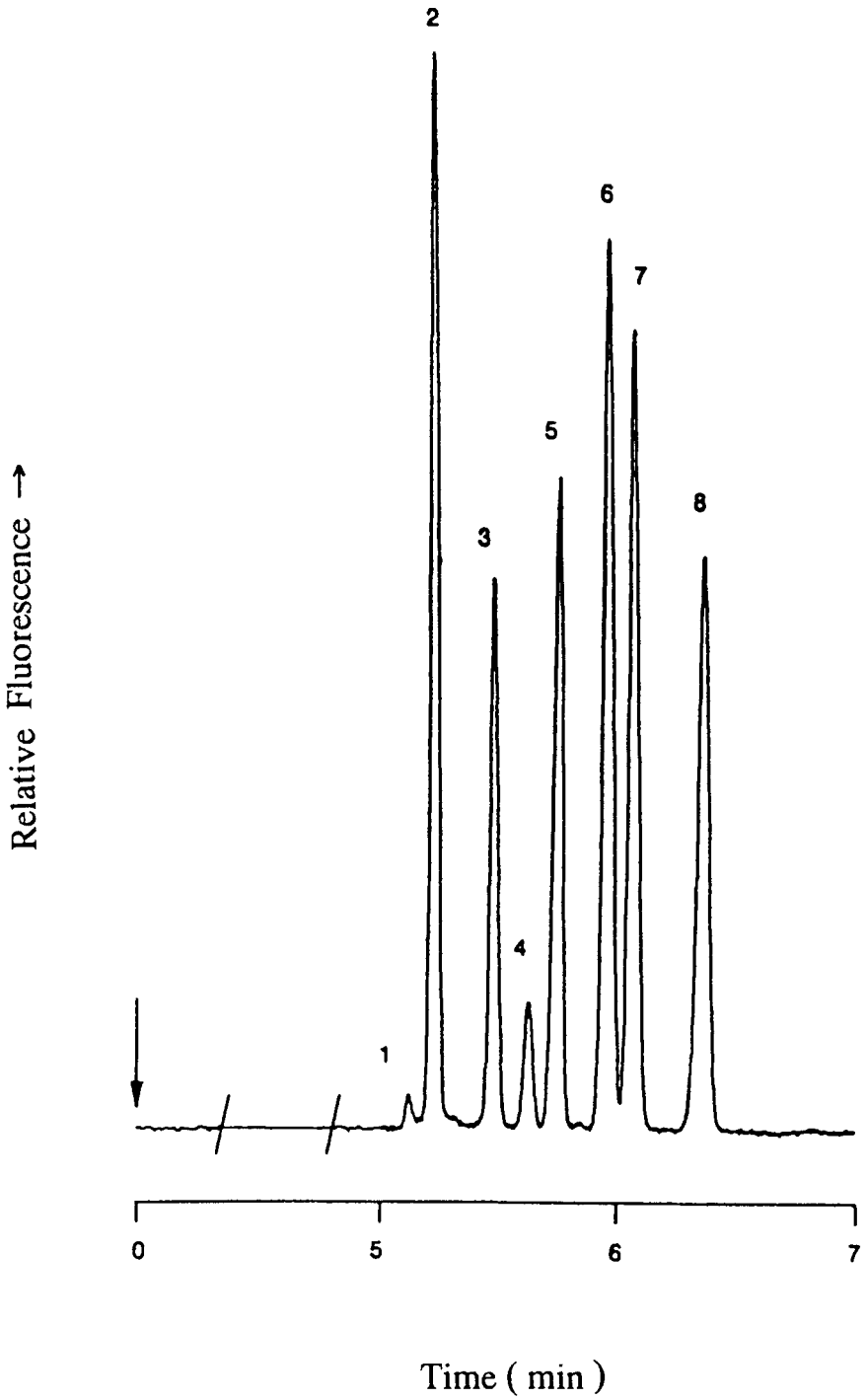

FIGURE 8. Electrochromatogram of eight polycyclic aromatics obtained with a 10 μm i.d. coated PSL–ODS capillary. Conditions: applied voltage, 20 kV; total length of capillary, 49.0 cm with 26.5 cm separation distance; current, 0.7 μA; temperature, 25°C; electrokinetic injection, 9 pl; mobile phase, 50 m*M* phosphate buffer (pH = 7.0)- methanol (1:1); on-column laser-induced fluorescence detection. Peaks: 1 = naphthoquinone (k′ = 0); 2 = 9-anthracenemethanol (k′ = 0.03); 3 = 9-anthracenecarbonitrile (k′ = 0.06); 4 = anthracene (k′ = 0.09); 5 = 7,8-benzoflavone (k′ = 0.12); 6 = fluoranthene (k′ = 0.16); 7 = pyrene (k′ = 0.19); 8 = 9-vinyl-anthracene (k′ = 0.24). (From Bruin, G. J. M., Tock, P. P. H., Kraak, J. C., and Poppe, H., *J. Chromatogr.*, 517, 557, 1990. With permission.)

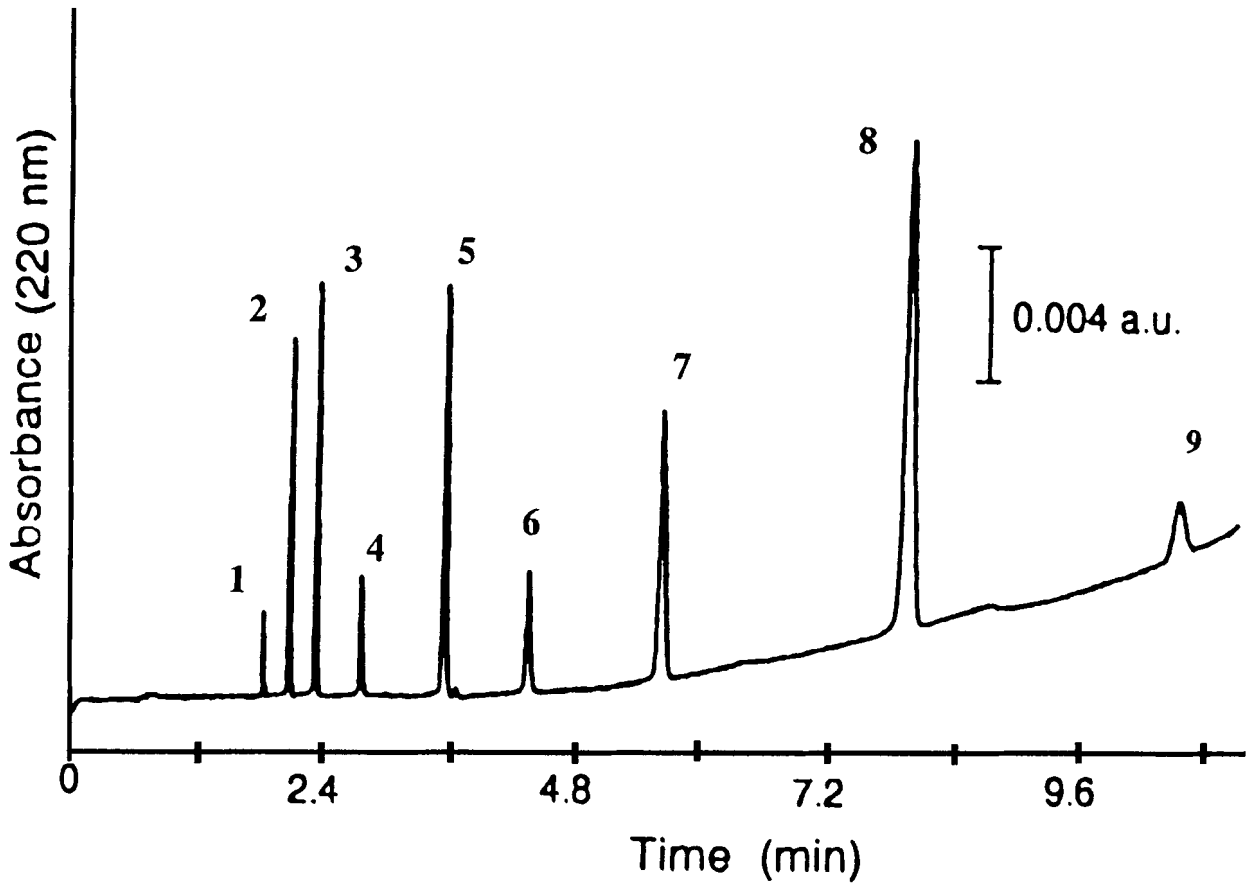

FIGURE 9. High-resolution analysis of benzene derivatives obtained with a PCC of 50 μm i.d. Conditions: applied voltage, 45 kV; length of columns 28.5 cm; packing, 3 μm Hypersil ODS; current, 2.0 μA; electrokinetic injection, 2.5 kV for 5 s; mobile phase, 2 m*M* sodium tetraborate/acetonitrile (20/80); EOF, 2.6 mm/s; on-column UV detection, 220 nm. Peaks: 1 = thiourea (N = 46,000, h = 2.0); 2 = benzyl alcohol (N = 54,000, h = 1.8); 3 = benzaldehyde (N = 56,000, h = 1.7); 4 = benzene (N = 47,000, h = 2.0); 5 = 1,2-dichlorobenzene (N = 54,000, h = 1.8); 6 = 1,2,3-trichlorobenzene (N = 52,000, h = 1.8); 7 = 1,2,3,4-tetrachlorobenzene (N = 49,000, h = 2.0); 8 = pentachlorobenzene (N = 43,000, h = 2.2); and 9 = hexachlorobenzene (N = 43,000, h = 2.2). (From Yamamoto, H., Baumann, J., and Erni, F., *J. Chromatogr.*, 593, 313, 1992. With permission.)

The highest efficiencies were obtained, however, at the expense of lower detection sensitivities caused by excessive light scattering from the packing particles. Reproducibility in peak heights was of an RSD <5%. As for the linearity, the response was found to be linear between 2×10^{-7} and 2×10^{-11} *M*, with a correlation coefficient of 0.9995. Reproducibility in the retention times over a period of 1 week with the same column were of an RSD <2%. The same mixture of PAHs has also been attempted by CZE[166] and MEKC,[167] but with poorer results.

B. Separations of Enantiomers in EC

The separation of enantiomers has long been a challenging field to analytical scientists. In recent years, interest for chiral separations has increased rapidly. Enantiomers of chiral bioactive molecules often differ in potency, toxicity, pharmacological actions, and metabolism.[168] Therefore, the ability to rapidly and accurately separate and determine the enantiomeric composition of chiral compounds is becoming increasingly important in pharmaceutical industry, food sci-

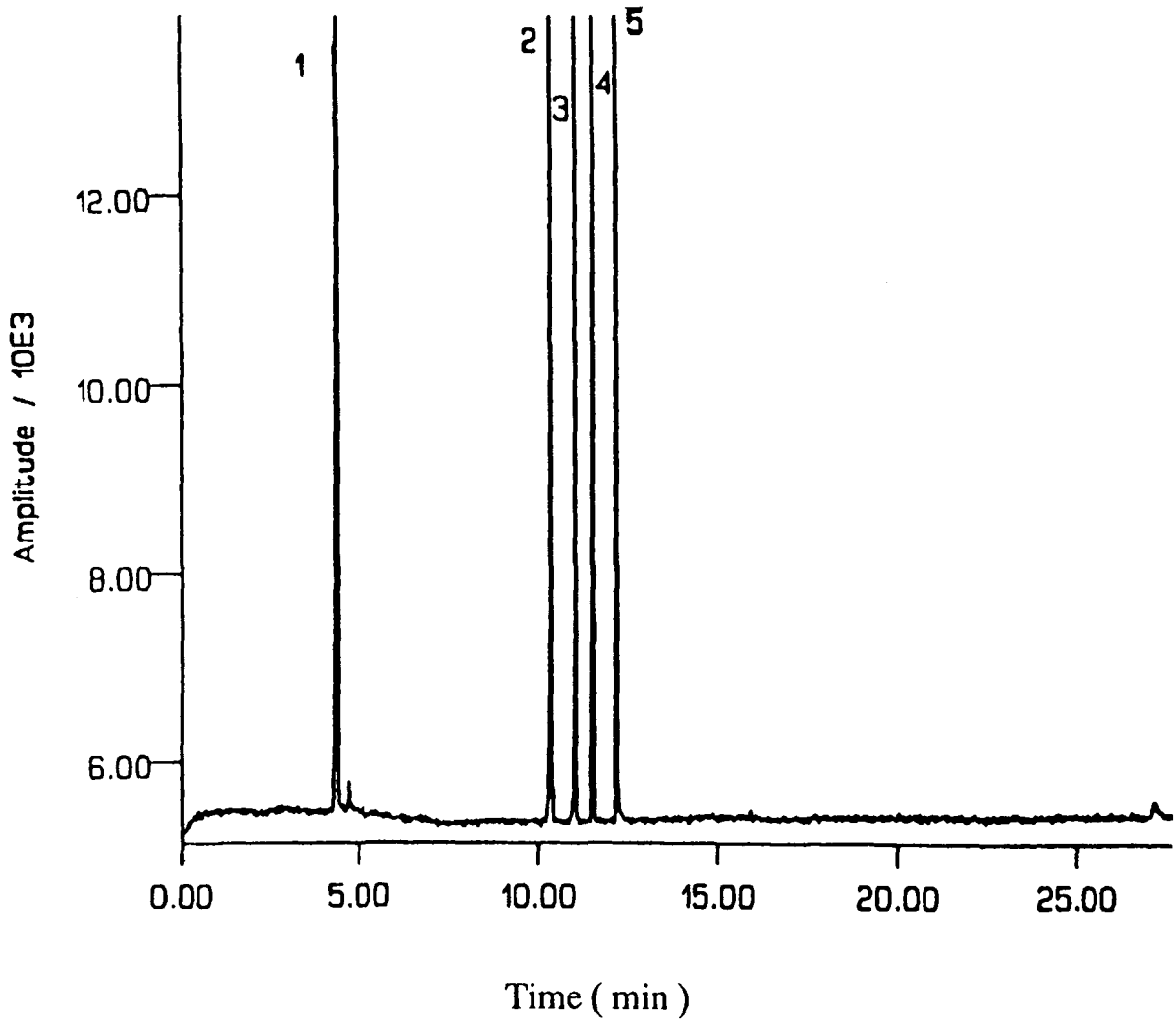

FIGURE 10. High-efficiency analysis of five drugs obtained with a PCC of 50 μm i.d. Conditions: applied voltage, 30 kV; capillary, 50 cm × 50 μm i.d. with 26 cm packed; packing, 3 μm Spherisorb ODS; current, 2.0 μA; temperature, 30°C; electrokinetic injection, 2.0 kV for 30 s; mobile phase, 50 m*M* NaH_2PO_4/acetonitrile (30/70) with apparent pH = 3.5; on-column UV detection, 210 nm. Peaks: 1 = bendroflumethiazide; 2 = nortriptyline; 3 = clomipramine; 4 = imipramine; 5 = methdilazine. (From Smith, N. W. and Evans, M. B., *Chromatographia,* 41, 197, 1995. With permission.)

ence, and agricultural chemistry, along with a tremendous eagerness for developing enantiomeric separation methods.

Chiral separation by HPLC using chiral stationary phases (CSPs) is currently very popular. Today, more than 50 different CSPs are commercially available for direct chiral separations. These CSPs may be broken down into different categories according to the immobilized protein used as the chiral selector.[169]

CZE has also been utilized for enantiomeric separations, but chiral separations are being undertaken by the use of chiral selectors in the running buffer (cyclodextrins),[170–174] chiral surfactants (bile salts),[175–178] and cyclodextrin-surfactant mixtures.[179,180]

The first reports on enantiomeric separations by EC described the use of CD derivative-coated capillaries. Mayer and Schurig[181] have reported several chiral separations in an OTC–EC system, obtained by

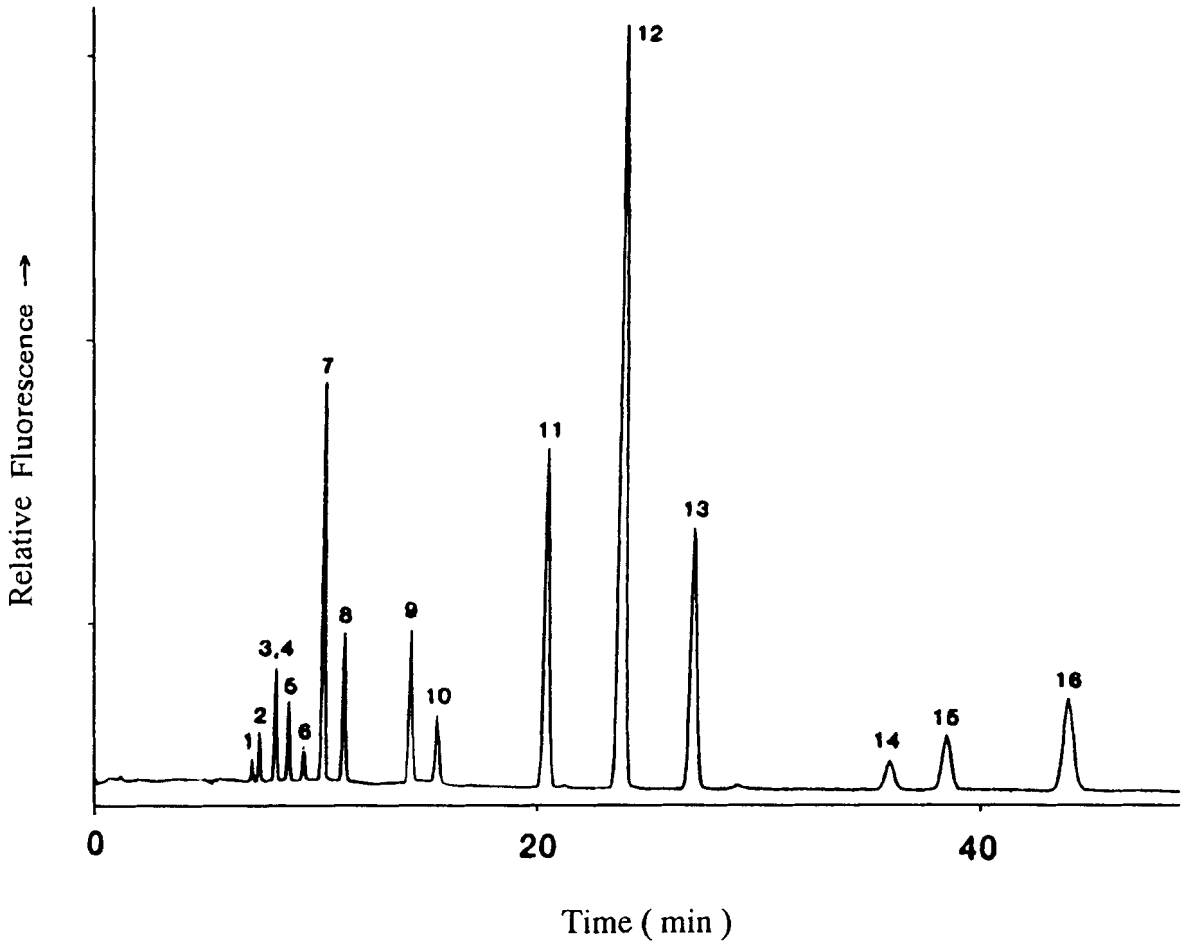

FIGURE 11. Electrochromatogram of the 16 PAH priority pollutants EPA obtained with a PCC of 75 μm i.d. Conditions: applied voltage, 15 kV; capillary, 43 cm × 75 μm i.d. with 33 cm packed; packing, 3 μm ODS-SynChrom (90%) and 1 μm silica-phase separation (10%); electrokinetic injection, 5 kV for 5 s; mobile phase, 4 m*M* sodium borate/acetonitrile (20/80); on-column laser-induced fluorescence detection. Peaks: 1 = naphthalene, 2 = acenaphthylene, 3 = acenaphtene, 4 = fluorene, 5 = phenanthrene, 6 = anthracene, 7 = fluoranthene, 8 = pyrene, 9 = benz[*a*]anthracene, 10 = chrysene, 11 = benzo[*b*]fluoranthene, 12 - benzo[*k*]fluoranthene, 13 = benzo[*a*]pyrene, 14 = dibenz[*a,h*]anthracene, 15 = benzo[*ghi*]perylene, and 16 = indeno-[1,2,3-*cd*]pyrene. (From Yan, C. and Rakestraw, D. J., *Anal. Chem.*, 67, 2026, 1995. With permission. Copyright American Chemical Society.)

coating a 50-μm capillary with Chirasil-Dex, a phase consisting of permethylated cyclodextrins attached to a dimethylsiloxane backbone. In this regard, Armstrong et al.[182] used a capillary coated with a derivatized β-CD and a organohydrosilane copolymer in EC. Racemic mephobarbitol was optically resolved at pH 7.8. The use of capillaries (50 μm i.d.) packed with β-CD CSP has also been described by Li and Lloyd[55] to separate several drugs.

Recently, the use of proteins as chiral selectors in capillary electrophoresis (CE) has been reviewed.[183] The results reported suggest that the wide variety of chiral selectors used in HPLC may be applied to EC using PCs with protein stationary phases. However, not all of them may be suitable as additives in CZE due to solubility, cost, or excessive detector response. The preliminary results in EC with a 50-μm i.d. capillary packed with α_1-acid glycoprotein (AGP) have been presented.[54] A number of chiral drugs could be fully or partially resolved by EC, including benzoin and some benzodiazepines. Separation efficiencies were generally somewhat higher than those observed in HPLC separations using conventional AGP PCs. New results have also been presented on the use of human serum albumin CSP immobilized (HSA CSP) in PCCs,[183] CSP initially developed for HPLC.[184,185]

EC can produce high-efficiency chiral separations;[55] however, with protein phases the efficiency seen so far is rather discouraging.[54,136] Optimum performance will only be accomplished with smaller packings, but it is difficult for EC with protein phases resembling the efficiency of other chiral selectors used in CE in solution. Nevertheless, the wide applicability of protein chiral selectors and the difficulty of their use as additives in CE make their utilization in EC an area worthy of further investigation.

Finally, Smith and Evans[56] proved the quality of EC using a PCCs (50 μm i.d.) with 3 μm ODS particles to separate the diastereoisomers of the E- and Z-isomer of the Cefuroxime axetil (cephalosporin antibiotic) (see Figure 12).

C. EC/MS Coupling

Mass spectrometry (MS) presents some advantages over the use of conventional detectors due to the unrivaled specificity of mass analysis. Coupling of electroseparation techniques to MS is readily achieved owing to the compatibility of flow rates in 25- to 100-μm i.d. capillaries with MS. However, separation of neutral analytes using MEKC cannot be coupled to MS because it relies on MS-incompatible quantities of compounds, typically anionic surfactants for micelle formation. EC coupled with MS thus is an alternative to MEKC for the separation of neutral analytes.

The first coupling between EC and MS was reported in 1994 by Gordon et al.[186] A mixture of steroids (aldosterone, hydrocortisone, and testosterone) was separated with a PCC of 50 μm i.d. and 3 μm ODS-silica in an online experiment to demonstrate the feasibility of coupling to MS. In this work, transfer capillaries were required to connect the separation columns with the MS resulting in loss of efficiency. This problem was solved by Schmeer et al.[187] using an electrospray system (ESI) in the coupling of the PCC to MS. Peptides were analyzed in a PCC with 1.5 μm ODS-silica particles. This EC–MS coupling enables routine detection of picomole quantities. Lord et al.[48] have reported the use of EC/ESI/MS for the analysis of some nonionic, water-insoluble, disperse textile dyes, particularly as an alternative to MEKC. Finally, Lane et al.[188] demonstrated the principles and potential of EC/ESI/MS and its application for the separation and characterization of some typical pharmaceutical compound mixtures.

In summary, EC/ESI/MS can be considered a very promising method for the analysis of neutral compounds. The small amount of sample and EC's high separation power in

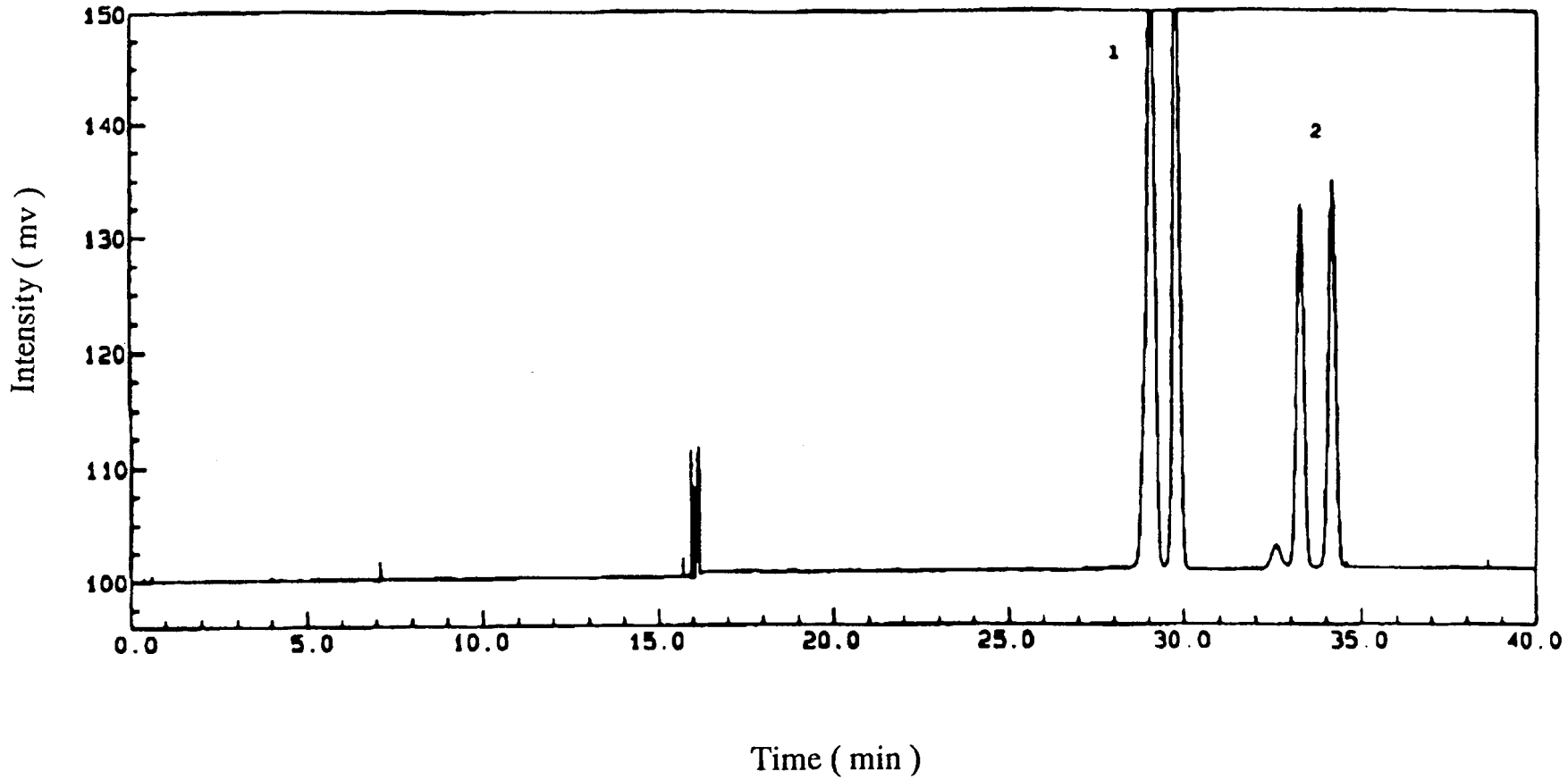

FIGURE 12. Electrochromatogram of the diastereoisomers of the Cefuroxime axetil (cephalosporin antibiotic) obtained with a PCC of 50 µm i.d. Conditions: applied voltage, 30 kV; capillary, ≈ 40 cm × 50 µm i.d.; packing, 3 µm ODS-1 phase separation; temperature, 30°C; electrokinetic injection, 20 kV for 12 s; mobile phase, 10 m*M* NaH_2PO_4/acetonitrile (50/50) with apparent pH = 9.5; on-column UV detection, 276 nm. Peaks: 1 = Z-isomers; 2 = E-isomers. (From Smith, N. W. and Evans, M. B., *Chromatographia,* 38, 649, 1994. With permission.)

conjunction with the information provided by MS represent an excellent combination, especially for microanalysis, and may offer wider applications in the biological, environmental, and forensic areas.

V. CONCLUSIONS

The EDF can be considered flat over all the tube bore or channel diameter when the inner diameter of the OTC is ≥0.5 μm or the particle size for PCC is ≥1.5 μm. In addition, under referenced conditions, its mean flow velocity will be irrespective of the tube bore or particle size.

The use of EDF provides three important advantages over pumped systems with PDF. First, the flow profile of EDF is much flatter than the parabolic flow profile of PDF, the efficiency of an OTC increases by a factor larger than 2, thus enabling the use of OTCs whose internal diameter is wider (10 to 20 μm) and whose instrumentation features are less stringent. Second, no pressure drop is generated in a PCC with EDF and hence, small particle size (1.5 μm) can be used with columns of great length (>50 cm). In addition, the EDF is irrespective of channel diameter over a wide range of particle sizes, and should therefore be nearly the same in all channels across a packed bed leading to a lower A-term. This way, at least one source of band broadening in PCCs is reduced. Third, sample injection is easier with EDF than with PDF systems. In techniques with OTCs and PDF, the sample introduction is usually carried out by split injection, requiring a relatively large sample volume. When the sample volume is extremely small, the split technique cannot be applied and another means of introducing the sample is necessary, pressure-pulse-driven stopped-flow injection, a very sophisticated and expensive system which complicates OTC–LC techniques. In techniques involving OTCs and EDF, the sample introduction is performed by means of electromigration or by the hydrodynamic method (vacuum or pressure). Both techniques are very simple and lend themselves to automation.

From predicted low efficiency values, it could be concluded that a chromatographic separation in OTC with a 50-μm i.d. is not a very attractive idea. Unfortunately, although in some ways of OTC–EC is experimentally simpler than OTC–LC, columns with an i.d. of <50 μm require the use of less commonly available detection methods like laser-induced fluorescence. However (note as future trends), considering resolution the ultimate goal of separation and not efficiency, acceptable results could be obtained with OTC of 50 μm i.d. The obvious advantage of these columns is that they do not require very specific and sophisticated detection systems and can easily be used with UV detectors adapted for commercial CE apparatus. In this case, separations will strongly depend on the intrinsic selectivity of the column, which could limit its application to specific areas such as chiral separations (i.e., immobilized cyclodextrin phases coating 50 μm i.d. capillaries). On the other hand, these columns could show great versatility, because the same column may be used not only in EC, but also in GC or supercritical fluid chromatography.

Finally, considering the growing interest for CE techniques, renewed research efforts in microdetection will certainly lead availability of commercial ultramicrodetectors with better sensitivity features in the near future.

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