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Review

Homogeneous gels for capillary electrochromatography

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This article is dedicated to the memory of Professor Csaba Horváth, whose excellent lectures and brilliant scientific explanations about electrochromatography have always been very stimulating and exploring.

Abstract

Homogeneous gels represent a new type of (electro)chromatographic media possessing unique separation properties unmatched with any other chromatographic beds. It is important to emphasize that they principally differ from continuous beds, polymer rods (better known as monoliths), which are particulate separation media with pores permitting hydrodynamic flow through the columns. Monoliths, thus, are more similar to beds conventionally packed with beads, although the particles building up monolithic columns are usually smaller in size (few submicometers) and covalently linked together. Consequently, homogeneous gels deserve better the term "monoliths" having a non-particulate structure formed by crosslinked free polymer chains (according to a dictionary a monolith is a non-modularized column). The goals of this minireview are to clarify the position of homogeneous gels among the separation media (including polymer solutions), to explain and to exemplify their outstanding (electro)chromatographic properties. This review gives hopefully a complete list of references to homogeneous gels developed for capillary electrochromatography.

Keywords: Homogeneous gels; Monoliths; Capillary electrochromatography; Microdevice

Contents

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1. History

Electrophoresis, the migration of electrically charged species in an electrical field [1–3] is used for the separation of various molecules ranging from bioparticles and biopolymers to low-molecular-weight compounds and ions. It can be conducted in different modes and in carrier-free or anticonvective media, such as polymer solutions or gels, which – if their pores are small enough – also have a size-sieving function.

The early history of electrophoresis is a search for sufficiently good anticonvective media. The analytical device of Tiselius [4], in which the sample ions are subjected to electrophoresis in free buffer, i.e., in the absence of an anticonvection medium, was supplemented in the late forties by the introduction of paper electrophoresis [5], a method which, in contrast to the moving boundary method, affords complete separation of proteins into discrete zones. The gel-based molecular-sieving anticonvection media soon followed the paper strips. Smithies was first to introduce such a medium, a starch gel [6], which, unfortunately, had a low but not negligible content of charged groups resulting in some adsorption of proteins and an electroendosmotic flow. The starch gel was soon replaced by polyacrylamide gels, introduced independently by Raymond and Weintraub [7], Davis and Ornstein [8] and Hjertén [9]. Hjertén [10] has demonstrated the unique properties of polyacrylamide gels and emphasized the importance of varying the pore size to attain optimum resolution. With polyacrylamide as a nearly ideal anticonvection medium the resolving power of electrophoresis was increased considerably, because diffusion was reduced and the sample components migrated as sharp zones. In 1961, Hjertén showed that gels of the neutral agarose are superior to the charged agar gels (which contain sulfate groups) for electrophoresis and immuno-electrophoresis [11,12]. Polyacrylamide and agarose gels are usually used for electrophoresis in a conventional slab or rod format but also in capillary electrophoresis.

The first papers on capillary gel electrophoresis were published in the 1980s by Hjertén [13] and Karger and co-workers [14,15]. They addressed the application area of protein separations in polyacrylamide gels in the presence of sodium dodecyl sulfate (SDS). Since then, it has become evident that almost every method developed for slab gel electrophoresis can easily be transferred to a capillary format with the advantages of fast analysis with high resolution and full automation. However, attempts to perform separations in polyacrylamide gel-filled capillaries have enjoyed only limited success because these gels are not stable during electrophoresis. Gel instability, i.e., bubble formation and clogging of the pores in the gels by precipitated proteins, limits the number of runs [16,17]. Although some research groups have described methods for the preparation of stable, bubble-free polyacrylamide gels for capillary gel electrophoresis [18–22], these have for unknown reasons not become widely used. Other types of cross-linked gels (mostly modified acrylamide matrices, such as poly(*N*-substituted-acrylamides) [23]) have also been introduced [24–26]. Bode showed that polymers entrapped in agarose gels had molecular-sieving properties [27], which certainly prompted the use of molecular-sieving polymer solutions in CE [21,28]. It should be emphasized that gels yield a higher resolution than do polymer solutions [29,30], which, however, have the advantage of being replaceable, i.e., they permit repeated automated analyses, as do methylated agarose gels.

In many respects, capillary electrochromatography (CEC) is a hybrid separation technique with advantages from both high-performance capillary chromatography and capillary electrophoresis [31,32,14]. The earliest use of electroendosmosis in a liquid chromatography experiment was reported by Strain [33] who separated dyes in an alumina column. Electrochromatography in its present form, i.e., for transport of the mobile phase was introduced by Synge and co-workers [34,35]. However, two decades was to pass until the first successful application of electrochromatography in columns conventionally packed with beads was published [36,37]. The current tremendous interest in CEC is probably due to a series of papers by Knox et al. [38–41]. Its popularity has only increased by the introduction and common use of monolithic columns able to generate high electroosmotic flow (EOF) [42]. However, the great advantages of monoliths, compared to conventional packed beds, are that they can easily be prepared in narrow-bore tubes and can be covalently attached to the tube wall, i.e., no disturbing frit is required to support the bed.

Few review articles about capillary electrochromatography and its technology deal with homogeneous gel beds [43–45]. Although the review [43] dedicates a separate section to “Soft gels” it does not differentiate between homogeneous gel beds, particulate monoliths and open tubular CEC. Two other reviews treat some of the papers dealing with homogeneous gels in the sections “Fritless columns” [44] and “Polyacrylamide-based technologies” [45] but do not distinguish clearly between monoliths and gels probably because the conventional in situ polymerization method is employed for both types of beds. Therefore, I will also clarify the differences between homogeneous gels and monoliths.

2. Theoretical considerations

2.1. Capillary electrochromatography

In capillary electrochromatography an electroendosmotically-driven flow is used instead of a pressure-driven flow to propel the mobile phase through the column. The separation mechanism in CEC is primarily based on differential interaction (e.g., partition between two phases). If the solutes are charged their migration velocities also are influenced by the electrical field. CEC offers the same stationary phases with different chromatographic properties and broad range of retention mechanisms and selectivities typical of chromatography without requiring an expensive HPLC pump. As

in free zone capillary electrophoresis, small inner diameter (10–100 μm) columns can be employed to minimize thermal gradients originating from Joule heating, thereby reducing zone broadening significantly.

CEC provides several important advantages over μHPLC. The electroendosmotic flow is generated uniformly along the capillary, so there is no pressure drop in the column. However, the flow velocity profile is not plug-like [46], as is often stated (a perfect plug flow occurs only in open capillaries). The electroendosmotic flow velocity is virtually independent of the capillary diameter or, for packed capillaries, the diameter of the beads. In contrast, the pressure-driven flow velocity in a packed bed is proportioned to the square of the particle diameter and inversely to the column length.

In open tubular CEC the stationary phase is attached in a thin layer to the inner wall of the capillary. The method has a low capacity, but gives a high resolution when the inner diameter is small, which, however, gives low sensitivity in on-tube UV-detection due to the very short light path [47–50]. Alternatively, the capillary column can be packed with a particulate stationary phase (packed-CEC), which generally consists of inorganic particles (e.g., silica beads) [38,40]. Packed beds have a high loading capacity, but the classical chromatographic zone broadening effects are relatively large. Additionally, (i) none of the standard methods to pack columns with small beads give sufficiently uniform beds in narrow bore tubes, (ii) frits are needed to support the bed and (iii) pressurized electrode chambers are required to avoid bubble formation. Many of these drawbacks are eliminated when the stationary phase is made up of a polymeric network (continuous beds [46,51,52], also called continuous polymer rods, monoliths [53,54] and continuous column supports [55,56] or gels [57–59]). One of several reasons for the good chromatographic properties of continuous beds is that the particles are very tiny (0.2–0.5 μm) which means a small (but not negligible) eddy diffusion (*A*) in the van Deemter equation:

$$H = A + \frac{B}{v} + Cv \quad (1)$$

where B/v is the longitudinal diffusion and Cv is the resistance to mass transfer [60]. Eddy diffusion often gives a relatively large contribution to the total plate height, but is smaller the smaller the beads, i.e., from this point of view the continuous beds are preferable to conventional packed beds which usually consist of beads with diameters in the range 3–5 μm. Moreover, the homogeneous gels are preferable to the continuous beds for electrochromatography because they are non-particulate supports.

2.2. Distinction between particulate and homogeneous media

It is difficult to make a stringent definition of homogeneous media. For practical purposes a less rigorous definition is acceptable: a homogeneous separation medium is one which is visually transparent, i.e., there are no elements large enough to be visible in a light microscope or/and cause sufficient light scattering to make the medium opalescent (on the molecular level all media are heterogeneous). According to this definition, solutions of non-charged or charged polymers, such as polyacrylamide, dextran, polyvinylpyrrolidone, methylcellulose, polyvinylalcohol, and non-charged or charged gels, for instance, those obtained by crosslinking of these and other polymers, are homogeneous.

According to theoretical considerations [61,62], not only polymer solutions [63–65] but also gels [57–59] such as, cross-linked agarose and polyacrylamide behave as ideal chromatographic media since they are not made up of particles, thus being physically and macroscopically homogeneous. Therefore, the eddy diffusion should be zero in gels. The effective pore size in homogeneous gels is much smaller than the average diameter of the channels between the (non)porous beads in packed beds and, thereby, the residence time of the analytes in the mobile phase is shorter, i.e., the resolution is higher. Observe also that the mobile phase cannot be transported through a homogeneous gel by a hydrodynamic flow (because of the high back pressure) but only by an electroosmotically generated flow in the gel.

The *principal* difference between homogeneous gels and continuous beds (continuous polymer rods, silica rods, monoliths) is larger than that between packed beds and the continuous beds. It should be emphasized that homogeneous gels and continuous beds (continuous polymer rods, silica rods, monoliths) represent two structurally completely different separation media. Gels have the disadvantage, compared to polymer solutions, not to be replaceable, highly methylated agarose gels being an exception [66].

2.3. Polymer solutions

The extensive use of polymer solutions necessitates some characterization of these media. Although polymer solutions cannot be described as gels they belong to the category of homogeneous media, thus having very similar properties as the gels [27]. It should be emphasized that the often-used terminology, “chemical gels” or “solid gels” (high viscosity crosslinked matrix) and “physical gels” or “liquid gels” (low viscosity non-crosslinked sieving medium), corresponding to “real” gels and polymer solutions, respectively, is confusing.

Polymer solutions formed from non-crosslinked linear or slightly branched polymers have a flexible, dynamic pore structure. They can easily be replaced in the separation channel provided that the viscosity is not excessively high. The pore size of this type of medium can be varied, even during the separation, by simply changing the column temperature if the polymer is thermosensitive, for instance those synthesized from isopropylacrylamide. Polymer solutions are popular in separations of both DNA and proteins. It has been shown that concentrated polyacrylamide solutions provide excellent single-base resolution of single-stranded DNA fragments more quickly than does slab gel electrophoresis [67,68]. Since then several types of polymers have been employed

as DNA separation media for capillary electrophoresis, including polyacrylamide [69], methylcellulose [70], hydroxyethylcellulose [71], hydroxypropylcellulose [72], polyethylene glycol [73], polyethylene oxide [74], liquefied agarose [75], and polyvinyl alcohol [73]. Non-crosslinked linear polyacrylamide was the first polymer employed in CE separations of SDS–protein complexes [76–78]. Later, other non-crosslinked polymers, such as slightly branched dextran [79], linear polyethylene oxide [80] and pullulan [81] were used for the same purpose.

2.4. Continuous beds (monoliths)

The first successful method to prepare this type of beds was published as late as 1989 [42]. The continuous beds were later called continuous polymer rods [53], silica rods [56] or monoliths [54]. The beds, as prepared by Hjertén's group, possess the property that the resolution increases upon an *increase* in flow rate when proteins were eluted by a gradient. All these beds are built up of small *particles*, as are conventional packed beds, but with the difference that the particles are covalently linked. In the capillary format they are attached to the column tube wall to avoid supporting frits, which cause zone broadening and in electrochromatography also bubble formation.

2.5. Why is a homogeneous medium an ideal electrochromatographic stationary phase?

Eddy diffusion [corresponding to the first term in Eq. (1)] often gives a relatively large contribution to the total plate height in chromatography, but it is smaller the smaller the beads. Therefore, the continuous beds are preferable to conventional packed beds. Homogeneous gel beds, for instance, those of agarose and polyacrylamide, have the advantage that the eddy diffusion is zero.

The total experimental standard deviation in seconds (σ^2_{exp}) measured at $0.68c_0/2$ (c_0 is the peak concentration of the analyte) as compared to that calculated for free diffusion, using the Einstein equation $\sigma^2_{diff} = 2Dt$ [82]. The calculated diffusional broadening (σ_{diff}) was found to be higher than the measured experimental total broadening of the boundaries (σ_{exp}), which indicates that the originally plane boundary between the acetone phase and the gel phase at the start of the run became distorted during the run only by longitudinal diffusion, but not by eddy diffusion (Fig. 1). The longitudinal diffusional broadening (the second term in the van Deemter equation) is smaller than that in free solution, i.e., is restricted by the presence of the gel matrix.

In addition, the distance between the walls of a gel pore is much smaller than the average distance between the beads in a packed bed or in a continuous bed. Therefore, the time required for an analyte molecule to diffuse from one interaction site to another is much shorter in a gel than in the other two beds. In other words, the resistance to mass transfer is smaller or differently expressed: the third term in the van Deemter equation is smaller. It was found experimentally that the resistance to mass transfer dominates the performance of homogeneous gels (Fig. 2).

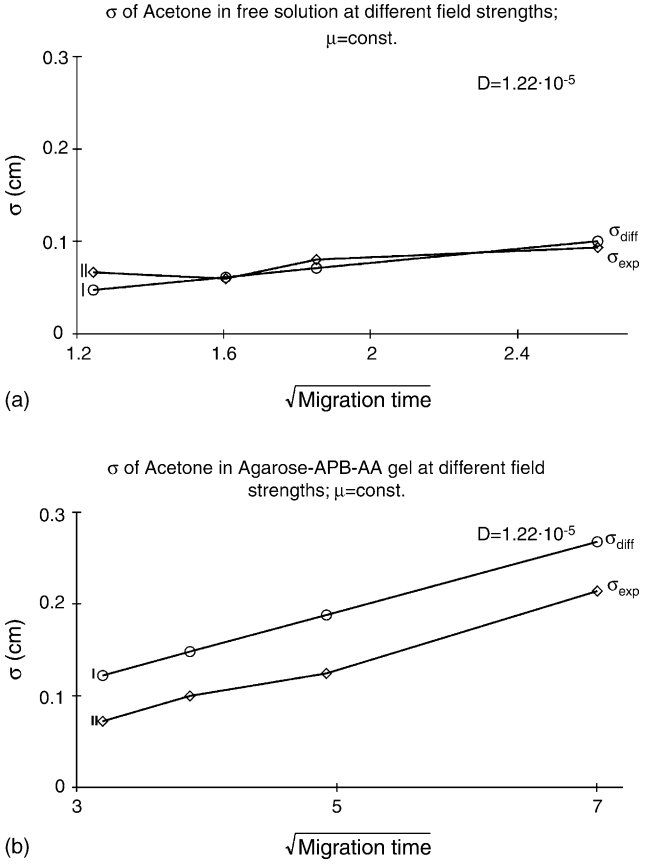

Fig. 1. Plots of the calculated diffusional broadening (σ_{diff}) and the measures experimental total broadening of the boundaries (σ_{exp}) in frontal analysis of acetone vs. the square root of the migration time. Medium: (a) acetone, free solution; (b) agarose, 3-(acrylamido)phenylboronic acid (APB)-acrylic acid (AA) gel. Buffer: TAPS, pH 8.2: (a) 25 mM; (b) 5 mM. Voltage: 4, 3, 2 and 1 kV. Observe that $\sigma_{diff} = \sigma_{exp}$ in (a), and ($\sigma_{diff} > \sigma_{exp}$ in (b). Reprinted from ref. [82], with permission.

Interestingly, the total plate height of an enantiomer of a neutral drug (tropicamid) was found to be independent of the electroendosmotic flow velocity [83]. A prerequisite for this desirable behavior is that the on/off kinetics is fast and that the distance an analyte must diffuse from one interaction site to another is short. In homogeneous gels this distance is equal to the average diameter of the gel pore. In many cases the on/off kinetics is very slow due to high ligand density or strong interactions. The performance can be improved by decreasing the number of ligands or the strength of the interaction. Addition of organic solvents can decrease such interactions (Fig. 3). The influence of organic solvent (acetonitrile) on the electroendosmotic mobility is similar to that in chromatographic reversed-phase separations [58], although the separation mechanisms may be different [84]. To summarize, the homogeneous gels approach the ideal chromatographic medium both in theory and in practice and deserve further study.

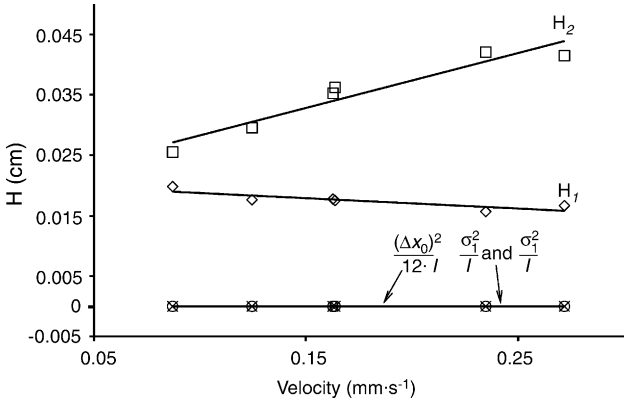

Fig. 2. The van Deemter plot. H_1 and H_2, the measured plate heights for the fast and slow enantiomers of tropicamid, respectively. $(\Delta x_0)^2/12l$, and σ_2^2/l are the expressions used for the calculation of the plate heights for the widths of the starting zone and longitudinal diffusion of the fast and slow enantiomers, respectively. Eq. (1), where $A = 0$, shows that the term Cv dominates. Reprinted from ref. [83], with permission.

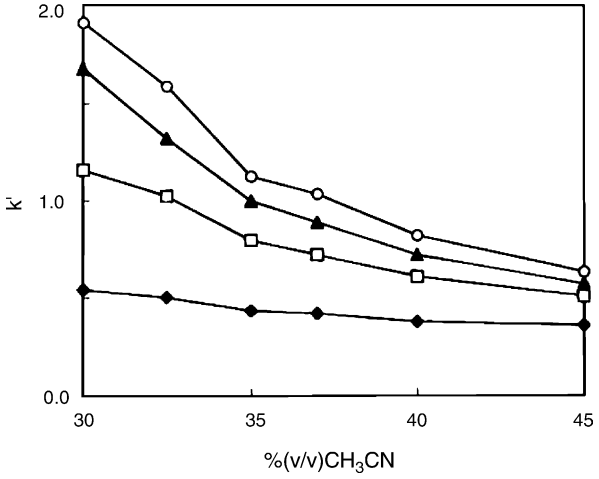

Fig. 3. Capacity factor (k') of PAHs on a poly(AMPS-co-IPAAm) hydrogel column as a function of the % (v/v) of acetonitrile: (◯) naphthalene, (▲) fluoranthene, (□) benz[*a*]anthracene, and (◆) benzo-[*a*]pyrene. Conditions: varying amounts of acetonitrile in 100 mM Tris-150 mM boric acid (pH 8.1) buffer; capillary, 46.9 cm × 50 μm i.d. (21.9 cm effective length); %*T*, 6.9; %*C*, 5.8; %*S*, 5.5; applied voltage, 15.0 kV. Reprinted from ref. [59], with permission.

3. Applications

3.1. Separation of low-molecular-weight compounds

Fujimoto was the first who showed that it is possible to obtain reasonably fast EOF ($\sim 4.5 \times 10^{-5}$ cm^2 V^{-1} s^{-1} = 4.5 T [85]) in polyacrylamide gels [57–59,86], which is somewhat surprising since neutral polyacrylamide gels have small pores. In these gels acrylamide was co-polymerized with a charged monomer, 2-acrylamido-2-methyl-1-propanesulfonic acid (AMPS), and crosslinked with *N*,*N*′-methylenebisacrylamide (Bis) utilizing the free radical mechanism with the catalyst system ammonium persulfate and *N*,*N*,*N*′,*N*′-tetramethylethylenediamine. The well-known

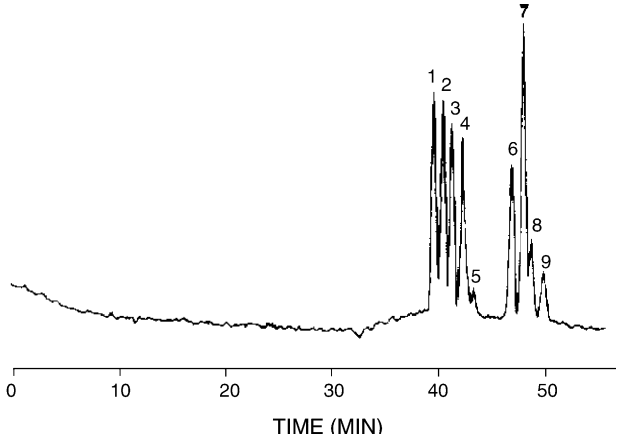

Fig. 4. Electrochromatogram of ketones on a charged polyacrylamide gel column: mobile phase, 100 mM Tris-150 mM boric acid (pH 8.1); capillary, 65.0 cm × 0.075 mm i.d. (50.0 cm effective length); %*T*, 13.7; %*C*, 5.1; %*S* 10.0; applied voltage, 20.0 kV; detection wavelength, 254 nm. Sample: (1) dimethyl ketone, (2) methyl ethyl ketone, (3) methyl *n*-propyl ketone, (4) methyl *n*-butyl ketone, (5) methyl *n*-pentyl ketone, (6) methyl phenyl ketone, (7) ethyl phenyl ketone, (8) *n*-propyl phenyl ketone, and (9) *n*-butyl phenyl ketone. Reprinted from ref. [57], with permission.

nomenclature for the composition of polyacrylamide gels (%*T*, total monomeric concentration and %*C*, crosslinker concentration [10]) was extended by formulating a third equation for the charged monomer concentration (%*S*). Mixtures of neutral organic compounds, like ketone homologues, acetone, acetophenone, thiourea, benzyl alcohol, mesityl oxide and benzaldehyde, were separated (Fig. 4) and the separation mechanism was studied. The migration velocity of acetone, used as a neutral EOF marker, increased almost linearly by increasing the concentration of the charged monomer when the *T* (=6%) and *C* (=9.2%) were kept constant. Assuming that the replacement of acrylamide by AMPS did not change the effective pore size, it was estimated to be 18–48 Å and, therefore, could be adequately small to restrict the migration of low-molecular-weight compounds through the gel. However, it should be recalled that low-molecular mass compounds, but not proteins and nucleic acids, will interact with most gels by so-called “aromatic adsorption” [87]. Surprisingly, these negatively charged homogeneous gels were stable and their separation performance was constant over a long period of time. This important and attractive feature of a separation matrix is in contrast to the behavior of neutral and negatively charged non-crosslinked (linear) polyacrylamide polymers [58]. Usually, good separation efficiency was achieved, which outperformed the conventionally packed and monolithic (continuous beds) electrochromatographic beds. One reason is the flat (or almost flat) profile of the endosmotic-driven flow (see Fig. 5), which was demonstrated with a rhodamine B sample plug in a polyisopropylacrylamide gel [59]. The performance of these hydrophobic gels was exemplified by highly efficient separation of steroids and polyaromatic hydrocarbons (PAHs). The reversed-phase mechanism proposed is supported by the fact that the

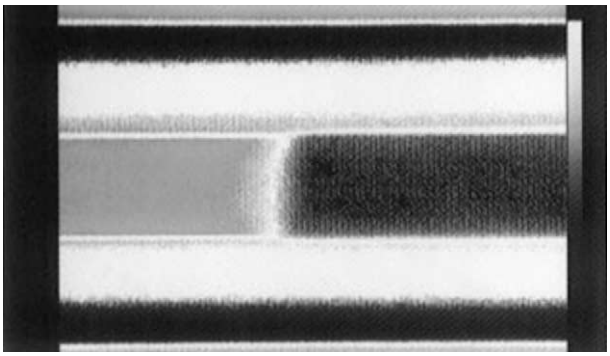

Fig. 5. Digitized images of zone front of EOF. A single image recorded on videotape was digitized using an image processing technique. Sample solution, 1.5 mM rhodamine B in methanol; applied field strength, 285 V cm^{-1}; current, 7 μA. Reprinted from ref. [59], with permission.

addition of organic solvents (such as acetonitrile) to the background electrolyte decreased the separation factor (*k*) of these compounds significantly (see Fig. 3). However, the influence of "aromatic adsorption" should not be neglected. In a later paper, Fujimoto has suggested positively charged gels for electrochromatography, produced by replacing AMPS with tertiary amines or quaternary ammonium bases [86].

Agarose gels as electrochromatographic stationary phases, possessing charged moieties and ligand groups providing specific interactions with the sample components, has also been introduced [82]. It is not a trivial problem to derivatize agarose because no gel will form if the classical methods to attach ligands to an agarose *solution* are employed (the formation of hydrogen bonds between the polymer chains is a prerequisite for gel formation, but for sterical reasons derivatized polymer chains cannot come so close to each other that hydrogen bonds form). Therefore, a preformed polymer with the desired ligands was entrapped in an agarose gel: (i) carboxylic groups to generate electroendosmosis (to propel the mobile phase through the homogeneous gel); (ii) boronate groups to create electroendosmosis and form bonds with compounds containing two OH-groups in *cis*-orientation. Ribonucleosides were separated (they are neutral at pH 7.8) with high efficiency (plate numbers were between 100 000 and 350 000 m^{-1}).

Yet another gel, polyvinyl alcohol (PVA) crosslinked with borate ions is useful for capillary electrochromatography [82]. The bed was prepared in a piece of coated capillary with highly suppressed EOF filled with a 4% PVA solution and sodium borate was used as buffer in the electrode vessels [88]. Upon electrophoresis, borate ions migrated through the capillary and reacted with the polymer chains of PVA, forming a gel in about an hour. Borate ions served as crosslinkers and as charged groups generating EOF. These gels were then employed for the separation of neutral low-molecular-weight compounds (alkyl substituted *p*-benzoic acids). High plate numbers (160 000–250 000 m^{-1}) and symmetrical peaks were obtained. Disadvantageously, the crosslinking step was time-consuming and the lifetime of the gel was limited.

However, stable polyacrylamide gels with β-cyclodextrin ligands could be synthesized from acrylamide, Bis, AMPS and 2-hydroxy-3-allyloxypropyl-β-cyclodexrin (allylated β-CD) [83]. The most attractive feature of these gels is the extended lifetime: they could be used repeatedly for several weeks without any decrease in performance. Additionally, the separation efficiency does not decrease by increasing the field strength (see Fig. 4 in ref. [83]), a finding contrary to gels described by Fujimoto [58]. The electroendosmotic mobility has been measured at different concentrations of the crosslinker (allylated β-CD) in a negatively charged polyacrylamide gel [83]. The continuous decrease in the EOF mobility is probably due to the changes in the pore size of the gel: the smaller the pores the slower the EOF.

Recently, new agarose-based homogeneous gels were introduced suitable to reversed-phased capillary electrochromatographic separations [89]. Hydrophobic functional ligands and charged groups were attached to agarose in solution by the chemical reactions with either 3-chloro-2-hyroxypropyldimethyldodecylammonium chloride or 3-chloro-2-hydroxypropyldimethylstearylammonium chloride. Although, these gels are thermally replaceable, they could be used repeatedly without loss of resolution. Additionally, they are UV transparent (providing possibility in column/in-gel detection), require no covalent attachment to the capillary inner wall (or microchip channel), and are suitable for isocratic or gradient operation in the aqueous–organic mobile phases at high field strengths (up to 500 V cm^{-1}).

3.2. Enantiomer separations

The first studies on electrochromatographic enantiomer separations in charged homogeneous gels were certainly inspired by the works dealing with neutral α-, β-, and γ-cyclodextrins (CDs) either physically entrapped in [90] or covalently bound to (allyl carbamoylated β-CD [91]) a neutral polyacrylamide matrix. Therefore, only charged enantiomers could be separated. These approaches are very interesting from the point of view that they represent extreme cases of capillary electrophoresis or capillary electrochromatography, being at the border between them. If we define electrochromatography as a chromatographic separation method in the presence of EOF then these approaches should rather be called capillary electrophoresis or capillary gel electrophoresis utilizing a (pseudo)stationary phase for chiral recognition. Unfortunately, bubble formation was commonly observed in these gels and consequently, short lifetime was reported [91].

Koide and Ueno have tested the same approach utilizing Fujimoto's charged polyacrylamide gels with physically entrapped β-CDs (2,6-di-*O*-methyl-β-CD and β-CD polymers [92,93]). Baseline separations of racemic terbutaline and benzoin were accomplished when β-CD polymers (poly β-CD with M_w = 3000–50 000 and a carboxymethyl β-CD

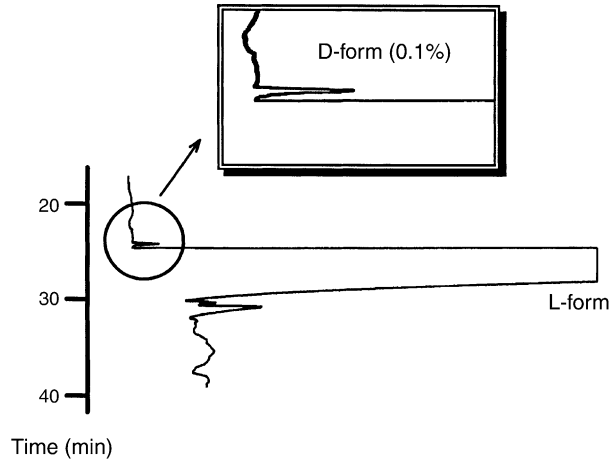

Fig. 6. Optical purity test of L-alanine-2-naphthylamide with a (+)-tetraallyl 18-crown-6 carboxylate-bonded negatively charged polyacrylamide gel-filled capillary. Sample: L-alanine-2-naphthylamide spiked with ca. 0.2% of DL-alanine-2-naphthylamide. Conditions: applied voltage (current), 239 V cm^{-1} (6 μA). Reprinted from ref. [97], with permission.

polymer with M_w = 1000–10 000) were used. The resolution was impaired upon repeated runs, probably due to slow leakage of the chiral selector molecules. The monomeric β-CD (2,6-di-*O*-methyl-β-CD) did not give rise to any separation because the selector was easily moved from the matrix by the strong EOF. Not unexpectedly, these gels were further developed by attaching the chiral selectors covalently to the matrices [94–96]. Allyl carbamoylated β-CD was co-polymerized with acrylamide, a charged monomer (either AMPS for negatively or *N*-(2-acrylamidoethyl)triethylammonium iodide for positively charged gels resulting in catodic or anodic EOF, respectively) and crosslinked with Bis. The structure of these gels was proposed to be similar (Fig. 2 in ref. [94]).

At the same time another type of charged polyacrylamide gel (either negative or positive) was developed for enantiomer separation using allylated β-CD (2-hydroxy-3-allyloxypropyl-β-CD) as chiral selector [83]. Enantiomers of neutral and somewhat charged drug compounds have been separated electrochromatographically with efficiencies outstanding in some cases (plate numbers of half a million per meter). These gels have the additional attractive feature that the plate heights do not increase upon increased field strength—a finding contrary to the properties of Fujimoto's gels (cf. Fig. 3 in ref. [58] with Fig. 4 in ref. [83]).

Similarly to previous approaches homogeneous charged gels were synthesized for enantiomer separations with covalently attached crown ethers as chiral selectors [97]. Primary amino compounds were separated with high resolution and efficiency for optical purity tests (Fig. 6).

In a review article from 2002 [98] a separate section is dedicated to homogeneous gel columns, summarizing the achievements in the field but limited to enantiomer separations.

3.3. Gel electrochromatography in a miniaturized separation system

A recent trend in analytical techniques is to miniaturize the equipment and to make small-sized (disposable) separation platforms, so-called microchips. Beyond the "futuristic" efforts there are several "down-to-earth" reasons for following this path. An obvious advantage of these devices is that extremely small amounts of sample and reagents are required for an analysis. The approach holds the promise of integration of an entire laboratory on one plate, which has been already demonstrated in simple cases. However, much remains to be done, because (i) today's technology to make microchips, which involves sophisticated photolithography and chemical wet-etching is very expensive at the developmental stage, (ii) detection sensitivity is limited, which affects the separation performance, and (iii) the entire apparatus around the microchip is still complicated and incomparably large (e.g., a mass spectrometer), which limits the use to well-educated researchers with great funds.

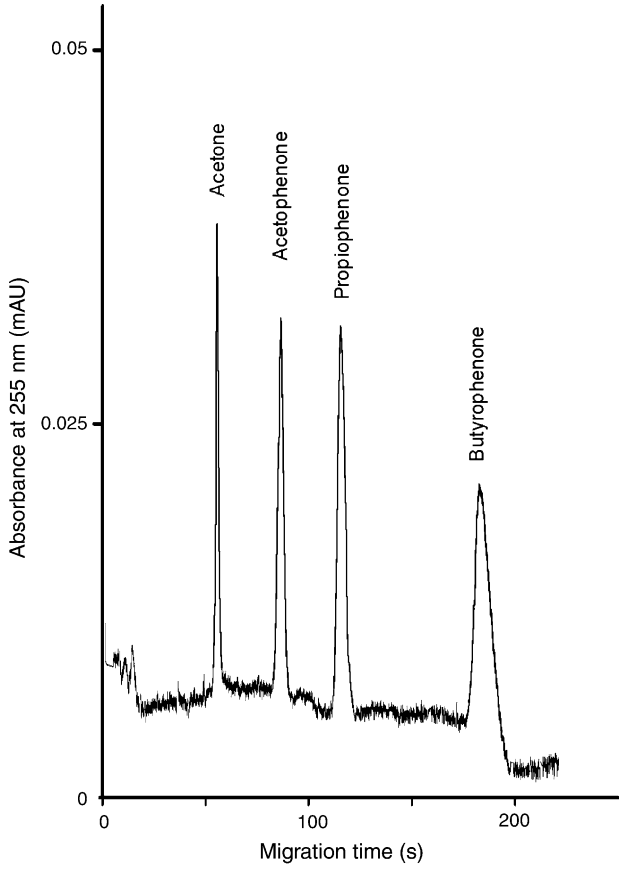

Fig. 7. Electrochromatographic separation of acetone, acetophenone, propiophenone and butyrophenone in a microchip channel (l = 2.8 cm) filled with an AA3-AMPS1-CD10 gel. Buffer: 20 mM Tris-30 mM boric acid, pH 8.2. Injection: 200 V (F = 47 V cm^{-1}) for 10 s; running voltage: 2 kV. Reprinted from ref. [84], with permission.

A new class of microdevices has been recently designed [84], composed of a supporting plastic (PVC) plate integrated with a groove for a piece of fused silica capillary (the separation channel), a slit for on-tube detection, an "islet" for the application of sample, electrode vessels and platinum electrodes. It combines the accumulated knowledge of conventional capillary electrophoresis with an easy-to-fabricate design based on inexpensive materials (PVC, ceramics), including fused silica capillaries. It permits electrophoretic, electrochromatographic and chromatographic separations with universal on-tube UV detection. The design can be characterized as a hybrid between capillary electrophoresis and microchip electrophoresis, containing the best features of both methods.

Neutral compounds (acetone, acetophenone, propiophenone and butyrophenone) can be separated electrochromatographically in a negatively charged homogeneous gel (Fig. 7). The separation is mostly due to interactions between the hydrophobic moieties of the analytes and the β-cyclodextrin cavity. Additionally, "aromatic adsorption" can contribute to the resolution. Addition of increasing concentrations of acetonitrile to the mobile phase decreased significantly the mobility of EOF. The gel structure was stable even at relatively high (40%) acetonitrile concentration, although the resolution decreased significantly. The similarity to reversed-phase chromatographic supports is obvious.

4. Summary

The described homogeneous charged gels represent a new category among stationary phases for capillary electrochromatography, being the only non-particulate beds, in sharp contrast to monolithic and conventionally packed columns, which both consist of particles. They approach the ideal separation properties in theory, which was experimentally supported. For instance, they permit very high resolution and separation efficiency. The usefulness of homogeneous gels in electrochromatographic applications was explored only recently in a few research groups. The outstanding stability of some of these gels makes them very attractive for analysis not only in capillaries but also in microchip formats, including the hybrid microdevices.

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