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Review

Stationary phases for capillary electrochromatography

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Abstract

This review summarizes the variety of stationary phases that have been employed for capillary electrochromatography (CEC) separations. Currently, about 70% of reported CEC research utilizes C_{18} stationary phases designed for liquid chromatography, but an increasing number of new materials (e.g., ion-exchange phases, sol–gel approaches, organic polymer continuous beds) are under development for use in CEC. Novel aspects of these different materials are discussed including the ability to promote electroosmotic flow, phase selectivity and activity for basic solutes. In addition, new column designs (polymer continuous beds and silica–sol–gel monoliths) are described.

Keywords: Reviews; Stationary phases, CEC; Electrochromatography

Contents

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1. Introduction

Capillary electrochromatography (CEC) combines aspects of capillary electrophoresis (CE) with liquid chromatography (LC) to achieve a unique separation methodology. The primary factor motivating interest in this rapidly developing field is the promise of high efficiency liquid phase separations. As with conventional LC, retention is defined by solute–stationary phase interactions and is usually predictable. Unlike conventional LC, in CEC the stationary phase plays a dual role and provides the basis for electroosmotic pumping of the mobile phase in addition to chromatographic separation.

In this review we present a comparison of the main categories of stationary phases that have been utilized for CEC separations. CEC separations are influenced by a number of parameters including the applied voltage, mobile phase composition, type of buffer, column temperature, capillary dimensions, and most importantly, the stationary phase. The stationary phase is the 'heart' of this technique and a variety of bonded phases have been used. Categories include modified silica gel, sol–gel monoliths, organic polymers (continuous beds) and ion-exchange materials. To date, most (about 68%, see Table 1) of the published research in CEC has been based on C_{18}-modified silica gel. These stationary phases are primarily intended for LC, but very high efficiencies (up to 400 000 plates/m) have been reported for CEC applications. A smaller fraction of research has been devoted to the development and utilization of stationary phases intended specifically for CEC. Such phases are designed to promote electroosmotic flow (EOF) or to address efficiency and stability issues. The use of bonded phases with permanent charges offers certain advantages to CEC. These special materials are usually not commercially available (with the exception of some cation-exchange stationary phases/resins) and it is anticipated that research will expand in this area. In 1997, Robson et al. presented an overview of stationary phases and applications in CEC [1], and recently Fujimoto reviewed CEC applications on various stationary phases and focused on organic polymer (continuous beds) packed columns [2]. Because of rapid developments in this field it is useful to update this information and provide more detail on stationary phase nature and suitability. Our primary emphasis is on silica-based stationary phases and their use and potential for CEC. We further discuss how selectivity and silanol activity of various materials influence CEC separations compared with LC. Finally, new column designs (organic polymer continuous beds and silica–sol–gel monoliths) are presented including an outlook for more efficient and more selective materials. We make a distinction here between electrochromatography with packed capillaries and open-tubular (OT) CEC [3–5]; the latter approach is not discussed in this review.

2. Design of CEC packing materials[1]

Categorization of the various packing materials described in about 180 CEC publications yields data provided in Fig. 1 and Table 1. CEC is still a growing area of research as can be concluded from the increasing number of publications. C_{18} stationary phases constitute the largest fraction of stationary phase types (about 70% of all applications); however, the fraction of other materials has increased over the last 5 years. An increasing number of research articles present applications with ion-exchange (IX) stationary phases.

2.1. Influence of particle size and porosity

Initially, most CEC studies were carried out with C_{18} phases prepared on 5-μm particle size silica [6–11]. As smaller particles (3 μm, 1.5 μm) have become more widely available, CEC columns have been prepared with these sorbents to further enhance separation efficiency. Using 3-μm particles, column efficiencies have approached 400 000 plates/m (equivalent plate height of ~2.5 μm) [12,13]. Very fast and efficient separations have been achieved with 1.5-μm C_{18} phases (mostly non-porous silica) and a number of applications have been presented

[1]Certain commercial equipment, instruments, or materials are identified in this report to specify adequately the experimental procedure. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the materials or equipment identified are necessarily the best available for the purpose.

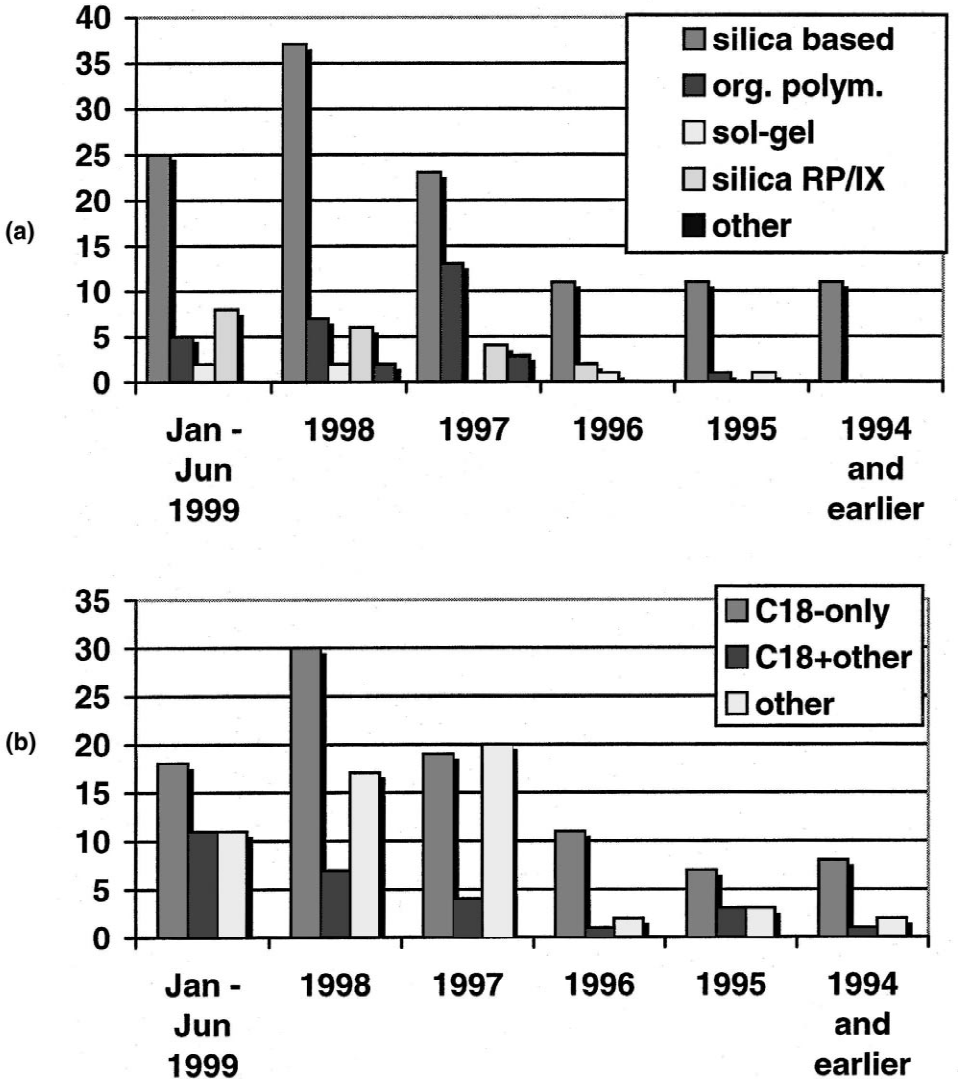

Fig. 1. Overview of the different materials that have been utilized for CEC. (a) Annual number of publications in the respective material categories. (b) Annual number of research papers with respect to use of C_{18} phases.

Table 1
Percentage of published CEC applications using C_{18} phases exclusively (a), utilization of C_{18} silicas together with other materials (b), all C_{18} stationary phases (c), and non-C_{18} materials (d)

	Total	June 1999	1998	1997	1996	1995	1994 and earlier
(a) C_{18}-only	53	45	56	44	78	54	73
(b) C_{18}+other	15	27.5	13	9	7	23	9
(c) C_{18} total	68	72.5	69	53	85	77	82
(d) Remaining	32	27.5	31	47	15	33	18

[7,14–18]. Separations of nitroaromatic compounds on 1.5-μm C_{18} non-porous silica were achieved in <2 min and plates numbers reached 500 000/m [18]. Dadoo et al. described the separation of 16 priority pollutant PAHs in <10 min under isocratic conditions, and average plate numbers ranged from 300 000 to 400 000/m [17]. A set of five PAHs could be separated in <5 s on a 6.5-cm packed bed; 10% bare silica gel was added to the bonded phase for EOF enhancement. By use of on-column fluorescence detection, efficiencies of up to 750 000 plates/m have been achieved.

Even smaller silica particles in the submicrometer range have been utilized in CEC. Lüdtke et al. described a modified procedure of the Stoeber synthesis for preparation of 0.5-μm silica particles [19]. Tetraethoxysilane (TEOS) and octadecyltrimethoxysilane were subject to hydrolysis and polycondensation in a water–ethanol–ammonia mixture to yield monodisperse reversed-phase (RP) silica spheres. The material was calcinated at 550°C to create pores by burning off the organic moieties. Using the C_{18} silane as porogen, mean pore sizes of 3.4 nm were obtained and the surface area was 308 m^2/g. Finally, the porous particles were modified with C_8-dimethylchlorosilane. The surface coverage was calculated to be 2.1 $\mu mol/m^2$, which is reasonably high considering the rather small pore diameter available for C_8 ligands. As expected, very fast separations could be achieved; however, efficiencies (N=80 000–290 000 plates/m) were lower than those obtained with 1.5-μm particles. Reduced column efficiency was attributed to the B term of the van Deemter equation. To date, particles as small as 0.2 μm have been investigated for their potential use in CEC [20]. For a series of silica spheres ranging from 0.2 to 3 μm particle diameter, it was demonstrated that EOF is independent of the particle size [20]. Choudhary and Horvath extensively studied a series of silica RP and polystyrene phases of different particle diameters in terms of EOF production [21]. No correlation between particle size and flow velocity could be established. This observation was attributed to the different nature of the utilized materials, which cause different zeta potentials.

The influence of pore size on efficiency was investigated by Li and Remcho [22]. Van Deemter curves for 7-μm RP silica particles with pore sizes from 10 to 400 nm were evaluated (Fig. 2). Plate heights (h) were shown to decrease with increasing pore diameter, and a minimum value of $h \approx 10$ μm was calculated for a 400-nm pore size substrate. The slope of the van Deemter curves was found to decrease with increased pore size at high linear flow velocities. The enhanced efficiencies are attributed to perfusive flow characteristics in these wide-channel materials and are also apparent for operation in LC mode (see also Section 2.4.2).

2.2. Materials with permanent charges for increased EOF

Conventional C_{18} bonded silicas are mostly prepared by monomeric surface modification using monofunctional C_{18} silanes. Polymeric phases are synthesized by use of a trifunctional silane in the presence of water. The latter materials are highly shape selective and are utilized for separations of PAHs, carotenoids, vitamins and steroids [23]. Monomeric phases are relatively easy to prepare and are often endcapped to reduce residual silanol activity. Because these residual silanols are required for EOF, endcapped phases may not be suitable for CEC. Some manufacturers (e.g., Waters, Hypersil, and others) now offer C_{18} phases that are optimized for CEC in the sense that the materials are not endcapped and/or may have a lower surface coverage of alkyl ligands. This results in an increased number of surface silanols intended to promote EOF. Fig. 3 illustrates the separation properties of a variety of C_{18} materials [24]. It is evident that the base deactivated material produces a low EOF (due to the reduced quantity of surface silanols) and therefore exhibits long retention/migration times. The fastest separations have been achieved with the CEC C_{18} phases. A series of C_{18} phases was also investigated by Zimina et al. [25], and bonded phase properties together with EOF mobilities are summarized in Table 2. Similar to Fig. 3, differences in EOF are apparent and can be attributed to differences in surface area, endcapping, synthesis procedure for and purity of silica gel, and silanol activity.

2.2.1. Strong cation-exchange (SCX) materials

The cation-exchange group used for silica gels is usually a sulfonylalkyl silane, and a variety of SCX

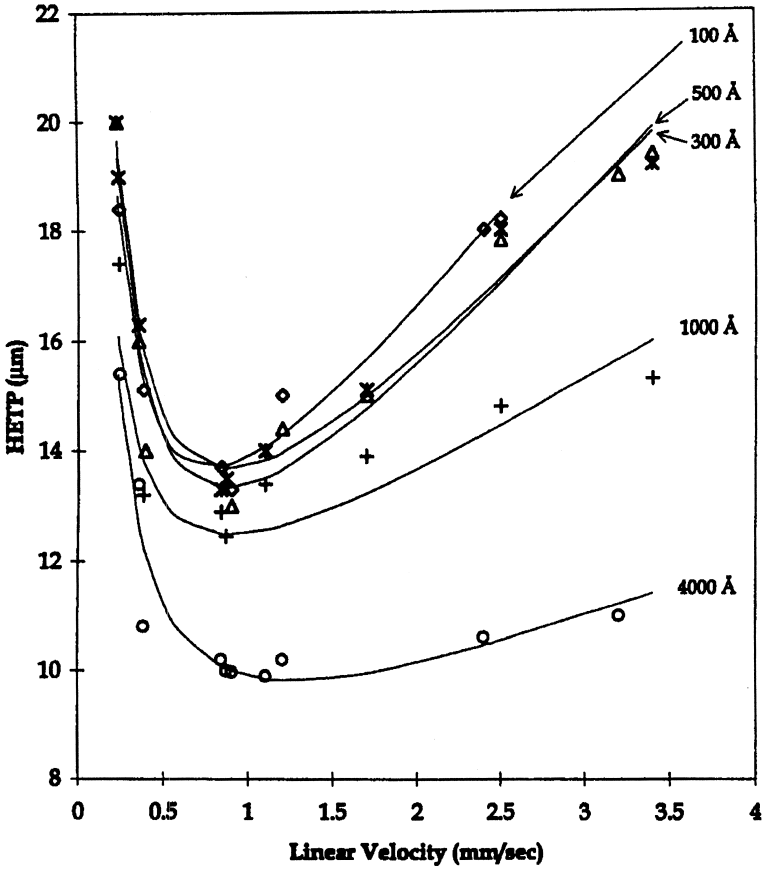

Fig. 2. Plate height as function of linear velocity for different pore sizes (adapted from Ref. [22]).

materials exist, including C_6-SCX, C_3-SCX, phenyl-SCX, and the so-called mixed-mode phases C_{18}-SCX. The sulfonyl groups (pK_a of sulfonic acid is −2.2) are ionized over a wide pH range and are able to promote EOF within this interval. Over the last 5 years, there has been a considerable increase in CEC research with these stationary phases (Fig. 1). Smith and Evans presented the first application of materials with SCX properties for CEC separations of basic pharmaceutical compounds [26]. Tricyclic antidepressants like nortriptyline or clomipramine were found to exhibit strong peak tailing on conventional C_{18} phases. Separations of these compounds on SCX silica resulted in excellent resolution with extremely high efficiencies (millions of plates/m). Other groups also confirmed these results [27]; however, reported difficulties in the reproducibility of separations could not be explained. A description was given in terms of a 'focusing effect' that leads to efficiencies higher than theoretical values. Very recently, Moffatt et al. observed such abnormally high efficiencies under certain conditions even with a 'conventional' C_{18} stationary phase [28]. These very high efficiencies for neutral–anionic solutes are believed to result from discontinuities between electrolyte and solvent system (isotachophoretic effect) when the sample is injected in water instead of in the mobile phase.

Very recently, Cikalo et al. examined a variety of SCX materials in terms of the influence of pH, acetonitrile content, effect of ionic strength, and influence of voltage on EOF using a neutral solute (thiourea) as an indicator [29]. In contrast to results of other groups [30,31], they observed rather lower linear velocities and currents with the SCX phase compared to the C_{18} material under the given conditions. Therefore it still remains unclear how the

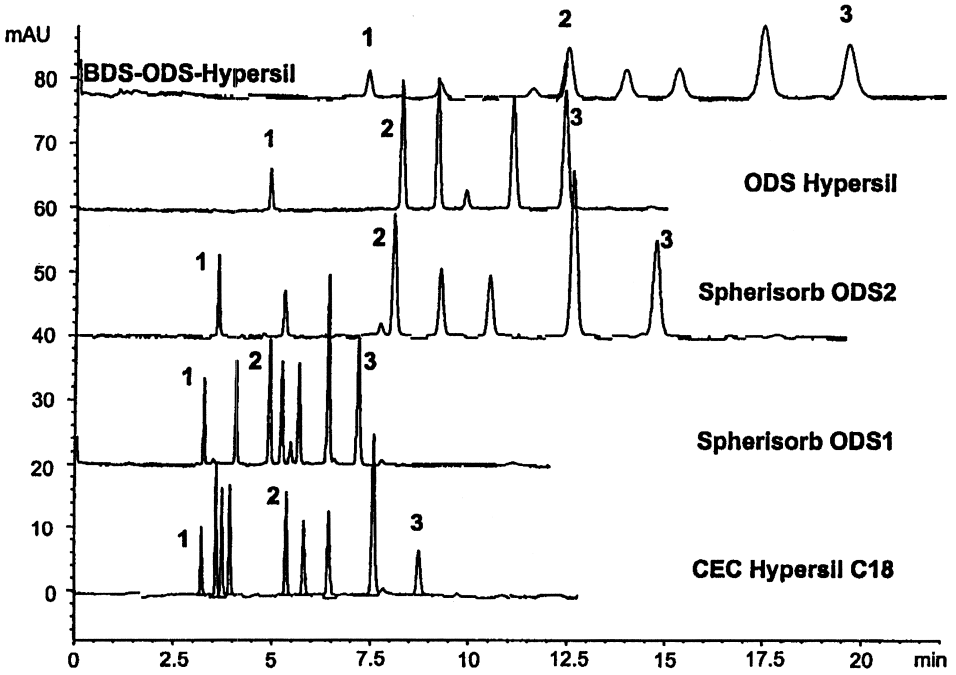

Fig. 3. Separation of PAHs on different C_{18} stationary phases. Samples contained thiourea (1), naphthalene (2) and fluoranthene (3) (adapted from Ref. [24]).

SCX materials behave in CEC and more research is needed in this area to further develop this technique.

A series of different SCX materials (propyl-SCX, phenyl-SCX, mixed-mode-SCX, and Symmetry SCX (Waters Spherisorb)), together with a C_{18} bonded phase, has been evaluated by Smith and Evans [31]. Different elution orders for tricyclic antidepressants have been observed among the various SCX materials. For a Symmetry SCX phase, focusing effects for basic solutes were observed even at high pH. Due to the strong hydrophilic character of the sulfonyl groups, the retention of the hydrocarbon-SCX phases (sulfonyl groups attached to alkyl chains) is low and these phases appear to have limited CEC applications for neutral compounds of similar hydrophobicity. The mixed-mode phase (separated octadecyl and SCX ligands) provided sufficient retention and similar EOF properties over a wide pH range, as well as good efficiency. The combination of pure alkyl ligands with SCX ligands appears worthy of further

Table 2
Properties and electroosmotic mobilities for a series of C_{18} stationary phases; EOF mobilities were determined using thiourea as dead time marker and acetonitrile–25 m*M* 3-cyclohexylamino-2-hydroxy-1-propanesulfonic acid (70:30, v/v) pH 9.53; (adapted from Ref. [25])

Bonded phase	Endcapping	Surface area (m^2/g)	Electroosmotic mobility ($\mu_{eo} \cdot 10^4$ $cm^2\,V^{-1}\,s^{-1}$)
Nucleosil 5 C_{18}	Endcapped	350	1.56
LiChrospher RP-18	Uncapped	450	1.45
Zorbax BP-ODS	Endcapped	350	0.68
Spherisorb S5 ODS2	Endcapped	220	0.50
Hypersil ODS	Endcapped	170	0.14
Partisil 5 ODS3	Endcapped	350	<0.01
Purospher RP-18	Chemically treated	500	<0.01

research for the development of optimized CEC stationary phases.

Zhang and El Rassi reported the synthesis of a SCX material [30,32], consisting of octadecyl sulfonated silica (ODSS) prepared through a three-step synthesis on silica. An initial hydrophilic layer (glycidylsilane) was prepared, and sulfonyl and octadecyl groups were subsequently attached. As with other SCX materials, separations were faster (38% increase in EOF velocity) than with a corresponding C_{18} material. In separations of nucleosides and bases, differences in selectivity were observed and were compared to the conventional C_{18} material. A slightly different approach was pursued by Wei et al. [33]. They attached a polymeric layer containing sulfonic acid groups to silica. Plate numbers for a set of basic compounds varied between 40 000 and 900 000/m, and changes in elution order were achieved by variation of the pH. However, overall peak shape was not as good compared to other published work on SCX phases.

2.2.2. Anion-exchange materials

Anion-exchange (AX) stationary phases have received much less attention in CEC than their cation-exchange counterparts. Lämmerhofer and Lindner described the preparation of a weak anion-exchange (WAX) material for enantiomeric separations of N-derivatized amino acids [34]. The ligand consists of a quinine group that is positively charged at pH less than 6.3. Selectivity was slightly altered compared with LC separations on the same material, but efficiency was improved by a factor of 2 to 3. An interesting separation of iodine, iodate and perrhenate was performed on an AX silica [35]. In comparison with CE, selectivity was different and sample loading capacity was increased, lowering the limit of detection by a factor of about 20.

A separation of myoglobin peptides has been reported using a mixed mode phase, which consists of a 1:1 ratio of C_{18} and dialkylamine groups [36]. The EOF velocity was considerably increased compared to a C_{18} bonded phase, as indicated by the migration time of thiourea. This was especially apparent at low pH where the amine groups are fully protonated. The positively charged surface results in electrosmotic flow in the opposite direction compared to conventional RP and SCX materials, where surfaces are negatively charged. Selectivities for myoglobin digests differed between the mixed-mode phase and the C_{18} material, but column efficiencies were comparable. This approach may be applicable in other areas involving the separation of basic compounds, especially for drugs, where SCX columns are problematic in terms of reproducibility.

2.3. Alteration of selectivity through stationary phase variation

Considerable effort has been expended in the comparison of selectivity for CEC and LC separations. For silica-based RP materials, selectivity is usually similar for both approaches whereas efficiency is greatly enhanced for CEC [10,27,37–46].

Lurie et al. compared C_8 and C_{18} stationary phases for the separation of drugs (cannabinoids) [47]. CEC separations utilizing the C_{18} phase led to enhanced resolution, shorter migration times and slightly enhanced selectivity for these compounds. Phenyl, C_8 and C_{18} phases have also been investigated for the separation of polar neutral pharmaceutical compounds [48]. Under optimized conditions the C_{18} modified silica was superior to C_8 and phenyl phases. It provided the necessary retention and also enhanced efficiency and selectivity. A series of barbiturates was also analyzed with phenyl, C_8 and C_{18} phases whose selectivities differed slightly for the same mobile phase composition [49].

The separation of structural isomers such as PAHs, vitamins, carotenoids, terpenes, etc. is often difficult with monomeric C_{18} columns. In general, better separations of such compounds can be achieved with ordered stationary phases (e.g., polymeric alkyl phases and/or long alkyl chain length phases). C_{30} stationary phases have been utilized by Sander et al. [46] and Roed et al. [50] for the separation of carotenoid isomers and retinyl (vitamin A) esters, respectively. Compared with C_{18} phases, the longer alkyl ligands exhibit excellent shape selectivity making the C_{30} phase a useful alternative for difficult separations. In Fig. 4 gradient elution CEC separations of carotenoid isomer mixtures are demonstrated on monomeric C_{18}, polymeric C_{18} and polymeric C_{30} phases. These isomers range from polar xanthophylls (lutein, zeaxanthin, echinenone) to nonpolar hydrocarbons (α- and β-carotene). As

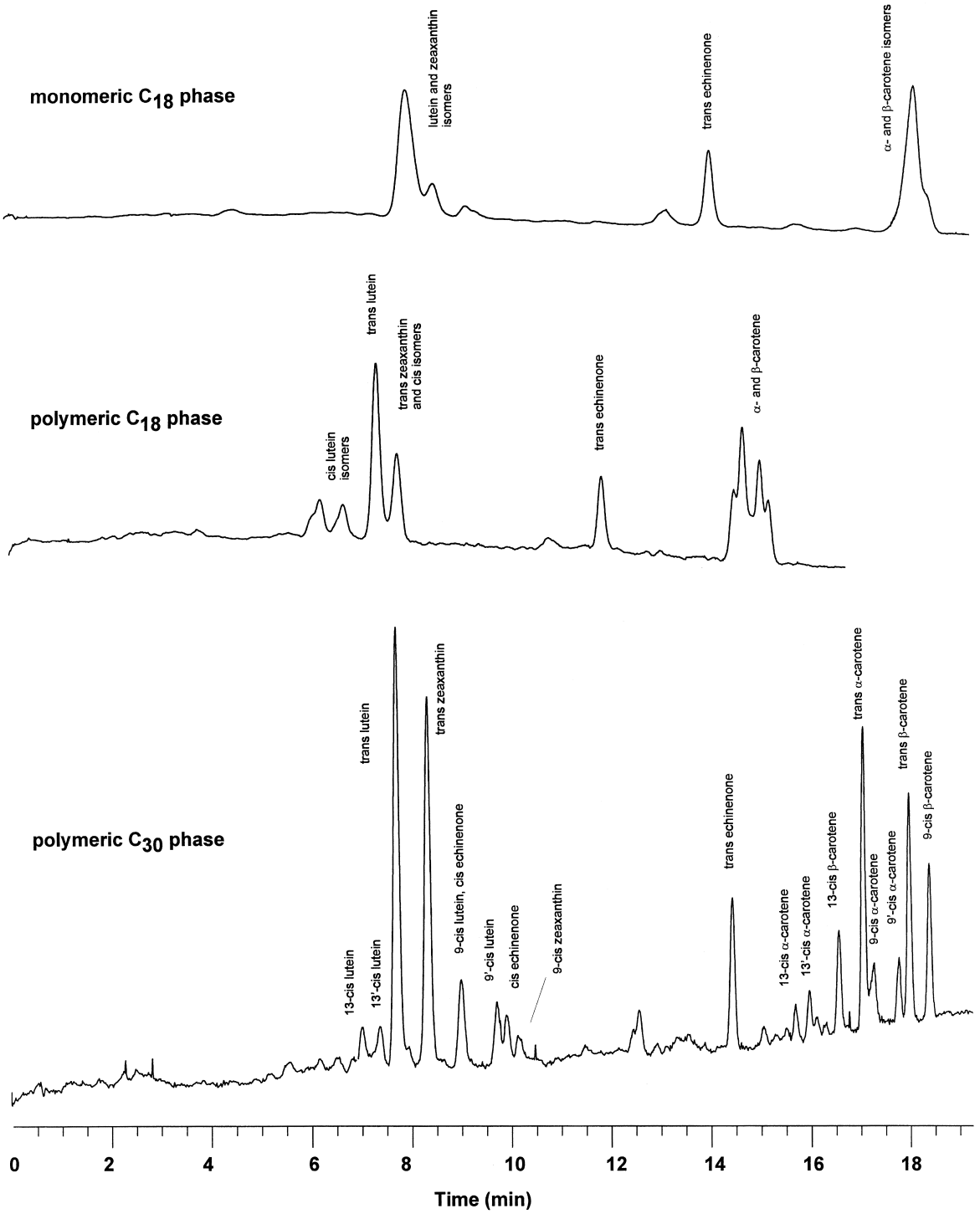

Fig. 4. Gradient elution CEC separations of carotenoid isomers on monomeric C_{18}, polymeric C_{18} and polymeric C_{30} stationary phases. The bare silica utilized was YMC Gel, particle size 5 μm, pore size 20 nm.

can be seen in Fig. 4 the 'conventional' monomeric C_{18} phase provides poor separation of the isomers since only the polar and nonpolar groups are separated. Better results were obtained with a polymeric C_{18} material (which has a higher ligand density and order), but still many isomers coelute. Separations are greatly improved with the C_{30} stationary phase, and all major *cis*- and *trans*-carotenoid isomers are separated. Despite the polymeric nature of the C_{30} phases, column efficiencies approached 280 000 plates/m for isocratic separations, which is comparable with plate numbers that have been reported for monomeric C_{18} bonded phases.

2.4. Column design

For capillaries filled with particulate substrates, the necessity of column bed stabilization presents significant problems in column design. Unlike with conventional LC or even micro-LC, external column fittings cannot be used to retain the packing material in CEC due to restrictive dead-volume requirements. Instead, frits are typically manufactured in situ to define the inlet and outlet of the column. A variety of approaches to frit preparation have been reported. Approaches include heating processes [10,19,51,52], heating in combination with a silicate solution to 'glue' silica particles [9,25], use of microchannel capillary arrays as porous plugs [53], and frit fabrication from a silicate–formaldehyde medium [14]. Regardless of the approach, good quality frits can be difficult to achieve, especially when small particles (i.e., <3 μm particle size) are used. Ideally CEC column endfrits should provide mechanical stability to the packed bed without degrading chromatographic performance or introducing flow irregularities. Many of the problems reported for CEC (including bubble formation, loss in column efficiency, and retention variability) have been attributed to poor frit quality. It should also be noted that columns become very fragile after the polyimide coating has been removed during the frit making process.

The interface of frit and unpacked capillary appears to be a major source for bubble formation, which reduces EOF [9]. Carney et al. have found that the probability of bubble formation increases with increased length of the frit [54]. In addition, recoating of the frits with C_{18} silane reduced the occurrence of bubbles. Recently CEC separations using fritless capillaries packed with 1.5- and 3-μm particles has been reported by Mayer et al. [55]. With this approach, the inlet of the capillary was tapered and an outlet frit was not necessary because the applied electric field attracted the packed bed towards the capillary inlet (anode). Very good results in terms of efficiency and stability have been achieved.

The preparation of inorganic (sol–gel) or organic (polymer) continuous bed columns circumvents the frit problem, as a continuous packed bed is covalently attached to the capillary wall, making a frit unnecessary.

2.4.1. Porous organic polymers (continuous beds)

Continuous beds were first prepared by Hjerten et al. in 1989 for use in LC [56], later a similar procedure was applied by Svec et al. [57]. The first continuous bed prepared for CEC was reported by Hjerten [58]. Since then, a number of reports have been published describing capillaries prepared by in situ polymerization of organic monomers [59–62]. Table 3 illustrates the different properties of these organic polymers. Efficiencies ranged from 120 000 to almost 400 000 plates/m and appear to be slightly lower than silica-based columns on an average basis. In general, the procedures for column preparation follow a similar strategy. The polymer backbone usually consists of either polyacrylamides or polymethacrylates–acrylates. If necessary, hydrophobicity can be enhanced by adding alkyl functionalities [60,62]. EOF is promoted by the incorporation of ionizable functional groups such as acrylic acid or sulfonic acid monomers within the polymerized mixture. For reversed EOF, ammonium-functionalized monomers have been utilized [63]. In the case of rigid continuous beds, porogens in form of a ternary solvent mixture (water, propanol and 1,4-butanediol) are added [61]. Properties of these polymers are adjusted by variation of the relative amount of backbone monomers, porogens, EOF promoting groups and alkyl functionalities. A detailed discussion of the influence of various reaction parameters on pore size and column efficiency has been presented by Peters et al. [64]. Using a chiral methacrylate as monomer, Peters et al. designed the column properties for the separation of enantiomers [65].

Table 3
Properties of various organic polymer continuous beds for CEC separations.

Research group	Materials	EOF promoter	Retentive functionalities	Maximum efficiencies, Comments
Fujimoto [59,95]	Acrylamides	Acrylamido-2-methylpropane sulfonic acid (AMPS)	Hydrogels, high aqueous mobile phases	160 000 plates/m
Hjerten [60,63]	Acrylamides, methacrylates	Vinyl sulfonic acid, dimethylallyl ammonium chloride	C_4 and C_{18} methacrylates	260 000 plates/m For column without C_{18} ligands
Svec [61,64]	Methacrylates	AMPS	Organic backbone	210 000 plates/m
Novotny [62]	Acrylamides, polyethyleneglycol (PEG)	Vinyl sulfonic acid, acrylic acid	C_4, C_6 and C_{12} acrylates	400 000 plates/m Increased efficiency through addition of PEG to polymer mixture

Molecular imprint polymers (MIPs) constitute a growing area of materials for CEC [66–68] and have been recently reviewed [69]. MIPs can be prepared in situ through (co)polymerization of monomers (usually methacrylates) in the presence of an imprint molecule (e.g., propranolol or phenylalanine anilide). After the reaction the imprint species are washed off the polymer leaving cavities that have a strong affinity for the templates. Acrylic acid has been used as an EOF promoting agent. These materials are suited for chiral separations and a number of applications have been demonstrated. Schweitz et al. demonstrated a very fast separation of propranolol enantiomers, achieved in about 120 s [66]. Overall, efficiencies for MIPs have been relatively low, but improvements are expected as synthetic approaches are refined.

2.4.2. Silica–sol–gel monoliths and silica continuous beds

Silica–sol–gel monoliths and continuous beds present an inorganic alternative for fritless packed columns. We are going to use the term ‘monolith’ for those materials, which are produced from bare silica or RP materials, with the addition of alkoxysilanes using sol–gel conditions. Recently a series of different approaches has been reported. A summary is given in Table 4. Dittmann at al. have prepared a monolithic column by thermal treatment of a capillary packed with C_{18} modified silica [70]. The thermal treatment was carried out by use of a heated wire in a manner similar to the frit making process. The heated wire was drawn along the capillary and the drawing speed was varied. EOF was observed to increase for slower rates of drawing. In addition, retention was observed to decrease as indicated by a reduction of k' for *o*-terphenyl. This suggests that a portion of the C_{18} ligands are removed from the silica surface due to the influence of heat. Asiaie et al. detailed the preparation of sintered octadecylsilica to obtain a different type of monolithic column, followed by resilanization with a C_{18} monochlorosilane [71]. Scanning electron microscopy (SEM) was used to evaluate the extent of monolith forma-

Table 4
Overview of the different approaches for silica–sol–gel monoliths utilized in CEC

Research group	Monolith preparation	Introduction of RP properties	Maximum efficiencies (plates/m)
Horvath [71]	Sintering of 6-μm C_{18} particles	Resilanization	125 000
Zare [72]	Tetraethoxysilane	3- and 5-μm C_{18} particles	80 000
Dittmann [70]	Thermal treatment	3-μm C_{18} particles	Not available
Remcho [73]	Potassium silicate	5-μm C_{18} particles	160 000
Lee [74]	Mixture of alkyl- and alkoxysilanes	5-μm C_{18} particles	130 000
Tanaka [77]	Silica rod, in situ column preparation	Silanization	Not available

tion. The sintering of the bonded phase particles was performed in the presence of sodium bicarbonate at 360°C. At this temperature, the pore structure remained open. The result was a column with enhanced mechanical stability compared to conventionally packed columns. Minimum plate heights have been evaluated with CEC (8 μm) and μ-LC (16 μm) measurements, and CEC separations have been more efficient. Because of the absence of frits, bubble formation was alleviated. It was suggested that resilanization of frits of conventionally packed capillaries may have a similar effect. This hypothesis was tested recently by Carney et al. [54].

Dulay et al. utilized TEOS to form a sol–gel matrix for immobilization of C_{18} silica particles [72]. The sol–gel–silica slurry was packed into capillaries under vacuum, and monolith formation was allowed to proceed at 100°C with the evaporation of ethanol. It was observed that the presence of bonded phase particles enhanced the stability of the sol–gel matrix. An optimum C_{18} particle concentration of 300 mg/ml TEOS solution gave the best column properties with respect to retention and selectivity. Plate numbers of 80 000/m were achieved for separation of PAH solutes with a 3-μm C_{18} sol–gel capillary, and these were lower plate counts than other monolith approaches discussed here.

Chirica et al. described the entrapment of a packed bed (C_{18} bonded silica) with a silicate (Kasil) solution at temperatures up to 160°C [73]. Separations of PAHs were compared to a column without entrapment, and retention times were considerably reduced for the monolith, possibly due to a partial blocking of the pores with the silicate solution. The utilization of potassium silicate as a 'glue' results in additional surface silanols and increased EOF. Such surfaces are very active and may not be suitable for basic compounds.

Tang et al. proposed a slightly different preparation strategy [74]. Monolith formation was achieved with a mixture of tetramethoxysilane and ethyltrimethoxysilane. Separation efficiencies of 130 000 plates/m with 5-μm C_{18} particle–sol–gel monolith were reported and are similar to those obtained with the sintering approach of Asiaie et al. [71]. Peak shapes were very symmetric (asymmetry factor *A* ranged from 1.03 to 1.09 for aromatic compounds and PAHs), whereas Chirica and Remcho observed some peak tailing (PAH solutes) for their monolithic column. Evaluation of the *C* term of the van Deemter equation suggested that the pores are partially filled with the sol–gel matrix.

Approaches for the preparation of continuous silica beds that were subsequently derivatized with C_{18} silanes have been described by Minakuchi et al. [75] as well as by Fields [76]. A sol–gel process was performed in-column by acid-catalyzed hydrolysis and polycondensation of TEOS [75]. These materials have mesopores and through-pores on the order of 2–20 nm and 0.5–8 μm, respectively, thus allowing for fast mobile-phase flow usage in LC. Plate numbers approached 40 000/m. In a related publication Ishizuka et al. described the preparation of silica rods in capillaries [77]. These materials exhibited low EOF in CEC, which was attributed to high surface coverage and high purity of the sol–gel matrix. A different approach has been published based on the polymerization of a potassium silicate solution [76]. The average pore size of the resulting continuous xerogel is about 2 μm. Preliminary LC studies revealed low efficiencies on the order of about 13 000 plates/m. So far, no CEC data using these continuous silica materials have been published, but their potential due to perfusive flow properties (see Section 2.1) is quite obvious.

2.5. Special applications

2.5.1. Other silica-based materials

The separation of basic drugs can be accomplished with bare silica gel, as demonstrated by Wei et al. [78]. Retention mechanisms have been studied regarding the influence of acetonitrile content, buffer ionic strength, and pH. In addition to electrophoresis, three different separation mechanisms can take place on bare silica. These include normal-phase, ion-exchange, and surprisingly, reversed-phase mechanisms under varied conditions. The latter mechanism is believed to originate from the presence of siloxane groups on the silica surface.

Various materials have been applied to chiral separations in CEC. Among them, β-cyclodextrin (β-CD) functional groups have been widely used either attached to silica [8], embedded in polyacrylamide gels [79–81] or as mobile phase additives

[82–85]. Wistuba et al. recently described a permethylated β-CD immobilized on silica for the separation of barbital enantiomers [86]. The same solutes were investigated by Dermaux et al. using vancomycin coated silica gel [87]. Using naproxen-derived and Whelk-O chiral stationary phases, Wolf et al. achieved high efficiency separations (up to 200 000 plates/m) of various neutral enantiomers [88]. Recently Krause et al. described two chiral materials on 1000-Å pore size silica gel, which was coated with derivatized polyacrylamide or cellulose carbamate [89]. Separations have been carried out in the normal- and reversed-phase modes using nano-LC and CEC. Compared to nano-LC, CEC separations had only similar efficiency.

2.5.2. Particulate organic polymers

Ion-exchange polymer particles (resins) have also been utilized in CEC. Kitagawa et al. described separations of a series of ions such as sulfite, sulfate and thiosulfate; alkali ions; or lanthanides with cation and anion-exchange resins [90]. They reported that ions were not retained much on C_{18}, C_8 or cyano bonded silica, making these ion-exchange columns valuable separation media. An interesting application utilizing an ethylene chlorotrifluoroethylene polymer was presented by Alicea-Maldonado and Colon [91]. Although this material does not possess ionizable groups, EOF can be generated within the capillary. Origins for EOF include the fused-silica capillary wall, silica frits and the unpacked section, which can act as a pump. Linear flow velocities of 1 mm/s were measured for a mobile phase without buffer. Flow velocities of 2.5 mm/s were generated when trifluoroacteic (TFA) acid was utilized as a mobile phase additive. TFA is believed to adsorb on the surface of the fluoropolymer, creating an ionic layer.

MIP polymers have been prepared by grinding and sieving of bulk material to yield particles of 10-μm diameter or less for CEC applications, and chiral separations of D,L amino acids [92]. Other CEC columns have included cellulose based packing materials, such as cellulose acetate fibers [93], or octadecylated cellulose for normal-phase CEC operation [94].

3. Conclusion

This review illustrates the variety of packing materials that are currently used in CEC. Although C_{18} phases are dominant, an increasing number of new materials are being introduced for CEC columns. These include ion-exchange materials, mixed-mode phases (reversed-phase and ion-exchange properties), organic polymer continuous beds and silica–sol–gel monoliths. To date, the highest reproducible column efficiencies have been achieved with 1.5-μm C_{18} bonded phases. New materials, which incorporate permanent charges, need to be designed to further enhance selectivity and efficiency in CEC. These materials will include variations of existing ion-exchange and mixed-mode materials (different alkyl and other ligands), as well as improvements in preparation of organic continuous beds and inorganic monolithic columns.

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