Review

Monolithic organic polymeric columns for capillary liquid chromatography and electrochromatography[☆]

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Dedicated to the memory of Professor Zdeněk Deyl.

Abstract

This review briefly summarizes the present state of the preparation and use of capillary monolithic columns for liquid chromatography (LC) and electrochromatography (EC). Most important approaches to the preparation of monolithic stationary phases based on organic polymers are outlined and the properties of the monoliths obtained are compared with those of classical particulate phases. A few selected applications of monolithic columns are shown to demonstrate the most important advantages of monolithic capillary columns. It is concluded that both the monolithic and particulate capillary columns are important and that judicious choice of the type suitable for a particular application requires careful consideration of the purpose of the separation and the properties of the solutes to be separated. Monolithic columns are substantially younger than packed ones and thus will require further theoretical and experimental study to further improve their preparation and to enable reliable prediction of their properties and applicability; nevertheless, they are very promising for the future.
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Keywords: Monolithic capillary columns; Capillary liquid chromatography; Electrochromatography; Packed capillary columns; Comparison; Review

Contents

1. Introduction

The extremely rapid development of science and technology and, indeed, of all aspects of our material life, places great demands on analytical chemistry and thus also on analytical separations. The mixtures to be separated are more and more complex, the range of characters and sizes of the separated species are widening, the number of separations needed is increasing and it is simultaneously required that the separations be as fast and as cheap as possible. The analysts respond to these requirements in two principal ways: they are developing new separation systems and are scaling down their size. In this review, we deal with separations in the liquid phase and are concerned with new, monolithic stationary phases for classical liquid chromatography (LC) and electrochromatography (EC), as applied to capillary columns, i.e., to the CLC and CEC methods.

The first stainless steel packed capillary columns (1 mm I.D.) were introduced in LC in 1967 [1,2] and the field was elaborated, e.g., [3–5]. Stainless steel capillaries were gradually replaced by fused silica ones and their internal diameter decreased, down to 20 μm [6]. Capillaries for electrophoresis appeared even earlier, in 1958 [7], and were gradually developed, e.g., [8–11]. The liquid chromatographic separation in a packed capillary, driven by electroosmosis, capillary electrochromatography (CEC), appeared in 1974 [12] and further works on it, e.g., [11,13,14] led to its extensive application to pharmaceutical and biochemical fields. An excellent critical survey of CEC can be found in the monograph by Deyl and Švec [15]. The use of capillary columns in LC and EC is now quite well established but the preparation of reliable packed capillary columns is still difficult. A very promising alternative appeared by the invention of monolithic columns. In fact, it has been stated that the development of monolithic columns is one of the most important breaks in the history of chromatography since the Tswett's discovery [16].

The first, early attempts (1967–1974) at producing monolithic columns consisting of a single piece of a sorbent [17–21] were not particularly successful. Only in 1989 with the introduction of a soft polyacrylamide gel as a continuous sorbent bed in LC [22] started rapid development which led to rigid, macroporous polymer bed [23]. This was followed by silica monoliths that could be functionalized at the pore surface [24,25]. It was shown that a silica monolith could also be prepared by sintering bare silica particles along a packed column [26]. For CEC separations, monoliths were also prepared and used [27–33] including charged polyacrylamide gels for uncharged solutes [34,35]. The best general treatment of all important aspects of monolithic materials can be found in the book edited by Švec, Tennikova and Deyl [36]. A special issue of Journal of Separation Science is devoted to monolithic stationary phases, both polymeric and silica based [37].

Both packed and monolithic capillary columns for LC and EC are now commercially available. This field is quite wide and thus we further discuss merely monolithic organic polymer capillary columns and refer to packed capillaries only for the sake of comparison.

2. Preparation of monolithic organic polymer columns

These are obtained by radical polymerization or copolymerization of monomers containing one or more double bonds. The polymerization mixture contains a functional monomer determining the resultant polarity of the monolith and a cross-linking agent (monomer with two or more double bonds), further there are an initiator and a porogenic agent determining the size and distribution of the pores. The formation of pores during polymerization depends on the thermodynamic quality of the porogenic agent, the temperature and the content of the cross-linking agent [38]. For more detailed description see, e.g., [36,39].

Common polyacrylamide monoliths that belong among highly polar ones are obtained by copolymerization of acrylamide, methacrylamide or their derivatives with the cross-linking agents methylenebisacrylamide or piperazine
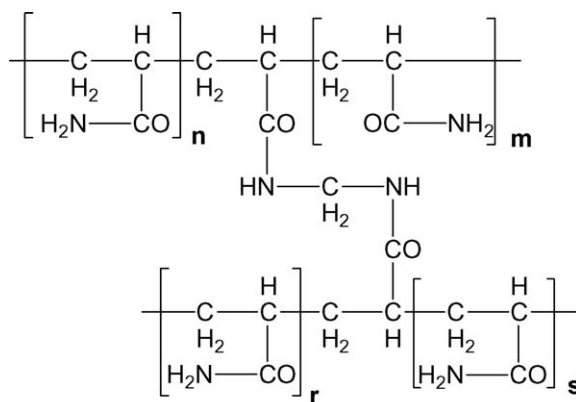

Fig. 1. Chemical structure of a highly polar polyacrylamide monolith.

diacrylamide (Fig. 1) [22,40,41]. Polystyrene monoliths that belong among strongly hydrophobic materials are prepared by polymerization of styrene and its derivatives with divinylbenzene as the cross-linking agent (Fig. 2) [42–44]. Non-polar monoliths involve the recently prepared polynorbornene, cross-linked by hexahydrodimethanonaphthalene (1,4,4a,5,8,8a-hexahydro-1,4,5,8-exo,endo-dimethanonaphthalene) (Fig. 3) [45,46]. This kind of polymerization is a living polymerization which allows flexible surface grating of various chromatographic ligands. Medium polar monoliths based on methacrylic acid esters can be synthesized by polymerization of butyl methacrylate or other methacrylic acid esters with ethylene dimethacrylate cross-linking agent (Fig. 4) [47–51]. Some esters of methacrylic acid, e.g., 2-hydroxypropyl methacrylate and 2-hydroxyethyl methacrylate, can be used to cover the internal wall of the capillary with a hydrophilic polymeric film [52], to suppress adsorption of some solutes. Methacrylate monoliths
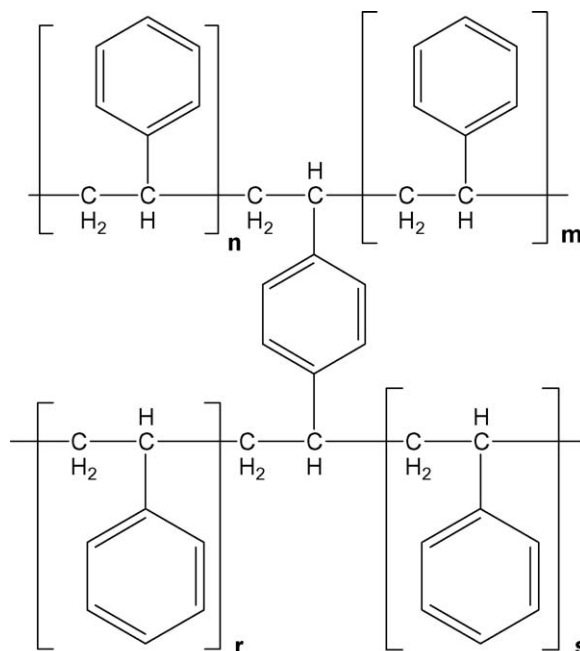

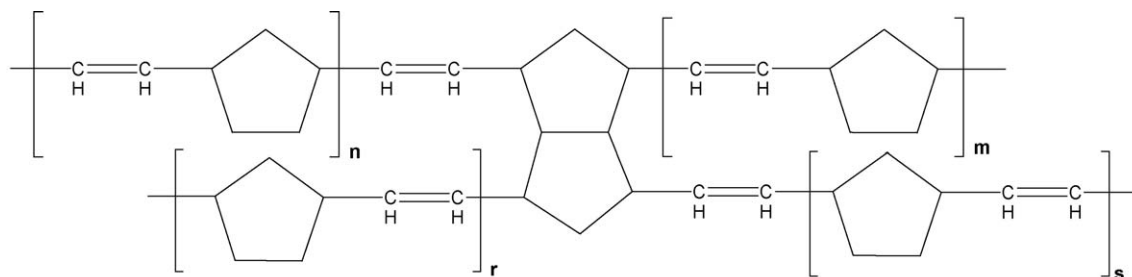Fig. 2. Chemical structure of a strongly hydrophobic polystyrene monolith.

Fig. 3. Chemical structure of a non-polar polynorbornene monolith.

for micro-HPLC and CEC are described, e.g., by Buszewski and Szumski [53].

The monolith formation by radical polymerization can be started in four ways, i.e., by UV radiation, increased temperature, gamma rays and chemical agents. First, from the point of view of the monolith homogeneity, UV radiation initiation is recommended [54,55] which, however, has a limitation in that UV transparent liquids must be used and that brown polyimide-coated capillaries, impenetrable for UV rays, are excluded. Second, the most common initiation is based on increased temperature, also applicable to polyimide-coated capillaries, where a substance is added to the polymerization mixture which decomposes to form free radicals on a temperature increase, e.g., α, α'-azobisisobutyronitril [28,50,51,56]. Third, the gamma radiation can be used but it requires strict safety measures. This approach has two important advantages, namely, no need for addition of initiators and a great versatility permitting the preparation of monoliths of a certain chemical structure in a wide range of sizes, shapes and porous characteristics. The optimum doses and dose rates range from 20 to 40 kGy and 10 to 16 kGy/h, respectively [57].

Monolithic columns prepared after thermal initiation may exhibit somewhat poorer homogeneity compared to monoliths obtained with UV initiation [55], owing to a radial pore size distribution caused by a radial gradient of the degree of polymerization. Initiation by UV radiation and thermal initiation are applicable to preparation of monoliths of differing polarity because the initiation agents have various polarities and can be dissolved in various polar solutions. The oldest way is

chemical initiation at laboratory temperature, using a number of chemical agents, such as ammonium peroxodisulfate with N,N,N',N'-tetramethylethylenediamine catalyst that is readily soluble in polar solvents [58] and has also been used to prepare medium polar poly(butyl methacrylate) monoliths at laboratory temperature [59].

So far, monolithic columns based on organic polymers are mostly prepared in laboratories, but a commercial monolithic capillary based on poly(styrene) is available from LC Packings/Dionex, Amsterdam.

3. Properties of organic polymer monoliths and their comparison with packed columns

Monoliths are separation media that can be compared to a single large particle whose shape and volume fills completely the interior of a separation column. Organic polymeric monoliths, formed in a single step, produce pores with sizes continuously varying within a certain range (for a comparison of the shape and morphology of silica particles and of organic and silica monoliths see Fig. 5 [60]). Therefore, it is at present impossible to formulate a general model analogous to that for silica monoliths, in which the pore sizes are more rigorously defined, and thus the description of the properties of organic polymeric monoliths and their prediction are much more empirical and subject to greater uncertainty. Individual parameters of such monoliths must be studied individually for various polymer types.

It has been found that the separation efficiency and selectivity are virtually independent of the method of initiation of polymerization of the poly(butyl methacrylate) monolith [59]. However, the retention does depend on it, as demonstrated, e.g., on the retention of hydrophobic aromates on this monolith which is greater for thermal initiation, compared to chemical initiation. Chemical initiation leads to a 10% decrease in the Walters hydrophobicity index, defined as the ratio of the retention factors of anthracene and benzene in the mobile phase of 65:35 (v/v) acetonitrile–water (Fig. 6). The hydrophobicity can basically be controlled by changing the length and/or the density of the alkyl-chain, while permeability of a rigid polymer depends on the composition and the amount of the porogenic solvent.

A number of published works deal with testing of columns in terms of standard experimental parameters, such as the separation efficiency, optimum linear flow rate, HETP, time of analysis, pressure gradient, etc. For example, important chro-
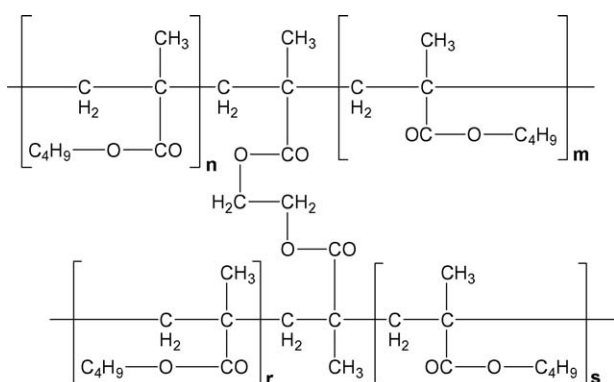

Fig. 4. Chemical structure of a medium polar poly(butyl methacrylate) monolith.
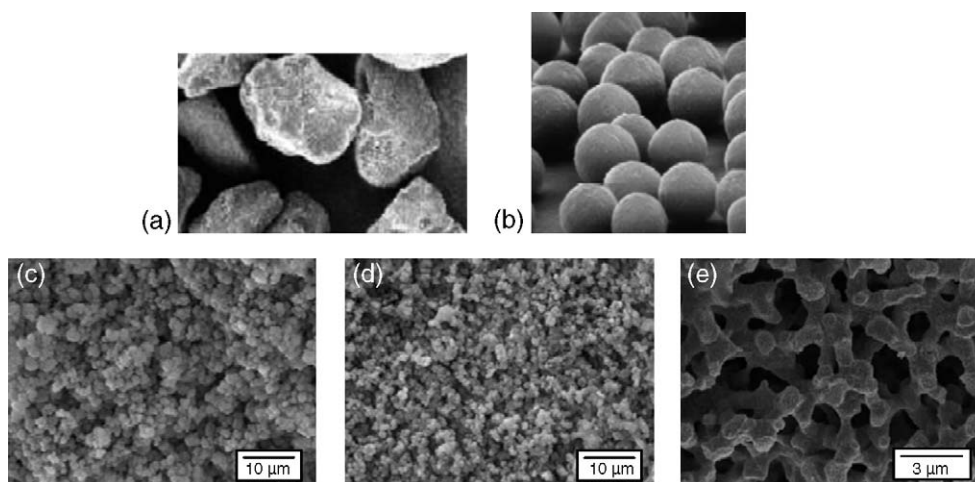
Fig. 5. Scanning electron microscopy images of different types of porous chromatographic materials: (a) irregularly-shaped silica particles; (b) spherical silica particles; (c) organic polymer monolith A (UNO S); (d) organic polymer monolith B (CIM Disk); and (e) silica-based monolith (Chromolith). Reproduced with permission from [60].
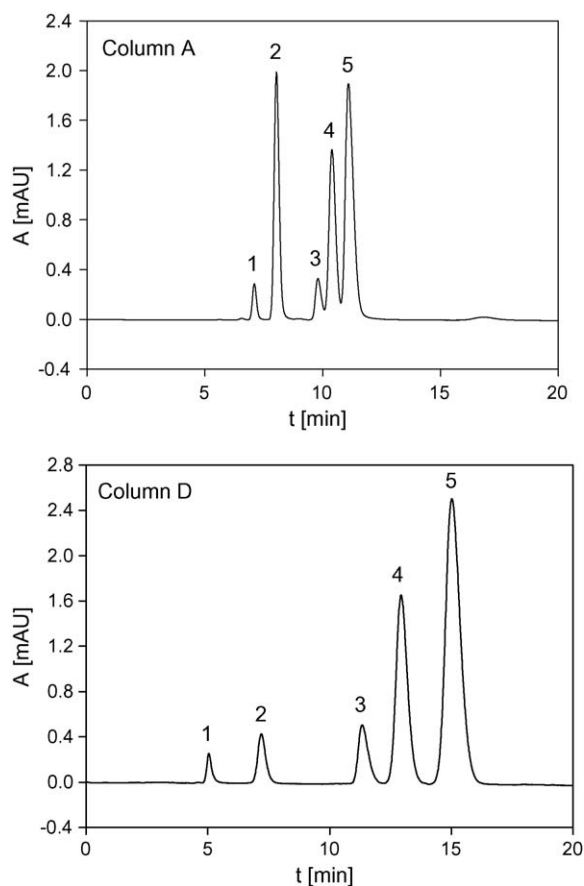

Fig. 6. Chromatograms of the separation of uracil (1), phenol (2), benzene (3), toluene (4) and ethylbenzene (5) on columns A (chemically initiated by ammonium peroxodisulfate) and D (thermally initiated by α,α'-azobisisobutyronitrile). Poly(butyl methacrylate) monolithic column of 320 μm I.D.; mobile phase, acetonitrile–water (65:35, v/v); flow rate, 2 μl/min; detection, 214 nm. Reproduced with permission from [59].

matographic parameters of monolithic capillary columns prepared by copolymerization of styrene and divinylbenzene inside a 200 μm I.D. fused silica capillary using a mixture of tetrahydrofuran and decanol as the porogen have been characterized and critically compared with the properties of columns of the same dimensions, packed with microparticulate, octadecylated poly(styrene-co-divinylbenzene) (PS–DVB–C18) particles (Table 1) [44]. The permeability of the monolithic column is slightly higher than that of the PS–DVB–C18 packed one and invariant up to an inlet pressure of 250 bar, indicating its high pressure stability. Different permeabilities of this monolithic column in different solvents indicate the monolith swelling. Interestingly, monolithic columns exhibit a 3.6 times better separation efficiency for oligonucleotides than microparticulate ones. To study differences in the molecular diffusion processes occurring in microparticulate and monolithic columns, van Deemter plots have been used in the above paper. All kinds of diffusional band broadening are reduced two to five times in monolithic columns, due to their favourable pore structure. Using inverse size-exclusion chromatography with tetrahydrofuran and polystyrene standards, a total porosity of 70% has been determined for the monoliths studied, consisting of 20% intraparticle and 50% interparticle porosity. The observed fast mass transfer and the resulting high separation efficiency suggest that the surface of the monolithic stationary phase is rather rough and does not contain large pores accessible to macromolecular analytes such as polypeptides or oligonucleotides. The maximum analytical loading capacity of monolithic columns for oligonucleotides has been found in the region of 500 fmol, which is analogous to the values for microparticulate columns. Batch-to-batch reproducibility obtained with commercial microparticulate stationary phases is better than that obtained with monolithic stationary phases, as the latter are prepared individually.

Organic polymer monoliths are especially suitable for separations of large solutes, e.g., proteins, primarily because of rapid mass transfer within the column. The performance of nano-LC-monolithic columns based on polystyrene has been compared

Table 1

Comparison of monolithic and microparticulate columns based on styrene–divinylbenzene copolymer (I.D. 200 μm, 60 mm long, monolithic column is prepared in situ, microparticulate column is octadecylated (commertical packing DNASep, particle size 2.1 μm) Data taken from [44]

Parameter	Monolith	Microparticulate
Pressure drop at flow rate of 3 μl/min (bar)	90–120	150
Batch-to-batch reproducibility (RSD of retention times of 7 homologous oligothymidylic acids in size from 12 to 18 nucleotides (%))	9.5	4.2
Run-to-run reproducibility of 17 columns (RSD of retention times of 7 homologous oligothymidylic acids in size from 12 to 18 nucleotides (%))	0.5–3	0.3–3
Average retention times	−24 s compared to microparticulate	
Specific permeability B_0 (m^2)		
Acetonitrile	2.9×10^{-15}	
Water	3.5×10^{-15}	
H_{min} (μm) at optimal flow rate	8.6 at 0.97 μl/min.	30.8 at 0.59 μl/min.
Parameteres of van Deemter curves		
A (μm)	3.0	15.7
B (μm/(mm/s))	0.9	3.6
C (μm/(mm/s))	6.1	13.5
Loading capacity for oligonucleotide (dT)$_{16}$	500 fmol, i.e., 2.4 ng	500 fmol, i.e., 2.4 ng
Porosity		
Intraparticle porosity, ε_p	0.19	0.185
Interparticle porosity, ε_i	0.52	0.285
Total porosity, ε_T	0.71	0.47
Average pore diameter (mm)	55	25
Specific surface (m^2/g)	43	96

$\varepsilon_p = V_p/V_c$; V_p, pore volume, V_c, volume of the empty separation column; $\varepsilon_i = V_i/V_c$; V_i, interstitial volume; $\varepsilon_T = \varepsilon_p + \varepsilon_i$.

with that of a Vydac-C8 particulate column with 30 nm pores, which is commonly used in protein separations [61]. Nano-LC polystyrene monolithic columns have been found to be preferable to conventional phases in analyses of protein molecules, because of a one-step fabrication process, faster analysis times and lower limits of detection.

A certain problem of organic polymer monoliths lies in the great dependence of their properties on the composition of the polymerization mixture. The effect of the content of co-monomeric ligand in the preparation of an acrylamide monolith in a capillary has been studied in detail (Table 2) [62]. The spe-

Table 2

Data for porous acrylamide monoliths in dependence on the co-monomer content

	Co-monomer content (%)		
	0	25	50
Total cumulative pore volume (ml/g), V_g	1.27	0.28	0.19
Specific surface area (m^2/g), S_g	73.9	35.9	0.7
Total porosity (%)	62.3	26.7	19.6
Mean pore radius (nm)	34.4	15.9	7.4
Apparent (mercury) density (g/cm^3)	4.8	1.8	2.1
Bulk (mercury) density (g/cm^3)	0.67	1.20	1.49
Weight swelling ratio, q_w	9.0		
Volume swelling ratio, q_v	6.9		
Capacity factor for G$_7$	0.74	0.75	0.79
Separation efficiency for G$_7$ (theoretical plates/m)	152000	68000	26000

Monolith AAm/Bis (T 5%, C 60%, TRIS-AAm/Bis x%), 60% of acetonitrile. Reproduced with permission from [62]. N-[tris(hydroxymethyl)methyl]-acrylamide (TRIS-AAm), acrylamide (AAm) and N,N′-methylenebis-acrylamide (Bis), %T refers to the total monomer concentration (g/ml × 100) and %C refers to the degree of cross-linking, i.e., the Bis/AAm ratio (g/g × 100).

cific surface area (BET) of the acrylamide monolith is around $S_g = 74$ m^2/g which is comparable with common monolithic materials [63], similar to the total pore volume $V_g = 1.2$ ml/g, obtained by mercury intrusion porosimetry. In the dry state, the pores occupy around 62% of the total polymer volume. The globular character gradually disappears with increasing content of the TRIS-AAm copolymeric ligand in the polymerization mixture. The values of mass (q_w) and volume (q_v) swelling degrees provide information on the internal structure of the porous network: the greater the difference between q_w and q_v, the greater is the total pore volume in the monolith. In pure water, the acrylamide matrix increases its weight as much as nine times ($q_w = 9.0$) and its volume almost seven times ($q_v = 6.9$) compared to its dry state; therefore, the monolith expands almost twice in all directions (for the discussion of swelling see also [44]). The porosity calculated in the swollen state equals $P_s = 69\%$, but the real values may be different. Electron microscopic images in cryoscopic arrangement indicate that swelling of acrylamide monoliths leads to a mushroom structure, characteristic of macroporous hydrogels with communicating pores; the pore size is estimated at about 0.5 μm from the visual recording.

Butyl and lauryl acrylate polymer monoliths for CEC have been compared [64]. A small percentage of more hydrophobic lauryl acrylate monomer in the polymerization mixture leads to expected enhancement of the retention of neutral solutes. However, its greater content has led to a decrease in the retention, due to non-uniform polymerization. Methacrylate-based monolithic columns with various functional groups (butyl, lauryl, octadecyl and isobornyl) have been prepared and tested in micro-HPLC and CEC [53], obtaining a good reproducibility of the synthesis, a high bed homogeneity and a high separation efficiency (90,000

and 140,000 plates per metre for micro-HPLC and CEC, respectively). A high efficiency of various acrylate-based monoliths in CEC and nano-LC (more than 300,000 plates per metre) has been demonstrated [65], confirming that the mobile phase ionic strength may significantly affect the separation efficiency. The influence of the nature of the mobile phase organic modifier (ACN or methanol) on EOF, retention, efficiency, and selectivity has been studied.

In an effort to further improve monolithic column selectivity, molecularly imprinted polymers (MIPs) have been introduced [66]. In this first work, a template compound and an initiator were dissolved in a mixture of a functional monomer (methacrylic acid or 2-(trifluoromethyl) acrylic acid), a cross-linker (ethylene dimethacrylate), and porogenic solvents (cyclohexanol and 1-dodecanol), and the mixture was degassed and poured into a conventional stainless steel column. Polymerization was performed in situ in a water bath. On completion of polymerization, the template and porogenic solvents were washed away with methanol–acetic acid. The selectivity and efficiency of columns prepared in this way were still not particularly high and the hydrodynamic resistance of the columns was great. To improve the performance, a new in situ molecular imprinting polymerization process was introduced employing solvents of low polarities (toluene and 1-dodecanol) [67]. These stationary phases have larger pores and thus exhibit low back-pressures, leading to accelerated separation of enantiomers and diastereomers at elevated flow rates.

A MIP monolithic capillary column was first used for CEC, obtaining a higher separation efficiency than when using a MIP in HPLC [68]. The authors attained a better quality of imprinting by using a non-polar solvent, toluene, with UV initiation at a low temperature ($-20\,^{\circ}\text{C}$). It has been recommended to use high concentrations of monomers in the prepolymerization mixture to improve the solubility of the polar imprinted molecule and to reduce interference during the complex formation [69]. Imprinted monolithic columns for HPLC and CE were recently reviewed [70].

4. A few selected applications of monolithic columns in CLC and CEC

The applications of capillary monolithic columns in micro-HPLC and CEC are numerous and involve both low- and high-molecular solutes. Certain technical problems must be solved when monoliths are to be applied to real-life analyses (on the other hand, the monoliths do not require frits, necessary to prevent loss of stationary phase particles under the pressure of the mobile phase with packed columns). The monolith for CLC must be covalently bound to the capillary wall to prevent its ejection by the mobile phase [71]. This is attained by using silanization agents with double bonds that can subsequently take part in the radical polymerization and be built into the monolith structure. When using a monomer containing an oxirane ring in the polymerization mixture, the ring is hydrolyzed by the silanol groups on the capillary surface and is spontaneously attached to the surface. The monoliths for CEC need not always be attached to the capillary surface because the driving force, the electroosmotic

flow (EOF), is generated within the monolith and thus does not exert any external pressure on it.

Monoliths for CEC, in contrast to CLC monoliths, must usually be provided with ionizable functional groups capable of generating EOF [28]. This is attained by using reagents containing a double bond capable of radical polymerization and a sulfonic acid group undergoing dissociation. The dissociated sulfonic acid (or trimethylammonium) groups on the monolith surface then generate the zeta-potential leading to EOF. Because their dissociation constant is high, a sufficiently rapid and reproducible EOF can be obtained within a wide pH range, leading to rapid analyses even at low pH values. Sulfonic acid groups can also be chemically bonded to the surface of silica particles to provide a stable EOF, as shown in many mixed-mode phases ($\text{SO}_3\text{H/RP18}$). The generation of EOF in packed silica CEC columns is much more complicated and less reproducible, as the silanol groups, a weak acid, on the surface of silica particles dissociate only at pH values above ca. 5, are affected by many experimental factors and give rise to a slow EOF.

However, with charged solutes, ionizable functional groups need not always be present, as demonstrated, e.g., [31] on CEC using a neutral hydrophobic polymer, prepared by in situ copolymerization of lauryl methacrylate and ethylene dimethacrylate without any charged monomers in the reaction mixture for classification of basic drugs based on their acidity and charges.

Some authors [42,72,73] used the same monolithic column for separation of the same solutes by both CLC and CEC. They have found that the HETP values are lower in CEC and the corresponding van Deemter curves are flatter, so that the separation efficiency does not deteriorate even at higher mobile phase flow rates. The van Deemter curves are also flatter than those obtained with packed columns [74,75].

Important applications of monolithic columns for bioseparations have been reviewed [76]. For example, monolithic media are the best solution for CEC separation of viruses and bacteria. The diffusion of viruses into the pores of conventional media is extremely slow. In monoliths, large bacteria or viruses are transported by convection. Separation of bacteria has extensively been studied by Buszewski and his group (e.g., [77,78]).

Numerous and very important applications are in the field of analyses of medium-size and large biologically active molecules, typically peptides and proteins. A rapid gradient CLC separation of proteins on a home-made non-polar monolithic capillary column is depicted in Fig. 7 [42]. A monolithic organic polymer stationary phase was successfully applied to identification of peptides in complex mixtures using HPLC-ESI-MS/MS [79]. An exceptionally high performance attained with the $100\,\mu\text{m}$ I.D. monolithic column can be explained by a combination of the high chromatographic efficiency of the monolithic stationary phase with a remarkably small column I.D. Another application of monolithic columns concerns separation and detection of phosphorylated and nonphosphorylated peptides in liquid chromatography–mass spectrometry using acidic or alkaline mobile phases [80].

An efficient and rapid separation of peptides by CEC on an organic polymer monolith of medium polarity can be seen in Fig. 8 [55]. To identify proteins in proteomics, it is important
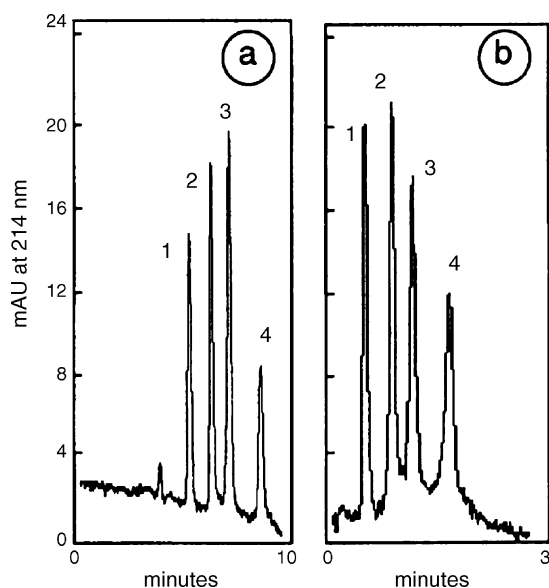
Fig. 7. A chromatogram of standard proteins obtained by CLC with a polystyrene monolithic capillary column (75 μm × 27/38 cm); linear gradient from 20 to 75% acetonitrile in water containing 0.1% trifluoroacetic acid; flow rate, 0.34 μl/s (a) and 1.5 μl/s (b). Peak identification: 1, ribonuclease A; 2, cytochrome C; 3, lysozyme; 4, β-lactoglobulin B (1 mg/ml of each in buffer). Reproduced with permission from [42].

to separate specific peptides obtained by trypsin digestion of the protein to be identified; the CEC separation can be carried out on a poly(vinylbenzyl chloride) monolithic capillary column, derivatized by *N*,*N*-dimethylbutylamine with formation of a tetraalkylammonium group positively charged and of hydrophobic *n*-butyl chains [81] (Fig. 9). The monolithic porous stationary phase was prepared in [81] in silanized fused silica capillaries of 75 μm I.D. by in situ copolymerization of vinylbenzyl chloride and ethylene dimethacrylate in the presence of propanol and formamide as the porogens. The chloromethyl

Fig. 9. Capillary electrochromatograms illustrating the separation of tryptic digest of cytochrome c obtained by isocratic elution at 25 and 55 °C. Column, 40 cm (effective length 30 cm) × 75 μm, fused silica with styrene-based monolith having quaternary ammonium functions. Mobile phase, 40% CH₃CN in 50 mM phosphate buffer, pH 2.5; applied voltage, −30 kV; detection, 214 nm. Reproduced with permission from [81].

groups at the surface of the porous monolith were reacted with *N*,*N*-dimethylbutylamine to form a positively charged chromatographic surface with fixed *n*-butyl chains.

An ion-exchange monolithic stationary phase, prepared by derivatization of poly(glycidyl methacrylate-co-divinylbenzene) with diethylamine [82] (Fig. 10) allows a fast and highly efficient separation of a homologous series of phosphorylated oligothymidylic acids [d(pT)12–18]. Analysis of biological and synthetic ribonucleic acids by liquid chromatography–mass spectrometry using monolithic capillary columns has been published [83].

CEC with a methacrylate-based monolithic column has successfully been applied to rapid separations (less than 8 min.)
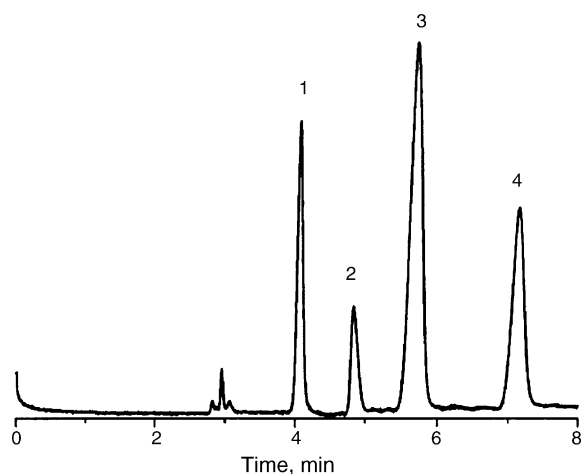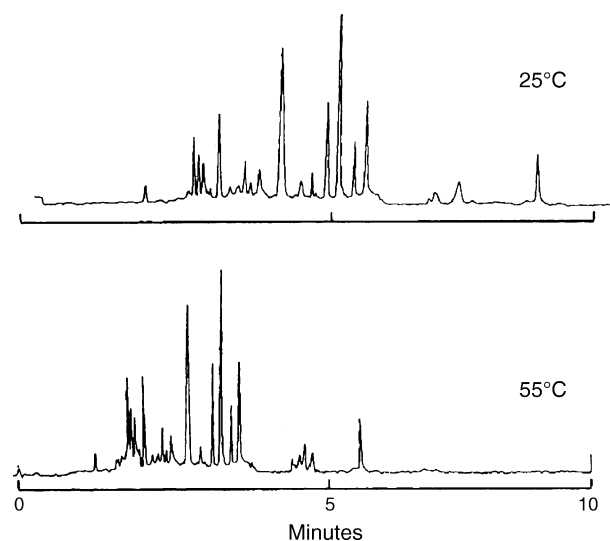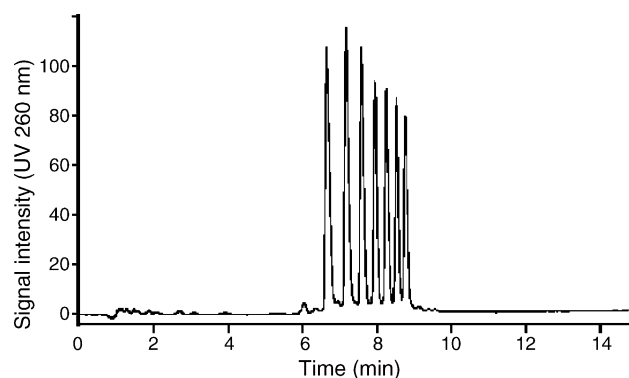

Fig. 8. Electrochromatographic separation of 1, Gly-Tyr; 2, Val-Tyr; 3, methionine enkephalin, and 4, leucine enkephalin on a monolithic capillary column with a pore size of 492 nm. Conditions: mobile phase, 10% of aqueous 10 mmol/l sodium 1-octanesulfonate and 90% of a 2:8 mixture of 5 mmol/l phosphate buffer, pH 7.0, and acetonitrile; UV detection, 215 nm; total sample concentration, 1 mg/ml. Reproduced with permission from [55].

Fig. 10. Separation of phosphorylated oligothymidylic acids (d(pT)12-18) on poly(3-diethylamino-2-hydroxypropylmethacrylate-co-divinylbenzene) monolithic capillary column. Experimental conditions: column, 80 mm × 0.2 mm I.D.; mobile phase, A, 20 mM KH₂PO₄, 20% ACN, pH 7.0; B, 1 M NaCl in A; gradient, 25–55% B in 2 min, 55–100% B in 7 min; flow rate, 2.3 μl/min) 1; room temperature; detection, UV, 260 nm. Reproduced with permission from [82].
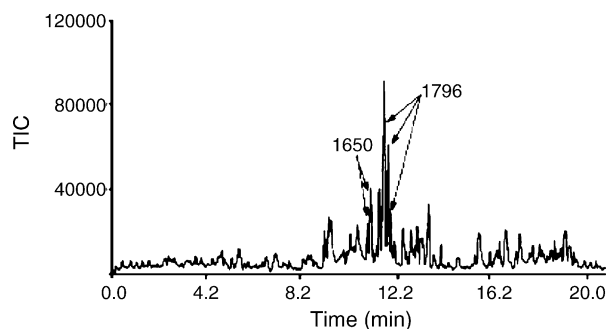
Fig. 11. A mass electrochromatogram of a complex fraction of the *O*-linked glycans chemically released from bile-salt-stimulated-lipase. Experimental conditions: monolithic amino column 28 cm, field strength, 500 V/cm, mobile phase, acetonitrile–water–ammonium formate buffer (240 mmol/l, pH 3.0, 55:44:1, v/v/v). ESI-Ion Trap MS–MS: the sheath liquid, containing 1% formic acid and 1 mM sodium acetate in the acetonitrile–water mixture (50:50, v/v), was introduced at a flow rate of 0.5 μl/min for an effective electrospray ionization of carbohydrates in the positive-ion detection mode. Tandem mass spectrometry was performed using the reverse-then-forward scan. Collision-induced dissociation (CID) of the ions formed during the MS/MS experiments was performed at $q_z = 0.2$. Reproduced with permission from [85].

of polyphenols, flavanones and flavanones-7-*O*-glycosides [84]. The chemical and mechanical stabilities of the monolithic column over a wide range of buffer pH (2–10) and time are satisfactory.

An application of a monolith to separation of *O*-linked glycans chemically released from bile-salt-stimulated-lipase, with mass spectrometric detection, is shown in Fig. 11 [85]. Highly permeable methacrylate-based monolithic stationary phases of different hydrophobicity have been prepared, using thermally initiated radical polymerization of methacrylate ester monomer with different alkyl-chains (C2, C4, C6, C12, C18) and ethylene dimethacrylate (EDMA), and applied to rapid reversed-phase liquid chromatographic separation of alkylbenzenes at high flow rates and an elevated temperature [86].

5. Conclusions

All the aspects discussed above can be summarized as follows:

- Introduction of monolithic columns is a highly innovative contribution to liquid chromatography and electrochromatography.
- Monolithic capillary columns permit separations of solutes of any size and polarity; they are especially suitable for separations of medium-sized and large solutes, typically peptides, proteins and glycoproteins, as well as synthetic polymers and microorganisms.
- A great advantage of monoliths lies in their relatively simple and cheap preparation in the laboratory; this provides possibilities of tailoring stationary phases for particular tasks.
- Monoliths permit rapid analyses at low pressure gradients, preserving a sufficiently high separation efficiency and a good selectivity; the reliability is satisfactory.

- Separations on capillary monolithic phases are suitable for inclusion in multidimensional techniques.
- CEC with monoliths brings many advantages, especially in enhanced separation efficiency and selectivity.
- Monoliths can be prepared in various shapes, such as rods, disks, tubes, etc., depending on the purpose.
- Silica-based monoliths are reproducible; their performance can directly be compared with silica particulate phases and described by a general model.
- Organic polymer monoliths are described much more empirically, there may be problems with reproducibility of their properties, but they offer great possibilities for tailoring stationary phases for given purposes.
- Some monoliths cannot be exposed to high pressures (1–5 MPa for most of organic ones and up to 20 MPa for silica-based ones), consequently, there may be problems with adhesion of the monolith to the capillary wall.
- Low back-pressures in monolithic columns cause some problems with radial sample injection.
- Swelling of organic polymer monoliths brings some limitations, especially in the selection of the mobile phase composition.

Finally: there is room for both packed and monolithic columns. They must be chosen on the basis of detailed consideration of all aspects of the problem in hand. The young monolithic columns bear great promise for the future but will require further work to obtain sufficient experience in tackling the tricky problems presented to analysts by their scientific and technological colleagues.

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