Monolithic Phases for Ion Chromatography

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Abstract

Monolithic media are continuing to increase in popularity in chromatographic applications, and the ongoing use of commercially available materials in ion chromatography (IC) has made monoliths a viable alternative to packed-bed columns for routine use. We discuss different strategies for the synthesis of polymeric and silica monoliths with ion-exchange functionality, such as direct incorporation of ion-exchange functionality during monolith preparation and different postpolymerization alterations such as grafting and coating. The formulations and strategies presented are focused on materials intended for use in IC. We also discuss strategies for materials characterization, with emphasis on nondestructive techniques for the characterization of monolith surface functionality, especially those with applicability to in situ analysis. Finally, we describe selected IC applications of polymeric and silica monoliths published from 2008 to 2010.

1. INTRODUCTION: ION CHROMATOGRAPHY

IC: ion chromatography

Ion chromatography (IC) is a modern form of ion-exchange chromatography in which charged analytes are retained and separated. The charged analytes are adsorbed electrostatically onto a stationary phase carrying charge functionalities of the opposite polarity and are thereby retained. Elution is achieved through displacement of analyte species by ionic species in the eluent that carry the same charge polarity as the analyte. IC is a very versatile chromatographic mode that is used for the separation of analytes ranging from small ionic species to large biomacromolecules. Early work in IC showed great success in separating small inorganic ions, and the introduction of suppressors for conductivity detection led to a large advance in the field. However, the inherent "softness" of the eluents typically used in IC, and the nondenaturing conditions at which the separations take place, have made IC a popular technique in the modern bioanalytical laboratory (1, 2). In addition, IC has become increasingly popular in two-dimensional separations, thanks to its orthogonal selectivity compared with that of reversed-phase (RP) chromatography (3, 4).

As with all chromatographic separations, the end result for any given analyte depends on a large set of factors, including the column and eluent used. The choice of eluent composition depends on the combination of column and detection mode and also varies with the analyte or application. For the analysis of inorganic anions using high-capacity anion-exchange columns and suppressed conductivity detection, hydroxide or carbonate eluents are often utilized. In protein analysis using UV detection, the eluent is typically a high–ionic strength salt-containing buffer. Many possible modifications can be made to an eluent system with the aim of optimizing or altering a separation, but without a suitable interaction between the analyte and the stationary-phase material, the separation cannot take place. Hence, although many chromatographic supports for IC are available, there remains a need for further development. In general, the suitability of a chromatographic stationary-phase material depends on a combination of suitable porosity and surface area, as well as on the type and extent of surface functionality. In IC, a charged surface functionality is necessary to allow analyte–stationary phase interactions. Commonly used ion exchangers in IC applications include both silica-based and polymeric support materials, and their surface charge can originate from a number of different functionalities. However, it is not only the charge on these functionalities that is important; different functionalities that have the same nominal charge frequently provide large differences in the strength of the analyte–stationary phase interactions. The characterization of the surface functionality is therefore a key component in column selection and optimization of any given IC separation.

2. MONOLITHS: BENEFITS AND DRAWBACKS

As is the case for other chromatographic modes, monolithic stationary phases have found increasingly widespread application in IC. A monolith is a one-piece entity, which in chromatography usually translates to a single piece of porous polymer or silica used as a chromatographic stationary phase. Although the name monolith did not gain popularity until the mid 1990s, polymer-based continuous supports had already surfaced in the late 1980s under the name continuous-bed supports (5), as well as in the early 1990s under the similar name continuous-rod materials (6). The silica-based counterpart of these supports was introduced at around the same time (7, 8, 9). The use of monolithic media in IC began with the introduction of monolithic media in general, and the first polymeric monoliths presented were used in IC of proteins (5, 6).

These early polymeric monoliths with ion-exchange functionality exemplify two of the major approaches used to impart ion-exchange functionality to a monolith; both approaches are described in more detail below. The cation-exchange functionality of the continuous bed arose from the

monomers (acrylic acid and *N,N′*-methylenebisacrylamide) used in its preparation (5), and the reaction of surface epoxy groups with diethylamine was used to introduce anion-exchange surface functionality to a methacrylate-based monolith (6). In the first applications of a silica monolith for IC, the ion-exchange functionality was imparted onto the silica monolith through the use of semipermanent coating with surfactants. In an early example, the separation of inorganic anions was realized on an RP (C18) monolithic column coated with didodecylmethylammonium bromide (10).

Although they are prepared in different ways and contain vastly different chemistries, polymer-based and silica-based monoliths have some distinct similarities. The greatest advantage of both silica and polymeric monoliths relates to their high porosity and continuous structure. Both monoliths have large through-pores that, when used in flow-through mode, result in convective mass transfer, which differs from the diffusion-governed mass transfer in packed-bed columns. This convective mass transfer usually ensures that, in a chromatographic separation, efficiency is not significantly reduced when the flow rate is increased (11, 12). The large through-pores also allow the monolithic columns to be operated at relatively high flow rates without a correspondingly high back pressure. Together, these characteristics make monolithic columns ideal for use in high-throughput applications.

Another distinct advantage of monolith media is the possibility of in situ manufacturing, which allows a monolith to be prepared in its column housing without the need for a packing procedure or retaining frits. This advantage is especially important in microchip channels or narrow-bore capillaries, into which packing of particulate separation materials can be difficult. However, although such in situ synthesis has been demonstrated for formats ranging from capillaries (13) to normal-bore columns (6) for polymeric monoliths, the silica monolith can essentially only be prepared in situ in capillaries (14). This is because of the substantial shrinkage that accompanies the formation of a silica monolith (15). For larger-bore columns, the silica monolith is prepared in a mold, and once prepared, it is transferred to its column housing or is shrink-clad in tubing such as polytetrafluoroethylene (16).

Other advantages of monolithic materials are specific to each type of monolith. The same is true for some of their disadvantages; in some ways, these monolithic media have characteristics that are complementary to each other. For example, the advantages of the polymeric monoliths include their mechanical stability and high tolerance of a wide range of pH conditions. A distinct disadvantage of silica-based materials is their pH instability, which renders them incompatible with both acidic and alkaline eluents. The simplicity of preparation is another key advantage of the polymeric monoliths, as is the degree of control of the material's physical characteristics that can be achieved through variation of the composition of the polymerization mixture and the polymerization conditions. Possible disadvantages include potential swelling or shrinkage of the monolith, the extent of which depends on the composition of the monolith as well as on the solvents used in a chromatographic application.

A further drawback of the polymeric monoliths is the relatively low surface area that arises directly from the high porosity of these materials. Polymeric monoliths show lower separation efficiencies than do modified silica monoliths, especially for small molecules and ions. Generally, silica monoliths perform best for small molecules, and polymeric monoliths perform best for large molecules. This finding can be illustrated by the applications for which the two monolith types have been used (**Table 1**). The reasons for this difference lie in the structure of the respective monoliths. Silica monoliths have a porous structure with rod-like features (**Figure 1*a***), whereas traditional polymeric monoliths are composed of microglobular structures (**Figure 1*c–e***). The skeletons of polymeric monoliths typically contain macropores and micropores, whereas silica monoliths contain macropores and mesopores. These mesopores are accessible for analyte-surface interaction, unlike the micropores in polymer monoliths. For large analytes, such as proteins, this is

Table 1 Selected applications of monoliths in ion-exchange chromatography

Monolith	Modification(s)	Ion-exchange chromatography mode(s)	Applications/analytes	Reference
Silica				
Hybrid silica VTMS-*co*-TMOS-*co*-MPAC	—	SAX	Enrichment of phosphopeptides prior to MALDI TOF MS	79
Hybrid silica	Grafting with different monomers for AX functionality	WAX	Nucleotides and inorganic anions, protein separation, tryptic digestion of BSA	89
TMOS-*co*-MTMS	DMAPAA-Q, DMAEA-Q, DMAPAA, DMAEA, DAHMA, HMPMA With and without MAS anchor group	SAX		
Hybrid silica TEOS-*co*-AEAPMDMS	None	WAX	Extraction of genomic DNA from blood from the crucian carp	120
Hybrid silica	On-column copolymerization of DMAPAA-Q	WAX	LC and CEC of inorganic anions and nucleotides	87
Polymeric				
Bio-Monolith QA	—	SAX	Adenovirus type 5 particles Separation from proteins and DNA	125
CIM mini-DEAE	—	WAX	Fast column switching SEC × IC ICP MS, albumin and transferrin cisplatin adducts	122
CIM QA	—	SAX	Concentrating rotaviruses from water samples	126
DNASwift SAX-1S	—	SAX	Identification of RNA linkage isomers from phosphodiesterase II digests	127
ProSwift^{TM} WCX	—	—	Online peptide enrichment coupled to MS	128
UNO Q	—	—	One-step purification of β-1,3–1,4-glucanase from the fungus *Laetiporus sulphureus* var. *miniatus*	129
GMA-*co*-EDMA	Two-step photografting: PEGMA plus AMPS or acrylic acid	SCX WCX	Separation of proteins and peptides Protein digest	57
GMA-*co*-EDMA	Polyethyleneimine modified Polyethyleneimine with molecular weights of 0.6, 2, 20, 30, and 100 kDa	AX	Separation of lipase isoforms from *Candida* spp. 99–125	121
GMA-*co*-EDMA	UV grafted with META	SAX	Separation of inorganic anions	117

(*Continued*)

Table 1 (*Continued*)

Monolith	Modification(s)	Ion-exchange chromatography mode(s)	Applications/analytes	Reference
DEAEMA-*co*-PEGDA	None	WAX	Protein separation	42
AETAC-*co*-PEGDA	None	SAX	Analysis of protein fraction from *Escherichia coli* DH5 lysate	
GMA-*co*-PEGDA	Chemical modification			
NBE-*co*-DMN-H6	Living grafting of ONDCA	WCX	Separation of standard peptide mixture	59

Abbreviations: AEAPMDMS, *N*-(*b*-aminoethyl)-*c*-aminopropylmethyldimethoxysilane; AETAC, 2-(acryloyloxy)ethyl trimethylammonium chloride; AMPS, 2-acrylamido-2-methyl-1–propanesulfonic acid; AX, anion exchanger; BSA, bovine serum albumin; CEC, capillary electrochromatography; CIM, convective interaction media; DAHMA, 3-diethylamino-2-hydroxypropyl methacrylate; DEAEMA, 2-(diethylamino)ethyl methacrylate; DMAPAA-Q, *N*-[3-(dimethylamino)propyl]acrylamide methyl chloride–quaternary salt; DMN-H6, 1,4,4a,5,8,8a-hexahydro-1,4,5,8-exo,endo-dimethanonaphthalene; EDMA, ethylene glycol dimethacrylate; GMA, glycidyl methacrylate; HMPMA, 2-hydroxy-3-(4-methylpiperazin-1-yl)-propyl methacrylate; ICP, inductively coupled plasma; LC, liquid chromatography; MALDI TOF MS, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry; MAS, 3-methacrylamidopropyltriethoxysilane; META, [2(methacryloyloxy)ethyl] trimethylammonium chloride; MPAC, [3-(methacryloylamino)propyl] trimethylammonium chloride; MTMS, methyltrimethoxysilane; NBE, norborn-2-ene; ONDCA, 7-oxanorborn-2-ene-5,6-carboxylic anhydride; PEGDA, poly(ethylene glycol) diacrylate; PEGMA, PEG methacrylate; SAX, strong anion exchanger; SCX, strong cation exchanger; SEC, size-exclusion chromatography; SPMA, sulfopropyl methacrylate; TEOS, tetraethoxysilane; TMOS, tetramethoxysilane; VTMS, vinyltrimethoxysilane; WAX, weak anion exchanger; WCX, weak cation exchanger.

not a large drawback, but the limited surface area significantly limits the applicability of polymeric monoliths for the separation of small analytes. Silica monoliths are therefore more advantageous for such applications. A recently devised strategy that has been tested to improve the usefulness of polymeric monoliths for small-molecule separations is the use of postpreparation hyper-cross-linking, which substantially increases the surface area (17).

In addition to simple polymer- and silica-based materials, numerous other organic and inorganic materials have been prepared as monoliths and used in chromatographic applications. These include titania-based monoliths (**Figure 1*b***), cryogel materials (**Figure 1*g***), and high–internal phase emulsion polymers (**Figure 1*b***). Below, we briefly describe the general manufacturing of these monolithic materials. Some strategies are employed for both silica and polymeric monoliths, but there are distinct differences in the preparation of ion-exchange materials that are mostly due to the characteristics of the support materials themselves. Some of the strategies used for monolith functionalization have been adopted from the developments made in particle modification, whereas others have been developed specifically for monolithic media.

3. PREPARATION OF POLYMERIC MONOLITHS

The general procedure used in the preparation of a polymeric monolith is illustrated in **Figure 2*a***. The process begins with a polymerization mixture composed of a mixture of monomers, pore-forming solvents, and an initiator. This mixture is transferred to a mold, such as a column or capillary, and is then polymerized. Most polymerizations are radical polymerizations induced either with UV irradiation (18) or with heat (19, 20). In addition, initiation using γ radiation (21) or microwave irradiation (22) has been demonstrated. Monolithic media described as cryogels are also prepared by free radical polymerization, but the polymerization is performed at subzero temperatures through the use of ice crystals as the pore-forming elements (23). Apart

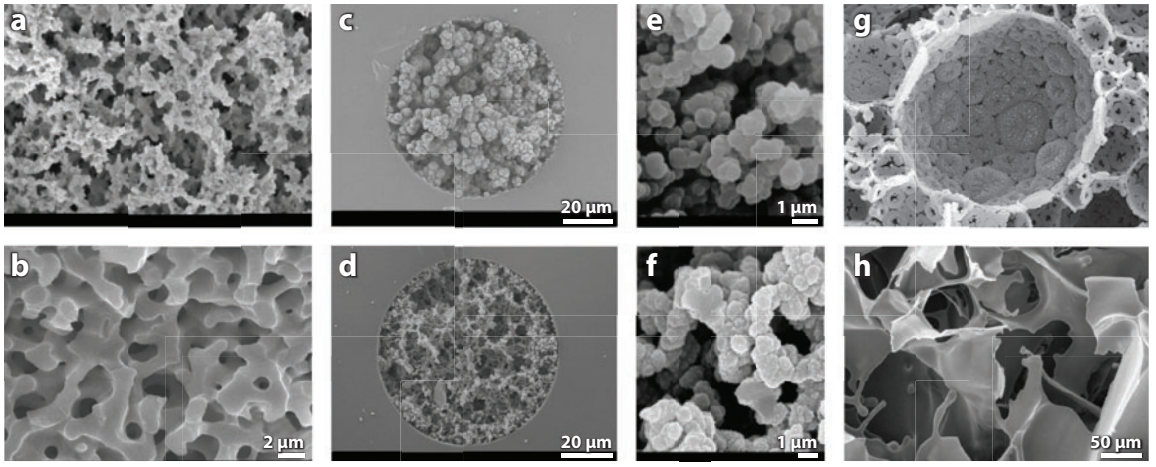

Figure 1

Scanning electron micrograph (SEM) images of monolithic materials. (*a*) SEM photographs of 100-μm monolithic silica columns (MOP-DMAPAA-Q) at 2,500× magnification. Reprinted with permission from Reference 87. (*b*) Titania monolith. SEM images of dried gels prepared with *f* 0.775. The dried gels were derived via the aging treatment at 60°C. Reprinted with permission from Reference 130. (*c*) SEM photographs of a poly(SEMA) monolith. Reprinted with permission from Reference 38. (*d*) Polymeric monolith. SEM photographs of poly(VS) monoliths. Reprinted with permission from Reference 38. (*e*) Polymeric monolith. SEM image of a BuMA-*co*-EDMA-*co*-AMPS monolith. Reprinted with permission from Reference 62. (*f*) Polymeric monolith E coated with latex. SEM image of a latex-coated BuMA-*co*-EDMA-*co*-AMPS monolith. Reprinted with permission from Reference 62. (*g*) SEM image of GMA-EGDMA polyHIPE at 3,000× magnification. HIPE has a 90% pore volume. Reprinted with permission from Reference 131. (*h*) SEM of pDMAEMA-grafted pAAm cryogel (6%). The cryogel samples were fixed in 2.5% glutaraldehyde in 0.12 M sodium phosphate buffer, pH 7.2, overnight. The samples were then dehydrated in ethanol (0%–50%–75%–99.5%) and critical-point dried. Reprinted with permission from Reference 53. Abbreviations: AMPS, 2-acrylamido-2-methyl-1–propanesulfonic acid; BuMA, butyl methacrylate; EDMA, ethylene glycol dimethacrylate; EGDMA, ethylene glycol dimethacrylate; GMA, glycidyl methacrylate; HIPE, high–internal phase emulsion; DMAPAA-Q, *N*-[3-(dimethylamino)propyl] acrylamide methyl chloride–quaternary salt; pAAm, polyacrylamide; pDMAEMA, poly[2-(dimethylamino)ethyl] methacrylate; SEMA, sulfoethyl methacrylate; VS, vinylsulfonic acid.

from radical polymerizations, several other polymerization types have been used in the preparation of polymeric monoliths; these include nitroxide-mediated living free radical polymerization (24, 25) and ring opening metathesis polymerization (ROMP), in which a metal catalyst is used to initiate polymerization (26). The use of both living free radical polymerization and ROMP has the advantage that the initiator remains on or within the material, which allows the additional possibility of postpolymerization modification (25–27). Another recently presented initiation approach, which also allows continued living polymerization initiated from the monolith surface, is atom-transfer radical polymerization (ATRP) (28). Emulsion polymerization is another approach by which epoxy-based materials have recently been prepared (29–31). In a thermally induced dissolution/precipitation approach, monolithic media have been prepared from linear aliphatic polyamides (32), including polyamide sourced from fishing line (33).

Monomers included in the polymerization mixture contribute to the resulting functionality of the polymer monolith. The common general requirement for monomers used in radical polymerizations is the presence of polymerizable vinyl groups, which allow styrene-, methacrylate-, and acrylamide-based materials to be prepared. Through use of the ROMP approach, the range of possible monomers, and therefore the range of attainable materials, is restricted because cyclic olefins such as norborn-2-ene and cyclooctene, which has a substantial ring strain, are required (34). In the polymerization mixture, pore-forming solvents aid in the formation of the porous structure

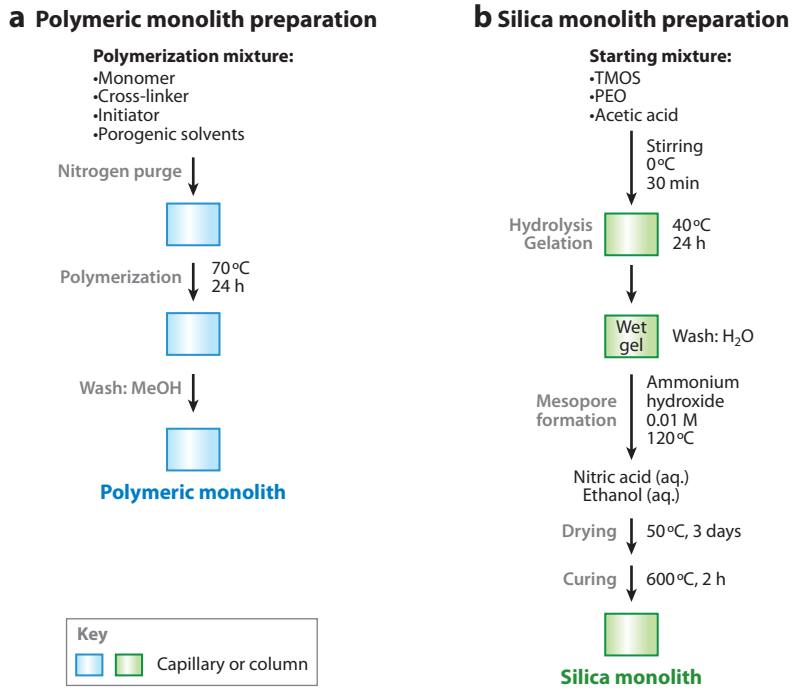

Figure 2

The preparation of (*a*) polymeric and (*b*) silica monoliths. Abbreviations: PEO, poly(ethylene oxide); TMOS, tetramethoxysilane.

of the monolith, and a mixture of solvents is often used to control the resulting porous properties of the monolith. The final characteristics of the formed monolith depend on the starting polymerization mixture and the conditions at which polymerization was performed (35). Although the extent to which all of the different factors affect the monolith formation is not completely known, some factors have been studied in considerable detail. Readers interested in the factors affecting polymeric monolith formation should refer to Reference 36, in which most of the findings are summarized systematically.

3.1. Polymeric Monoliths in Ion Chromatography

When preparing a polymeric monolith for use in IC, one can introduce ion-exchange functionality in one of many ways, some originating from earlier work performed on particulate materials and some having been developed solely for monoliths. All of these approaches rely either on the direct incorporation of functionality during polymerization of the monolith or on some form of postpolymerization modification. Examples of strategies used for the preparation of polymeric monolithic ion-exchange stationary phases are illustrated in **Figure 3*a***.

3.2. Functionality by Copolymerization

In a monolith, the monomers form an integral part of the formed monolith; thus, their characteristics influence the resulting material characteristics, including surface functionality. The use

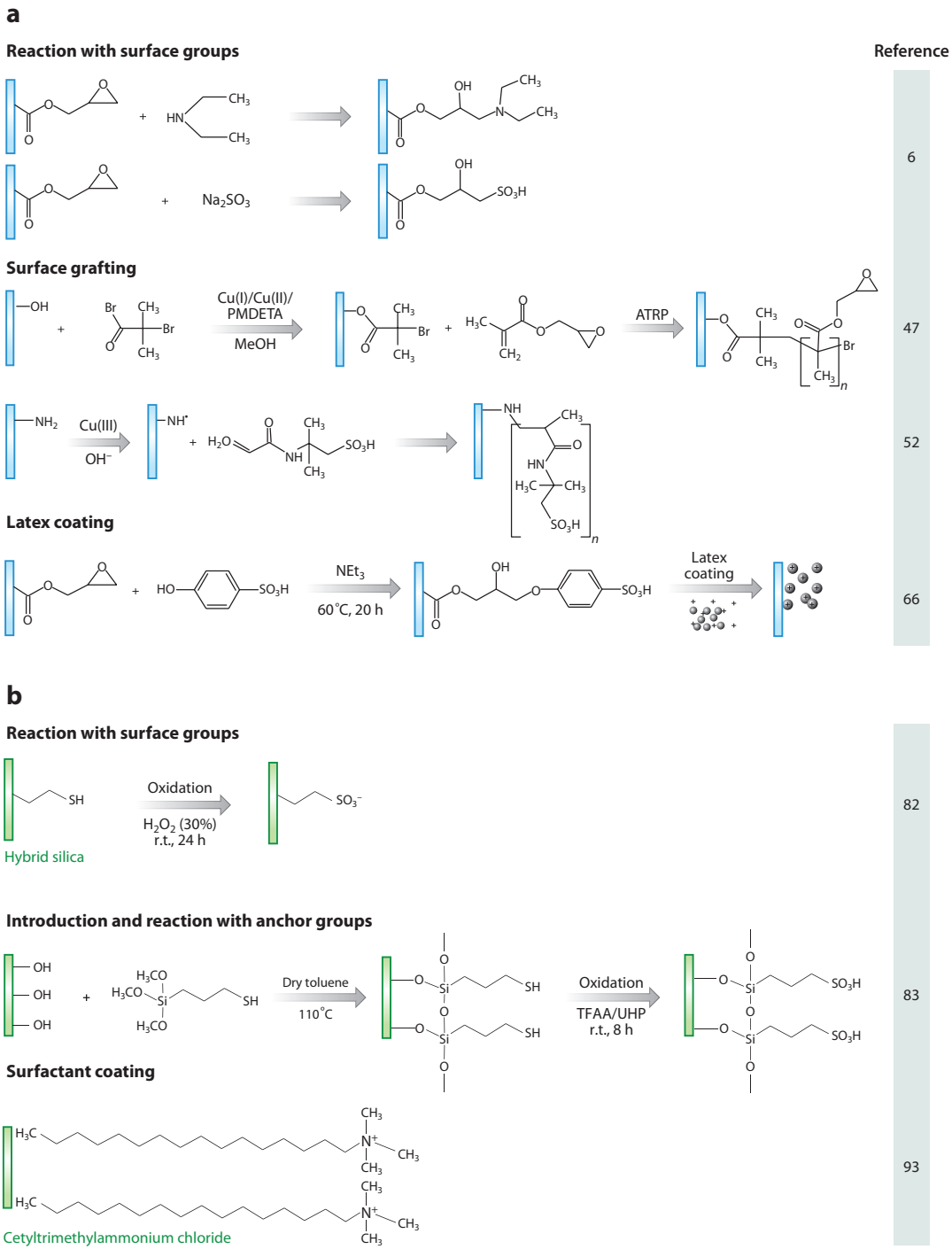

Figure 3

Functionalization strategies used in the preparation of monolithic ion-exchange chromatographic stationary phases. (*a*) Examples used for polymeric monoliths. (*b*) Examples used for silica and hybrid silica monoliths.

of copolymerization as a means of introducing ion-exchange functionality is a seemingly straightforward approach, as the outcome relies only on the functionality of the functional monomer. The advantage of this approach is that no subsequent modifications are necessary. The large number of available monomers suggests almost endless possibilities, but although this approach is straightforward and convenient, it also has drawbacks. When altering the monomers used in the polymerization mixture to introduce a desired functionality, a renewed optimization of the polymerization conditions—including the components and amounts used in the polymerization mixture—is needed. This optimization sometimes involves a relatively simple adjustment to a porogenic solvent composition, but this is not always the case. The factors affecting the properties of the resulting materials are often interlinked, which can make the optimization of a monolithic material a complicated task. In addition, because both the monomers and cross-linkers become part of the final monolithic structure, not all of the added functionality becomes available on the surface of the monolith. The amount of functionality obtained at the surface is important to know, but it is extremely difficult to predict.

AMPS: 2-acrylamido-2-methyl-1–propanesulfonic acid

Monomers that have been used for the incorporation of ionic or ionizable anionic functionalities in the preparation of cation-exchange materials for IC or capillary electrochromatography (CEC) include 2-acrylamido-2-methyl-1–propanesulfonic acid (AMPS) (37), sulfoethyl methacrylate (SEMA) (38), and vinylsulfonic acid (VSA) (38), as well as phosphoric acid 2–hydroxyethyl methacrylate (PAHEMA) (39), bis[2-(methacryloyloxy)ethyl] phosphate (BMEP) (39), and ethylene glycol methacrylate phosphate (40). Monomers used for the preparation of anion-exchange materials include methacryloyloxyethyl trimethylammonium chloride (41), 2-(diethylamino)ethyl methacrylate (42), and 2-(acryloyloxy)ethyl trimethylammonium chloride (42).

In ion-exchange separations of biological molecules, such as proteins or peptides, the hydrophobicity of the monolithic material is likely to affect retention as a result of nonspecific interactions. Such interactions are undesirable because they can cause peak broadening or inactivation of biologically important compounds due to denaturation. Conventional polymeric monolith formulations typically yield substantially hydrophobic monoliths due to the hydrophobic nature of styrenic or methacrylate monomers and cross-linkers. To reduce the hydrophobicity of these materials, investigators have explored the use of cross-linkers that are expected to be more hydrophilic. Gu et al. (37, 38) attempted to increase the hydrophilicity of a monolith by incorporating the cross-linker poly(ethylene glycol) (PEG) diacrylate (PEGDA). In the initial study, PEGDA was tested in combination with AMPS, SEMA, and VSA, all of which have sulfonic acid functionality; this study produced materials intended for use in cation-exchange chromatography of peptides and proteins. Although the results showed evidence of incorporated cation-exchange functionality, addition of a substantial amount of acetonitrile to the eluent was required in order to suppress hydrophobic interactions between the analytes and the monolith. The most promising results were obtained with VSA: The test peptides were eluted in a reasonable time without the addition of acetonitrile. The range of monoliths prepared with PEGDA as a cross-linker has been recently extended to anion exchangers; the monomers used were 2-(diethylamino)ethyl methacrylate and 2-(acryloyloxy)ethyl trimethylammonium chloride (42). In a recent study on the use of PEG-based cross-linkers, Chen et al. (39) incorporated another PEG-based cross-linker: PEG acrylate (PEGA). The phosphoric acid ion-exchange functionality was incorporated by use of PAHEMA or BMEP, in combination with PEGDA or PEGA, respectively. Synthetic peptides were eluted from both monoliths in an ion-exchange mode, without the need for addition of acetonitrile to the eluent. Another material in which phosphate groups were used as ion-exchange sites was prepared by thermally initiated polymerization using ethylene glycol methacrylate phosphate and bisacrylamide as the cross-linker (40). This monolith was used as a trap column for peptide-protein digestion in the

first dimension of a multidimensional liquid chromatography (LC)–mass spectrometry system in which the second dimension consisted of an RP gradient separation.

GMA: glycidyl methacrylate

SAX: strong anion exchange(r)

EDMA: ethylene glycol dimethacrylate

3.3. Functionality by Postpolymerization Reactions

A less direct, but possibly more convenient, way of imparting functionality into a polymeric monolith relies on some form of postpolymerization modification. Such a modification process has the distinct advantage of allowing for independent optimization of porous properties (and other support monolith characteristics) and surface functionality. Thus, the preparation of the support monolith needs to be optimized only once, which also allows the same monolithic support material to be used as a base for various surface functionalities and stationary-phase materials. Prepared materials can thus be compared and conclusions made that relate primarily, if not solely, to their surface characteristics. Numerous strategies can be used for the postpreparation modification of a polymeric monolith, but the usefulness of some of these strategies is limited. Postpolymerization modifications that have been successfully employed for polymeric monoliths include reaction of functional reagents with the monolith surface (43–49); grafting of monomer to or from the surface (25, 47, 50–61); and coating procedures, including the use of latex particles (62–66).

3.3.1. Reaction with surface groups. Modification by reaction with monolith surface groups is popular in monolith functionalization and has been used to produce both anion- and cation-exchange materials. The use of various chemical reactions or additions to a monolith surface often relies on the availability of an anchor group or reactive site. A suitable monomer in the preparation of methacrylate-based monoliths is therefore glycidyl methacrylate (GMA), which leads to the introduction of epoxide functionality. Indeed, one of the first examples of a methacrylate-based monolith for use in IC used GMA, and ion-exchange functionality was introduced through a reaction between the surface-bound epoxide and diethylamine (43) This approach has been very popular and has been extended through the use of different amines in the final reaction step (44). Strong anion-exchange (SAX) quaternary ammonium functionality can be obtained from surface epoxide groups of a poly(GMA-*co*-DVB) monolith (DVB refers to divinylbenzene). The two-step procedure involves ring opening of the epoxide group with diethylamine and subsequent alkylation of the formed tertiary amine with diethyl sulfate (46). Following a similar approach, a monolith with SAX functionality was prepared through further functionalization of the backbone of an epoxide-based monolith. The monolith was prepared by emulsion polymerization of an epoxy resin oil phase and a polyfunctional amine water phase, which created a monolith with both hydroxyl groups and a tertiary amine present on the monolith surface. The tertiary amine was further modified to a quaternary amine through iodomethane quaternization, and the resulting SAX functionality was utilized for the IC of proteins (47).

In a more significant alteration, investigators prepared a material for CEC in which epoxide groups were reacted with a synthesized macrocyclic polyamine. Starting with a poly(GMA-*co*-EDMA) monolith (where EDMA stands for ethylene glycol dimethacrylate) prepared by thermally initiated polymerization, the introduced positively charged moieties enabled generation of electroosmotic flow and produced ion-exchange sites for retention of anionic analytes (48). The modification with the macrocyclic amine was also found to increase the hydrophilicity of the monolith surface, as evidenced by reduced retention of the hydrophobic analyte toluene on the modified column used in the LC mode. The postpolymerization modification was achieved by filling the monolithic column with a solution of the macrocyclic amine in methanol and allowing reaction to take place for 12 h at 70°C. Modification of a poly(GMA-*co*-EDMA) monolith by reaction with polyethyleneimine followed by quaternization of the amine groups using methyl

iodide has also been reported in a monolith used for analysis of inorganic anions (e.g., F^-, Cl^-, NO^{2-}, NO^{3-}, Br^-, I^-, ClO_4^-, and SO_4^{2-}) (67). Ring opening reactions of epoxide groups result in the formation of C–N bonds, which are typically hydrolytically stable. This stability ensures that the functionality of such stationary phases is maintained, even when they are subjected to alkaline eluents or a high concentration of alkali in column-cleaning cycles (44).

WCX: weak cation exchanger

SCX: strong cation exchanger

Ring opening of epoxide groups with amines can also be used as the first step in the preparation of cation exchangers. The epoxide groups of a poly(GMA-*co*-EDMA) monolith were reacted with ethylenediamine, followed by monochloroacetic acid, to yield a weak cation exchanger (WCX) (68). Alternatively, epoxide-containing monoliths are suitable starting materials for strong cation exchangers (SCXs). For example, a methacrylate-based polymeric monolith used for the separation of Na^+, NH_4^+, K^+, Mg^{2+}, and Ca^{2+} was prepared by sulfonation of a poly(GMA-*co*-EDMA) monolith via ring opening of surface epoxide groups using sodium sulfite (49). A 1-*M* solution of Na_2SO_3 was passed through the monolithic column at a flow rate of 3 μl min^{-1}, and sulfonation proceeded at 75°C. The reaction time for the sulfonation influenced the resulting ion-exchange capacity (49). Baseline separation of Na^+, K^+, NH^{4+}, Mg^{2+}, and Ca^{2+} was achieved on a 151 μeq ml^{-1} capacity, 0.25 × 150 mm monolith, when 10 mM $CuSO_4$ was used as the eluent, at a flow rate of 3 μl min^{-1}. In addition, use of a flow gradient decreased the overall run time without having detrimental effects on the peak resolution. The same monolith formulation was later used for the separation of different tetraalkylammonium ions (69).

3.3.2. Functionality by surface grafting. Surface grafting of different monomers onto a monolith is another way to alter the surface characteristics. The introduction of surface functionality through a grafting strategy can increase the density of functional groups on the monolith surface and can yield materials with high ion-exchange capacities. However, grafting can also result in swelling of the grafted polymer and a reduction in permeability (50). In addition, the experimental parameters must be chosen so as to avoid a low grafting yield.

Grafting can be initiated and performed in different ways: The initiator can be present on the monolith surface or added with the monomer in solution. An example of an external initiator is potassium diperiodatocuprate, which was employed in the grafting of macroporous polyacrylamide cryogels with *N*,*N*-dimethylaminoethyl methacrylate (51). In this study, the investigators explored two different approaches to the preparation of the grafted material, namely (*a*) premixing of the monomer and initiator and (*b*) activation of the cryogel with the initiator prior to the introduction of the monomer solution. In the latter approach, the grafting is performed by pseudo-surface-initiated polymerization, which results in higher grafting yields—presumably because fewer polymers form in solution. The same approach was recently used for the grafting of cryogels with the monomer AMPS (52), as well as with 2-(dimethylamino)ethyl methacrylate, 2-(methacryloyloxy)ethyl trimethylammonium chloride, and acrylic acid (53).

Some factors that influence the grafting of the monomer AMPS onto a cryogel were recently investigated (54). The grafting yield, illustrated as the protein-binding capacity of the final material, increased with an increase in concentration of AMPS and Cu(III). Increased grafting temperature and grafting time also increased the protein-binding capacity (54). The use of UV-initiated grafting of monolith surfaces was also reported, and its potential to control surface properties was demonstrated (55, 56). Pucci et al. (56) used photoinitiation and suitable masking devices to achieve exquisite control of grafting location in the preparation of a monolith with a gradient of AMPS functionality.

In addition to adding desired ion-exchange functionality to the surface of a monolith, grafting can also shield undesired secondary interactions that could be detrimental to the separation of bioactive analytes such as proteins. Such shielding is exemplified by the preparation of

monolithic columns for use in cation-exchange chromatography of proteins that, prior to being grafted with ion-exchange functionality, were grafted with the hydrophilic monomer PEG methacrylate (57). Ion-exchange functionality was introduced via photografting of AMPS and acrylic acid onto a poly(GMA-*co*-EDMA) monolith, yielding strong and weak cation-exchange functionality, respectively (57). The highest binding capacities, ranging from 20 to 30 mg ml^{-1}, were achieved for columns prepared using the two-step shielding and functionalization approach. However, no secondary interactions were observed for either type of material.

Researchers have also used living polymerization approaches for surface grafting. For example, through the use of capped, stable free radicals on the surface of a material prepared by nitroxide-mediated, stable free radical polymerization to initiate further polymerization, poly(styrene-*co*-DVB) monoliths were grafted with the monomer sulfopropyl methacrylate to yield a material for cation-exchange chromatography (25). Similarly, in ROMP synthesis, if the initiator used to prepare the support monolith is not removed after polymerization, it can be employed to further modify the monolith surface (58, 59). The living nature of ROMP allows for control of the grafting outcome, which is the main advantage of this approach. This process was used to prepare ion-exchange monoliths with two different functional monomers: 2-(*N*,*N*-dimethylaminoethyl) norborn-5-ene-2-yl-carboxylic amide (58), which yielded an anion exchanger, and 7-oxanorborn-2-ene-5,6-carboxylic anhydride (ONDCA), which yielded a cation exchanger (59). Grafting to produce the cation exchanger was performed by flushing the monolith with a saturated solution of the monomer ONDCA. The monolith was thereafter end-capped, and in a final step, the anhydride was hydrolyzed (59).

ROMP can also be used solely in the grafting step by recharging a prepared monolith with attachment of the initiator for ROMP following monolith formation (60). Grafting can then be performed through the use of a living polymerization to impart functionality onto the monolith surface. More recently, the use of ATRP was demonstrated for the surface modification of monolithic materials (47, 61). Cation-exchange materials for use in IC of proteins were prepared via an elaborate route involving (*a*) activation of the monolith surface with 2-bromoisobutyryl bromide, (*b*) grafting of GMA onto the monolith surface with ATRP, and finally, (*c*) a post-ATRP modification of the GMA to yield strong and weak cation-exchange functionalities (47). Sulfonation of the epoxide groups of the GMA polymer brushes by $NaHSO_3$ led to SCX functionality, whereas reaction with iminodiacetic acid resulted in WCX functionality (47). The success of each step was verified by X-ray photoelectron spectroscopy, and the final materials had protein-binding capacities of approximately 10 mg ml^{-1}. Both the SCX and the WCX were used for separation of a protein mixture, although baseline separation was achieved only when stepwise gradients were used (47). This study also illustrates the diversity of the epoxide-based support material, whose backbone contains both tertiary amines and hydroxyl groups. The tertiary amines can be modified into quaternary amines, as described above, and the hydroxyl groups can be modified following the grafting route. However, this approach is complex, and the authors of this study reported that both online ATRP modification and the optimization of parameters presented challenges (47).

3.3.3. Functionality by surface coating. The surface characteristics of a material can also be altered by either dynamic or static coating. For polymeric monoliths, coating with charged latex particles, which we introduced for use in both IC (62, 63) and CEC (64, 65), is a viable alternative to other surface-modification strategies. The latex coating of a material relies on electrostatic interaction between the latex and the material surface; such electrostatic interaction ensures stationary-phase stability. The prepared support monolith must therefore have a surface charge that is opposite to that of the latex particles. Such a charged support monolith can be prepared in the same fashion as conventional ion-exchange monoliths. Once the monolith is prepared, the

surface is coated with latex particles by pumping an aqueous suspension of the latex through the monolithic column (62). A latex-coated polymeric monolith for carbohydrate analysis was prepared by use of a poly(BMA-*co*-EDMA-*co*-AMPS) monolith as the support monolith. The monolith surface was coated with latex particles that had been quaternized through reaction with iodomethane (62). This approach was later extended to the preparation of an anion-exchange material used for separation of inorganic ions (63). By using two different latexes with different selectivities, this study demonstrates the versatility of this approach. In both examples, however, scanning electron microscopy (SEM) images revealed that the monolith surface was not completely covered with latex particles; the prepared material therefore exhibited limited ion-exchange capacity. The limited coverage by latex particles was attributed to limited sulfonate surface functionality. To rectify this limited capacity, we investigated different approaches to increase the extent of negative charge on the monolith (66). All the approaches we tested increased the ion-exchange capacity of the latex-coated monolith; the most promising results were obtained by ring opening the epoxide groups of a methacrylate-based monolith by use of sodium sulfite. The ion-exchange capacity of this monolith was 30 times that of the poly(BMA-*co*-AMPS-*co*-EDMA) monolith used in earlier experiments.

TMOS: tetramethoxysilane

4. SILICA MONOLITH PREPARATION

Silica monoliths are prepared by a sol-gel process that begins with an alkoxysilane such as tetraethoxysilane or tetramethoxysilane (TMOS). This multistep process (**Figure 2*b***) involves gel formation induced by hydrolysis and polycondensation, followed by aging and drying. The end result is a material whose structure is both macroporous and mesoporous. As the initial hydrolysis and polycondensation proceed, siloxane oligomers are created; they combine to form a gel network. This network facilitates the formation of macropores, whose extent and size depend on the composition of the starting solution, the catalyst and its concentration, and the reaction temperature and solvents (70, 71). Different additives have been tested to facilitate phase separation in order to control the characteristics of the silica monolith; these additives include surfactants and water-soluble polymers. PEG is a popular additive (72, 73), although other additives have been explored and found to affect the porous network formed (74).

In the resulting macroporous network, micropores are also present. Such micropores are used as starting points for the introduction of mesopores to the material. The mesopores are introduced through enlargement of the micropores, which was initially performed by treating the formed monolith with ammonium hydroxide (16). This enlargement has recently been achieved through the use of a combination of urea and heat (70). In this approach, urea is added to the initial reaction mixture. Once the monolith macroporous and microporous network is formed, the micropores can be enlarged simply by heating the monolith: Heating causes hydrolysis of urea, thereby producing ammonia. This approach has the benefit of a simple preparation, given that reagent does not need to be added postpreparation to achieve mesopore formation. The possibility of controlling macropore and mesopore formation independently is a key advantage of the silica monoliths. Readers interested in a more in-depth study of the factors affecting silica monolith formation are referred to reviews by Tanaka et al. (75) and Siouffi (15), as well as work by Nakanishi & Soga (7–9, 76, 77).

Various strategies to introduce functionality have been developed to allow the use of monolithic silica in different chromatographic modes. Methods to produce silica monoliths used in ion-exchange chromatography can be divided into two main approaches, namely functionality by copolymerization (hybrid silica) and postpreparation modification. Examples of strategies used in the preparation of monolithic silica materials for use in IC are illustrated in **Figure 3*b***.

4.1. Functionality by Copolymerization: the Preparation of Hybrid Silica

Similar to the copolymerization approach used to prepare polymeric monoliths, functionality can be introduced into a silica monolith during its initial preparation, which leads to the formation of hybrid silica. In a typical procedure, a mixture of silanes and phase-separation agent is combined with a functional monomer and an initiator (78). The formation of the monolith proceeds through an initial condensation reaction followed by polymerization. This approach has been used to prepare both cation and anion exchangers and is a convenient way to introduce functionality to a monolith. The resulting organic functionalities in the silica hybrids are covalently linked to the inorganic silica matrix through hydrolytically stable Si–C bonds. For the preparation of a monolith with SAX functionality (79), a mixture of the siloxanes TMOS and vinyltrimethoxysilane was combined with the functional monomer [3-(methacryloylamino)propyl] trimethylammonium chloride. After condensation followed by polymerization, the monolith was used to enrich phosphopeptides from a protein digest prior to matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI TOF MS) analysis. The use of hybrid silica may aim either to simply introduce functionality to the monolith or to change other characteristics of the monolith. Although hybrid silicas can be prepared with the desired ion-exchange functionality from a silane that contains that functionality, for every new silane used, reoptimization of the condensation and polymerization mixture may be needed. The preparation of hybrid silica with a functionality that easily lends itself to further modifications via various simple chemical reactions is an approach that allows for one-time optimization of the hybrid silica support monolith without the need for reoptimization for every desired functionality. One such versatile and reactive functionality is the thiol group (80).

4.2. Postpreparation Functionalization

Various postpreparation functionalization strategies have been developed and used for the preparation of monolithic silica ion-exchange materials. As is the case for polymeric monoliths, the number of attainable materials that use these strategies is essentially infinite. However, some strategies offer a great deal of versatility, whereas others require the existence of reactive sites or anchor groups for incorporation of functionality to be realized. In general, the strategies can be divided into the following approaches: introduction and reaction with surface groups (80–86), surface grafting (87–90), semipermanent coating (10, 91–96), and permanent coating (10, 65, 91, 97)—all of which can be used with both silica and hybrid silica monoliths.

4.2.1. Introduction and reaction with surface groups. A traditional approach to silica modification that is often used for particle modification is reaction of a chlorosilane with the silica surface; this strategy has been adopted for the preparation of RP monolithic silica (98). Hybrid silica can also be reacted further and used as a starting point for surface functionalization. In the preparation of columns for CEC, beginning with a hybrid silica with thiol functionality, peroxytrifluoroacetic acid was used to oxidize surface groups to sulfonic acids, which yielded a surface with SCX functionality (81). Similarly, hydrogen peroxide (30% weight/weight) was used to oxidize surface thiol groups to yield sulfonic acid functionality (82). The oxidation was performed by flushing the monolith with the hydrogen peroxide solution for 12 h at room temperature. The resulting monolith had both hydrophobic and SCX functionality and was used for in-line solid-phase extraction of sulfonamides in a milk matrix prior to high-performance liquid chromatography (HPLC)-UV analysis.

A more complex, multistep procedure that also takes advantage of the reactivity of the thiol group has been developed (83). Treatment with acid (HCl) maximized the number of

surface silanol groups in a monolith prepared from TMOS (84). Thereafter, reaction with 3-mercaptopropyltrimethoxysilane introduced thiol functionality. The introduced thiol groups were oxidized with anhydrous trifluoroacetic acid peroxide produced in situ within the capillary, which yielded sulfonic acid functionalities. In addition to the reaction steps, the process also required numerous washing steps between treatments. The authors of this study (84) identified a low conversion rate as a cause for concern in the oxidation step. Starting from a commercially available bare monolithic silica column (Performance Si from Merck KGaA, Darmstadt, Germany), the authors prepared a chelating ion-exchange column via an on-column modification in which a mixture of glycidoxypropyl trimethoxysilane and iminodiacetic acid was flushed through the monolith, in a recycling manner, at a flow rate of 0.5 ml min^{-1} at 70°C for 6 h (85). The same approach was later used to covalently attach lysine (2,6-diaminohexanoic acid) groups to a silica monolith (86). The prepared material had zwitterionic functionality, and the carboxylic acid responsible for the cation-exchange capacity was a WCX group, which allowed for pH control of the ion-exchange capacity.

4.2.2. Surface grafting. Surface grafting as a means of surface functionalization can be performed either directly onto the silica surface or through attachment of an anchor group and subsequent grafting using the anchor as the attachment point (88). To introduce the anchor group, a silane containing the desired anchor group functionality, such as an epoxide or vinyl group, is reacted with the silica surface, which introduces a reactive site to the silica surface. The introduction of anchor groups can also be achieved through incorporation of the anchor group functionality in the preparation of a hybrid silica monolith. A hybrid silica monolith prepared from a mixture of TMOS and methyltrimethoxysilane was modified in two steps to produce a monolith with SAX functionality for use in CEC and HPLC (87). First, the surface was functionalized by attachment of 3-methacryloxypropyl groups as anchor groups. Second, the anion-exchange functionality was introduced via polymerization of *N*-[3-(dimethylamino)propyl] acrylamide methyl chloride–quaternary salt (DMAPAA-Q) onto the 3-methacryloxypropyl anchor groups through the use of ammonium persulfate as the initiator. In a similar approach, anion- and cation-exchange functionalities were introduced by polymerization of 3-diethylamino-2-hydroxypropyl methacrylate (DAHMA), 2-(triethylammonium)ethyl methacrylate chloride (DMAEA), *p*-styrenesulfonic acid sodium salt (pSSA), and AMPS onto hybrid silica monoliths modified with 3-methacryloxypropyltriethoxysilane as the anchor groups (88). A combined anion and cation exchanger was also prepared by sequential grafting of pSSA, followed by DAHMA. The prepared materials were evaluated in IC mode using nucleic acids, nucleotides, and inorganic anions as test solutes. Through the use of 3-methacrylamido propyltriethoxysilane as the anchor group, hybrid silica monoliths for anion-exchange chromatography were prepared with various monomers containing tertiary amino or quaternary ammonium groups, including DMAPAA-Q, DMAEA-Q, DMAPAA, DMAEA, DAHMA, and HMPMA [2-hydroxy-3-(4-methylpiperazin-1-yl)-propyl methacrylate] (89). The column modified with HMPMA demonstrated better performance than the other materials in terms of selectivity for nucleotides and inorganic anions. When tested for separation of tryptic digest of bovine serum albumin, this column also performed better than a particle-packed column used for comparison. Surface grafting can be performed on both silica and hybrid silica monoliths, which may be expected to yield different grafting outcomes. Interestingly, in a study in which both silica and silica hybrid monoliths were prepared, reacted with *N*-(3-triethoxysilylpropyl) methacrylamide to introduce anchor groups, and thereafter grafted with acrylic acid, the investigators found no significant difference in the obtained efficiencies between the silica and silica hybrid monoliths (90).

4.2.3. Semipermanent and permanent coating. In addition to the procedure in which functionality is attached covalently to the silica surface, numerous coating strategies have been employed to alter the surface characteristics of silica monoliths, including the use of semipermanent (95, 99) and permanent coatings (97). The use of a semipermanent surfactant coating is a relatively straightforward way of changing the surface functionality of a silica monolith. In many experiments, either bare silica or RP-functionalized silica monoliths were coated with a surfactant, and the coated materials were used for IC involving separations of both anions (91) and cations (92). For example, in a study involving an RP silica monolith, the use of a semipermanent coating with the surfactant didodecylmethylammonium bromide resulted in a monolith suitable for IC separation of inorganic anions (10). Other surfactants employed for semipermanent coating of monolithic silica include cationic surfactants, such as cetyltrimethylammonium chloride (93) and cetylpyridinium chloride (94), and anionic surfactants, such as lithium dodecylsulfate (92). Zwitterionic surfactants (95), which allow tuning of retention properties, have also been used. For example, the zwitterionic surfactant (dodecyldimethylamino) acetic acid allowed for control of surface protonation and, therefore, the retentive properties of the monolith (96). A disadvantage of semipermanent coating is that a small concentration of the surfactant usually must be present in the eluent. This background concentration leads to a more complex eluent composition and also affects detection and sensitivity. However, when a longer-chained carboxybetaine surfactant, N-dodecyl-*N*,*N*-(dimethylammonio)undecanoate, was used to coat an RP silica monolith, the coating stability was maintained without the addition of surfactant to the eluent (95).

A more permanent coating of the monolith surface can be achieved by the use of latex particles, and this approach can significantly improve ion-exchange capacity. The permanent nature of the coating eliminates the need for eluent additives, thereby allowing the use of a simpler, more flexible eluent system; further, better detection limits can be obtained through this method, compared with the semipermanent-coating approach. Glenn et al. (97) have demonstrated the difference in stability between latex coating and surfactant coating. These authors compared a surfactant-coated RP monolithic silica stationary phase with a latex-coated bare monolithic silica stationary phase. The coating of the positively charged quaternary ammonium latex particles was achieved by electrostatic interaction with negatively charged ionized silanol groups on the monolith. The surfactant coating and latex coating were performed according to procedures reported elsewhere (10, 65). An earlier study reported that didodecylmethylammonium bromide coatings were stable for up to three weeks (91) but that a decoating and recoating procedure had to be performed every three to five days to ascertain reproducibility. The retentive properties of the surfactant-coated monolith were reduced by 10% after having been used for only 11 h, whereas the latex-coated monolith retained its retentive properties within 1% after 2.5 months of use. Thus, evidence from the anion separations performed over time led to the conclusions that (*a*) the stability of the latex coating is much higher than that of the surfactant coating and (*b*) a higher efficiency can be obtained with the latex-coated monolith (97).

5. ALTERNATIVE MONOLITHIC MATERIALS

In addition to the polymeric and silica-based monoliths discussed thus far, numerous other materials have been prepared as monoliths for use in chromatography. These materials include titania (100, 101), zirconia (102), hafnia (103), and graphitized carbon (104). Titania (101) and zirconia (102) monoliths were prepared via a sol-gel synthesis approach similar to that used in the preparation of monolithic silica. These two sol-gel synthesis approaches have the same disadvantages, including shrinkage of the monolith. Proposed advantages of using titania or zirconia in lieu of silica include these materials' thermal stability, high mechanical strength, and good pH stability

(103, 105, 106). Additionally (and perhaps importantly for the use of these materials in IC), titania and zirconia have surfaces with amphoteric hydroxide groups and, therefore, both anion- and cation-exchange capabilities. Titania also interacts with phosphorus-containing compounds, which has potential advantages in certain biological applications (107). The first monolithic graphitized carbon column, prepared in 2003, used silica beads to impart the macroporous structure of the material (104). These alternate monolith types are not as widely used in chromatographic applications as silica-based and polymeric monoliths, although in the future they may present a viable alternative to traditional monoliths.

6. MONOLITH CHARACTERIZATION

In chromatographic applications, both the bulk and surface properties of a stationary-phase material are crucial to the suitability and usefulness of the material. Factors such as mechanical and chemical stability are important and are related to the bulk of the material, and surface properties influence retention characteristics. Techniques for the characterization of these properties are therefore essential.

In the characterization of monolithic materials, an initial assessment of porous properties is often performed by SEM, which allows one to visualize the homogeneity and porosity of the monolithic materials. Other techniques used to assess porous properties include (*a*) mercury intrusion porosimetry for determination of the pore-size distribution and total porosity of a material (*b*) and nitrogen adsorption/desorption for the determination of surface area and porosity. Both techniques provide useful information and allow for material comparisons, but they can be used to characterize the material only in its dry state—they cannot be performed directly on the monolith in its capillary or column housing. The micro- and mesoporous properties of graphitized carbon monoliths (104) and the macroporous properties of both silica-based and polymeric monolithic materials (108) have also been assessed through transmission electron spectroscopy (TEM). Whereas some examples use crushed monolith as the sample (104), one study characterized the monolithic materials directly, via the capillary format, by using TEM and the aid of computational techniques (108). However, the need for embedding, staining, and microtomy destroys the sample.

The ability to prepare some monolithic columns in situ in the desired mold is a major advantage. However, in situ synthesis makes characterization of the monolith somewhat difficult, especially in small-diameter formats. To circumvent this difficulty, a second sample of monolith is often prepared in parallel in bulk format, for example in a sample vial, and is then used for characterization. Although this is often a convenient way of assessing the monolith properties and enables comparison between materials prepared in the same-sized mold, the characterization data obtained do not necessarily reflect the actual characteristics of the monolith prepared in the mold. A comparison between the porous properties of poly(styrene-*co*-DVB) monoliths prepared from the same polymerization mixture under identical conditions, but in three different-sized molds (250-μm-inner-diameter fused-silica capillaries, 100-ml microvials, and 2-ml glass vials), revealed significant differences in both the meso- and macroporous properties among the three formats (109). The samples were analyzed by nitrogen adsorption/desorption and mercury intrusion porosimetry and were visualized by SEM. The differences in porous properties that could be observed via mercury intrusion porosimetry and nitrogen adsorption/desorption data could not be seen in the SEM images of the samples. A reduction in the monolith mold size, from a 2-ml vial to a 250-μm-inner-diameter capillary, led to both a decrease in surface area, from 52.2 $\pm$ 4.7 to 34.6 $\pm$ 1.7 $m^2\ g^{-1}$, and an increase in median pore diameter, from 310 to 544 nm.

A technique that allows porous properties to be assessed in situ is inverse size exclusion. However, sets of suitable standards that do not undergo enthalpic interactions with the stationary phase are required. Recently developed alternatives to the use of SEM, nitrogen adsorption/desorption, and mercury intrusion porosimetry include near-IR spectroscopy, which has been used to characterize silica and polymer particles as well as monoliths (110). The advantages of the technique include its speed and that it is nondestructive. However, this study (110) does not clearly demonstrate the usefulness of this for monolithic materials. In addition, the ease of operation may be counteracted by the need for principal component analysis in data treatment. Another recent approach is the use of confocal laser scanning microscopy for the characterization of the macroporous structure of monoliths (111, 112). Following image acquisition and processing, statistical analysis is performed to extract information about the silica skeleton and the macroporous structure. The morphological information thereby obtained is comparable to that derived from TEM. The authors (111) claim that this information is more easily accessed than TEM data, presumably because there is no need for embedding and microtomy. However, the method requires the covalent attachment of a dye, such as V450-succinimidyl ester, and therefore requires attachment handles to be present on the silica. In this study, silica monoliths were amine-modified by use of 3-aminopropyltriethoxysilane (111).

Characterization of surface properties, including functional group content, yields vital information not only about possible interaction sites for retention but also about sites for potential further surface modification. The correlation between bulk and column monoliths may be even more complicated to assess when it comes to the material surface properties, especially when postpolymerization functionalization is employed. Although bulk preparation of a monolith is fairly straightforward, the adaptation of surface-modification strategies to a bulk material is not always easy, and therefore deductions drawn from results obtained for bulk material are difficult to transfer to the capillary format. A characterization that can be performed in situ and online would therefore be a very useful way to attain useful and interpretable results. Ideally, in monolith characterization, the characterization approach should be nondestructive so that a prepared and functionalized monolith can be characterized and then used for its intended purpose. To achieve this goal, some investigators prepare and use the monolith and perform material characterization after the chromatographic evaluation is completed. Although this procedure may be suitable for monolith development, it is hardly sustainable in the long run and certainly does not suit users aiming for long-term use of the monolithic column. Another possibility is the preparation of duplicate/replicate columns, some of which may be used for characterization and others for chromatography. Chromatographic evaluation of the finished, functionalized monolithic stationary phase may be the only truly informative way of assessing the surface functionality of the column as it is intended to be used. The retention of standard analytes can be used to provide column comparisons, whereas characteristics such as loading capacity and ion-exchange capacity can be assessed via various breakthrough experiments.

Chromatographic evaluation requires the user to perform sometimes lengthy tests on each prepared column to verify surface functionality. Obtaining an indication about the quality of the monolith prior to chromatographic testing is certainly useful for the chromatographer. Electron probe microscopy, in combination with electrochromatographic evaluation, has been used to characterize the obtained functionality of a monolithic column for use in CEC; the column was prepared by photografting and photomasking to produce a gradient of AMPS functionality along the column length (56). The investigators employed electron probe microscopy to verify the presence and position of sulfur atoms originating from the AMPS. Other successful examples of online characterization include the use of contactless conductivity measurements for the determination of column voids (113) and assessment of surface functionality homogeneity for both photografted

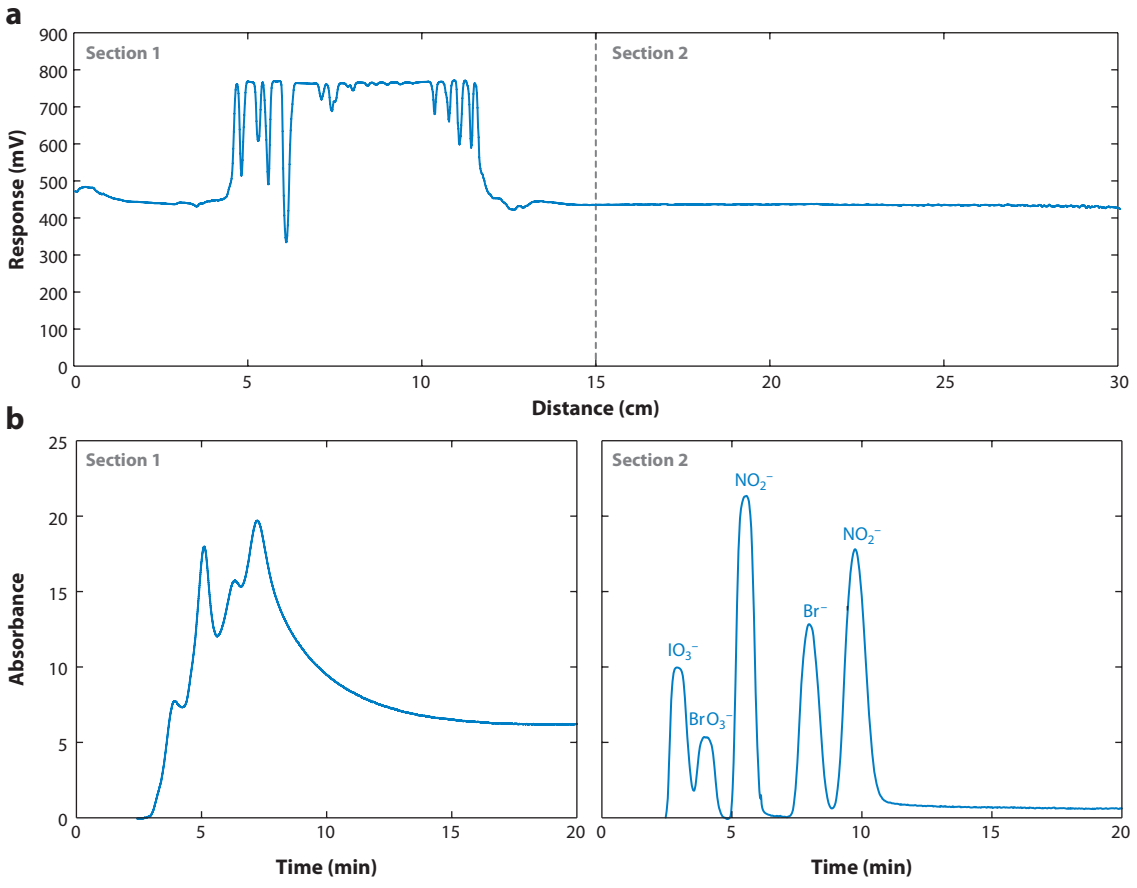

Figure 4

(*a*) Predicting column performance using contactless conductivity detection. Contactless conductivity measurement performed along the length of a latex-coated poly(styrene-*co*-DVB) monolith in a 30 cm × 200 μm inner-diameter capillary column. (*b*) Resulting separation performance of column sections. Ion chromatography separation of IO_3^-, BrO_3^-, NO_2^-, Br^-, and NO_3^-. The injection volume was 1 μl, the eluent was 1 *M* KCl, the flow rate was 2 μl min^{-1}, and the detection wavelength was 215 nm (116). Abbreviation: DVB, divinylbenzene.

(114) and surfactant-coated (115) monoliths. Contactless conductivity measurements have also been used to ensure sufficient material washing following material grafting (114). We recently observed that although conductivity measurements do not appear to correlate well with the pore size of the monolith, these measurements provide useful information about differences in bulk-material porosity and hence homogeneity (116). We employed scanning contactless conductivity measurements to verify column homogeneity, which was then related directly to chromatographic performance for IC. Scanning contactless conductivity measurements across the entire length of a 30-cm column indicated two distinct areas (**Figure 4*a***). After cutting the monolith in half at the point where the conductivity trace changed and performing a chromatographic evaluation of the two parts as IC columns, we observed a clear correlation between the difference in the conductivity trace and the separation performance (**Figure 4*b***). These findings thus suggest that contactless conductivity measurements can be correlated to chromatographic performance to perform quality assessment of monolithic columns.

7. FINDING THE MONOLITH THAT CAN DO THE JOB: APPLICATIONS OF MONOLITHS IN ION CHROMATOGRAPHY

The choice of chromatographic column depends on both the intended separation and the compatibility of the column with the desired eluent. The commercial availability of both polymeric and silica-based monoliths is significant in that it allows the use of monolithic columns to extend beyond research laboratories. The continued introduction of monolithic stationary phases is also positive, given that the availability of more columns allows the chromatographer greater choice when designing a new method. Both silica-based and polymeric monoliths for ion-exchange chromatography are commercially available. Silica-based monoliths with ion-exchange functionality include Metrosep Dual from Metrohm AG (Herisau, Switzerland) under license from Merck KGaA. RP-functionalized or bare silica monoliths that can be used as supports for a surfactant coating, as described above, include Chromolith® from Merck KGaA and Onyx from Phenomenex, Inc. (Torrance, California). Although these columns require somewhat complex modifications, their commercial availability allows for the broad use of the developed modification procedures throughout the scientific community. Commercially available polymeric monolithic columns include convective interaction media (CIM) discs from BIA Separations (Ljubljana, Slovenia); the ProSwift™, DNASwift™, and IonSwift™ columns from Dionex Corporation (Sunnyvale, California); UNO columns from Bio-Rad Laboratories (Hercules, California); and Bio-Monolith columns from Agilent Technologies, Inc. (Santa Clara, California). In addition, so-called cryogels used as support materials for some ion exchangers (53) are available from Protista (Lund, Sweden). The CIM discs and the Bio-Monolith, ProSwift, and DNASwift columns are based on a polymethacrylate matrix, whereas the UNO columns are based on polyacrylamide and the IonSwift column is based on a styrene-DVB backbone.

Monolithic media have been used for IC of such diverse analytes as small ionic species and biomacromolecules (**Table 1**). Although commercial monolithic columns are in use, many published studies involve the preparation and use of widely available in-house or modified materials. Apart from the recently introduced IonSwift, which is intended for the analysis of organic acids and inorganic anions, all commercially available polymeric monoliths are made for separations of larger biomacromolecules. As mentioned above, polymeric monoliths are more widely used for the separation of large analytes, such as proteins, and their silica counterparts are more often used for analysis of smaller ionic species. However, there are exceptions. For example, Ueki et al. (49) prepared a methacrylate-based polymer monolith for use in the separation of cations such as Na^+, NH_4^+, K^+, Mg^{2+}, and Ca^{2+}, and Connolly & Paull (117) used a methacrylate-based polymer monolith for the separation of small inorganic anions. Both support monoliths were poly(GMA-*co*-EDMA) materials prepared by thermally initiated radical polymerization. The cation exchanger was prepared by sulfonation via ring opening of surface epoxide groups using sodium sulfite (49), whereas the anion exchanger was produced by a grafting approach in which [2(methacryloyloxy)ethyl] trimethylammonium chloride was photografted onto the monolith surface using benzophenone as the initiator (117).

Monoliths for biological applications are common and include both cation and anion exchangers. The use of latex-coated monolithic supports has been extended to oligonucleotides (118, 119). DNA was extracted from whole blood with an efficiency of 52% through the use of an amino hybrid silica monolithic column in capillary format (120). In another recent example, a SAX hybrid silica capillary monolith was prepared for the enrichment of phosphopeptides from a tryptic digest of α- and β-casein prior to MALDI TOF MS analysis. (79). Elution using 5% formic acid solution as the elution buffer ensured compatibility with the matrix and satisfied other requirements of the MALDI TOF MS analysis. Although many in-house manufactured

monoliths intended for protein separations are still tested using only standard proteins, numerous more interesting applications have been presented. For example, four lipase isoforms were successfully separated on a poly (GMA-*co*-EDMA) monolith modified with 30 kDa polyethyleneimine (121).

Commercially available monoliths can be used for other challenging applications. With the aim of developing a fully automated system designed to screen the interaction of metallodrugs with proteins in biological samples, investigators used two monolithic discs (CIM mini-DEAE discs) in parallel in the second dimension, following a size-exclusion chromatographic (SEC) separation. The sample consisted of fetal calf serum incubated ex vivo with cisplatin, from which isolation of protein fractions was achieved in the SEC dimension. Elution from the anion exchangers was achieved with a gradient of NaCl in 20 mM tris-HCl, and detection was performed via inductively coupled plasma MS. The use of monolithic discs ensured that gradient elution was fast (3 min), and the use of two parallel discs enabled the second disc to be loaded with the next fraction of the size-exclusion column as gradient elution of the first fraction was performed on the first disc (122). Because the interactions of interest were both covalent and noncovalent, separation of proteins in their native state was essential and could be achieved by use of SEC $\times$ IC. In an attempt to extend the use of polymeric monolith media, we used commercially available ProSwift cation exchangers in combination with both salt and pH gradients for the characterization of monoclonal antibodies. Although a complete baseline resolution of antibody charge-state variants was not achieved, the results indicated that pH gradient elution may be a viable alternative for the characterization of monoclonal antibody charge-state heterogeneity (123). In our most recent work (124), we improved the resolution by extending the approach to other buffer systems. The use of simpler and more MS-compatible buffers in the creation of pH gradients is also promising (124). Monoliths have also proven useful for the separation and isolation of still larger analytes, such as viruses. A Bio-Monolith QA (where QA refers to quaternary amine) column was used to separate intact adenovirus type 5 from contaminating proteins and DNA (125).

8. CONCLUSIONS

A hybrid silica having anion-exchange functionality incorporated by grafting of functional monomers onto a 3-methacrylamidopropyltriethoxysilane anchor group illustrates the impact of surface chemistry on chromatographic performance (**Figure 5*a***). The resulting chromatograms reveal identical elution order for the four nucleotides but markedly different retention, resolution, and peak shape. This difference was attributed to the functional monomers used; a cyclic diamine yielded the monolith with the highest retentive powers (89).

The importance of surface chemistry, especially in the separation of analytes prone to secondary interactions, such as proteins, is not limited to ion-exchange functionality. The elution profile for four proteins in cation-exchange mode on polymeric GMA-*co*-EDMA monoliths photografted with AMPS (**Figure 5*b***) shows a difference in elution between a monolith grafted only with AMPS and one grafted with PEGMA prior to AMPS in order to decrease column hydrophobicity (57).

Monolithic media are distinctly different from particulate media in terms of porous structure and in situ manufacturing and modification strategies. There are advantages and disadvantages of both silica and polymeric monoliths, ranging from their preparation and characteristics to their possible modification strategies. For example, the pH stability and mechanical strength of polymeric monoliths exceed those of silica monoliths. The combination of macro- and mesopores in silica monoliths offers the advantage of increased accessible surface area compared with the macro- and micropores of polymeric monoliths. Regarding the introduction of surface

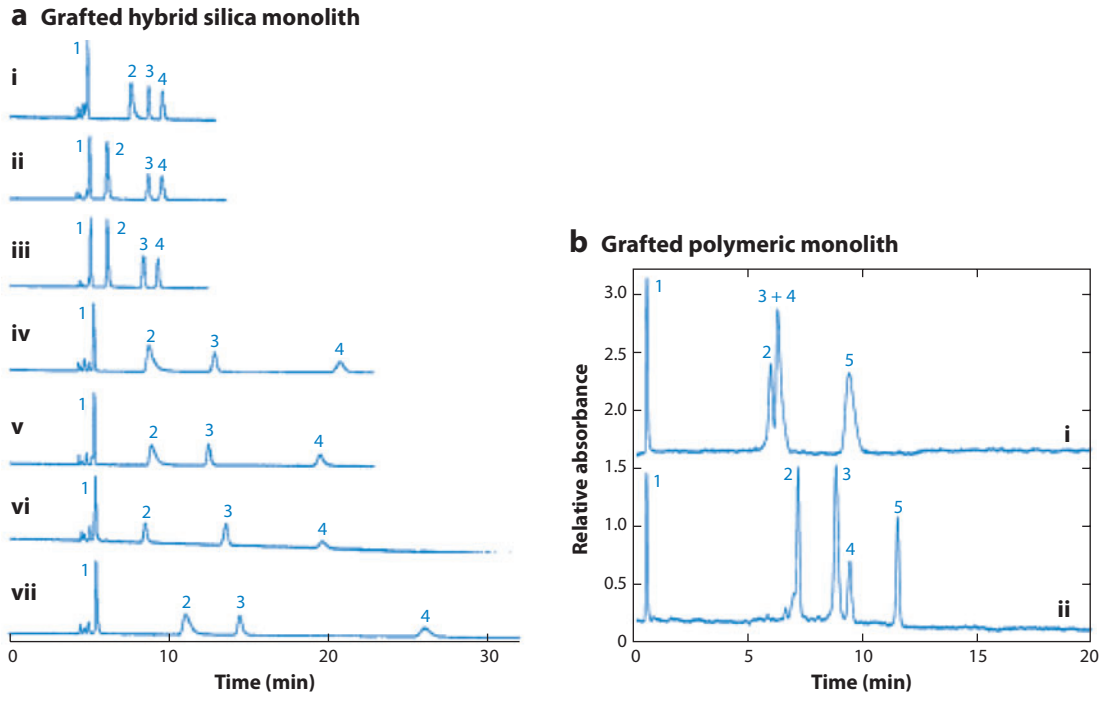

Figure 5

Influence of surface chemistry on chromatographic performance for (*a*) a grafted hybrid silica monolith and (*b*) a grafted polymeric monolith. Panel *a* reproduced from Reference 89. Panel *b* reproduced from Reference 57 with permission.

functionality, the number of possible strategies is large, and new strategies will be developed. The introduction of functionality to a monolith during its preparation (copolymerization or the preparation of hybrid silica) seems to be straightforward, but it has drawbacks arising from the need to reoptimize the polymerization mixture and conditions. The basic support structure (porous properties, mechanical strength, etc.) needs to be controlled carefully, or the material will lose its usefulness. In both polymeric and silica monoliths, more work remains to be done in the development of support materials.

To achieve polymeric monoliths with bimodal macro- and mesoporous structures similar to those in silica monoliths, further invention and optimization are required. For silica monoliths, a careful choice of hybrid silica components can significantly enhance the ease with which subsequent modifications can be performed. In terms of the introduction of specific surface functionality, for both silica and polymeric monoliths, postpreparation processes, such as grafting and coating, allow for the independent optimization of (*a*) support monoliths, including porous properties, and (*b*) surface functionality. This is a distinct advantage, given that the optimization of the preparation of support monoliths can be tedious. It also allows for more straightforward comparisons between materials on the basis of surface functionality alone.

The commercial availability of both silica- and polymer-based monoliths as ion-exchange chromatographic stationary phases is a positive development that has contributed greatly to the use of ion-exchange monoliths beyond research laboratories. Therefore, the range of applications of these media can be expected to expand.

SUMMARY POINTS

1. Ion-exchange chromatography is an important chromatographic mode whose applications range from analysis of inorganic anions and metal ions to the study of larger molecules such as peptides, proteins, and viruses. Monolithic media formed from both polymeric and silica materials are used in IC applications.
2. The surface functionalities necessary to allow for ion-exchange interactions can be introduced into a monolithic material either during preparation or during a postpreparation functionalization step.
3. Introduction of functionality during preparation is straightforward. In the case of polymeric monoliths, this approach relies on copolymerization of functional monomers, whereas for silica-based materials, hybrid silica is prepared. The major disadvantage of these methods is that reoptimization of preparation conditions is needed for every new surface functionality, which can often be a tedious task.
4. The use of postpreparation functionalization has the advantage of allowing for optimization of the characteristics of the support material, and optimization of the surface functionality, to be performed independently.
5. The range of possible strategies for the introduction of ion-exchange functionality into a specific material depends largely on the characteristics of that material, especially the availability of anchor groups to be used as reaction sites.
6. Surface grafting or coating can significantly alter the surface characteristics of a monolith. The use of living polymerization in the surface-functionalization step allows for control of the extent of grafting, and UV-initiated polymerization allows for the selective positioning of grafting in a chromatographic column. The use of hybrid silica is a convenient way to introduce anchor groups for subsequent grafting.

FUTURE ISSUES

1. In the development of monolithic media for IC applications, careful control of both support and surface properties is important. For biological applications, biocompatibility of the monolithic material continues to be a concern; therefore, we anticipate continued development within this area.
2. Polymeric and silica-based monoliths are generally used for different-sized analytes. Further development of the support monoliths might help bridge the gap between these two types of materials.
3. Strategies used in postpreparation functionalization can be expected to be optimized and extended beyond currently used approaches. The use of living polymerization has not been fully exploited, and the continued development of latexes and other coatings should allow for even more refined fine-tuning of surface functionality originating from the same support monolith.

DISCLOSURE STATEMENT

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LITERATURE CITED

1. Tang J, Gao MX, Deng CH, Zhang XM. 2008. Recent development of multi-dimensional chromatography strategies in proteome research. *J. Chromatogr. B* 866:123–32
2. Geng XD, Wang LL. 2008. Liquid chromatography of recombinant proteins and protein drugs. *J. Chromatogr. B* 866:133–53
3. Dugo P, Cacciola F, Kumm T, Dugo G, Mondello L. 2008. Comprehensive multidimensional liquid chromatography: theory and applications. *J. Chromatogr. A* 1184:353–68
4. Dong X, Wu R, Dong J, Wu M, Zhu Y, Zhou H. 2009. Recent progress of polar stationary phases in CEC and capillary liquid chromatography. *Electrophoresis* 30:141–54
5. Hjertén S, Liao JL, Zhang R. 1989. High-performance liquid chromatography on continuous polymer beds. *J. Chromatogr.* 473:273–75
6. Svec F, Fréchet JMJ. 1992. Continuous rods of macroporous polymer as high-performance liquid chromatography separation media. *Anal. Chem.* 64:820–22
7. Nakanishi K, Soga N. 1992. Phase separation in silica sol-gel system containing polyacrylic acid. 2. Effects of molecular weight and temperature. *J. Noncryst. Solids* 139:14–24
8. Nakanishi K, Soga N. 1992. Phase separation in silica sol-gel system containing polyacrylic acid. 1. Gel formation behavior and effect of solvent composition. *J. Noncryst. Solids* 139:1–13
9. Nakanishi K, Soga N. 1991. Phase separation in gelling silica–organic polymer solution: systems containing poly(sodium styrenesulfonate). *J. Am. Ceram. Soc.* 74:2518–30
10. Hatsis P, Lucy CA. 2003. Improved sensitivity and characterization of high-speed ion chromatography of inorganic anions. *Anal. Chem.* 75:995–1001
11. Liapis AI, Meyers JJ, Crosser OK. 1999. Modeling and simulation of the dynamic behavior of monoliths. Effects of pore structure from pore network model analysis and comparison with columns packed with porous spherical particles. *J. Chromatogr. A* 865:13–25
12. Skudas R, Grimes BA, Thommes M, Unger KK. 2009. Flow-through pore characteristics of monolithic silicas and their impact on column performance in high-performance liquid chromatography. *J. Chromatogr. A* 1216:2625–36
13. Nordborg A, Svec F, Fréchet JMJ, Irgum K. 2005. Extending the array of crosslinkers suitable for the preparation of polymethacrylate-based monoliths. *J. Sep. Sci.* 28:2401–6
14. Ishizuka N, Minakuchi H, Nakanishi K, Soga N, Nagayama H, et al. 2000. Performance of a monolithic silica column in a capillary under pressure-driven and electrodriven conditions. *Anal. Chem.* 72:1275–80
15. Siouffi AM. 2003. Silica gel–based monoliths prepared by the sol-gel method: facts and figures. *J. Chromatogr. A* 1000:801–18
16. Minakuchi H, Nakanishi K, Soga N, Ishizuka N, Tanaka N. 1996. Octadecylsilylated porous silica rods as separation media for reversed-phase liquid chromatography. *Anal. Chem.* 68:3498–501
17. Urban J, Svec F, Fréchet JMJ. 2010. Efficient separation of small molecules using a large surface area hypercrosslinked monolithic polymer capillary column. *Anal. Chem.* 82:1621–23
18. Viklund C, Pontén E, Glad B, Irgum K, Hörstedt P, Svec F. 1997. "Molded" macroporous poly(glycidyl methacrylate-*co*-trimethylolpropane trimethacrylate) materials with fine controlled porous properties: preparation of monoliths using photoinitiated polymerization. *Chem. Mater.* 9:463–71

19. Viklund C, Svec F, Fréchet JMJ, Irgum K. 1996. Monolithic, "molded", porous materials with high flow characteristics for separations, catalysis, or solid-phase chemistry: control of porous properties during polymerization. *Chem. Mater.* 8:744–50
20. Svec F, Fréchet JMJ. 1995. Temperature, a simple and efficient tool for the control of pore size distribution in macroporous polymers. *Macromolecules* 28:7580–82
21. Safrany A, Beiler B, Laszlo K, Svec F. 2005. *Polymer* 46:2862–71
22. Zhang YP, Ye XW, Tian MK, Qu LB, Choi SH, et al. 2008. Novel method to prepare polystyrene-based monolithic columns for chromatographic and electrophoretic separations by microwave irradiation. *J. Chromatogr. A* 1188:43–49
23. Arvidsson P, Plieva FM, Savina IN, Lozinsky VI, Fexby S, et al. 2002. Chromatography of microbial cells using continuous supermacroporous affinity and ion-exchange columns. *J. Chromatogr. A* 977:27–38
24. Meyer U, Svec F, Fréchet JMJ, Hawker CJ, Irgum K. 2000. Use of stable free radicals for the sequential preparation and surface grafting of functionalized macroporous monoliths. *Macromolecules* 33:7769–75
25. Viklund C, Nordström A, Irgum K, Svec F, Fréchet JMJ. 2001. Preparation of porous poly(styrene-*co*-divinylbenzene) monoliths with controlled pore size distributions initiated by stable free radicals and their pore surface functionalization by grafting. *Macromolecules* 34:4361–69
26. Sinner FM, Buchmeiser MR. 2000. A new class of continuous polymer supports prepared by ring-opening metathesis polymerization: a straightforward route to functionalized monoliths. *Macromolecules* 33:5777–86
27. Peters EC, Svec F, Fréchet JMJ, Viklund C, Irgum K. 1999. Control of porous properties and surface chemistry in "molded" porous polymer monoliths prepared by polymerization in the presence of TEMPO. *Macromolecules* 32:6377–79
28. Zhang R, Qi L, Xin P, Yang G, Chen Y. 2010. Preparation of macroporous monolith with three dimensional bicontinuous skeleton structure by atom transfer radical polymerization for HPLC. *Polymer* 51:1703–8
29. Nguyen AM, Irgum K. 2006. Epoxy-based monoliths. A novel hydrophilic separation material for liquid chromatography of biomolecules. *Chem. Mater.* 18:6308–15
30. Nguyen AM, Dinh NP, Cam QM, Sparrman T, Irgum K. 2009. Preparation and characterization of sizable macroporous epoxy resin-based monolithic supports for flow-through systems. *J. Sep. Sci.* 32:2608–18
31. Wang J, Zhang C, Du Z, Xiang A, Li H. 2008. Formation of porous epoxy monolith via concentrated emulsion polymerization. *J. Colloid Interface Sci.* 325:453–58
32. Nguyen AM, Nordborg A, Shchukarev A, Irgum K. 2009. Thermally induced dissolution/precipitation—a simple approach for the preparation of macroporous monoliths from linear aliphatic polyamides. *J. Sep. Sci.* 32:2619–28
33. Nguyen AM, Duc NT, Irgum K. 2008. Sizeable macroporous monolithic polyamide entities prepared in vlosed molds by thermally mediated dissolution and phase segregation. *Chem. Mater.* 20:6244–47
34. Sinner FM, Buchmeiser MR. 2000. Ring-opening methathesis polymerization: access to a new class of functionalized, monolithic stationary phases for liquid chromatography. *Angew. Chem. Int. Ed.* 39:1433–36
35. Peters E, Petro M, Svec F, Fréchet JMJ. 1998. Molded rigid polymer monoliths as separation media for capillary electrochromatography. 1. Fine control of porous properties and surface chemistry. *Anal. Chem.* 70:2288–95
36. Svec F, Tennikova TB, Deyl Z, eds. 2003. *Monolithic Materials: Preparation, Properties, and Applications*. Amsterdam: Elsevier
37. Gu B, Chen Z, Thulin CD, Lee ML. 2006. Efficient polymer monolith for strong cation-exchange capillary liquid chromatography of peptides. *Anal. Chem.* 78:3509–18
38. Gu B, Li Y, Lee ML. 2007. Polymer monoliths with low hydrophobicity for strong cation-exchange capillary liquid chromatography of peptides and proteins. *Anal. Chem.* 79:5848–55
39. Chen X, Tolley HD, Lee ML. 2010. Polymeric cation-exchange monolithic columns containing phosphoric acid functional groups for capillary liquid chromatography of peptides and proteins. *J. Chromatogr. A* 1217:3844–54

40. Wang F, Dong J, Jiang X, Ye M, Zou H. 2007. Capillary trap column with strong cation-exchange monolith for automated shotgun proteome analysis. *Anal. Chem.* 79:6599–606
41. Eeltink S, Hilder EF, Geiser L, Svec F, Fréchet JMJ, et al. 2007. Controlling the surface chemistry and chromatography properties of methyacrylate-ester-based monolithic capillary colums via photografting. *J. Sep. Sci.* 30:407–13
42. Li Y, Gu B, Tolley HD, Lee ML. 2009. Preparation of polymeric monoliths by copolymerization of acrylate monomers with amine functionalities for anion-exchange capillary liquid chromatography of proteins. *J. Chromatogr. A* 1216:5525–32
43. Svec F, Fréchet JMJ. 1995. "Molded" rods of macroporous polymer for preparative separations of biological products. *Biotechnol. Bioeng.* 48:476–80
44. Danquah MK, Ho J, Forde GM. 2007. Performance of R-*N*(R′)-R″ functionalised poly(glycidyl methacrylate-*co*-ethylene glycol dimethacrylate) monolithic sorbent for plasmid DNA adsorption. *J. Sep. Sci.* 30:2843–50
45. Yang G, Feng S, Liu H, Yin J, Zhang L, Cai L. 2007. On-line clean-up and screening of oxacillin and cloxacillin in human urine and plasma with a weak ion exchange monolithic column. *J. Chromatogr. B* 854:85–90
46. Wieder W, Bisjak CP, Huck CW, Bakry R, Bonn GK. 2006. Monolithic poly(glycidyl methacrylate-*co*-divinylbenzene) capillary columns functionalized to strong anion exchangers for nucleotide and oligonucleotide separation. *J. Sep. Sci.* 29:2478–84
47. Dinh NP, Cam QM, Nguyen AM, Shchukarev A, Irgum K. 2009. Functionalization of epoxy-based monoliths for ion exchange chromatography of proteins. *J. Sep. Sci.* 32:2556–64
48. Tian Y, Yang F, Yang X, Fu E, Xu Y, Zeng Z. 2008. Macrocyclic polyamine–modified poly(glycidyl methacrylate-*co*-ethylene dimethacrylate) monolith for capillary electrochromatography. *Electrophoresis* 29:2293–300
49. Ueki Y, Umemura T, Li J, Odake T, Tsunoda K-I. 2004. Preparation and application of methacrylate-based cation-exchange monolithic columns for capillary ion chromatography. *Anal. Chem.* 76:7007–12
50. Frankovič V, Podgornik A, Krajnc NL, Smrekar F, Krajnc P, Štrancar A. 2008. Characterisation of grafted weak anion-exchange methacrylate monoliths. *J. Chromatogr. A* 1207:84–93
51. Savina IN, Galaev IY, Mattiasson B. 2005. Anion-exchange supermacroporous monolithic matrices with grafted polymer brushes of *N*,*N*-dimethylaminoethyl-methacrylate. *J. Chromatogr. A* 1092:199–205
52. Yao K, Yun J, Shen S, Chen F. 2007. In-situ graft-polymerization preparation of cation-exchange supermacroporous cryogel with sulfo groups in glass columns. *J. Chromatogr. A* 1157:246–51
53. Savina IN, Galaev IY, Mattiasson B. 2006. Ion-exchange macroporous hydrophilic gel monolith with grafted polymer brushes. *J. Mol. Recognit.* 19:313–21
54. Chen F, Yao KJ, Shen S, Yun J. 2008. Influence of grafting conditions on the properties of polyacrylamide-based cation-exchange cryogels grafted with 2-acrylamido-2-methyl-1-propanesulfonic acid. *Chem. Eng. Sci.* 63:71–77
55. Rohr T, Hilder EF, Donovan JJ, Svec F, Fréchet JMJ. 2003. Photografting and the control of surface chemistry in three-dimensional porous polymer monoliths. *Macromolecules* 36:1677–84
56. Pucci V, Raggi MA, Svec F, Fréchet JMJ. 2004. Monolithic columns with a gradient of functionalities prepared via photoinitiated grafting for separations using capillary electrochromatography. *J. Sep. Sci.* 27:779–88
57. Krenkova J, Gargano A, Lacher NA, Schneiderheinze JM, Svec F. 2009. High binding capacity surface grafted monolithic columns for cation exchange chromatography of proteins and peptides. *J. Chromatogr. A* 1216:6824–30
58. Eder K, Huber CG, Buchmeiser MR. 2007. Surface-functionalized, ring-opening metathesis polymerization–derived monoliths for anion-exchange chromatography. *Macromol. Rapid Commun.* 28:2029–32
59. Gatschelhofer C, Mautner A, Reiter F, Pieber TR, Buchmeiser MR, Sinner FM. 2009. Ring-opening metathesis polymerization for the preparation of norbornene-based weak cation-exchange monolithic capillary columns. *J. Chromatogr. A* 1216:2651–57
60. Lubbad S, Buchmeiser MR. 2003. A new approach to high-capacity functionalized monoliths via post-synthesis grafting. *Macromol. Rapid Commun.* 24:580–84

61. Zhang R, Yang G, Xin P, Qi L, Chen Y. 2009. Preparation of poly(*N*-isopropylacrylamide)-grafted polymer monolith for hydrophobic interaction chromatography of proteins. *J. Chromatogr. A* 1216:2404–11
62. Hilder EF, Svec F, Fréchet JMJ. 2004. Latex-functionalized monolithic columns for the separation of carbohydrates by micro anion-exchange chromatography. *J. Chromatogr. A* 1053:101–6
63. Zakaria P, Hutchinson JP, Avdalovic N, Liu Y, Haddad PR. 2005. Latex-coated polymeric monolithic ion-exchange stationary phases. 2. Micro-ion chromatography. *Anal. Chem.* 77:417–23
64. Hutchinson JP, Zakaria P, Bowie AR, Macka M, Avdalovic N, Haddad PR. 2005. Latex-coated polymeric monolithic ion-exchange stationary phases. 1. Anion-exchange capillary electrochromatography and in-line sample preconcentration in capillary electrophoresis. *Anal. Chem.* 77:407–16
65. Hutchinson JP, Hilder EF, MacKa M, Avdalovic N, Haddad PR. 2006. Preparation and characterisation of anion-exchange latex-coated silica monoliths for capillary electrochromatography. *J. Chromatogr. A* 1109:10–18
66. Hutchinson JP, Hilder EF, Shellie RA, Smith JA, Haddad PR. 2006. Towards high capacity latex-coated porous polymer monoliths as ion-exchange stationary phases. *Analyst* 131:215–21
67. Kanatyeva AY, Viktorova EN, Korolev AA, Kurganov AA. 2007. Comparison of nonporous silica-based ion exchangers and monolithic ion exchangers in separation of inorganic anions. *J. Sep. Sci.* 30:2836–42
68. Yang G, Liu H, Zhang Y, Wang S, Yin J, et al. 2006. On-line simultaneous removal of human serum albumin and enrichment of doxazosin using a weak cation-exchange monolithic column. *J. Chromatogr. A* 1129:231–35
69. Yang B, Takeuchi M, Dasgupta P, Umemura T, Ueki Y, Tsunoda KI. 2007. Tailoring elution of tetraalkylammonium ions. Ideal electrostatic selectivity elution order on a polymeric ion exchanger. *Anal. Chem.* 79:769–72
70. Nakanishi K, Shikata H, Ishizuka N, Koheiya N, Soga N. 2000. Tailoring mesopores in monolithic macroporous silica for HPLC. *J. High Res. Chromatogr.* 23:106–10
71. Shi ZG, Feng YQ, Da SL. 2003. Study of the preparation conditions of silica monoliths for HPLC. *J. Liq. Chromatogr. Rel. Technol.* 26:2881–96
72. Hara T, Kobayashi H, Ikegami T, Nakanishi K, Tanaka N. 2006. Performance of monolithic silica capillary columns with increased phase ratios and small-sized domains. *Anal. Chem.* 78:7632–42
73. Sun YW, Wang YJ, Guo W, Wang T, Luo GS. 2006. Triblock copolymer and poly(ethylene glycol) as templates for monolithic silica material with bimodal pore structure. *Microporous Mesoporous Mater.* 88:31–37
74. Chamieh J, Zimmermann Y, Boos A, Hagäge A. 2009. Preparation and characterisation of silica monoliths using a triblock copolymer (F68) as porogen. *J. Colloid Interface Sci.* 340:225–29
75. Tanaka N, Kobayashi H, Nakanishi K, Minakuchi H, Ishizuka N. 2001. Monolithic LC columns. *Anal. Chem.* 73:421–29A
76. Nakanishi K, Soga N. 1992. Phase separation in silica sol-gel system containing polyacrylic acid. 3. Effect of catalytic condition. *J. Noncryst. Solids* 142:36–44
77. Nakanishi K, Soga N. 1992. Phase separation in silica sol-gel system containing polyacrylic acid. 4. Effect of chemical additives. *J. Noncryst. Solids* 142:45–54
78. Yan L, Zhang Q, Zhang J, Zhang L, Li T, et al. 2004. Hybrid organic-inorganic monolithic stationary phase for acidic compounds separation by capillary electrochromatography. *J. Chromatogr. A* 1046:255–61
79. Dong M, Wu M, Wang F, Qin H, Han G, et al. 2010. Coupling strong anion-exchange monolithic capillary with MALDI–TOF MS for sensitive detection of phosphopeptides in protein digest. *Anal. Chem.* 82:2907–15
80. Zheng MM, Ruan GD, Feng YQ. 2009. Hybrid organic-inorganic silica monolith with hydrophobic/strong cation-exchange functional groups as a sorbent for micro–solid phase extraction. *J. Chromatogr. A* 1216:7739–46
81. Hu J, Xie C, Tian R, He Z, Zou H. 2006. Hybrid silica monolithic column for capillary electrochromatography with enhanced cathodic electroosmotic flow. *Electrophoresis* 27:4266–72
82. Xu L, Lee HK. 2008. Preparation, characterization and analytical application of a hybrid organic-inorganic silica-based monolith. *J. Chromatogr. A* 1195:78–84

83. Xie C, Hu J, Xiao H, Su X, Dong J, et al. 2005. Preparation of monolithic silica column with strong cation-exchange stationary phase for capillary electrochromatography. *J. Sep. Sci.* 28:751–56
84. Xie C, Hu J, Xiao H, Su X, Dong J, et al. 2005. Electrochromatographic evaluation of a silica monolith capillary column for separation of basic pharmaceuticals. *Electrophoresis* 26:790–97
85. Sugrue E, Nesterenko P, Paull B. 2003. Iminodiacetic acid functionalised monolithic silica chelating ion exchanger for rapid determination of alkaline earth metal ions in high ionic strength samples. *Analyst* 128:417–20
86. Sugrue E, Nesterenko PN, Paull B. 2005. Fast ion chromatography of inorganic anions and cations on a lysine bonded porous silica monolith. *J. Chromatogr. A* 1075:167–75
87. Jaafar J, Watanabe Y, Ikegami T, Miyamoto K, Tanaka N. 2008. Anion exchange silica monolith for capillary liquid chromatography. *Anal. Bioanal. Chem.* 391:2551–56
88. Ikegami T, Ichimaru J, Kajiwara W, Nagasawa N, Hosoya K, Tanaka N. 2007. Anion- and cation-exchange micro-HPLC utilizing poly(methacrylates)-coated monolithic silica capillary columns. *Anal. Sci.* 23:109–13
89. Watanabe Y, Ikegami T, Horie K, Hara T, Jaafar J, Tanaka N. 2009. Improvement of separation efficiencies of anion-exchange chromatography using monolithic silica capillary columns modified with polyacrylates and polymethacrylates containing tertiary amino or quaternary ammonium groups. *J. Chromatogr. A* 1216:7394–401
90. Ikegami T, Horie K, Jaafar J, Hosoya K, Tanaka N. 2007. Preparation of highly efficient monolithic silica capillary columns for the separations in weak cation-exchange and HILIC modes. *J. Biochem. Biophys. Methods* 70:31–37
91. Pelletier S, Lucy CA. 2006. Achieving rapid low-pressure ion chromatography separations on short silica-based monolithic columns. *J. Chromatogr. A* 1118:12–18
92. Xu Q, Mori M, Tanaka K, Ikedo M, Hu WZ. 2004. Dodecylsulfate-coated monolithic octadecyl-bonded silica stationary phase for high-speed separation of hydrogen, magnesium and calcium in rainwater. *J. Chromatogr. A* 1026:191–94
93. Ito K, Takayama Y, Makabe N, Mitsui R, Hirokawa T. 2005. Ion chromatography for determination of nitrite and nitrate in seawater using monolithic ODS columns. *J. Chromatogr. A* 1083:63–67
94. Li J, Zhu Y, Guo YY. 2006. Fast determination of anions on a short coated column. *J. Chromatogr. A* 1118:46–50
95. Ríordáin CO, Barron L, Nesterenko E, Nesterenko PN, Paull B. 2006. Double gradient ion chromatography using short monolithic columns modified with a long chained zwitterionic carboxybetaine surfactant. *J. Chromatogr. A* 1109:111–19
96. Ríordáin CO, Nesterenko P, Paull B. 2005. Zwitterionic ion chromatography with carboxybetaine surfactant–coated particle packed and monolithic type columns. *J. Chromatogr. A* 1070:71–78
97. Glenn KM, Lucy CA, Haddad PR. 2007. Ion chromatography on a latex-coated silica monolith column. *J. Chromatogr. A* 1155:8–14
98. Zhang T, Khadra I, Euerby MR, Skellern GG, Watson DG, Tettey JNA. 2008. Simultaneous analysis of basic, acidic and neutral compounds on an endcapped octadecylsilane silica-based monolith by pressure-assisted capillary electrochromatography. *Electrophoresis* 29:944–51
99. Ríordáin CO, Gillespie E, Connolly D, Nesterenko PN, Paull B. 2007. Capillary ion chromatography of inorganic anions on octadecyl silica monolith modified with an amphoteric surfactant. *J. Chromatogr. A* 1142:185–93
100. Konishi J, Fujita K, Nakanishi K, Hirao K. 2006. Phase-separation-induced titania monoliths with well-defined macropores and mesostructured framework from colloid-derived sol-gel systems. *Chem. Mater.* 18:864–66
101. Randon J, Guerrin JF, Rocca JL. 2008. Synthesis of titania monoliths for chromatographic separations. *J. Chromatogr. A* 1214:183–86
102. Randon Jo, Huguet S, Piram A, Puy G, Demesmay C, Rocca J-L. 2006. Synthesis of zirconia monoliths for chromatographic separations. *J. Chromatogr. A* 1109:19–25
103. Hoth DC, Rivera JG, Colón LA. 2005. Metal oxide monolithic column. *J. Chromatogr. A* 1079:392–96
104. Liang C, Dai S, Guiochon G. 2003. A graphitized-carbon monolithic column. *Anal. Chem.* 75:4904–12

105. Nawrocki J, Dunlap C, McCormick A, Carr PW. 2004. Chromatography using ultra-stable metal oxide–based stationary phases for HPLC. Part I. *J. Chromatogr. A* 1028:1–30
106. Nawrocki J, Dunlap C, Li J, Zhao J, McNeff CV, et al. 2004. Chromatography using ultra-stable metal oxide–based stationary phases for HPLC. Part II. *J. Chromatogr. A* 1028:31–62
107. Sui R, Liu S, Lajoie GA, Charpentier PA. 2010. Preparing titania aerogel monolithic chromatography columns using supercritical carbon dioxide. *J. Sep. Sci.* 33:1604–9
108. Courtois J, Szumski M, Georgsson F, Irgum K. 2007. Assessing the macroporous structure of monolithic columns by transmission electron microscopy. *Anal. Chem.* 79:335–44
109. Byström E, Viklund C, Irgum K. 2010. Differences in porous characteristics of styrenic monoliths prepared by controlled thermal polymerization in molds of varying dimensions. *J. Sep. Sci.* 33:191–99
110. Petter CH, Heigl N, Bonn GK, Huck CW. 2008. Fast, noninvasive and simultaneous near-infrared spectroscopic characterisation of physicochemical stationary phases' properties: from silica particles towards monoliths. *J. Sep. Sci.* 31:2541–50
111. Bruns S, Mullner T, Kollmann M, Schachtner J, Hoeltzel A, Tallarek U. 2010. Confocal laser scanning microscopy method for quantitative characterization of silica monolith morphology. *Anal. Chem.* 82:6569–75
112. Hlushkou D, Bruns S, Tallarek U. 2010. High-performance computing of flow and transport in physically reconstructed silica monoliths. *J. Chromatogr. A* 1217:3674–82
113. Connolly D, O'Shea V, Clark P, O'Connor B, Paull B. 2007. Evaluation of photografted charged sites within polymer monoliths in capillary columns using contactless conductivity detection. *J. Sep. Sci.* 30:3060–68
114. Gillespie E, Connolly D, Paull B. 2009. Using scanning contactless conductivity to optimise photografting procedures and capacity in the production of polymer ion-exchange monoliths. *Analyst* 134:1314–21
115. Gillespie E, Macka M, Connolly D, Paull B. 2006. Evaluation of capillary ion exchange stationary phase coating distribution and stability using radial capillary column contactless conductivity detection. *Analyst* 131:886–88
116. Hilder EF, Schaller D, Haddad PR, Evenhuis CJ, Nesterenko PN. 2009. *Scanning contactless conductivity measurements for quality assessment of monolithic columns*. Presented at Int. Sep. Sci. Soc., Dublin, Irel.
117. Connolly D, Paull B. 2009. High-performance separation of small inorganic anions on a methacrylate-based polymer monolith grafted with [2(methacryloyloxy)ethyl] trimethylammonium chloride. *J. Sep. Sci.* 32:2653–58
118. Thayer JR, Rao S, Xie S. 2006. Resolution of oligonucleotides and their derivatives on ProSwift anion-exchange monoliths. *LC/GC N. Am.* June:15
119. Thayer JR, Flook KJ, Woodruff A, Rao S, Pohl CA. 2010. New monolith technology for automated anion-exchange purification of nucleic acids. *J. Chromatogr. B* 878:933–41
120. Liu L, Yu S, Yang S, Zhou P, Hu J, Zhang Y. 2009. Extraction of genomic DNA using a new amino silica monolithic column. *J. Sep. Sci.* 32:2752–58
121. Lv YQ, Fu DY, Tan TW, Wang MY. 2010. One-step purification of YLLIP2 isoforms from *Candida* spp. 99–125 by polyethyleneimine modified poly(glycidyl methacrylate-*co*-ethylene glycol dimethacrylate) monolith. *J. Mol. Catal. B Enzym.* 62:149–54
122. Hann S, Falta T, Boeck K, Sulyok M, Koellensperger G. 2010. On-line fast column switching SEC × IC separation combined with ICP-MS detection for mapping metallodrug-biomolecule interaction. *J. Anal. At. Spectrom.* 25:861–66
123. Nordborg A, Zhang B, He XZ, Hilder EF, Haddad PR. 2009. Characterization of monoclonal antibodies using polymeric cation exchange monoliths in combination with salt and pH gradients. *J. Sep. Sci.* 32:2668–73
124. Talebi M, Nordborg A, Wang Q, Hilder EF, Haddad PR. 2010. *Polymeric monolithic ion-exchange stationary phases in combination with pH gradient for the separation and purity profiling of monoclonal antibodies*. Presented at Drug Anal. 2010, Antwerp, Belg.
125. Whitfield RJ, Battom SE, Barut M, Gilham DE, Ball PD. 2009. Rapid high-performance liquid chromatographic analysis of adenovirus type 5 particles with a prototype anion-exchange analytical monolith column. *J. Chromatogr. A* 1216:2725–29

126. Gutiérrez-Aguirre I, Banjac M, Steyer A, Poljšak-Prijatelj M, Peterka M, et al. 2009. Concentrating rotaviruses from water samples using monolithic chromatographic supports. *J. Chromatogr. A* 1216:2700–4
127. Thayer JR, Puri N, Burnett C, Hail M, Rao S. 2010. Identification of RNA linkage isomers by anion exchange purification with electrospray ionization mass spectrometry of automatically desalted phosphodiesterase-II digests. *Anal. Biochem.* 399:110–17
128. Neubert H, James I. 2009. Online capillary weak cation exchange enrichment hyphenated to nanospray mass spectrometry for quantitation of a basic pegvisomant derived peptide. *J. Chromatogr. A* 1216:6151–54
129. Hong MR, Kim YS, Joo AR, Lee JK, Oh DK. 2009. Purification and characterization of a thermostable β-1,3–1,4-glucanase from *Laetiporus sulphureus* var. *miniatus*. *J. Microbiol. Biotechnol.* 19:818–22
130. Konishi J, Fujita K, Nakanishi K, Hirao K, Morisato K, et al. 2009. Sol-gel synthesis of macro-mesoporous titania monoliths and their applications to chromatographic separation media for organophosphate compounds. *J. Chromatogr. A* 1216:7375–83
131. Krajnc P, Leber N, Stefanec D, Kontrec S, Podgornik A. 2005. Preparation and characterisation of poly(high internal phase emulsion) methacrylate monoliths and their application as separation media. *J. Chromatogr. A* 1065:69–73

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