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Review

# Preparation and application of hydrophilic monolithic columns

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ABSTRACT

Hydrophilic interaction chromatography (HILIC) has experienced increasing attention in recent years. Much research has been carried out in the area of HILIC separation mechanisms, column techniques and applications. Because of their good permeability, low resistance to mass transfer and easy preparation within capillaries, hydrophilic monolithic columns represent a trend among novel HILIC column techniques. This review attempts to present an overview of the preparation and applications of HILIC monolithic columns carried out in the past decade. The separation mechanism of various hydrophilic monolithic stationary phases is also reviewed.



**Contents**



## 1. Introduction

The separation of polar and hydrophilic compounds can be a significant challenge when using reversed-phase HPLC, which is by far the most popular HPLC mode. In order to achieve adequate retention of polar analytes, highly aqueous mobile phases are often required, which can cause a number of issues such as stationary-phase collapse [1] and decreased sensitivity in ESI-MS [2]. Normal-phase HPLC is a useful separation technique for providing effective retention for polar molecules, but the poor solubility of polar analytes in non-aqueous mobile phases, together with lower peak efficiency, reduced selection of stationary phases, and decreased reproducibility, has greatly limited its application.

Hydrophilic interaction chromatography (HILIC), which was first investigated by Alpert [3], has proven to be a powerful and solid alternative for both separation modes. It has experienced enormous growth in recent years, which was evident in Lämmerhofer's report where the number of published papers on HILIC increased exponentially in the past two or three years [4]. Also, Pontén et al. reported that there are currently about 25 new HILIC papers published every month, with an annual increase of between 50% and 70% over the last five years [5]. HILIC separations are carried out using polar stationary phases and a high-organic, low-aqueous mobile phase in order to achieve retention of very polar compounds which are difficult to retain using reversed-phase methods. Such mobile phase properties largely solved the solubility problem of polar compounds when using normal-phase HPLC. Additionally, the organic-rich mobile phases used with HILIC can assist spray formation, improve ionisation efficiencies and thus enhance the detection sensitivity in detectors relying on vaporisation of the eluent. Therefore, HILIC is an excellent technique in combination with

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ESI-MS and evaporative light scattering (ELSD) or charged aerosol detectors [6–9]. Recent examples of the successful use of HILIC in pharmaceutical analysis, especially with polar metabolites analysis, is another big boost for this technique [4,10]. Several reviews have discussed both applications [10–12] and stationary phases [13] used for HILIC. So far, HILIC applications focused mainly on the separation of carbohydrates [14,15], peptides [11,16,17], proteins [18,19], natural product extracts [20], polar pharmaceuticals [21,22] and some small polar analytes [23]. Silica [24–26], cyano [27], amino [9,16,28], diol [29], zwitterionic [30–33] and polyhydroxyethyl aspartamide and cyclodextrin-based packings [3,20] are most often used as HILIC stationary phases. Most of these stationary phases are prepared by chemical modification of the silica gel surface and are currently available commercially.

Monolithic columns have presented an ongoing trend in the development of HPLC column technology since they were first introduced for capillary LC in 1989 by Hjertén et al. [34]. This was mainly attributable to their high permeability, low resistance to mass transfer, and fast and simple preparation within micro- or nano-formats [35–39]. The additional advantages of organic based monoliths, such as high stability even under extreme pH conditions and the wide selection of monomers available with different functional groups, further enhanced their attraction. Similar to hydrophobic or other types of monoliths, there have been increasing reports on the use of HILIC monoliths in the past five years. Most of these were focused on the preparation of silica based or polymer based HILIC monolithic capillaries. Many attempts have been carried out in order to introduce the above mentioned popular HILIC functionalities as well as some novel hydrophilic functionalities onto the surface of monolithic materials. These columns have been evaluated as HILIC stationary phases not only using standard small polar compounds but also by real more complex samples.

## 2. Preparation of hydrophilic monolithic columns

### 2.1. Silica based monolithic columns

Silica based monolithic columns were first developed by the team of Nakanishi and Tanaka in the early 1990s [40–45]. Since then, the field has experienced impressive progress and success in both column preparation and application, which has been reviewed by several groups [35,46,47]. Most of these reported silica based monoliths were prepared based on similar procedures, which consist of the hydrolysis of one or more silanes (mostly tetramethoxysilane) in acidic solution in the presence of a suitable porogen. The aging and the maceration of the gel in a basic solution leads to the formation of suitable mesopores, and this is followed by the drying and heating, and the final surface modification with selected ligands as required. Besides two major generic advantages of all monolithic columns, i.e. the high permeability and the low resistance to mass transfer, one specific advantage of the silica based monolithic column is that its macropores and mesopores can be tailored separately to obtain the best performance for given analytical objectives. Recently Guiochon mentioned in his review that most publications dealing with silica monolithic columns concerned applications based on commercial columns [35]. He thought that this was because certain steps (e.g. the drying and cladding of the rods) are difficult to overcome in academic laboratories and the manufacturer Merck has well-written patents set to block any loopholes [48–51]. Among these commercially available silica monolithic columns produced by Merck, the Chromolith Performance monolith has the potential for HILIC applications since it has a polar silica surface. However only one such HILIC application has been reported based on these Chromolith Performance materials. Pack and Risley used this column in the HILIC separation mode for the detection and quantitation of lithium, sodium and potassium using ELSD [52].

Besides commercially available Chromolith Performance silica monoliths, some pure silica based monolithic capillary columns have also been prepared in academic laboratories and used for HILIC. The first silica based monolithic capillary columns were prepared following the sol–gel process described by Tanaka and co-workers at the end of the 1990s [43–45,53,54]. However their preparation is time-consuming due to the drying and calcinations steps. Recently, Puy et al. replaced the 24 h calcination step with a 2 h water washing step, which can eliminate 70% of the poly(ethylene glycol) (PEG) [55]. The remaining PEG does not have a detrimental effect on chromatographic properties, morphology or stability of the monolith. Later, they also found that the hydrothermal treatment step at 120 °C was not necessary for pure silica based monolithic capillaries used for normal phase mode or HILIC mode. The suppression of the hydrothermal treatment did not impair efficiency in CEC and in nano-LC but did contribute to an increase in retention factors [56]. In both articles, the authors evaluated silica monolithic capillaries based on simplified methods in nano-LC or CEC in a HILIC mode. Neutral xanthines were baseline separated with very high efficiencies (110,000–230,000 plates per meter).

Although there are limited HILIC publications based on bare silica monoliths, there are some interesting reports based on such columns which have been surface modified through grafting with polar or ion-exchange functional groups, such as amino [57–59], amides [60,61], carboxylic acids [62,63], etc.

Xie et al. reported several amino bonded silica monolithic stationary phases for hydrophilic interaction CEC (HI-CEC) or with a mixed mode of HILIC and weak anion-exchange CEC [57–59]. The monolithic silica matrix synthesised from a sol–gel process was chemically modified by 3-aminopropyltrimethoxysilane, 3-(2-aminoethylamino)propyltrimethoxysilane [57] or diethylenetriaminopropyltrimethoxysilane [59] in order to produce a column for hydrophilic interaction applications. The surface modifications were simply carried out by pumping the solution of silane in anhydrous toluene through the bare silica monolithic capillary column for 1 h which was thermostated at 75 °C or 110 °C for various times. These amino silica monolithic stationary phases exhibited HILIC behaviour toward neutral solutes. However no comparative research between these columns and bare silica monoliths has been reported.

Recently, Ikegami et al. summarised a polymer coating procedure in his review article [64] (Fig. 1). In general, the bare silica based monolithic column was first modified with an anchor reagent to introduce methacrylamide or methacrylate functionality. This was followed by on-column polymerisation with a vinyl monomer in order to coat the silica surface with the required functionality. This modification method has several advantages: first, the final products maintained the high permeability and the separation efficiency of the original bare silica monolithic columns even after polymer modification; secondly, various polar functional groups can be introduced simply by changing the functional monomer type; and finally, the amount of functional groups can be controlled by modifying the polymerisation conditions.

Ikegami et al. first investigated a method of surface modification of monolithic silica capillary columns with polyacrylamide (PAAm) in order to improve the HILIC retention character of bare silica based monoliths [60]. The pre-prepared silica monolithic capillary columns were first modified with the bi-functional monomer *N*-(3-trimethoxysilylpropyl) methacrylamide. Then, a monomer solution of acrylamide and 3-aminopropyltriethoxysilane in water was introduced into the column and thermostated at 60 °C for 1 h for the polymerisation step. This PAAm-coated silica monolithic capillary column exhibited three times greater permeability and slightly higher column efficiency compared to a commercial amide-

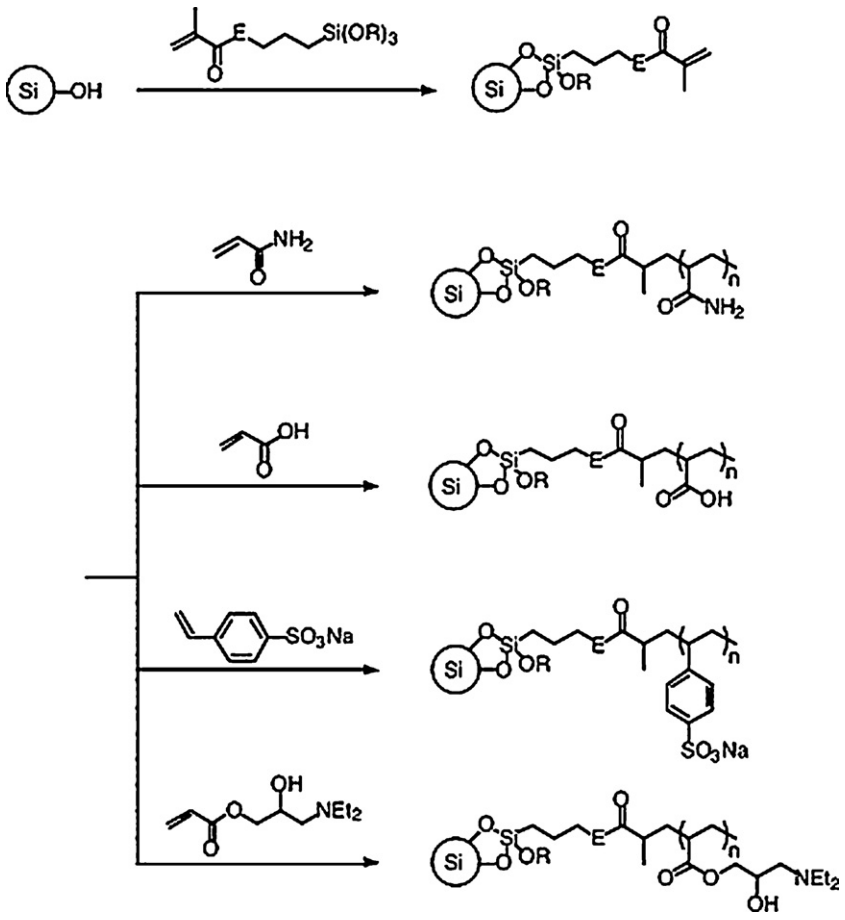


**Fig. 1.** Synthetic scheme of the polymer-coated monolithic silica columns [64].

type HILIC column packed with 5 μm particles (Fig. 2). A group of nucleic bases and nucleosides was selected for comparing the PAAm-coated silica monolithic capillary column with a bare silica monolithic capillary column and a commercial amide-type HILIC column. Clear HILIC mode retention behaviour was observed on all three columns. The retention factors of these solutes on the PAAm-coated monolithic silica capillary column were much larger than that on the bare monolithic silica capillary column, and showed good linear correlation of retention factor when compared with the commercial amide-type HILIC column.

Ikegami et al. subsequently significantly improved the column performance of PAAm-coated silica monolithic capillary columns by using a bare silica monolithic capillary column with a higher phase ratio, prepared following previously reported methods [61,65]. This high phase ratio PAAm-coated column exhibited a much higher efficiency ($H$ = 7 μm at a linear velocity of 1.0 mm/s) than that of the previously reported PAAm-coated silica monolithic capillary columns ($H$ = 16 μm at a linear velocity 1.0 mm/s). The authors compared the kinetic performance of this PAAm-coated monolithic silica column with three particle-packed columns, including the ZIC-HILIC column and two TSK-GEL Amide-80 columns (Fig. 3). These kinetic plots can be used to evaluate the limits of the separation efficiency considering the separation time under a constant backpressure. The results clearly proved that the PAAm-coated silica monolithic column can generate the same separation efficiency as particle-packed columns within a shorter time. For example, compared to Amide-80 columns packed with 3 μm particles, it can generate separations with the same efficiency but 1.7 times faster. This high efficiency PAAm-coated silica monolithic capillary column was successfully

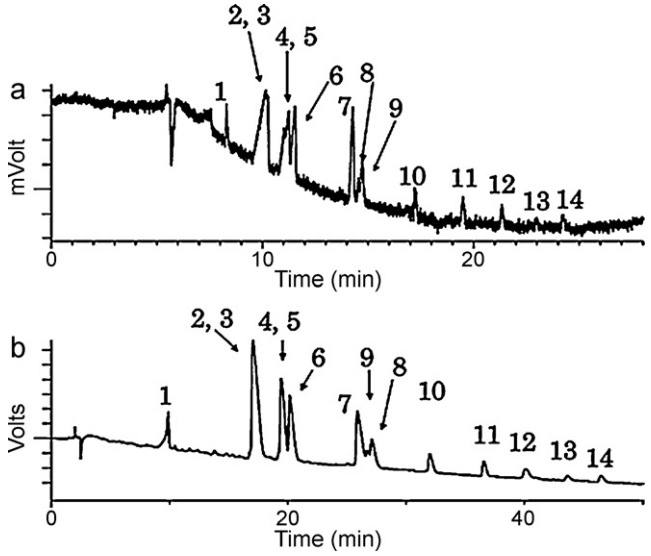


**Fig. 2.** Chromatograms of PA sugar derivative separations on a PAMS(100) (a), and an Amide-80 (b) column. HPLC conditions were ambient temperature, detection λ of 245 nm, mobile phase in ACN–water (0.2% formic acid), linear gradient of ACN 90–50% ($t_G$ = 30 min). a Column: PAMS(100)-1 (100 μm ID × 38 cm), linear velocity $u$ = 1.1 mm/s at $\Delta P$ = 2.1 MPa. b Column: TSKgel Amide-80 (2 mm ID × 15 cm), flow rate: 0.2 ml/min, linear velocity: $u$ = 1.0 mm/s at $\Delta P$ = 3.6 MPa. Solutes: (1) 2-aminopyridine, (2) PA-xylose, (3) PA-arabinose, (4) PA-glucose, (5) PA-mannose, (6) PA-galactose, (7) PA-maltose, (8) PA-lactose, (9) PA-cellobiose, (10) PA-maltotriose, (11) PA-maltotetraose, (12) PA-maltopentaose, (13) PA-maltohexaose, and (14) PA-maltoheptaose.
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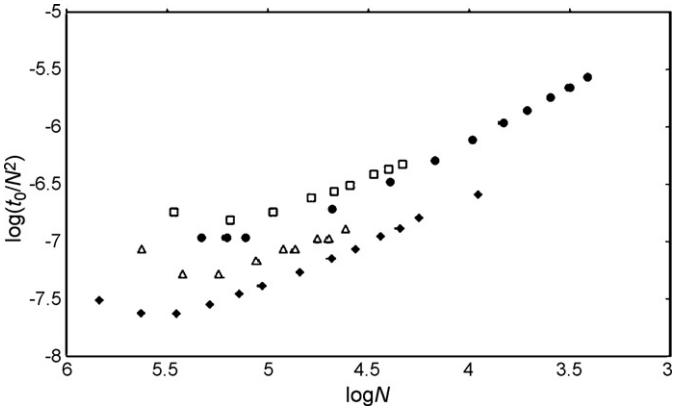


**Fig. 3.** Kinetic plot of $\log(t_0/N^2)$ versus $\log N$ with the assumed maximum pressure of 20 MPa for the (♦) MS-200T-PAAm, (●) the ZIC-HILIC, (□) the TSK-GEL Amide-80, 5 μm, and (△) the TSK-GEL Amide-80, 3 μm columns. Chromatographic conditions for TSK-GEL Amide-80 columns are as follows: column TSK-GEL Amide-80, 250 mm × 4.6 mm ID, 5 μm and 150 mm × 4.6 mm ID, 3 μm; mobile phase 75% $CH_3CN/H_2O$; temperature 40 °C; detection RI; solute mannitol ($k$ = 2.5); sample volume 10 μl injection.
Reproduced with permission from [61].

applied to the separation of underivatised carbohydrates using HILIC-ESI-MS.

In order to further improve the retention of pyridylamino (PA)-sugar derivatives on a PAAm-coated silica monolithic capillary column, Ikegami et al. also investigated poly(acrylic acid) (PAA)-coated silica monolithic capillary columns [62]. Due to the higher hydrophilicity and negative charge of acrylic acid functionalities, stronger retention was observed for the HILIC mode along with a weak cation-exchange mixed mode also expected. The same preparation procedure used for the PAAm coating was used for the PAA coating. *N*-(3-Triethoxysilylpropyl) methacrylamide was used as an anchor reagent in order to introduce methacrylamide functionality to the surface of the silica monolith. The monomer solution composed of acrylic acid and ammonium persulphate (APS) in water was forced into the column and then thermostated at 60 °C for 2 h in order for the reaction to proceed. The resulting PAA-coated columns exhibited high column efficiency ($H$ = 9–10 μm at a linear flow rate of 1.0 mm/s). These columns were evaluated for the HILIC mode separation of PA-sugars and peptides including a tryptic digest of bovine serum albumin (BSA). It showed greater retention toward PA-sugars than the PAAm-coated silica monolithic capillary columns prepared in the same manner. By increasing the concentration of functional monomer in the polymerisation mixture, the retentivity of PAA-coated silica monolithic columns could be further improved. In their following article [63], they systematically compared the gradient separation of peptides (or phosphorylase B tryptic digest) between the PAA-coated and the poly(octadecyl methacrylate)-coated monolithic silica columns using a capillary LC–ESI-MS system. Very different retention selectivity was observed. The HILIC mode separation based upon the PAA-coated silica represents an alternative to the reversed-phase mode for a wide range of compounds, especially for those of high polarity, in isocratic as well as gradient elution.

γ-Glycidoxypropyltrimethoxysilane (GPTMS) can also be used as an anchor reagent to introduce epoxy groups onto the surface of silica monoliths followed by modification. Recently, Huang et al. prepared a hydrophilic silica-based monolith for hydrophilic interaction pressurised CEC (HI-pCEC) through the on-column reaction of lysine and the epoxy groups [66]. The GPTMS-modified silica monolith was prepared by treating the bare silica monolith with the reaction solution of GPTMS and 2,6-lutidine (catalyst) in anhydrous toluene at 110 °C for 1 h. Lysine dissolved in 50 mM/l $NaH_2PO_4$ (pH 8.0) was then introduced into pre-modified columns and allowed to react at 75 °C for 1 h. A diol silica monolithic column was also prepared by simply treating the prepared epoxy monolith with 0.1 mol/l hydrochloric acid overnight and placing in an oven at 60 °C for 2 h in order to open the epoxy ring. Both columns together with the original epoxy monolithic column were evaluated using typical neutral non-polar and polar compounds in the HILIC mode. The results showed that the lysine monolith was much more hydrophilic than the diol monolith, which in turn exhibited less hydrophobic character than the epoxy monolith. Zhong and El Rassi also successfully used the same process to immobilise polar cyano functions onto the surface of silica based monoliths in order to perform normal phase chromatography of glycans [67].

Another approach to modify the silica monolithic surface is to use latex particles. The latex particle can be strongly attached to a bare silica surface through the electrostatic interaction between the negatively charged silanol groups and the positively charged functional groups on the latex particle [68–70]. Recently, Haddad's group prepared an agglomerated silica monolith by flushing a suspension of Dionex AS9-SC latex through a Chromolith Performance silica monolith (100 mm × 4.6 mm ID) [71]. The electrostatically attached latex is extremely rugged, able to withstand pH 0–14 and 1–100% (v/v) of common RP solvents in aqueous mixtures. The agglomerated column exhibited high efficiency for the anion exchange separation of inorganic anions with great chemical stability. Later, Ibrahim et al. also investigated the HILIC properties of the same latex-coated monolith [72]. Retention behaviour similar to a commercial HILIC column was observed for test analytes (naphthalene, phthalic acid and cytosine). The column efficiencies for the retained species (22–100 μm plate heights) on the latex-coated column are significantly higher than those achieved with the commercial Styros™ Amino HILIC material.

There have been other reports on silica monolithic columns being modified by polar functional groups such as β-cyclodextrin [73], amino acid [74], tertiary amine and quaternary ammonium [75], sulphonate [75,76], and phosphate groups [77]. However, their applications in the HILIC mode have not been investigated. Because of the high polarity of these functionalities, it is possible that they could be used for HILIC mode separation in the future.

### 2.2. Polymer based monolithic column

Polymer based monolithic materials include those that are styrene based, methacrylate based or acrylamide based. The actual polymerisation process is initiated either thermally or by photo induction of a mixture consisting of monomers and porogenic solvents together with an initiator. Many reviews have described the development and applications of polymer based monolithic materials in the past decade [35,36,39,78–81]. However, reports on hydrophilic polymer-based monoliths are still very limited. The lack of studies on polymer based monolithic columns for HILIC is the result of several factors. First, the limited solubility of very polar monomers in most commonly used porogens requires a completely new optimisation of the polymerisation solution mixture; second, the lack of commercially available polar monomers; and last, possible prejudices that polymer based monoliths are well-suited to biomacromolecule separations but not small molecules. Nonetheless, there has been some progress toward the fabrication of polymer based HILIC monolithic columns in recent years. Among the three types of polymer based monolithic columns, acrylamide-based monoliths show great potential for HILIC selectivity due to the hydrophilicity of the polyacrylamide backbone, which has been used successfully in normal phase separations [82,83].

Most reported acrylamide HILIC monoliths have been prepared based on a generic synthesis protocol, i.e. dispersion polymerisation in aqueous solution, which was developed by Hjertén and

co-workers in the middle of the 1990s [84]. In general, functional monomers, crosslinker and lyotrophic salt were dissolved in an aqueous rich phase. Instead of using the traditional organic solvents, which normally result in a somewhat hydrophobic monolith, water or water-formamide were used as solvents, leading to more hydrophilic materials. Piperazine diacrylamide (PDA) was normally used as the crosslinker due to its better solubility than methylene bisacrylamide (MBA) in water. A redox system consisting of *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TEMED) and ammonium persulphate were then added as initiator or catalyst and when conducting CEC, vinylsulphonic acid (VSA) was often added as a source of ligands which promoted an EOF. Polymerisation progressed at room temperature overnight. Following this procedure, Hjertén and co-workers evaluated a hydrophilic monolithic capillary column prepared by a simple one-step *in situ* co-polymerisation of isopropylacrylamide (and/or methacrylamide) with PDA [83]. The reaction was initiated using a mixture of 10% (v/v) TEMED and 10% APS (w/v).

Later, the group of Freitag thoroughly and systematically investigated the fabrication and separation mechanism of acrylamide-based monoliths using similar procedures [82,85–87]. As expected, the composition of the polymerisation mixture played a key role in the final monolith properties and porosity. For the monoliths with methacrylamide (MAA) and dimethylacrylamide (DMAA) as functional monomers, a transition from a gel type consistency to a hard polymer could be observed as the polymer formed when the cross-linker concentration was increased, but more so when the total monomer concentration was increased for a given combination of monomers [82,85]. A lyotrophic salt, often ammonium sulphate, is another crucial parameter in monolith preparation. For the dispersion polymerisation in aqueous solution, the initially produced gel-like network has to collapse at some point either into nodules or into bundles of polymer chains in order to form a porous structure. It was believed that the location of the pores may be "between the bundled chains" or "between the precipitated nodules". A lyotrophic salt was added into the reaction mixture in order to salt-out the polymerisation chain. Therefore, the nature of the solvent and the type and concentration of the salting-out agent can also significantly affect the porosity of the monolith. In general, the pore size increases with increasing amounts of salt [82]. For example, the average pore size of the poly(DMAA-*co*-PDA-*co*-VSA) monoliths prepared in the presence of 0, 20 or 120 mg/ml ammonium sulphate were 50, 800 and 1300 nm, respectively (Fig. 4) [82]. Hoegger and Freitag also compared the effect of another five salts with APS on the DMAA polymer [85]. No significant difference in the polymer consistencies, except in the case of magnesium chloride, was observed. Studies on the effects of solvent system showed that the polymerisation could be carried out in a mixture of water and DMF or formamide but not pure methanol. However, these monoliths were harder than those prepared in pure water. The influence of functional monomers was investigated by Hoegger and Freitag [85] with the aim of determining their ability to form hydrophilic stationary phases. A wide range of monomers, which included DMAA, MAA, VSA, allyl amine (ALAM), 2-hydroxy methacrylate (HEMA), butylacrylate, hexylacrylate, 2-hydroxyethyl acrylate (HEA), *N*-(hydroxymethyl)-acrylamide (HMAA), 3-amino-1-propanol vinyl ether (APVE) and 2-(acryloyloxy)-ethyltrimethylammonium methyl sulphate (2-AETMA) were selected. Two monomers, APVE and ALAM both failed to produce the necessary porous monoliths, apparently due to the low reactivity of allyl monomers compared to vinyl monomers. The monomers HEMA, HEA and HMAA all of which contain a carbonyl group, produced monoliths with the necessary rigidity and granular texture. Similar to Hjertén and co-workers' report [83], most of the above mentioned acrylamide based monolithic columns were shown to retain a series of polar aromatic compounds in micro-HPLC or CEC mode.

Around the same period, Novotny and co-workers [88] prepared an amino-phase hydrophilic monolith by co-polymerisation of acrylamide, MBA, PEG (MW 10 000), 2-AETMA (80 wt.%) and APVE (96%). A mixture of formamide/100 mM Tris–150 mM boric acid (pH 8.2) was used as porogen. The column produced was used for the analysis of bile acids and their conjugates by CEC/negative-ion ESI-MS with high column efficiencies (610,000 theoretical plates/meter for glycocholic acid) and low detection limits (~40 fmol for cholic acid). Even though it was referred to as a normal phase separation by the authors, the actual mobile phase composition (ACN/water/240 mM ammonium formate buffer (pH 3) 60:35:5, v/v/v) is more like HILIC conditions. Later, they prepared another cyano-phase hydrophilic monolith for CEC using a similar procedure [89]. The 2-AETMA and APVE in the polymerisation mixture were replaced with 2-cyanoethyl acrylate and VSA. This column together with the above-mentioned amino alkyl-phase monolithic column was used for the analysis of a wide range of neutral saccharides in a CEC-ESI-MS system. Tegeler et al. prepared the same cyano hydrophilic monolith and used it to separate complex glycan mixtures by CEC-MALDI MS [90].

Xie et al. reported molded macroporous monoliths with pore sizes up to 1000 nm prepared by co-polymerisation of the very hydrophilic monomers acrylamide and MBA, in the presence of the organic porogenic mixture DMSO and 2-heptanol. The composition of the porogenic solvent, the percentage of crosslinker as well as free radical initiator can be used to engineer monoliths with pores tailored for particular applications [91]. These hydrophilic monoliths can be used in the separation of biological polymers, solid-phase extraction, or for immobilisation of proteins.

Methacrylate based monoliths have also been developed for HILIC mode separations. Compared to acrylamide based monoliths, methacrylate based monoliths have two possible advantages. First, there is a broader range of commercially available hydrophilic methacrylate monomers with various functionalities and, second, there are plenty of previous published methods for modifying the methacrylate based monolith surface which could be used to introduce a hydrophilic functionality. Glycidyl methacrylate-based monoliths are possibly the most popular reactive monoliths for such two-step modification.

Wang et al. prepared a HILIC monolithic stationary phase by one-step *in situ* co-polymerisation of HEMA, ethylene dimethacrylate (EDMA), and MAA in a binary porogenic solvent consisting of toluene and 1-dodecanol [92]. The resulting monolith was evaluated as a HI-CEC stationary phase using pCEC. The separation of charged solutes was based on a mixed mode mechanism of hydrophilic interaction and weak electrostatic interaction as well as on their electrophoretic mobility, while the separation of neutral solutes was based solely on hydrophilic interaction at high ACN content. Later, they prepared another HILIC polymer based monolithic stationary phase by *in situ* co-polymerisation of HEMA and a polar cross-linker containing a hydroxy group, PETA, in the presence of a binary porogenic solvent consisting of cyclohexanol and dodecanol [93]. The hydroxy and ester groups on the surface of the monolithic stationary phase were thought to be the hydrophilic interaction sites as well as the EOF generator through attraction of ions from the mobile phase, imparting some zeta potential to the monolithic solid phase. A typical HI-CEC mechanism was observed on this neutral polar stationary phase for both neutral and charged analytes.

By *in situ* co-polymerisation of 3-sulphopropyl methacrylate (SPMA) and PETA in a binary porogenic solvent consisting of cyclohexanol/ethylene glycol, another hydrophilic polymethacrylate-based monolithic column was prepared by Xie and co-workers

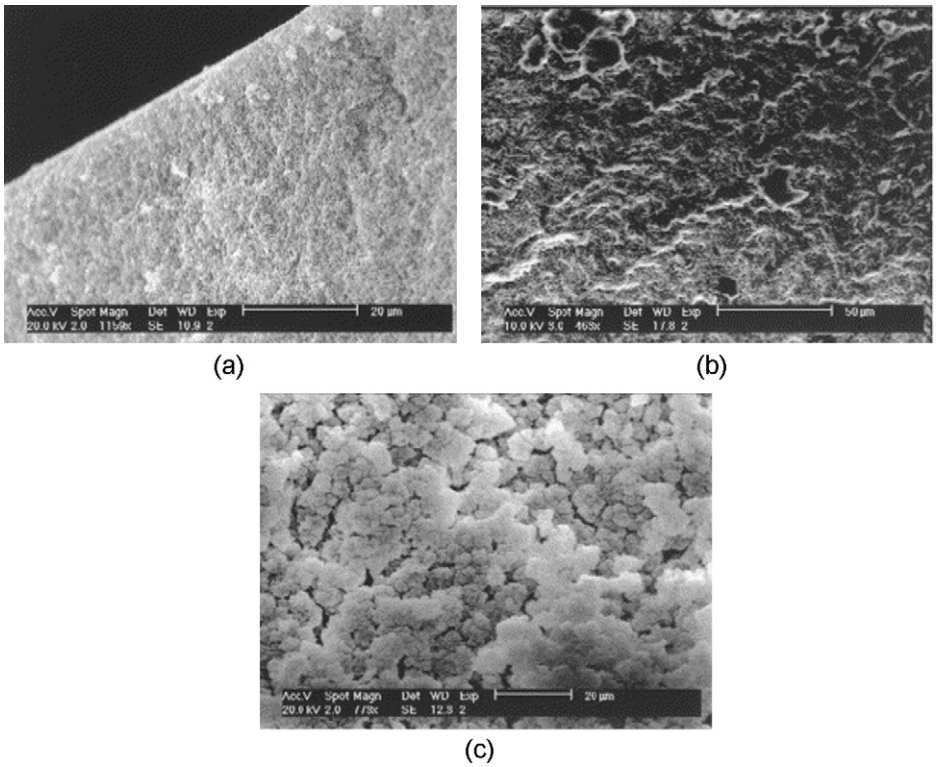


**Fig. 4.** Effects of ammonium sulphate concentration on the morphology of poly(DMAA–PDA–VSA) monoliths ($T$ = 29%, $C$ = 52%), prepared in the presence of (a) no, (b) 20 mg/ml, and (c) 120 mg/ml ammonium sulphate in 50 mM phosphate buffer, pH 7.
Reproduced with permission from [82].

[94,95]. The sulphonate and hydroxyl groups on the monolithic surface can provide hydrophilic interaction and strong cation-exchange interaction sites for small polar neutral and charged solutes. A typical HILIC mechanism was observed at higher organic solvent content (ACN > 70%) for polar neutral analytes. Very high column efficiencies were obtained on this monolithic column (>170,000 plates/m for pressure-assisted CEC and 105,000 plates/m for capillary LC).

Recently, materials with zwitterionic functional groups have been developed as HILIC stationary phases since they could take advantage of weak electrostatic interactions between charged analytes and zwitterionic functional groups combined with the high efficiency and selectivity of hydrophilic interaction [30–33]. For example, sulphobetaine type zwitterionic materials, possessing both positively charged quaternary ammonium and negatively charged sulphonate groups, have been used successfully for the separation of various hydrophilic molecules [96,23,97]. Viklund and Irgum described two approaches to synthesise the porous polymeric monoliths bearing zwitterionic sulphobetaine groups within DURAN glass columns (150 mm × 2.7 mm) using methacrylate monomers [98]. The first approach involved the one-step *in situ* photo-initiated co-polymerisation of *N*,*N*-dimethyl-*N*-methacryloxyethyl-*N*-(3-(sulphopropyl)ammonium betaine (SPE) and EDMA. Benzoin methyl ether was used as radical precursor. Methanol was selected since it could serve as a solvent for both the water-insoluble cross-linker and the water-soluble SPE. The second approach involved thermal initiated surface grafting of electrolyte responsive poly(SPE) on a rigid porous carrier poly(trimethylolpropane trimethacrylate) (poly-TRIM). The poly-TRIM was first prepared in an identical manner as for the above poly(SPE-*co*-EDMA) monolith. The column was then filled with a solution containing 10% of the zwitterionic monomer SPE in an aqueous solution containing 1% potassium peroxodisulphate with respect to the weight of the monomer and the reaction was allowed to proceed at 70 °C. The grafted monoliths exhibited an electrolyte responsive flow permeability, whereas the permeability of the co-polymerised monoliths was unaffected by changes in ionic strength in the interval tested. Both synthesis routines showed great potential for preparing HILIC methacrylate monoliths.

Jiang et al. later prepared a hydrophilic zwitterionic monolith within 100 μm capillaries by thermally initiated co-polymerisation of SPE and EDMA for use as a stationary phase in the micro-HILIC separation of small polar molecules [99]. The pore size distribution experiments showed that the optimised monolith has the majority of the pores with a diameter of 70–450 nm, in addition to a small number of mesopores. Typical HILIC retention was observed at high organic solvent content (ACN > 60%). This poly(SPE-*co*-EDMA) monolithic capillary column exhibited satisfactory selectivity for a range of neutral, acidic and basic test compounds in the HILIC mode. However due to the lower stationary phase ratio on this phase compared to that on the commercial ZIC-pHILIC packed column, less retention and lower resolution for test compounds were observed under the same separation conditions. By increasing the organic solvent content, a similar separation could be achieved. Another way to achieve the same separation is by enhancing the hydrophilicity of the stationary phase. We attempted to prepare HILIC monolithic columns by using the more hydrophilic crosslinker PEGDA, which was specially designed and used to decrease unwanted polymer backbone hydrophobicity, or the more polar functional monomer *N*,*N*-dimethyl-*N*-acryloxyethyl-*N*-(3-sulphopropyl) ammonium betaine. However, neither of these significantly improved the hydrophilicity of the monolith.

Several other groups also prepared poly-SPE based monolithic capillaries with a similar one-step *in situ* co-polymerisation procedure [100,101]. Xie and co-workers co-polymerised SPE, PETA, and VSA in a binary porogenic solvent consisting of cyclohexanol and ethylene glycol [100]. PETA was used to replace EDMA as

cross-linker because it is supposed to have a higher hydrophilicity due to its hydroxyl sub-layer. However, these columns did not show clear improvement on the hydrophilicity. They later prepared a cationic hydrophilic interaction monolithic stationary phase based on the co-polymerisation of 2-(methacryloyloxy) ethyltrimethylammonium methyl sulphate (META) and PETA in a binary porogenic solvent consisting of cyclohexanol/ethylene glycol. The hydrophilicity of this monolith increased with increasing META content in the polymerisation mixture. A typical HILIC mechanism was observed for the separation of neutral, basic and acidic polar analytes with this monolith [102].

Recently we reported a very hydrophilic zwitterionic monolith prepared by thermally initiated co-polymerisation of 1-(3-sulphopropyl)-4-vinylpyridiniumbetaine (4-SPV) and MBA [103]. $K_2S_2O_8$ was selected as initiator in this work. Due to the solubility limitation of the highly hydrophilic functional monomer 4-SPV, a low MeOH content co-solvent (or pure water) was used as porogen. The subsequent monolith exhibited high hydrophilicity with a low critical composition of the mobile phase (around 20% ACN in water) corresponding to the transition from the HILIC to the RP mode, which is a convenient measure of the degree of polarity of the HILIC stationary phase. A comparable separation of pyrimidines and purines was observed on both the commercial ZIC-pHILIC column and the poly(SPV-*co*-MBA) monolithic column at the same mobile phase composition, whereas the latter exhibited much higher permeability (backpressure 20 bar). Because of the high permeability, baseline separation can even be achieved on the poly(SPV-*co*-MBA) monolithic column in around 1 min with a backpressure of only 180 bar (Fig. 5). However the column-to-column reproducibility of this monolith was low because of the low solubility of the crosslinker MBA in the high water content porogen.

We also reported another zwitterionic hydrophilic methacrylate monolithic column prepared through a single-step co-polymerisation of the zwitterionic monomer 2-methacryloyloxyethyl phosphorylcholine (MPC) and the crosslinker EDMA [104]. A methanol/THF mixture proved to be a good porogen for preparing a highly cross-linked poly(MPC-*co*-EDMA) monolith, since it exhibited very good solubility for both the water-insoluble crosslinker EDMA and the water-soluble MPC as well as yielding monoliths exhibiting a uniform structure and good separation selectivity. This column showed similar hydrophilicity to the poly(SPE-*co*-EDMA) monolithic column. Interestingly, a good separation of small hydrophilic peptides, which was not observed on the poly(SPE-*co*-EDMA) monolithic column, was achieved on this monolithic column.

Hosoya et al. described a novel epoxy polymer based monolithic column prepared by reacting 4-[(4-aminocyclohexyl) methyl]cyclohexylamine (BACM) or trans-1,2-cyclohexanediamine with tris(2,3-epoxypropyl) isocyanurate (TEPIC) in PEG 300 [105]. Simple thermally initiated polycondensation in an appropriate porogenic solvent afforded an epoxy polymer, which showed different morphology compared with other polymer based monoliths. HILIC behaviour was observed on a poly(TEPIC-*co*-BACM) monolithic capillary column, which was attributed to a relatively hydrophilic polymer backbone containing OH as well as amine functional groups. Plate heights of 13–113 μm were observed for the separation of nucleosides in 90% ACN.

One-step *in situ* co-polymerisation procedures can take advantage of the broad variety of functional monomers available. However current optimisation of polymerisation conditions, especially the composition of the reaction mixture, is still semi-empirical. For each new set of functional monomers and cross-linkers, re-optimisation is required in order to obtain monoliths with the desired characteristics. In some aspects, a two-step procedure may represent a better option due to the simplicity

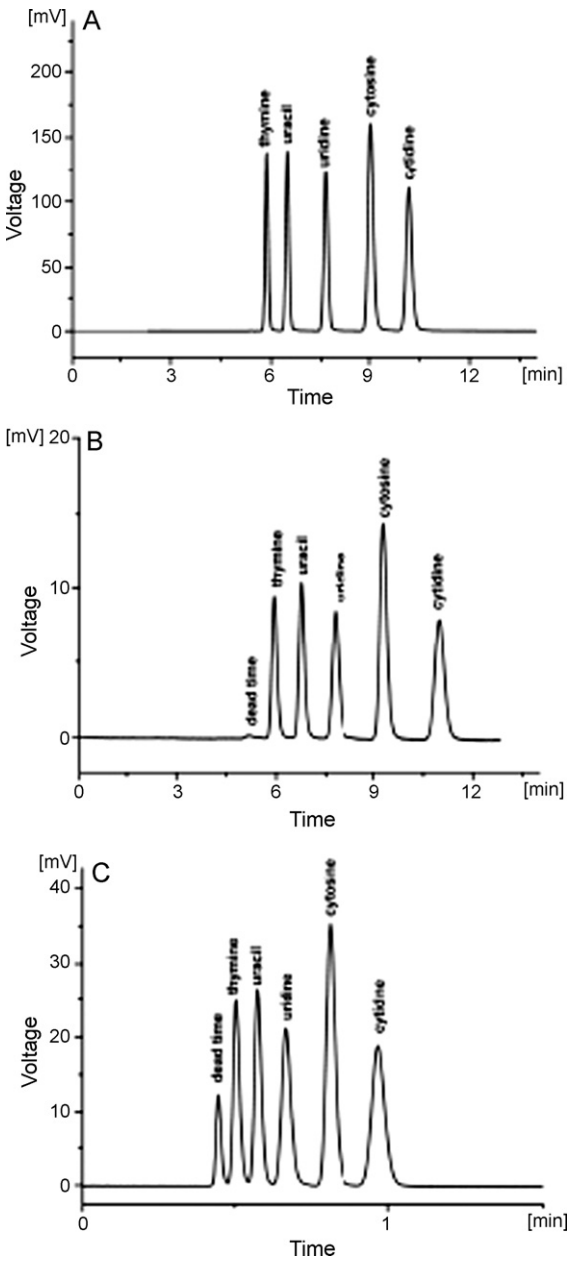


**Fig. 5.** Separations of pyrimidines and purines on two HILIC columns. Conditions: (a) commercial ZIC-pHILIC column 150 mm × 1.0 mm ID (3.5 μm); (b and c), poly(SPV-*co*-MBA) column 300 mm × 100 μm ID; mobile phase, ACN/H2O (80:20, v/v) containing 25 mM ammonium formate, pH 6.4; wavelength, 254 nm. Backpressure: a, 50 bar; b, 20 bar; c, 180 bar.
Reproduced with permission from [103].

of the single generic optimisation of polymerisation conditions of the monolith support with reactive monoliths. In the latter case, a polymeric matrix with optimised porous properties and bearing pendant reactive groups is modified via aminolysis or hydrolysis reactions in order to attain a surface with the desired selectivity. However, few HILIC polymer based monolithic columns have been prepared by this procedure [106]. An exception is that reported by Guerrouache et al., who prepared polymer-based monoliths for CEC with a zwitterionic surface character within capillary columns following a two-step approach [106]. The polymeric matrix was synthesised through a UV-initiated co-polymerisation of N-acryloxysuccinimide (NAS) and EDMA using toluene as porogen. The reactivity of the pristine monolith surface based on N-hydroxysuccinimide ester groups was used to introduce pri-

mary amino functionality via aminolysis with hexyldiamine and carboxylic acid groups via hydrolysis. Typical HILIC behaviour was observed on this column in the CEC mode.

Based on our knowledge, there have been very limited attempts at preparing styrene based HILIC monoliths. This could be attributed to the relatively high hydrophobicity of the styrene backbone. Huang et al. developed a hydrophilic poly(vinylpyrrolidinone-divinylbenzene) (VPDB) monolithic material for the extraction of polar phenols in a water matrix [107].

Compared to silica based monolithic columns, the current drawbacks of polymer based monolithic materials are the lower column efficiency, the lower phase ratio, the difficulty in controlling the size of both the macro- and meso-pores, and the lesser polarity of polymeric backbone compared with that of silica backbone. Much effort in the past few years has been aimed at addressing these problems including beam initiated polymerisation, polycondensation of epoxides, and polymerised high internal phase emulsions, etc. Svec recently published an excellent review describing the wide variety of methods enabling the preparation of polymer based monolithic columns [39].

### 2.3. Other monolithic columns

The limited pH stability of silica-based packing materials and the limited dimensional stability with changes in solvent (shrinking and swelling) of polymeric packings promoted studies in the early 90s into other inorganic materials for use as HPLC packings [108]. Similar efforts have been carried out on monoliths because of the expected benefits from both their monolithic structure and their stability in extreme environments [109–116].

Recently, Randon et al. reported two different strategies [109,110] for preparing zirconia based monolithic columns. The first was prepared by coating a classical silica based monolithic column with zirconium butoxide. Zirconium alkoxide was dissolved in dry ethanol and flushed through the monolith capillary. The remaining alkoxide groups on the zirconium coated surface were finally hydrolysed by flushing water through the capillary. Another zirconia monolith was prepared directly from zirconium alkoxide. The appropriate amount of liquid zirconium alkoxide was dissolved in dry ethanol. The hydrolysis solution was prepared with 0.01 M acetic acid, PEG and n-butanol and placed in an ultrasonic bath until complete dissolution was obtained at room temperature. After pouring the hydrolysis solution into zirconium alkoxide solution, the mixture was immediately introduced into a pre-treated capillary. The capillary was then kept at 30 °C for 24 h and then heated at 150 °C for 6 h. The HILIC retention mechanism was demonstrated on both a zirconia and zirconia coated monolith by the elution order of naphthalene, caffeine and theophylline. Compared to a silica based monolith, the zirconia based monolith exhibited much stronger theophylline retention, which could be attributed to the ligand exchange capabilities on the zirconia surface [117,118]. Therefore the selectivity of zirconia coated monoliths depends on the degree of zirconia coverage of the silica surface. A separation of three dimethylxanthine isomers (e.g. 1,3-, 1,7- and 3,7-dimethylxanthines), which were difficult to resolve using RPLC, was easily achieved.

Randon et al. also prepared a titanium monolith inside a capillary from a mixture of titanium propoxide, hydrochloric acid, formamide and water [111]. After optimisation of the hydrolysis ratio, porogen type, precursor concentration and drying step, a pure macroporous titanium monolith with a 10 times higher permeability when compared to a classic packed column was produced. A HILIC retention mechanism for xanthines was observed on this native titanium surface. At optimum conditions of the van Deemter plot (0.5 cm $s^{-1}$), the associated plate heights for test analytes was 15–30 μm. Several other efforts have been aimed at improving the chromatographic performance of titanium monoliths, in the past few years, even though their HILIC properties and applications were not investigated. Miyazaki et al. reported a titanium-coated silica monolith for liquid chromatographic separation [112]. This columns exhibited efficient separation with low pressure drop, which is a typical feature of monolithic structures, and also possessed phospho-selectivity, which is a unique property of the titanium surface. Konishi et al. developed reliable and reproducible synthetic pathways to fabricate $TiO_2$ monoliths using the alkoxy-derived sol–gel process accompanied by phase separation [113–116]. By modifying the starting compositions and applying a thermal treatment in the aging stage of wet gels, a crack-free, well-crystallised anatase $TiO_2$ monolith was obtained, having bimodal meso-macroporous structures with adjustable pore sizes and uniform pore size distributions. More recently, Sui et al. described a new approach for preparing nano-structured titanium based monoliths [119]. $TiO_2$ monolithic aerogels were synthesised in separation columns using *in situ* sol–gel reactions of titanium alkoxide in supercritical carbon dioxide instead of in heptanes and followed by calcination at 673 K. The supercritical $CO_2$ proved to be a better solvent for the sol–gel reactions in terms of lower shrinkage, less cracking and higher surface areas.

## 3. Separation mechanism

For neutral polar molecules, hydrophilic interaction is postulated to be the major mechanism in the HILIC separation. In order to evaluate the HILIC properties of new stationary phases, the influence of the organic solvent content (mostly ACN) on the retention of small molecules (e.g. toluene, thiourea and acrylamide) has often been investigated. HILIC stationary phases normally show typical HILIC behaviour at high organic solvent content, with the retention of polar compounds increasing with increasing organic solvent content, but returning to apparent RP properties when the organic solvent content is lower than a critical composition, if the stationary phases have some hydrophobic character. This critical composition of the mobile phase corresponds to the transition from the HILIC to the RP mode and can be used as a guide to the polarity of HILIC stationary phases [120,121]. Generally, with more polar stationary phases, the critical composition shifts to a lower concentration of ACN in the mobile phase, allowing the use of mobile phases with higher water content in the HILIC mode. Recently, we reported that the critical composition of ACN in water on the poly(SPE-*co*-EDMA) monolithic column is around 60% [99]. A similar critical composition was observed on several other monoliths, such as poly(MPC-*co*-EDMA) monolith [104], poly(SPMA-*co*-PETA) monolith [94] and poly(NAS-*co*-EDMA) monolith [106]. When the more hydrophilic META and SPV were used as functional monomers, the resulting zwitterionic poly(META-*co*-PETA) monolith and poly(SPV-*co*-MBA) exhibited a much lower critical composition of only 20% ACN.

For charged analytes, the mixed mode of hydrophilic interaction and ion-exchange was often observed on the HILIC monolithic column. This could be attributed to the charged functionalities on the stationary phase surface. For example, weak cation-exchange (WCX)/hydrophilic interactions were observed on PAA-coated silica monolithic columns [62], strong cation-exchange/hydrophilic interaction with poly(SPMA-*co*-PETA) monolithic columns [94], and weak anion-exchange/hydrophilic interaction on amino-bonded silica monolithic columns [57–59]. Although zwitterionic monoliths ostensibly have the same amount of both positively charged and negatively charged groups, it has been found that many of these materials have a negative surface charge over a wide pH range [33,104,122]. Electrostatic interaction has often been found to be a major contribution to the overall retention

of charged analytes on these zwitterionic monolithic columns [99,100,103,104,122].

Recently, Alpert proposed a new term, electrostatic repulsion–hydrophilic interaction chromatography (ERLIC) [122]. The combination of electrostatic repulsion and hydrophilic interaction offers unique selectivity for charged polar analytes. Similar ERLIC behaviour has been observed on many HILIC monolithic columns. Ibrahim et al. investigated the effect of salt concentration in the mobile phase on the retention of two amino acids on a AS9-SC latex coated silica monolith [72]. For the basic amino acid histidine, increasing methylphosphonate concentration suppresses the electrostatic repulsion between the cationic analyte and the cationic latexes, increasing its retention. However for the acidic amino acid aspartic acid, increasing methylphosphonate concentration shields the electrostatic attraction, decreasing its retention.

A quite interesting mixed mode separation mechanism was also investigated on the acrylamide based monolithic columns. These rather polar stationary phases were normally evaluated using a series of polar aromatic compounds. An increase in the polarity of the mobile phase decreased the retention factors of the better-retained compounds dramatically, as expected for HILIC. However, the elution order was not simply the inverted form of the reversed-phase separation since some elution orders also changed, suggesting other mechanisms may be in place other than simple hydrophobic differences. The authors explained that polar and charged analytes were retained by a mixed mode of mechanisms including hydrophilic interaction, electrostatic interaction, H-bridging interaction, $\pi$–$\pi$ interaction as well as possible aromatic adsorption [82,83,86].

## 4. Applications

Separation of highly polar compounds with low molecular weights such as peptides, nucleotides, nucleosides, amino acids, and sugar derivatives plays an important role in life sciences. Over the past two decades, HILIC has proved to be a solid alternative to other separation modes in this application area. Even though HILIC monolithic columns have not been used as extensively as commercially available HILIC packed columns in many application laboratories, their potential has been proven by the number of scientific publications.

Nucleic acid bases and nucleosides are polar compounds of significant biological and pharmaceutical interest. They have been used extensively as model compounds for evaluating polar stationary phases in HILIC mode. Some HILIC monolithic columns showed good selectivity for these specific test analytes [58,99,100,103,104]. Ikegami et al. reported a rapid separation of nucleosides within 1 min on a PAA-coated silica monolithic column, which suggested that it should be suitable for second dimension separation in two-dimensional comprehensive capillary HPLC, where very fast second dimension separations are required [62]. Holdšvendová et al. used a poly(N-(hydroxymethyl)methacrylamide-*co*-EDMA) monolithic column for the separation of oligonucleotides by capillary LC in the HILIC mode using 100 mM triethylamine acetate in ACN and water as eluents. Oligonucleotides differing in sequence and length, even by as little as a single base, were separated with gradient elution in a reasonable time of 35 min [123].

Carbohydrate analysis has been an analytical challenge due to difficulties in both their separation and detection. Recently, applications using HILIC monolithic columns for the separation of derivatised carbohydrates have been reported [60]. Ikegami et al. described the separation of 2-aminopyridine-labeled sugars on the PAAm-coated monolithic silica capillary column [60]. Although the phase ratio of this column is smaller than that of the commercial amide-type HILIC column, better separation was observed on the former (Fig. 2). The authors attributed this to the high permeability and higher column efficiency of the long PAAm-coated monolith. Later they investigated the separation of pyridylamino-sugars using both PAAm-coated or PAA-coated silica based monolithic columns in the isocratic and gradient mode. The PAA-coated monolith showed greater retention for the sugar derivatives than the PAAm-coated monolith. They concluded that PAA-coated columns are suitable for the fine separation of oligo sugars, while PAAm-coated columns are useful for the separation of longer polymers of sugar derivatives [62].

One advantage of the HILIC mode for the separation of polar solutes is the use of an organic-rich mobile phase, which is MS-friendly for ESI-MS detection. In order to avoid the derivatisation of carbohydrates, Ikegami et al. coupled micro-HPLC to an ESI-MS system using the PAAm-coated monolithic silica capillaries with high phase ratios for the analysis of underivatised carbohydrates. Fast and efficient separation of mixtures containing mono-, di- and trisaccharides was achieved within 5 min. Subpicomol-level detection (200 pg for oligosaccharides) and subfemtomol-level detection (3.2 ng/ml for nonreducing saccharides) were observed on the LC–ESI-MS system and the LC–ESI-MS/MS system, respectively. The system was successfully applied to the detection of disaccharides in extracts of plants such as corn, soybean, and *Arabidopsis thaliana* (Fig. 6) [61]. Novotny and co-workers also used cyano- or aminoderivatised polyacrylamide based monolithic columns for the analysis of complex oligosaccharide mixtures using a CEC-tandem MS system [89]. Low-femtomol sensitivities with the use of an ion trap mass spectrometer were obtained for the detection of neutral saccharides. This column provides a nearly universal selectivity for a wide range of carbohydrates, mono- and oligosaccharides with the intact reducing end, as well as saccharide alditols. Tegeler et al. prepared a hydrophilic monolith by co-polymerisation of acrylamide, methylene bisacrylamide, 2-cyanoethyl acrylate, and VSA and used it to separate complex glycan mixtures by CEC [90]. A special sample deposition device was constructed and optimised for interfacing CEC and capillary LC columns to MALDI mass spectrometry. The glycans released from selected glycoproteins are first separated by a hydrophilic-phase CEC and deposited onto standard MALDI plates together with a suitable matrix as densely spaced sample dots. The monolithic CEC columns are run isocratically under the conditions of an MS-compatible mobile phase.

Ikegami et al. systematically investigated the selectivity of PAA-coated silica monolithic capillary columns and C18-coated silica monolithic capillary columns for peptides (Fig. 7) [62]. Good separation of the tryptic digests of BSA and phosphorylase B in the HILIC mode was observed using the PAA-coated column (Fig. 8). The HILIC separation produced a peak capacity similar to the reversed-phase mode for the separation of peptides. These results suggested that the PAA-coated column has selectivity orthogonal to the commonly used C18 column, and it would be useful for increasing peak capacity in multidimensional micro-HPLC systems. Tegeler et al. also described a successful separation of a tryptic digest of ovalbumin using capillary LC–MALDI MS based on a polyacrylamide organic monolithic column [90].

Pharmaceutical analysis may be the most important driving force for the development of HILIC methods. Such applications have been reviewed recently by Dejaegher and Heyden [10]. According to Pontén's statistical data in 2010, almost 46% of HILIC literature in the period between January and August 2009 were in the area of pharmaceutical and clinical research [5]. HILIC monoliths have been used for such applications. One example is the investigation of metabolic pathways of new drug candidates, which is an important step in the drug development process. Recently, the FDA required that any human drug metabolites formed at greater than 10 percent of the parent drug systemic exposure at steady

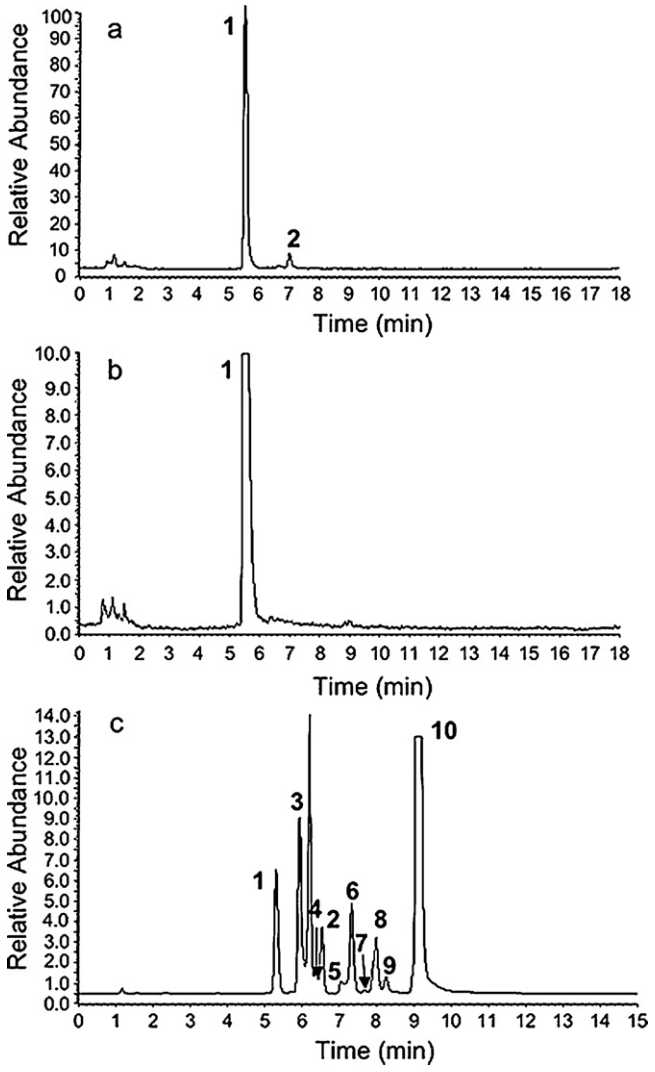


**Fig. 6.** LC–ESI-MS/MS SRM chromatogram of plant extracts. Samples a: a corn extract, b: a soybean extract, and c: an *Arabidopsis thaliana* leaf extract; peaks 1 sucrose, 2 trehalose, 3 maltose (α and β isomers), 4–10 unknown disaccharides. Reproduced with permission from [61].

state should be subject to separate safety testing, which involves synthesis of this metabolite and administration to test animals (http://www.fda.gov/downloads/Drugs/GuidanceCompliance-RegulatoryInformation/Guidances/ucm079266.pdf). HILIC coupled with MS has great potential in this application. For example, biosynthesis of bile acids is a major route of cholesterol metabolism in most species other than humans, and their analysis has a significant biomedical rationale. Novotny used CEC coupled with ion-trap MS for the detection and identification of bile acids in biologically important mixtures, based on surface modified silica monolithic capillary columns [88]. The hydrophilic amino-phase monolith columns appear highly desirable for this type of analysis, as they provide the necessary separation efficiencies together with a tolerance to biological extracts and other crude mixtures.

Recently Zheng and co-workers reported a simple, rapid, and sensitive method based upon an online combination of a polymer monolith microextraction (PMME) technique with HILIC/MS for monitoring three β2-agonist traces in human urine. The extraction was performed with a poly(MAA-*co*-EDMA) monolithic column while the subsequent separation was carried out on a Luna silica column by HILIC. The LOD's (S/N = 3) of the method were found to be 0.05–0.09 ng/ml of β2-agonists in urine. The recoveries of three β2-

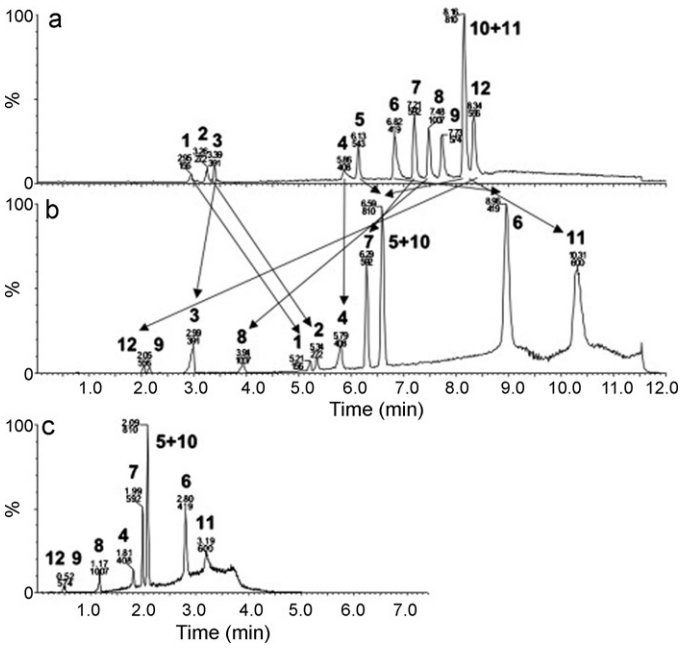


**Fig. 7.** LC–ESI TOF MS total ion chromatograms of peptides. (a) Column: MS-200T-$C_{18}$, 224 mm × 200 μm ID, mobile phase: 5–50% ACN (0.2% formic acid) in 10 min linear gradient; (b) Column: MS-200T-PAA, 190 mm × 200 μm ID, mobile phase: 90–10% ACN (0.2% formic acid) in 10 min linear gradient; (c) same as (b) but with 3 min linear gradient followed by 0.5 min hold. Detection: ESI-TOF-MS (3 kV, negative). Solutes: (1) γ-EH, (2) DSDPR, (3) VGSE, (4) bradykinin (1–5), (5) [$Arg^8$]-vasopressin, (6) bradykinin, (7) LHRH, (8) oxytosin, (9) Met-enkephalin, (10) bombesin, (11) substance P, (12) Leu-enkephalin for (a) and (b), samples 4–12 (9 peptides) for (c). Sample concentration: 2.5 μg/ml; injection volume: 50 nl. Reproduced with permission from [63].

agonists spiked in five different urine samples ranged from 79.8% to 119.8%, with RSD's less than 18.0% [124]. The same method has also been used for the determination of traces of thirteen sulphonamide antibacterial compounds in milk and eggs [125]. Good linearity was obtained for thirteen sulphonamides with the correlation coefficients ($R^2$) above 0.997. The LOD (S/N = 3) of the method were found to be 0.4-5.7 ng/ml of sulphonamides in whole milk and 0.9–9.8 ng/g of sulphonamides in eggs. The recoveries of thirteen sulphonamides in two matrices ranged from 80.4% to 119.8%, with relative standard deviations less than 11.8%.

Tetracycline antibiotics are extensively used to control bacterial infections in both humans and animals. Ye et al. reported a successful separation of tetracyclines using a lab-made silica

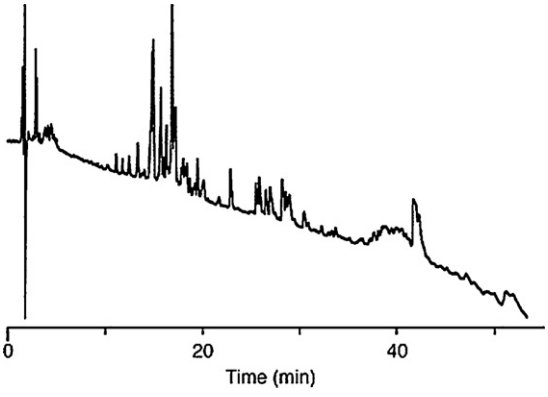


**Fig. 8.** Separation of tryptic digest of BSA. Column: 200T-MAS-AA, 200 μm ID × 22 cm. Mobile phase: A: water (0.2% $HCO_2H$); B: ACN; linear gradient in 60 min, 90–10% B; λ: 220 nm, injection volume: 50 nl. Reproduced with permission from [62].

based monolithic column with diethylenetriaminopropyl functional groups by HI-pCEC [59].

Li et al. described a method for the separation and determination of five major opium alkaloids (narcotine, papaverine, thebaine, codeine, and morphine) in *Pericarpium papaveris* by pCEC with a hydrolysed poly(GMA-*co*-EDMA-*co*-SPMA) monolithic column [5,126]. LOD's of these analytes were 1.5–6.0 μg/ml, and average recoveries were 79.0–95.9%, with RSD less than 4.6%. The proposed method was successfully applied to the analysis of *P. papaveris* samples.

In the pharmaceutical industry, it is important to detect and quantify the counter-ions from pharmaceutical salt forms. Pack et al. demonstrated the application of a Chromolith Performance SI silica column operated in the HILIC mode for the detection and rapid quantitation of lithium, sodium and potassium using ELSD [52]. An acceptable linearity of sodium concentration ($R^2$ = 0.999) across the working-standard range was observed and the calculated LOD was 0.1 μg/ml. High reproducibility (RSD = 1.6% on average) on the counter-ion determination from three pharmaceutical sodium salts was obtained. The accuracy of the counter-ion prediction was within 3% of theoretical when the salt content was corrected for potency.

## 5. Conclusions

In this review, the preparation and applications of HILIC monolithic columns over the past decade were discussed as well as the separation mechanism based on these columns. It is evident that HILIC materials have gained increased attention over the past three years. Monolithic materials, both silica based and organic based, are now being developed with a much greater understanding of the parameters that control their properties. As a result, whereas earlier monoliths were fabricated with poor permeability and performance properties in terms of plate numbers, this is no longer the case. What is needed now is a concerted effort by the pharmaceutical industry to put these materials through their paces so that their true worth *vs.* conventional materials can be evaluated.

## Conflict of interest

The authors have declared no conflict of interest.

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