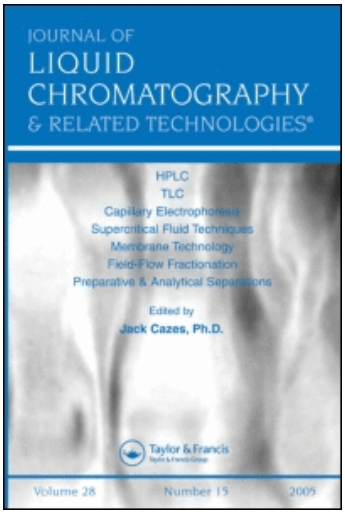

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Application of Monolithic Columns in High Performance Liquid Chromatography

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Abstract: Monolithic columns, consisting of continuous beds with through pores of organic or inorganic matrix, have found increasing applications for HPLC separations. The main advantage of the monolithic technology is the ability to use high flow rates without significant loss of efficiency, leading to very fast separations. The size and shape of the monolith depends on the application. For HPLC, rods and discs made of silica or polymer have been applied. The silica monoliths have bimodal pore structure and large surface area. These have found applications for small molecule analysis. The polymer monoliths have smaller surface area and have been primarily used for the separation of macromolecules[1] This paper discusses the recent applications of monolithic columns for HPLC separations.

Keywords: Silica monoliths, Polymer monoliths, HPLC applications

INTRODUCTION

In conventional HPLC columns, the stationary phase, consisting of microparticulate porous silica or polymers, is packed. The separation quality depends on the nature of the bonded ligand as well as particle size distribution and morphology (irregular, spherical, etc.). The packing method can also

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influence column performance. A commonly used parameter to evaluate separation performance is the efficiency, which is measured by the reduced plate height, h, and the plate number, N. For packed columns, the plate number is inversely proportional to the particle diameter, and hence, increased efficiency can be obtained by using small particles. However, this is limited due to the high back pressure generated, and for practical purposes short columns or low flow rates are employed. Ultra-high pressure liquid chromatography (UPLC) seeks to overcome this limitation[2,3] and such commercial instruments are just entering the market. Monolithic capillaries for capillary electrochromatography (CEC) are not addressed in this report as several excellent reviews have covered this topic.[4–10]

High flow rates can be used with the monolithic columns without loss of efficiency due to the nature of the monolithic media. Interconnected pores allow for convective flow, resulting in enhanced mass transfer between the stationary and mobile phases. In packed columns, slow diffusional mass transfer of solutes into the stagnant mobile phase in the pores of the stationary phase leads to peak broadening and decrease in column efficiency. Therefore, the major advantages of the monolithic columns include decreased analysis times and increased productivity, resulting in a reduced cost per analysis. Monolithic columns also offer greater mechanical stability versus polymer particle packed columns, where the combination of high back pressure with large-pore beads can lead to the collapse of particles. The stable monolithic chromatographic beds also do not develop voids.[11] The limitation of monolithic columns lies in the fabrication. Radial heterogeneity can affect efficiency, and hence, the monoliths are limited to a few mm in diameter.[12,13]

Silica Monoliths

The silica monoliths consist of a single rigid rod of continuous monolithic porous silica obtained via a sol-gel process. The procedure involves hydrolysis followed by polycondensation of the alkylsilanes in the presence of organic additives to form the macroporous structure, followed by solvent exchange and aging to form the mesopores.[14–18] The resulting monolith has a bimodal pore distribution consisting of large through pores of approximately 2 μm, comparable to the interstitial voids of particle packed columns, and mesopores on the order of 120 μm.[19] The through pores allow for the application of high flow rates, whereas the mesopores provide the surface area for stationary phase coverage and chromatographic interactions. Nakanishi et al. varied the post-gelation temperature and age time to tailor the pore size distribution of the mesopores on the surface of the monolithic skeleton.[15,20,21] Mesopores of greater than 100 nm were constructed. These may be advantageous for separation of high molecular weight polymers.[21] Applications of such large pore size in packed columns is limited due to loss of mechanical strength.

The structure and the geometry of the pores in the monolithic columns impart lower flow resistance for these columns.[22] The packed bed columns suffer from inherently slow diffusional mass transfer. For faster analysis, small particles (higher optimum linear velocity) and short columns are used, since the slope in the high velocity region of the van Deemter plot for the plate height vs. linear velocity is shallower.[22] For monolithic columns, the band broadening due to the stagnant mobile phase mass transfer is reduced because of its high permeability, and hence, efficiency is maintained at high linear velocities. The total porosity of the monolithic silica column has been reported to be 81%.[23] In comparison, the packed bed columns had a porosity of 66%, and similar porosity results have been reported elsewhere.[19,24] The high permeability allows for the operation at high flow rates and short diffusion paths, resulting in fast and efficient separations. With monolithic columns, the plot of plate height versus linear velocity is relatively flat, as illustrated in Figure 1.[15,21,23]

Selectivity for a column is determined by the surface chemistry, and hence, the retention properties of monoliths are similar to the conventional HPLC packings.[1] Guiochon's group studied the surface properties of the monolithic and particle packed C_{18} columns. Using frontal analysis and modeling, they determined that binding constants were similar for both types of columns, indicating similar adsorption energies.[12,13,25] However, the adsorption capacity of the monolithic column was found to be 1.4 times greater than that of the packed column, even though the surface area and the carbon content were comparable. This suggested that the accessible

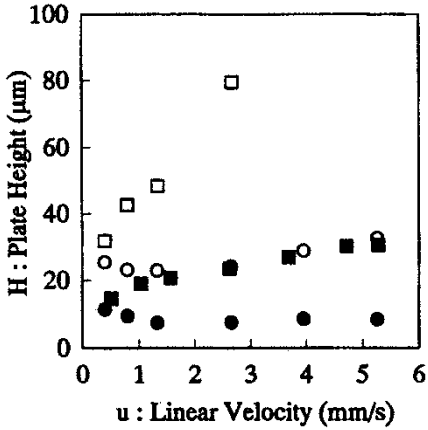

Figure 1. Van Deemter plots for C_{18} silica rod (closed symbols) and Capcellpak C_{18} SG (open symbols) with amylbenzene (●, ○) and insulin (■, □) as solutes. Mobile phase: 80% methanol with amylbenzene and acetonitrile-water mixture with insulin (30 : 70, v/v) for rod column and (32 : 68, v/v) for Capcellpak C_{18} SG in the presence of 0.1% TFA. From Ref.[15] with permission.

surface area of the monolithic column was larger than that of the packed column, likely due to the differences in the structure of the pore network.

Practical Considerations

The narrow peak widths generated with the use of monolithic columns require high sampling rates, typically greater than 10 Hz.[26,27] In addition, to truly capitalize on the reduction in analysis times, the autosampler and data acquisition needs to be fast. Henion's group reported on the use of an autosampler and software with a lag time of less than 17 sec between consecutive injections.[27]

If a monolithic column is employed for separation prior to mass spectrometric detection, the column effluent needs to be split prior to the MS, as current interfaces are limited to less than 2 mL/min.[22] This can be achieved using a valve post-column or through flow programming.[28–32] In coupling the monolithic separation column with LC-ion trap MS for the analysis of compounds of forensic interest, Pihlainen et al. reported a lack of data points due to the relatively long (400 ms) time required for the MS scan.[33] This can be overcome if quadruple or TOF-MS is employed.

Silica Rod Applications

Silica rod monolithic columns have been prepared in fused silica capillaries as well as clad in plastics such as PEEK or PTFE.[24,34] The capillary columns allow for applications in both micro-HPLC (μ-HPLC) and CEC modes. The only commercially available silica monoliths for HPLC are C_{18} revered phase columns based on the chemistry developed by Tanaka and co-workers.[14] The majority of the applications discussed herewith utilized either the 25, 50, or 100 mm columns from Merck KgaA (Chromolith Flash, SpeedRod, and Performance RP18e). They have been applied to a variety of small molecule applications.

Dolezalova et al. compared the reversed phase HPLC separation on a C_{18} silica monolith with micellar electrokinetic chromatography for the analysis of phenoxymethylpenicillin and ampicillin.[35,36] For the analysis of impurities in the drug, excellent correlation was obtained between the two methods. Although the MEKC separation was completed in 10 min, whereas the monolith method was 18 min, the precision and limit of quantitation ($<0.1\%$ w/w) was better with the HPLC method. It should be noted that this is still faster than conventional gradient HPLC methods. Rocheleau et al. applied the monolithic column for the impurity profiling of an analog of Taxol and found it to be comparable to the traditional silica packed columns.[37] The analysis time was reduced by 30%, and all except one impurity were separated. A validated stability indicating ion-pair HPLC method for p-aminosalicylic acid and its degradation product m-aminophenol

in pellets has also been reported.[38] Reversed phase chromatographic methods using aqueous buffer/acetonitrile mobile phase and C_{18} monoliths have been described for the determination of active ingredients in various pharmaceutical preparations and drugs of abuse in illicit tablets.[39,40]

The application of silica monoliths for quantitative structure-retention relationship (QSRR) screening to predict membrane permeability was investigated by Detroyer et al.[41] The elution behavior of a variety of basic pharmaceuticals on monoliths with and without micelles was similar to the particulate columns. However, the analysis was faster, and the QSRR between the log P and the monolithic method at times was better than HPLC with packed columns. Structure-retention correlation has also been determined for oxicam drugs using a RP-18e monolithic silica column.[42] Dissociation constants for the drugs were estimated and the octanol/water partition coefficients were obtained from extrapolation of the capacity factors.

The size and complexity of the combinatorial library analysis requires a technique with high sample throughput. For such applications, a 50 mm C_{18} silica rod column coupled to LC-MS provided a fast and powerful tool for the analysis of products of combinatorial chemistry.[43] The monolithic separation was completed in 5 minutes, whereas the same analysis on a 55 mm particulate column took 10 minutes. By increasing the flow rates from 1 mL/min to 9 mL/min, the analysis time was reduced from 6 min to less than 1 min for a separation of five β-blocking drugs (Figure 2). In addition to conventional solvents gradients, Cabrera et al. reported on the use of flow gradients for the separation of K-vitamins.[19] Flow gradient had the added advantage of further reduction in the total analysis time since re-equilibration between each analysis is not required. A single monolithic method using flow gradient from 2 to 5 mL/min provided adequate separation of paracetamol, caffeine, and brompheniramine, active ingredients in a medicine, in 2.5 min, whereas the original analysis required two separate HPLC methods.

Two-dimensional chromatography using normal phase in the first mode followed by reversed phase monolithic separation in the second was demonstrated by Dugo and coworkers.[26] In this approach, the first microbore column was operated isocratically at 20 μL/min, while the second monolithic silica column performed gradient analysis at 4 mL/min with a run time of 1 min. An automated switching valve allowed for the transfer of fractions from the first column to the second. Separation of oxygen heterocyclic components in citrus oil was demonstrated, and the 2D chromatographic contour plots provided additional details. By careful optimization of the method, this approach may be applicable for the separation of complex mixtures containing a range of polarities and hydrophobicities. Other plant oils, steroids, and flavanoids have been separated in the reversed phase mode using monolithic C_{18} silica columns.[4–46]

Bamba et al. demonstrated the separation of natural polyphenols, homologs and geometric isomers on a monolithic C_{18} silica column. By

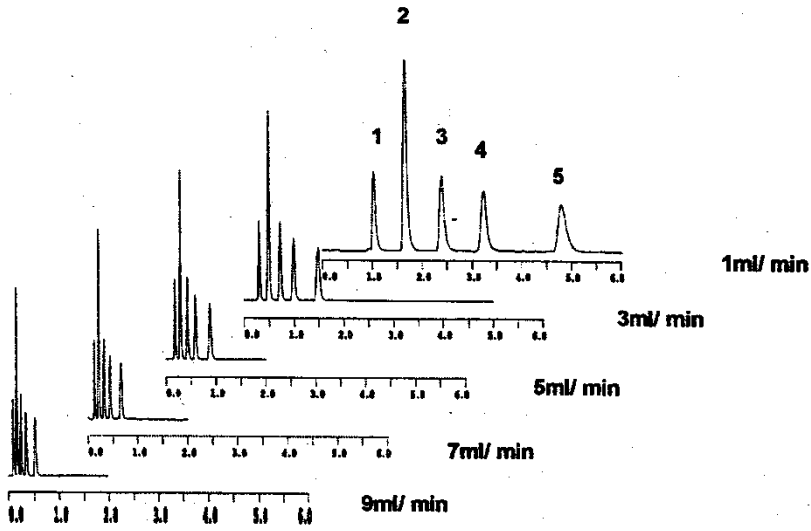

Figure 2. Chromatographic separation of five β-blocking drugs on a silica rod column at different flow rates: Column: silica sod column RP 18e, 50 × 4.6 mm; mobile phase: acetonitrile/0.1% TFA in water (20/80 v/v); flow rate: 1–9 mL/min, detection: UV 254 nm, samples: 1. atenolol, 2. pindolol, 3. metoprolol, 4. celiprolol, 5. bisoprolol. From Ref.[43] with permission.

coupling ten 10 cm monolithic columns good separation of over 60-mers was obtained and further optimization resulted in detection of 90-mers.[47] Application of monolithic columns for the separation of phenolic compounds in wine[48] and fruit[49] have also been reported. The mycotoxin, ochratoxin A, in wines was quantitated using solid phase extraction followed by HPLC using a SpeedROD column coupled with MS detection.[50] The chromatographic run time was reduced by a factor of three when compared to a traditional RP C_{18} column and the results for both columns were similar.

Trace determination of pesticides in water was accomplished by combining on-line solid phase extraction with LC-MS.[51] Organic polymer, silica, and carbon based turbulent flow columns were used as extraction cartridges for analyte enrichment followed by monolithic C_{18} silica columns for separation. Re-mixing of the solid phase extraction (SPE) eluate prior to introduction into the separation column was required for efficient band focusing. APCI-MS/MS provided selective and sensitive detection.

Silica monoliths modified with didodecyldimethylammonium bromide (DDAB) have been applied for the anion exchange separation of inorganic ions (Figure 3).[52,53] The coating was stable for up to 5500 column volumes and the columns could be regenerated by removing the old coating with 100% acetonitrile and recoating. The problem of high background and

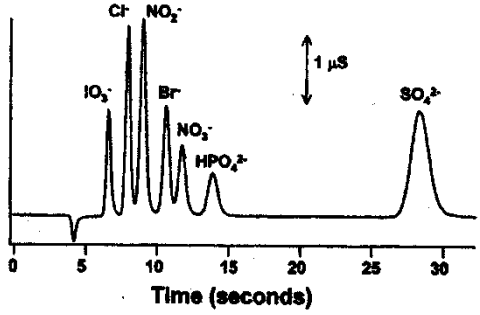

Figure 3. A 30-s separation of seven anions. Experimental conditions: DDAB-coated column, 6 mM o-cyanophenol (pH 7.0) at 10 mL/min, 20-μL injection, suppressed conductivity detection. Eluent flow with Waters 590 pump. Concentration of analytes, ~50 μm. Reprinted with permission from reference[53]. Copyright 2003. American Chemical Society.

system peaks resulting from the use of direct conductivity detection was minimized by using suppressed conductivity detection. Detection limits from 4 to 30 ppb were reported. A packed bed suppressor was used, which allowed for flow rates up to 10 mL/min.

The application of monolithic C_{18} silica columns for sequential injection analysis (SIA) provided an alternative to HPLC for determination of small molecules.[54–56] The use of a syringe pump in place of the high-pressure LC pump simplified the instrument and reduced the cost. The method was validated for various pharmaceuticals and parabens, and linear calibration was reported.

Bioanalytical Applications

Several groups have demonstrated the feasibility of monolithic columns for fast HPLC separations of drugs and metabolites in biofluids. The use of monolithic columns for the determination of the cyclooxygenase II inhibitors in human plasma was demonstrated by Vallano et al.[22] The monolithic method resulted in up to a five-fold reduction in analysis time and excellent reproducibility was demonstrated for up to 1600 injections using several columns. Bugey and Staub reported on the analysis of benzodiazapines in whole blood using monolithic columns with HPLC and diode array detection[57] for forensic toxicology applications. The samples were pre-treated using liquid-liquid extraction and the method was satisfactorily validated. Quantitation of therapeutic and toxic concentrations was achieved for eight of the commonly encountered benzodiazapenes in under 4 min. See Figure 4.[57]

The short analysis times possible with the monoliths provide a distinct advantage and allows for the use of HPLC for high-throughput profiling and

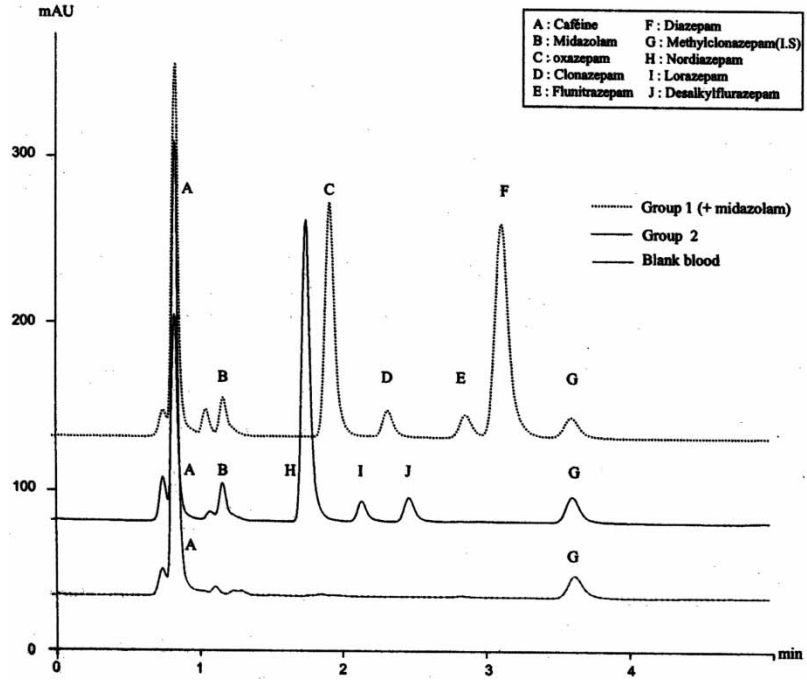

Figure 4. Chromatograms corresponding to the blood extracts of different groups of benzodiazepines studied. Chromatographic conditions: mobile phase phosphate-buffer-acetonitrile (65/35), Chromolith Performance^{TM} column RP-18 (12.5 × 4.6 mm), 20 μL injection, flow-rate 2 mL/min. From Ref.[57] with permission.

metabonomic studies.[58] Ideally, run times on the order of 10 minutes or less, including column reequilibration, are desired. Pham-Tuan and coworkers compared non-porous polymer based columns with small particles, short and normal length porous silica-based columns, and small bore porous silica-based columns to silica monoliths. The authors reported the monoliths to provide the most details for metabonomic fingerprinting of urine samples (Figure 5). The non-porous polymer phase was too weak for the retention of hydrophilic species and suffered from poor loadability resulting in band broadening for the later eluting hydrophobic components. The short silica packed column did not provide sufficient plates for the separation, whereas with the longer column complete elution was not achieved in 5 minutes. In addition, by switching from acetonitrile to a weaker methanol based mobile phase, the hydrophilic metabolites were further retained and separated. The application of monoliths for plasma samples with and without protein precipitation was also demonstrated by the same group. However, the authors caution against the protein accumulation on columns leading to blockage and reduced performance if sample pretreatment is not employed.

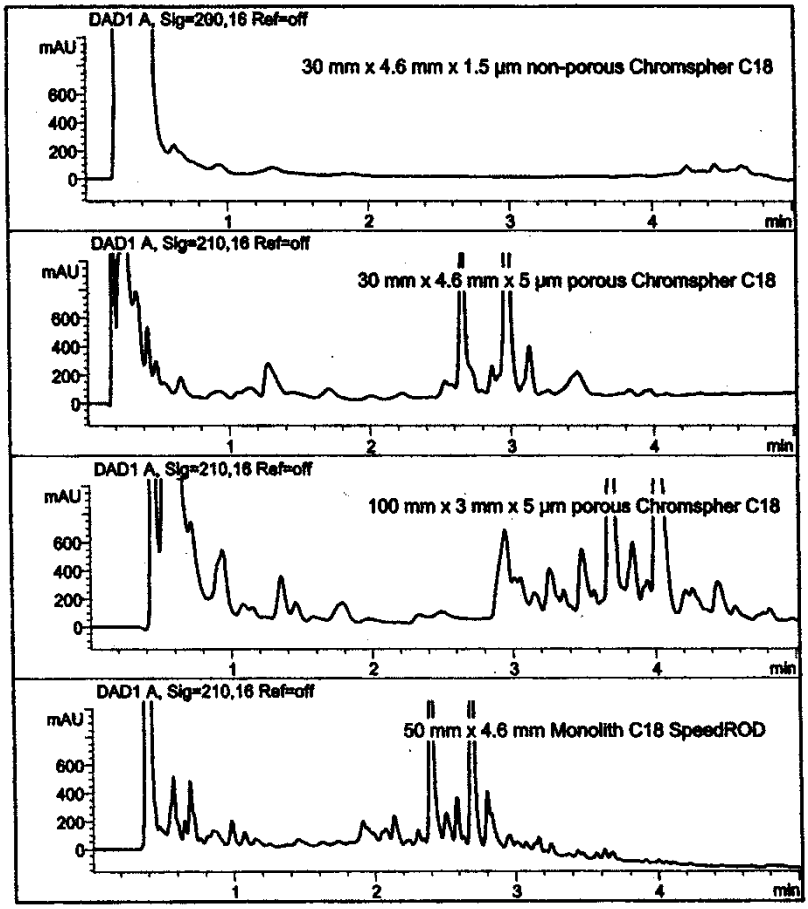

Figure 5. Urine profile on different reversed-phase HPLC columns. Injection volume 10 µL. Solvents: A = 0.1% HFm in Milli-Q water, B = 0.1% HFm in MeOH. Gradient 2% B(0–0.5 in) to 90% B(4.5–5 min), flow rate 1 mL/min. From Ref.[58] with permission.

Combining the use of monolithic columns with mass spectrometric detection provides a powerful tool for drug discovery. In these applications, chromatographic separation is usually performed prior to the MS analysis in order to minimize metabolic and endogenous interference as well as matrix effects. The time frame for the ion separation in the MS is on the order of milliseconds, whereas the HPLC separation takes minutes. A faster separation technique on the front-end may allow for elimination of the bottleneck for LC/MS and LC/MS/MS methods.[28] Wu et al. separated rat plasma extracts of a mixture of tempazepam, tamoxifen, fenfluramine, and

alprozolam in under a minute using a 50 mm silica rod column and a flow rate of 6 mL/min.[28,29] The flow from the separation column was split down to 0.4 mL/min using a purge valve before introduction to the mass spectrometer. The gradient LC/MS/MS method was shown to be rugged. The reproducibility of the retention time was less than 1% RSD and the coefficient of variation for the peak response for 250 ng/mL standard was less than 10%. Efficiencies ranging from 69,000 to 465,000 plates/meter were reported for the analytes studied under different flow rates. For the simultaneous quantification of a parent drug compound along with its glucuronidation and O-dealkylation metabolites, a lower limit of quantitation of 2.5 nM was achieved.[28] Over 200 dog plasma extract samples were analyzed in less than 4 hours. A similar tandem MS pharmakokinetic screening approaches have been reported by several groups.[27,30–32,59] The separation of the metabolite from the dosed drug eliminated the potential for interference from ion source fragmentation of the metabolite during quntitation of the parent drug. Selected or multiple reaction monitoring provided improved sensitivity.

Hsieh and coworkers reported on direct injection of diluted plasma using 96 well plates with minimal loss of column performance. The monolithic column provided on-line extraction, and recoveries of greater than 90% were demonstrated for the test analytes.[31,32] In addition, instead of post-column flow splitting to the LC/MS, flow programming was used reduce the flow rate during analyte elution. Ionization suppression due to matrix components was not an issue. Hefnawy and Aboul-Enein reported on a 5 minute method for the analysis of mianserin and its metabolites in human plasma using solid phase extraction followed by analysis on a C_{18} silica monolith.[60] The method was validated and the limit of detection ranged from 2.5 to 15 ng/mL. Monolithic columns were also employed for the fast quantitation of aromatic hydrocarbon metabolite carboxylic acids in human urine.[61] Statistical analysis indicated no significant difference in the results obtained with the monolithic column versus the existing HPLC method using a LiChrosorb C_{18} column.

Minakuchi and coworkers reported on the separation of polypeptides, including lysozyme, BSA, and trancferrin, using a C_{18} monolithic silica rod and compared to a variety of packed columns.[15] The resolution of the monolithic column was less affected by the gradient slope and the flow rate, compared to the non-porous silica and polymer columns.

Enantiomeric Separation

Lubda and Lindner prepared a novel tert-butylcarbamoylquinine chiral stationary phase by in situ modification of monolithic silica support.[62] The reproducibility of the separation was demonstrated and the dependence of efficiency on flow rate was compared for the monolith and the particulate CSPs. Baseline resolution of the enantiomers was achieved for a variety of N-derivatized amino acids using hydro-organic and polar-organic mobile

phases. By coupling columns in series, the resolution of Suprofen enantiomers was improved from 2.10 (1 column) to 3.45 (6 columns), while the back-pressure was still acceptable at 45 bar. An epoxy derivatized silica monolith was also prepared by covalent immobilization of penicillin G acylase and characterized for chiral applications.[63]

Chen, Hobo, and coworkers prepared capillary columns containing L-amino acid amide modified silica monoliths for μ-HPLC and CEC applications.[64,65] Dansyl amino acids were separated based on ligand-exchange mechanism with Cu(II) complexes.

Polymer Monoliths

Monolithic columns based on polymeric supports have also been investigated. These can be readily prepared inside fused silica capillaries to allow for μ-HPLC as well as CEC application.[66,67] The pore size is controlled by the selection of appropriate porogenic solvents and the fabrication conditions.[68] Both temperature and photo-initiated polymerizations have been reported.[69,70] The variety of stationary phase modification chemistry developed for the particulate polymer phases has been employed.

Polymer stationary phases often suffer from shrinking and swelling in the presence of different solvents. In addition, they are fragile and application of high pressures can lead to particle collapse in the case of packed columns. Although cross-linking imparts mechanical strength to the polymer phases, the use of high flow rates is still limited. Monolithic polymer columns allow for the use of high flow rates due to decreased flow resistance resulting in low back pressures. Oberacher and coworkers reported on the preparation and evaluation of octadecylated poly(styrene-co-divinylbenzene) monolithic columns.[66] The 60 cm $\times$ 200 μm ID fused silica columns were shown to be stable for up to 250 bar column inlet pressure and provide greater than three times the separation efficiency compared to a PSDVB packed pellicular column for oligonucleotides. The total porosity of the monolith was determined to be 70% using inverse size exclusion chromatography and the loading capacity was comparable to a similar packed column.

Macromolecule Analysis

Frechet and Svec's groups have reported on the size based separation of macromolecules using poly(styrene-co-divinylbenzene), poly(glycidyl methacrylate-co-ethylene dimethacrylate), and poly(2,3-dihydroxypropyl methacrylate-co-ethylene dimethacrylate) monoliths.[71,72] Polystyrene and poly(methyl methacrylate) standards were separated by a precipitation and redissolution mechanism using a solvent/non-solvent mobile phase gradient. Differences in the separation on the three phases were attributed to differences in porous properties and surface chemistry. The use of high flow

rates (20 mL/min) and steep solvent gradient with PSDVB monolithic rods, 50 mm and 4.6 or 8 mm ID, allowed for ultrafast separation of three polystyrene standards of molecular weight 9200, 34,000, and 980,000 within 40 sec (Figure 6).[73] Columns packed with non-porous PSDVB beads suffer from high back pressure at these flow rates. Although columns packed with macroporous beads allowed for high flow rates and provided good separation, the resolution decreased with a decrease in gradient volume. Therefore, they are not well suited for very fast separations.

Hydrophobic interaction chromatography was used for the separation of proteins on acrylamide-methacrylate based monoliths and the hydrophobic component of the polymerization mixture was varied to control the surface hydrophobicity.[74] Cytochrome c, ribonuclease, carbonic anhydrase, lysozyme, and chymotrypsinogen were separated using a 10% butylmethacrylate column and a mobile phase gradient of ammonium sulfate and sodium phosphate. This approach appears to be promising for large-scale preparative purification of proteins.

Horvath's group reported on the reversed phase μ-HPLC separation of proteins on a PSDVB monolith with a trifluoroacetic acid/acetonitrile gradient.[67] The 270 mm × 75 μm ID columns were prepared using in-situ polymerization and flow rates from 0.34 to 1.5 μL/sec were applied

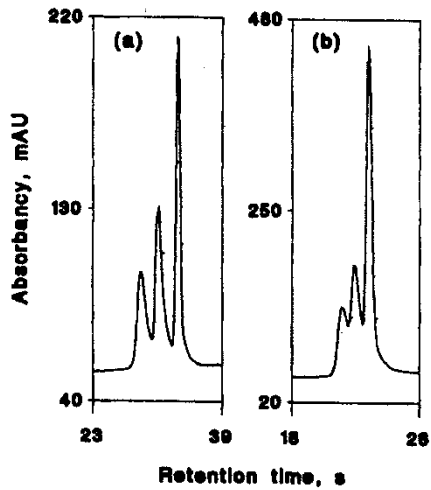

Figure 6. Effect of gradient steepness on the very fast separation of polystyrene standards. Conditions: Column: 50 × 8 mm I.D., molded poly(syrene-co-divinylbenzene) monolith; mobile phase, linear gradient from 100 to 0% methanol in tetrahydrofuran within 30 (a) and 12 s (b); flow rate, 20 mL/min; analytes, mol. weight 9200 (1), 34,000 (2), and 980,000 (3), 3 mg/mL of each standard in tetrahydrofuran; injection volume, 20 μL; UV detection, 254 nm; dead volume of the chromatographic system, 6.5 mL. From Ref.[73] with permission.

providing high efficiency separations. The reversed-phase μ-HPLC method coupled to MS detection may prove useful in proteomics where sample size is often limited. Premstaller et al. demonstrated such applications by coupling to ESI-MS and performing tryptic mapping of 1 and 5 pmol human transferrin and bovine catalase, respectively, on a PSDVB monolith.[68] The loadability of the monolith was determined to be in the 0.4 to 0.9 pmol range on a 60 × 0.2 mm ID capillary column. For small polypeptides with Mr $<$15,000, the loading capacity was significantly less than with particle packed columns. However, the dramatic decrease in loading capacity with increased molecular weight of the analyte observed with the porous packed columns, was not observed with the monolithic column. The loading capacity for large proteins of Mr $>$50,000 was comparable to the porous particle packed columns. Femtomolar detection limits were achieved for proteins with ESI-MS detection and UV detection provided 200–500 amol sensitivity for protein and polypeptide, respectively. Henion's group fabricated surface alkylated PSDVB monoliths for improved resolution in peptide analysis.[11] Flow contacting surfaces of the monolith were octadecylated using a Friedel-Crafts catalyst and 1-chlorooctadecane. The resulting columns were coupled to ESI-MS and applied for separation of peptide and protein standards, as well as tryptic digests of cytochrome c and myoglobin.

Cation exchange displacement chromatography of proteins on a monolithic column was demonstrated by Schmidt et al.,[75] Monolithic and particle packed columns containing strong cation exchange groups, SO_3^-, were prepared and poly(diallyl-dimethylammonium chloride) (PDADMAC) was used for displacement. Adsorption isotherm experiments indicated that for the lower molar mass PDADMAC, the capacity of the two columns was comparable. There were significant differences in the capacity and affinity of the two columns, however, when higher molar mass PDADMAC was used. This was attributed to the differences in pore size and accessible surface.

The mechanically stable polymer monolith matrices have also been applied for the affinity purification of proteins, and provide an alternative to the soft polymer gel systems.[76] A urea-formaldehyde monolith was prepared by in-situ condensation polymerization in a 100 × 10 mm ID stainless-steel tube. Cibacron blue F3GA dye was subsequently immobilized and the columns were used for affinity separation of human serum albumin, ovalbumin, lysozyme, and newborn calf serum. The use of convective interaction media (CIM) monoliths for affinity purification of proteins has also been reported[77–79] and will not be discussed here as it has reviewed in detail elsewhere.[80,81]

Construction of large volume monolithic columns with uniform structure poses a challenge due to radial inhomogeneities and practical limitations on length. Podgornik et al. investigated the effect of the exothermic glycidyl methacrylate-ethylene dimethacrylate polymerization process on the monolith pore structure.[82] Mathematical modeling was used to predict

the maximal thickness of the annulus having a uniform structure and a special PTFE housing was designed to provide radial uniform flow distribution. Fast, gradient elution preparative separations of a mixture of proteins were achieved on a column modified with weak anion exchanger, diethylaminoethyl (DEAE) groups. Flow rates from 40 to 240 mL/min were applied.

Carbon Monoliths

Liang et al. reported on a novel graphitized-carbon monolith for HPLC separations.[83] The column was prepared by first forming a precursor phenolic resin rod embedded with silica beads via an acid catalyzed polymerization, followed by carbonization and removal of the silica beads and catalyst. The resulting rod, 80 × 3.4 mm ID, was clad in PTFE and inserted into a stainless steel tube. The total porosity for the graphitized carbon monolith was determined to be ~90%, whereas the silica monolith had a total porosity of ~81%. Analysis of alkylbenzene was promising, with five components resolved in under 3 min.

CONCLUSIONS

Applications ranging from pharmaceuticals to synthetic polymers and biopolymers have been reported using either silica or polymer based monoliths. With the introduction of commercially available monolithic columns for HPLC just a few years ago, the number of applications continues to increase. The major advantage of monolithic columns lies in the ability to apply high flow rates to reduce chromatographic run times, while maintaining adequate separation. Since the columns contain flow-through pores instead of porous particles, mass transfer restrictions to the chromatography of large molecules are not observed. This, combined with the short equilibration time, on the order of 1 min or less, and the ability to perform flow gradients, make ultrafast monolithic separations attractive for high throughput analysis schemes, such as in proteomics, metabonomics, and combinatorial library screening. The monolithic columns may also prove useful for real-time monitoring of in-process samples, where fast methods are requisite.

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