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Recent developments and applications of polymer monolithic stationary phases

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Abstract

This review highlights the current trends and the most recent advances in the field of preparation and application of organic polymer-based monolithic materials and covers literature published in 2020. A short historical background is provided and protocols to anchor monoliths covalently to the wall of the column/separation device are discussed. Furthermore, advances in tuning the macroporous structure and establishing its link to separation performance are conferred. Finally, method development and key applications using novel monolithic columns are discussed.

KEYWORDS
bioanalysis, column technology, review, stationary phase

1 | HISTORICAL BACKGROUND

One of the earliest communications postulating macroporous interconnected column structures dates back to 1952, when Mould and Synge discussed the use of a porous block of hydrogel for the separation of large molecular weight biomolecules.[1,2] However, the electroosmotic flow would need to be utilized to propel solvent through the medium as the use of hydrostatic pressure would likely result in phase collapse. Experimentally, organic polymer monoliths were initially developed and tested by Kubin and colleagues in 1965 who used copolymerization of 2-hydroxyethyl methacrylate and ethylene dimethacrylate monomers to separate a homologous series of high molecular weight polysaccharides.[3] Following this initial proof of concept a few additional studies throughout the 1970s highlighted the production of organic monoliths from other polymers such as polyvinylchloride[4–7] and polyurethane.[8–10] It was not until the late 1980s that compressed polyacrylamide gel structures and crosslinked non-porous agarose were explored by Hjerten et al.[11,12] Also in the late 1980s, Tennikova and colleagues developed thin polymer monolith disks based on poly(glycidyl methacrylate-*co*-ethylene dimethacrylate).[13,14] These disks were cut from blocks of the copolymers and were stacked in a cartridge. Applications included the separation of proteins using hydrophilic interaction, ion exchange, as well as reverse-phase chromatography. Later, Svec and Fréchet reported the preparation of rigid polymer structures first based on polymethacrylates in a large 8 mm i.d. column[15] and furthermore on poly(styrene-*co*-divinylbenzene).[16] Early studies demonstrated the suitability of these monoliths to conduct fast separations of macromolecules when applying steep solvent gradients at high volumetric flow rates.[17] Figure 1 highlights the good performance of chromatography using a monolithic column and applying a steep solvent gradient to achieve the separation of intact proteins using reverse-phase mode in less than 20 s.[17] In the following years, monolithic structures were also synthesized in capillary column formats.[18,19] To advance the resolving power and enlarge the applicability of monolithic columns many efforts have been directed over the years to the tuning of the macropore structure[20,21] and developing novel surface chemistries.[22,23]

2 | COVALENT ANCHORING OF MONOLITHS TO THE WALL

To avoid channeling, that is, flow spillage through the void between the monolith and the column wall that would hinder the separation,

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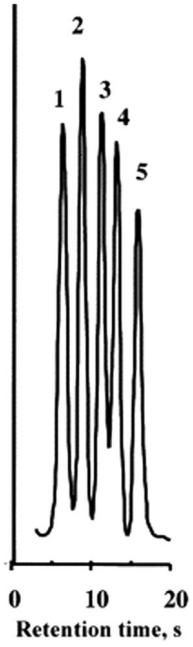

FIGURE 1 High throughput separation of intact proteins on a 4.6 mm i.d. × 50 mm monolithic column at a flow rate of 10 mL/min applying a 0.35 v% aqueous ACN gradient. Peak identification: ribonuclease (1), cytochrome *c* (2), bovine serum albumin (3), carbonic anhydrase (4), chicken egg albumin (5). Reprinted with permission from ref. [17]

monoliths need to be covalently bonded to the inner surface of the housing. Fused-silica capillaries are typically silanized using 3-((methacryloyl)-oxypropyl)trimethoxysilane (γ-MAPS). In 2006 Courtois et al. systematically investigated numerous reported etching and silanization protocols.[24] They demonstrated that some protocols frequently cited in literature were not giving satisfactory results. A sol-gel approach that deposits a thin layer of γ-MAPS gel on the capillary surface did not lead to covalent attachment but was rather retained by hydrophobic attraction, which ultimately led to channeling under increasing pressure. Using toluene as a solvent for the functional silane was the best to enable covalent attachment of the monolith to the fused-silica wall. This protocol has later been successfully applied to create monolithic capillary columns that tolerated ultra-high pressure of up to at least 800 bar.[25,26] Silanization has also been successfully applied to establish a covalent linkage between a polymer monolith and the wall of titanium oxide alloy.[27] The covalent attachment of monoliths to polymer substrates such as tubes, chips, and plates is more complex. Stachowiak et al. demonstrated a two-step sequential approach in which a UV-initiated proton abstraction from the polymer surface was required to create free radicals at the polymer surface for subsequent grafting of benzophenone.[28] In the next step, a thin poly(ethylene dimethacrylate) layer rich in double bonds was covalently linked to the surface. These double bonds served as anchors for covalent attachment of the monolith. This process was recently extended by do Nascimento et al. to the in situ preparation of methacrylate-based monoliths inside fluorinated poly(ethylene-*co*-propylene) tubes.[29] The surface functionalization was confirmed using attenuated total reflectance Fourier transform infrared spectrometry that revealed the presence of carbonyl, alkyl, and vinyl groups at the inside wall surface. The column designed for the reversed-phase separation was stable even at pressures of 70 bar and successfully applied for the analysis of mixtures of intact proteins. In a recent study, do Nascimento described the synthesis of (glycidyl methacrylate-*co*-ethylene glycol dimethacrylate) monoliths in situ in polypropylene ink-pen tubes.[30] The parent monolith was subsequently functionalized with Na_2SO_3 or iminodiacetate to produce strong and weak cation exchangers, respectively. A similar two-step approach was successfully applied in poly(ethylene-*co*-tetrafluoroethylene) tubes by the group of Herrero-Martinez.[31]

Abdulhussain et al. reported the fabrication of monoliths using a thermal polymerization in molds from polypropylene (PP) and its glass-reinforced counterpart. The PP was pretreated by depositing a layer of crosslinking monomer on the inner surface without any further pretreatment, to create free radicals.[32] For the glass-reinforced PP, a classical silanization was conducted prior to in situ synthesis of the poly(styrene-*co*-divinylbenzene) monolith. Attachment of monolith to the wall was investigated with scanning electron microscope microscopy and evaluation of the permeability was carried out in flow experiments. They stated that monoliths were successfully fabricated in both types of housings with a good attachment to the wall. Although they sometimes observed voids between the wall and the monolith, it appeared that monoliths were attached to the wall deeper in the PP-housed monoliths. The mechanical stability of glass-reinforced PP monoliths was assessed by flushing the column with different organic solvents. A linear trend between flow and pressure was generally observed up to a pressure of 30 bar. A nonlinear relation between flow rate and pressure was monitored when using THF as a solvent.

Concurrently, Carrasco-Correa et al. conducted a systematic study of different routes to establish covalent attachment of porous monoliths to the inner surface of acrylate-based photopolymerized microfluidic devices created via stereolithography 3D printing.[33] While previously published approaches did not lead to good results, the optimal procedure they demonstrated incorporated methacryloyl moieties onto the inner surface. Figure 2 shows the individual reaction steps. The first step involved hydrolysis of the methacrylate moieties to carboxylic acid groups. In the second step, they reacted them with a solution containing (1-ethyl-3-(3-dimethylaminopropyl)carbodiimide and N-hydroxysuccinimide (NHS). The third step consisted in exchanging the NHS moieties with hexamethylendiamine. Next, an amidation reaction is performed, followed by an attachment of glycidyl methacrylate. All reaction steps were carefully monitored and validated using attenuated total reflectance - Fourier transform infrared spectrometry. Their microfluidic devices integrating acrylate-based photopolymerized resins were successfully applied for automatic solid-phase extraction of anti-microbial agents, plastic additives, and monomers as models for emerging contaminants.

Peng et al. reported the synthesis of a poly(ethylene glycol phenyl ether acrylate-*co*-ethylene glycol dimethacrylate) monolith stationary phase directly in a 4.6. mm i.d. × 50 mm long stainless-steel column formats without additional surface modification. As loading was carried out applying aqueous samples, a shrinkage of the monolith and a

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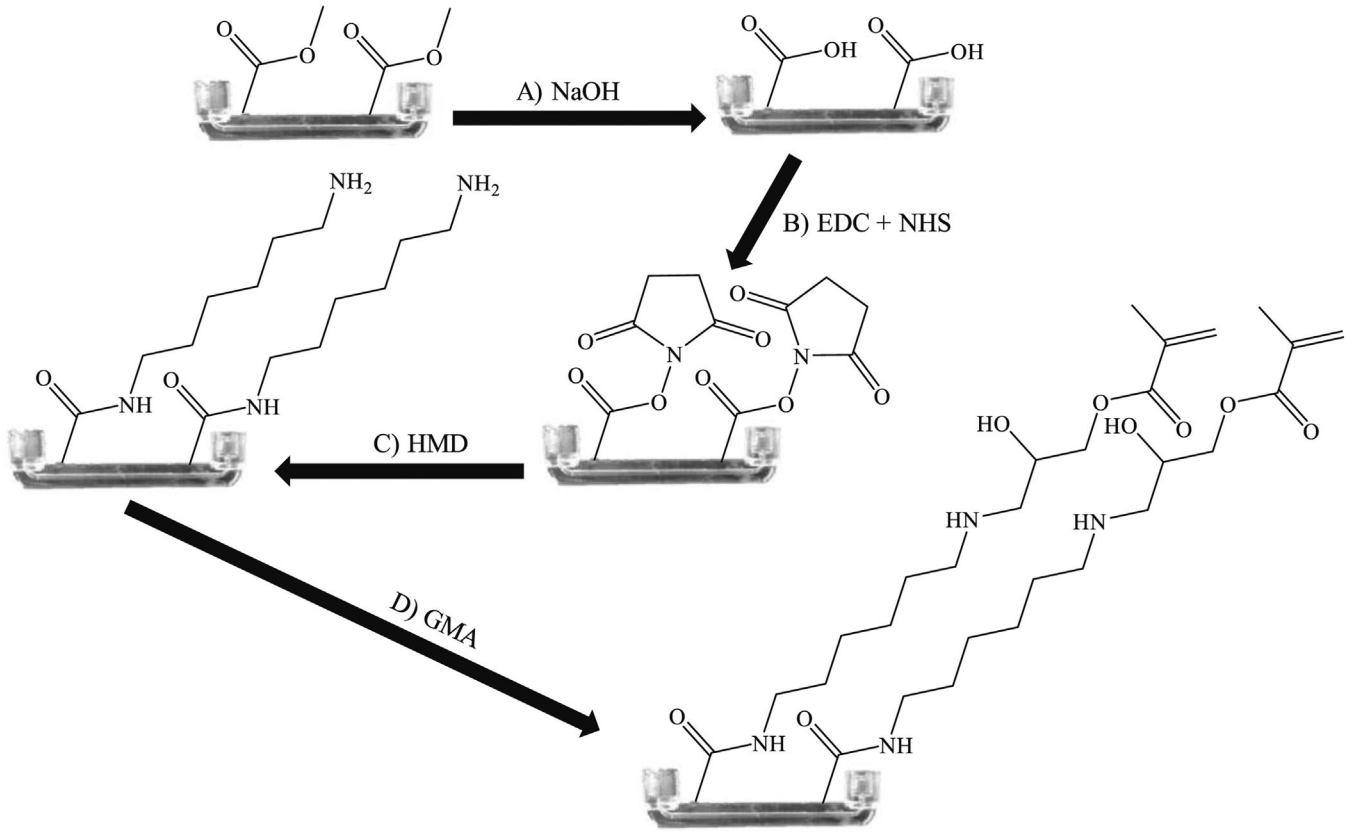

FIGURE 2 Scheme Sequential chemical modification of the inner surface of acrylate-based 3D-prints prior to in situ monolith preparation. The reagents used for the modification of the inner surface of the 3D printed support are: EDC: 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide; NHS: N-hydrosuccinimide; HMD: hexamethylenediamine and GMA: glycidyl methacrylate. Reprinted with permission from ref. [33]

significant channeling could be expected. However, none of these problems were reported while using the device as a cartridge in an on-line solid-phase extraction–liquid chromatography setup.[34]

3 | TUNING OF THE MACROPOROUS MONOLITH STRUCTURE

Having accurate control of the morphology is the key to control the kinetic performance limits of the corresponding stationary phase. Ideally, the external porosity is maximized, while the size of the globules and macropores is tuned. This control can aim at high resolving power (monoliths with small globules but larger macropores allowing the use of long columns) or fast separation speed (short columns with small globules and small macropores to reduce diffusion distances). Landmark papers discussing the optimization of the macropore structure were published by Svec and Fréchet[35,36] and by the group of Irgum.[37] Typically, the functional monomer, the crosslinker, and the initiator were first dissolved in a binary or ternary porogen solvent after which the polymerization was initiated. Viklund et al. also systematically investigated key variables including the composition of the porogen mixture, the content of cross-linking monomer, and polymerization temperature on the resulting macropore size distribution.[38] The porogen composition strongly affected the macropore size, with larger pores and large polymer globules being obtained in a poor solvent due to an earlier onset of phase separation. Increasing the proportion of the cross-linking monomer led to a decrease in average pore size because of the early formation of highly cross-linked globules with a reduced tendency to coalesce. These studies significantly contributed to the understanding of tuning monolith morphologies and paved the way for follow-up research. Recently, we reported guidelines for regulating the macropore structure of polymer monolithic columns.[39] We discussed the need to further reduce both diffusion distances by decreasing the macropore size and the eddy-dispersion contribution affected by macropore and globule sizes and also to reduce column heterogeneity. Mansour et al. and Wu et al. have also reviewed that factors are important in controlling and tuning the morphology of macroporous polymer monolithic materials.[40,41]

Jiang et al. recently reported on the development of monolithic columns containing graphene oxide prepared from high internal phase emulsion (HIPE) to target the enrichment of estrogens in the urine.[42] The high internal phase emulsions are generally defined as concentrated systems in which the volume fraction of the internal phase exceeds 0.74 and results in dispersed phase polyhedral droplets separated by thin films of the continuous mostly aqueous phase. They prepared the monolith using a single-step polymerization of HIPE comprising 2-ethylhexyl acrylate, glycidyl methacrylate, and divinylbenzene doped with graphene oxide. The recovery rates of

estrogens ranged between 85 to 106%. The intrinsic benefit of this adsorbent was the small pore size suggesting that these monoliths can prove useful in fast separations. However, the homogeneity of these monoliths must be addressed.

Catalá-Icardo et al. investigated the effect of the type of photoinitiator and irradiation time on morphology of monolithic columns and resulting chromatographic performance.[43] The initiators they used were azobisisobutyronitrile, benzophenone, 2,2-dimethoxy-2-phenylacetophenone, and 2-methyl-4′-(methylthio)-2-morpholinopropiophenone. The last produced monolith with the best performance was characterized with a very modest minimum plate height of 38 µm for alkyl benzenes and RSD of less than 11% for chromatographic parameters such as retention time and plate number. The fast polymerization rate was assigned to a high yield of dissociation and extinction coefficients and led to the formation of monoliths consisting of small globules within a 5 min short irradiation time while preserving a high permeability.

It is also critical for advancing column characteristics that the relation between column structure and resulting separation performance is well understood. This requires that good quantitative characterization of the morphology is needed. Different physical characterization approaches were applied to elucidate globule size and macropore size distribution. Irgum et al. pioneered structural characterization by computational assessment of macroporous monoliths with transmission electron microscopy, investigating the macropore size distribution via chord length distribution.[44] The results obtained were in agreement with mercury intrusion porosimetry data, and provided more information about the anisotropic porous structure and inherent heterogeneity. Müllner et al. reported on micrographs taken by serial block-face scanning electron microscopy (SEM). They then reconstructed the 3D structure of a polymer monolith synthesized in a 100 µm i.d. capillary to assess structure heterogeneity.[45] Nitrogen adsorption together with the Brunauer–Emmett–Teller equation is typically applied to analyze the micro- and meso-scale porosity and to obtain information concerning the surface area. Wouters et al. reported different representations of mercury intrusion porosimetry data and assessed structure homogeneity.[46] They also conducted argon gas-adsorption experiments and obtained more accurate information on the micro- and mesopores size distribution in dry state while applying the non-local density function theory model. To obtain information on the mesopore size distribution in wetted monoliths, inverse size-exclusion chromatography can be utilized.

Insights into van Deemter parameters needed to be established to link structural characteristics to dispersion and subsequent chromatographic performance. Information related to eddy dispersion (*A*-term) can be obtained by injecting a column dead-time marker and applying different flow rates. The mass transfer resistance of small molecular weight analytes is strongly affected by diffusion into the gel layer of the monolith (gel porosity),[47,48] which generally limits the applicability of polymer monoliths for small-molecule separations.

Macromolecule separations are typically conducted in gradient mode as there is a strong dependency between retention and mobile-phase composition. In a recent study, Fernández-Pumarega et al. assessed the effect of flow rate and gradient duration on peak capacity for intact proteins.[49] The highest peak capacity was achieved at an approximately 20-fold higher flow rate compared to the van Deemter optimum flow rate depending on the gradient duration applied and the molecular weight of the proteins. The optimum flow velocity increased with decreasing gradient time. The optimal flow rate was a compromise between the magnitude of the mass-transfer contribution, affected by molecular diffusion, and an increase in the peak capacity induced by the more favorable gradient volume. Dores-Sousa et al. reported tuning of the morphology of high-porosity poly(styrene-*co*-divinylbenzene) polymer monoliths in capillary column formats.[50] Whereas the external porosity of packed columns is fixed due to the sphere-shaped packing, the porosity of monoliths can be tuned by optimizing the monomer to porogen ratio. Monoliths with a 75% porosity had good structural integrity and the desired mechanical stability at a high pressure. The globule size and macropore size were fine-tuned. Effects of monomer to porogen ratio, porogen composition, polymerization temperature, and initiator content were systematically assessed. Decreasing globule and macropores size led initially to a strong decrease in eddy-dispersion (*A*-term) and mass transfer resistance (*C*-term) contribution. The high column permeability and low dispersion contributions of their monoliths produced a separation impedance as low as 976. An increase in polydispersity was observed for monoliths containing very small macropores and globules that resulted in a rise in the *A*-term. Columns are ideally operated above their van Deemter optimum flow rate to benefit from the gradient volume effect on peak capacity. The monoliths prepared by Dores-Sousa et al. could be used to establish high-efficiency gradient separations. Thus, they had the potential to outperform packed columns especially in proteomic research where long separation times at low flow rates are customary. The high resolving power was demonstrated both with intact proteins (Figure 3A) and a tryptic digest of *E. coli* cell lysate (Figure 3B). The potential for high-throughput separations of intact proteins shown in Figure 3C was confirmed using a 70 mm short capillary monolithic column while applying a 6 s ballistic gradient. It is important to note that this study demonstrated that it is not possible to accurately control the morphology of polymer monoliths with globule sizes below 250 nm using the conventional approach, i.e., free radical polymerization with optimized porogen ratio.

An alternative approach to the creation of three-dimensional structures, with fine control over the size of globules, as well as their shape, position, and aliment, has emerged during the past decade with the introduction of additive manufacturing or 3D printing technology. Fee et al. demonstrated proof of principle in 2014 and printed ordered column structures with globule sizes and microchannels in a range of 110–150 µm.[51] In a follow-up study published in 2020, Simon et al. reported the application of digital light processing to initiate a polymerization preprinting mixture of an acrylate crosslinker and [2-(acryloyloxy)ethyl] trimethyl ammonium chloride dissolved in a ternary porogen mixture of cyclohexanol, dodecanol, and water.[52] They 3D printed a macroporous cylindric structure on a multi-mm scale while on the microscale, a polymer monolithic structure with pores in the 100–300 nm range. Also, a macroporous monolithic Schoen-gyroid

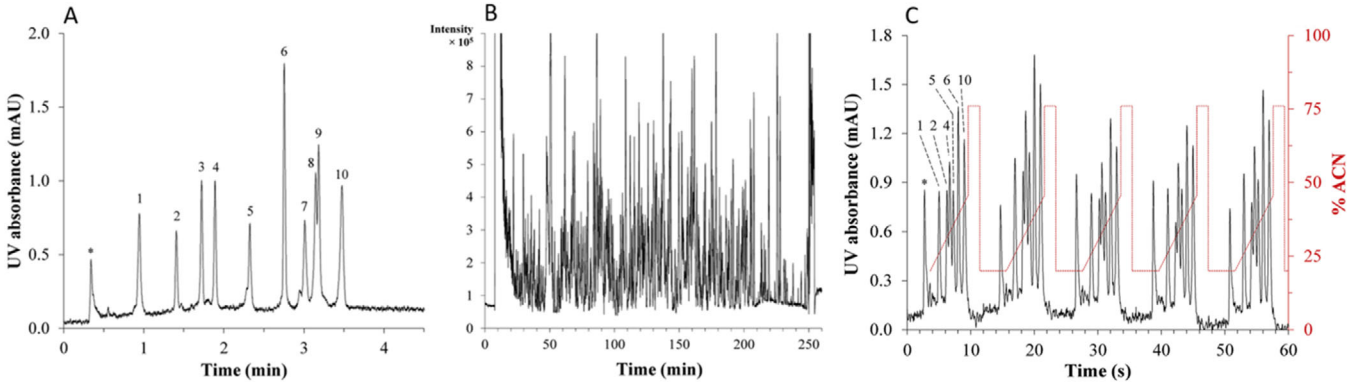

FIGURE 3 Examples high resolving power (A and B) and throughput (C) using monolith chromatography for biomolecule separations using high-porosity nanostructured monolithic capillary columns. (A) Gradient RP-LC separation of a mixture of 10 intact proteins. Peak identification: (*) injection solvent, (1) ribonuclease A, (2) insulin, (3) cytochrome *c* equine, (4) cytochrome *c* bovine, (5) trypsin, (6) α-lactalbumin, (7) α-chymotrypsin A, (8) α-chymotrypsinogen A, (9) myoglobin, and (10) carbonic anhydrase, (B) Gradient separation of a tryptic digest of *E. coli* on a 920 mm long capillary monolithic column with a 240 min gradient, (C) ballistic gradient separation of 7 intact proteins in a total cycle time of 12 s. Reprinted with permission from ref. [50]

structure was prepared and used for the analysis of two intact proteins using an ion-exchange mechanism. Two partly overlapping peaks were monitored over a time period of 20 min. This experiment highlights the importance of creating much smaller features with macropores in the sub-micrometer scale to advance the resolving power. Matheuse et al. demonstrated the possibility to print highly ordered monolithic structures with a total porosity of 80%, a 1 µm skeleton size, and 1.5 µm through pores using two-laser initiated photopolymerization.[53] While this approach represents a promising technology, the process is rather slow and expensive. Also, these structures must be still integrated in pressure-resistant microdevices to enable pressure-driven separations.

4 | METHOD DEVELOPMENT AND KEY APPLICATIONS

4.1 | Novel chemistries and methodologies

Advancing the selectivity by tuning the surface chemistry is the most powerful approach to enhance the resolution. The best straightforward approach for making monolithic columns is single-step copolymerization of functional monomers yielding the desired column structure and surface chemistry. Alternatively, post-polymerization functionalization strategies, for example, applying grafting approaches or using reactive monomers for subsequent modification can also be utilized. Two recent reviews on post-synthesis modification of the surface chemistry of polymer monoliths were published by Ribeiro et al.[22] and Alharthi et al.[54] A recent development involves the preparation of hydrophilic polymer monoliths based on poly(2-hydroxyethyl methacrylate) containing magnetic nanoparticles and amine-modified carbon nanotubes via photopolymerization of a HIPE described by Fresco-Cala et al.[55] They claimed that incorporation of more than 40 wt% of nanoparticles provided a new functionality, magnetism. However, they did not discuss the effect of such a high percentage of particulates on the macroporous structure. Also, the authors stated that the incorporation of amine-modified carbon nanotubes led to an increase in extraction efficiency through an increase in specific surface area and extra π–π interaction with the target compounds anti-inflammatory drugs.

Ganewatta et al. reported the incorporation of bare and cyano-modified silica nanoparticles in poly(glyceryl methacrylate-*co*-ethylene dimethacrylate) based monoliths for use in hydrophilic interaction chromatography.[56] Mixtures containing small neutral polar analytes, nucleobases, and organic acids were separated. However, a strong increase in peak width was observed with an increasing retention factor. This is likely to be related to a stationary mass transfer effect as earlier described by Huo et al.,[47] later also referred to as a gel porosity effect.[57–59]

Zajickova et al. evaluated the use of a commercially available 4.6 mm i.d. poly(styrene-*co*-divinylbenzene) monolithic column as separation media for the analysis of a mixture of pentadecapeptide antibiotics gramicidin A, B, and C and their corresponding isoforms using supercritical chromatography (SFC) coupled to mass-spectrometric detection with carbon dioxide/methanol as the mobile phase.[60] A steep methanol gradient from 2% to 40% and 0.1% formic acid enabled the separation of gramicidin isoforms within 3 min (Figure 4). Although the isoforms were not fully resolved for all gramicidins, selected masses characteristic of each isoform were detected and confirmed using selected ion monitoring in positive mode. While the used monolithic column was characterized by relatively large macropores and globules, the SFC separation might be improved using monoliths with smaller feature sizes.

Komendova et al. explored different retention models considering regression quality and statistical significance of individual regression parameters to model the dual retention mechanism of dopamine-related compounds using zwitterionic sulfobetaine functionality containing monolithic stationary phases.[61] They found that the

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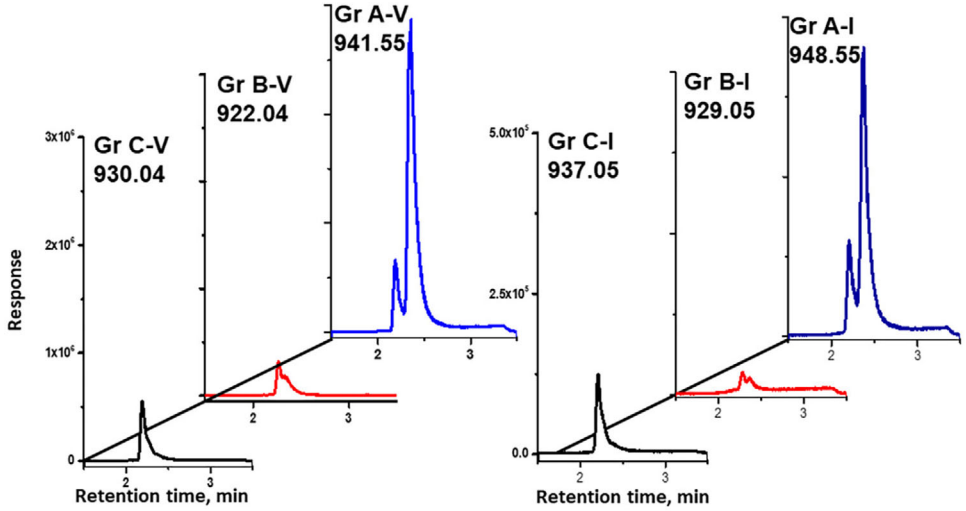

FIGURE 4 Proof-of-concept of the use of a monolithic column in SFC mode targeting the separation of gramicidin isoforms. SIM chromatograms of valine (V) and isoleucine (I) isoforms of gramicidin C, B, and A, with corresponding $[M + 2H]^{2+}$ ions. Reprinted with permission from ref. [60]. Copyright (2020) American Chemical Society

number of experimental points required to fit four-parameter models, such as the Neue-Kuss and Jandera models, limits robustness of regression analysis. Horvath's three-parameter retention model that describe retention of ionic analytes on non-polar stationary phases provided robust regression of experimental data and allowed extraction of structural characteristics of dopamine-related compounds.

4.2 | Enrichment/solid-phase microextraction

One of the attractive characteristics of polymer monoliths is their unique macropore structure and limited number of meso- and micropores. As a result, carry-over is less of an issue compared to classical silica-based fully-porous and core-shell particle-packed columns and also silica monolithic stationary phase used in the biomolecule analysis.[62] Recent research targeting monoliths for sample enrichment involves the development of an organic-hybrid monolith incorporating titanium dioxide nanotubes and polymerizable hydrophilic deep eutectic solvents (DES) introduced by Zhang et al. for the specific recognition of proteins via solid-phase microextraction.[63] DES can interact with protein through hydrogen bonding. The synergistic effect of nanotubes and hydrophilic DES chemistry allowed isolation of proteins of interest according to the *pI* of the target protein and enabled specific enrichment of albumin class proteins. Potential use of this work could be seen in the on-line depletion of albumin in clinical proteomic studies that is a current bottleneck in high throughput studies.

Qin et al. described the preparation of a zirconium arsenate-modified monolithic device for selective enrichment of phosphopeptides.[64] First, an arsenate functionalized monolithic column was prepared using a single-step copolymerization of 4-methacryloylaminophenylarsonic acid and ethylene dimethacrylate. The second step included attachment of Zr^{4+}metal ions via metal-chelate complex formation with the arsenate groups. The selectivity of the enrichment columns was assessed for capturing phosphopeptides from β-casein and BSA. Figure 5A and B shows the MALDI mass spectra of the digests before enrichment, and Figure 5C and D present corresponding spectra after enrichment. Clearly, high selectivity has been achieved as spectra after enrichment predominately show phosphopeptides.

Bickham et al. developed a monolithic solid-phase microextractor with reversed-phase properties based on lauryl methacrylate within the confines of a 3D printed microfluidic parallel channel structure.[65] The effectiveness was tested using nine fluorescently labeled preterm birth biomarkers varying in hydrophobicity. They found the monoliths to be tunable to give highly specific enrichment. Qi *et al.* described a solid-phase microextractor incorporating silica nanoparticles modified with tricontyl (C_{30}) alkyl chains in a polyacrylonitrile monolithic matrix.[66] Recoveries and limits of detection for carotenoids and fat-soluble vitamins were better than those achieved with commercial C_{18} cartridges.

Zhang *et al.* reported the preparation of a metal–organic framework (MOF) containing monolith for the on-line enrichment of aristolochic acid in medicinal plants.[67] The MOF was first functionalized to incorporate a polymerizable double bond functionality and then dispersed in the polymerization mixture containing methylolacrylamide monomer. The mixture was introduced in a 4.6 mm i.d. × 50 mm long stainless-steel column housing and polymerization was carried out for 3.5 h at 30 °C. No problems with channeling were reported, although the monolith was not covalently anchored to the column wall. While the MOF can exhibit unique microporous properties and hence selectivity due to is regular octahedral porous structure formed by coordination of tetravalent zirconium and 2-aminoterephtalic acid, it is likely that the micropores will be largely blocked as monomer diffuses into the porous structure and polymerizes. The increase in surface area is therefore likely explained by the effect of added MOF to the polymerization mixture affecting the macropore structure.

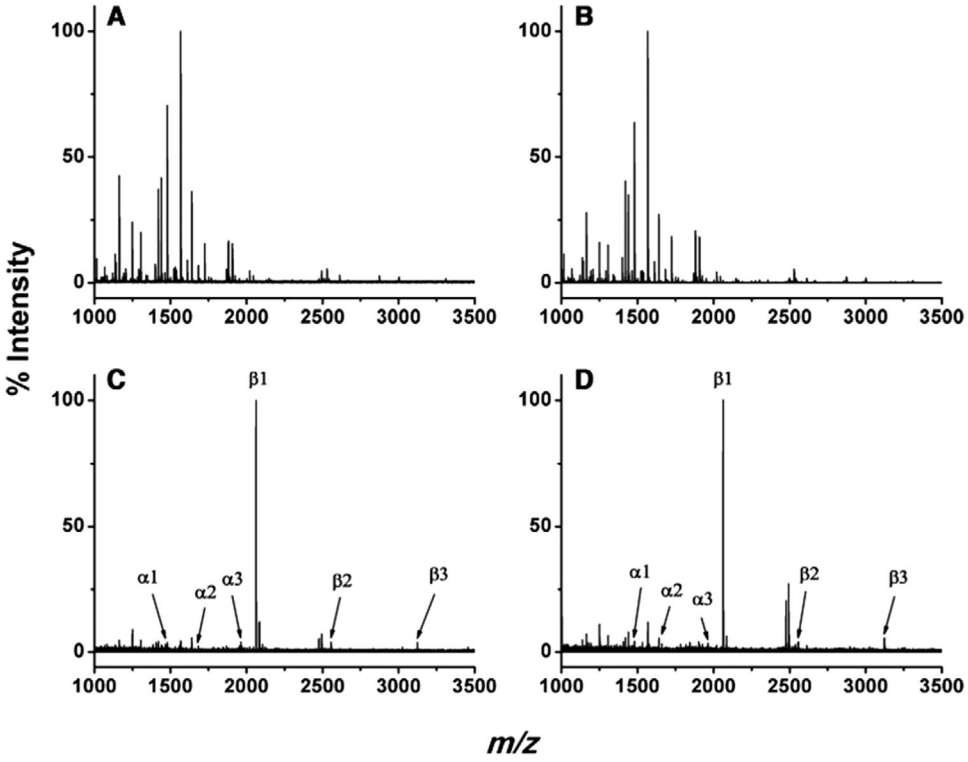

FIGURE 5 MALDI mass spectra of the tryptic digests of a mixture of β-casein and BSA obtained by direct analysis (A, B), and after enrichment of phosphopeptides using a zirconium arsenate-modified monolithic column (C, D). Molar ratio of β-casein to BSA, 1:500 (A, C), and 1:1000 (B, D). Reprinted with permission from ref. [64]

Glycidyl methacrylate-based poly(HIPE) monolith containing pipette tips for sample enrichment targeting malachite green and leucomalachite green were developed by Jiang et al.[68] Their process involved post-functionalization of the monolith with 6-aminocaproic acid via ring-opening of epoxy groups.

Mompo-Rosello *et al*. reported on the development of in-syringe hybrid monoliths modified with gold nanoparticles for selective extraction of glutathione in saliva and urine.[69] First, glycidyl methacrylate-based monolith was prepared *in-situ* in a polypropylene syringe, its pore surface modified using different ligands including ammonia, cystamine, and cysteamine, and subsequently functionalized with gold nanoparticles.

4.3 | Molecularly imprinted monoliths

Molecular imprinting involves the formation of cavities with a complementary geometric and/or chemical structure within the stationary phase and targets selective sample enrichment. Practical aspects in the synthesis of monolithic molecularly imprinted phases (MIP) were recently reviewed by Zheng et al.[70] and Pichon et al.[71] A groundbreaking research paper was also published in 2017 by Liu *et al*. who reported the development of imprinted porous monolithic materials for selective trapping of phosphopeptides.[72] Their approach involved systematic optimization and detailed characterization of the porous properties of the melamine-formaldehyde monolithic structures. The preparation of MIP monoliths followed using phosphorylated N-Fmoc protected ethyl esters of serine and tyrosine as templates. Unique selectivity could be established as polar *O*-phosphorylated side chains group were oriented towards the surface during the imprinting. Successful recognition and separations of phosphorylated peptides suggested that imprinted hydrophilic monolith could be useful in complex phosphoproteomic analysis.

More recently, Feng et al. reported MIP monolith based on a vinyl ester resin for screening of mycophenolate mofetil and mycophenolic acid, which is its cleavage product.[73] This approach was successfully applied for the selective enrichment of the target analytes in a pharmacokinetic study using patient plasma. Marchioni et al. developed MIP monolith using hydrogenated cannabidiol as template molecule for selective enrichment of cannabidiol and Δ9-tetrahydrocannabinol from plasma.[74] The monolithic capillary enrichment device was reused over fifty times without observing significant effect on extraction efficiency.

Fang et al. reported a new method combining magnetism-reinforced in-tube solid-phase microextraction based on molecular imprinting for the determination of trace aldehydes.[75] Their magnetized imprinted methacrylate-ester-based monolith was synthesized using 2,4-dinitroanaline as template molecule encapsulated by methacrylic acid monomer, before being mixed with the Fe_3O_4 ferrofluid and EDMA to polymerize. The MIP containing monolith in a capillary eliminated excess of 2,4-dinitrophenylhydrazine from the derivatized aldehyde solutions under the exertion of a magnetic field.

A methacrylate-ester-based monolith composed of 2-hydroxyethyl methacrylate, 2- (dimethylamino)ethyl methacrylate, piperazine diacrylamide, and N',N'-methylene bisacrylamide was imprinted by Mehta et al. with human serum albumin as template molecule in an extraction column housing made from polydimethylsiloxane.[76] Human serum albumin was adsorbed from diluted human plasma with a selectivity exceeding 98%. A slight decrease in the adsorption capacity was observed in the second cycle. Thereafter, the adsorption capacity remained constant.

4.4 | Enzymatic microreactors

Polymeric monolithic structures have been frequently used to immobilize proteolytic enzymes by covalently linking of the enzyme. The major application area is its use as immobilized enzyme reactor (IMER) allowing on-line digestion. Another area of application is its used in bioaffinity separations. Several excellent review papers on polymer monolithic IMER technology have appeared in literature in the past.[77–79] Recently, the Liu research group reported on IMERs using a poly(trimethylolpropane trimethacrylate) monolithic support and protease covalently attached via a thiol-ene click reaction.[80-84]

Wei *et al*. designed a microfluidic chip allowing to reduce and alkylate proteins, respectively, that integrated on-line mixer and a microchannel where trypsin was immobilized on pore surface of a poly(trimethylolpropane trimethacrylate) monolith.[80] Long-term stability tests performed with BSA digestion revealed only a 13.8% decrease in enzyme activity after 2-months use. Fan *et al*. and Zhao *et al*. applied the same approach and created IMER in capillary format.[81,82] In a follow-up paper, a microfluidic platform was reported by Wei *et al*., integrating online protein fractionation, denaturation, digestion, and peptide enrichment using a lysine-glycine-glycine imprinted monolith for extracting the tripeptide from the protein digests of MCF-7 cell.[83] The removal percentage of 94.6% for MCF-7 cell protein and the recovery of 90.8% peptide were reported.

Jiao et al. developed a polyHIPE monolith from glycidyl methacrylate and divinylbenzene that was sequentially modified with ammonia and gold nanorods.[84] In the next step they immobilized trypsin on the gold surface forming Au-S bonds. Digestion efficacy was tested with bovine serum albumin. The authors also performed a global proteome study and were able to achieve the identification of over 1000 proteins from rat liver tissue after only 2 min incubation in the IMER. This result confirmed efficient enzyme kinetics in comparison to conventional overnight in-solution digestion methods.

5 | CONCLUDING REMARKS

This review provides an overview of the recent developments of monolithic stationary phases mostly considering reports published in 2020. Tuning the morphology of monoliths such to achieve high separation efficiency, that is, a highly permeable structure having small macropores and small globules, is not straightforward. This is due to the complex interplay of polymerization precursors and conditions on the gelation and phase separation, and also includes polymer growth after phase separation that strongly affects the resulting macropore structure. Dores-Sousa et al.[50] demonstrated that maintaining column homogeneity starts to be problematic when downscaling dimensions of polymer globules below 200 nm. Note that the good monolith chromatography is exclusively reported for biomolecule analysis as gel-porosity effects are negligible.

A popular approach to advance the selectivity of polymer monoliths appears to be the incorporation of nanostructures such as particles, tubes, and fibers in the polymerization mixture. Obviously, this leads to changes in the macropore structure and affects the extent of the pore surface area. The same effect can also be achieved via carefully optimizing the monomer-to-porogen ratio and porogen composition. The selectivity is unlikely to change too much as most or even all surface of these nanostructures is covered with polymer. Microporous structures such as MOF were added in the polymerization mixture with an aim at using the micropore structure to enhance the quality of separations. It is important to note though that monomers are likely to penetrate the pores and locally polymerize. Hence, the selectivity can be lost to a large extent. Moreover, a significant increase in stationary phase mass transfer can be expected as the internal pore space in MOF can be at least partially blocked.

The number of applications of polymer monoliths is steadily rising. Their potential was demonstrated for the first time with separations using SFC mode and also with variety of interesting MIP monolithic materials that have been introduced over the last year. Selective sample enrichment is another application field for which unique chemistries of monoliths can be created. Unfortunately, some aspects such a specificity in case of the sample enrichment, mass loading, recovery, and carryover are not always consistently addressed making judgment of applicability difficult.

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CONFLICT OF INTEREST

The authors have declared no conflict of interest.

DATA AVAILABILITY STATEMENT

Data sharing not applicable to this article as no datasets were generated or analyzed during the current study

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