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Guidelines for tuning the macropore structure of monolithic columns for high-performance liquid chromatography

Dedicated to Frantisek Svec on the occasion of his 75th Birthday

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Abstract

The ability to control the external porosity and to tune the dimensions of the macropore size on multiple length scales provides the possibility of tailoring the monolithic support structure towards separation performance. This paper discusses the properties of conventional polymer-monolithic stationary phases and its limitations regarding the effects of morphology on kinetic performance. Furthermore, guidelines to improve the macropore structure are discussed. The optimal monolithic macropore structure is characterized by high external porosity (while maintaining ultra-high-pressure stability), high structure homogeneity, polymer globule clusters in the submicron range, and macropores with a diameter tuned towards speed (small diameter in the 100 - 500 nm range using short beds) or efficiency (larger macropores in the range of 500 nm - 1 μm allowing the use of longer column formats). Finally, promising approaches to control the morphology are discussed.

Keywords: polymer monoliths; column technology, UHPLC, review, stationary phase, morphology

1. Introduction

Advances in separation science are to a great extent driven by new developments in column technology. Columns packed with silica particulate materials have been the preferred packing material throughout the history of HPLC. The first commercially-available columns packed with 10 μm silica C_{18} particles were introduced in 1973 [1] and since then the particle diameter has been systematically decreased to realize faster separations and also to increase the separation efficiency [2,3]. A disadvantage of packed columns is that the external porosity is fixed due to the spherical packing. As a result, column designs, in which characteristic size (such as particle diameter) and porosity can be controlled independently, have the potential to yield intrinsically better chromatographic performance. Consequently, polymer-monolith stationary phases have emerged as attractive alternative for packed columns and find unique applicability in biomolecule analysis [4-7].

One of the first discussions on monolithic stationary phases dates back to 1948 when Nobel laureates, A.J.P. Martin, A. Tiselius, and R. Synge, proposed to use electro-endosmosis to transport a solution through a continuous block of porous gel structure [8]. From this time, several protocols have been published describing the synthesis of hydrogels [9,10], porous polymer foams [11-13], and other monolith type materials. It was in 1989 when Stellan Hjertén stated that a "continuous polymer bed" can be considered a good chromatographic column if it permits for a hydrodynamic flow at typical LC flow rates without indication of bed compression or damage [14]. Hjertén also developed the first crosslinked polyacrylamide gels for ion-exchange chromatography of intact proteins [14,15]. In 1993 the term 'monolith' was introduced to describe a stationary-phase support composed of a single piece of functionalized cellulose [16]. A major achievement was realized by Tennikova, Svec, and Belenkii who introduced rigid macroporous polymer stationary phases as thin disks for liquid chromatography [17]. An excellent historical overview describing the development of monolithic columns is provided by Svec in [16].

Many of the ongoing developments in the field of polymer-monolithic stationary phases are built on the pioneering work of Svec and coworkers. The most important research highlights of his group are as follows. In 1993, the Svec and Fréchet group reported on the development of 8 mm i.d. poly(styrene-*co*-divinylbenzene) monolithic columns with an average macropores size of 1 μm, yielding a stationary phase with high permeability [18]. Operating at a flow rate of 25 mL/min, a reversed-phase gradient separation of 4 intact proteins was achieved in only 30 s. Shortly after, the groups of Svec and Irgum published a series of papers describing different approaches to control the macropore structure [19-22], and monoliths were created incorporating charged moieties in the polymer backbone allowing for ion-exchange separations [23,24]. Based on this technology, Peters *et al.* developed methacrylate-based stationary phases in capillary column format applicable to capillary electrochromatography [25]. The Svec group also developed monolithic materials with a wide range of different surface properties, including monoliths coated with latex nanobeads [26] and gold nanoparticles [27-30], reactive polymer supports for high-throughput bioreactors [31,32], monoliths with superhydrophobic surfaces [33], monoliths incorporating metal-organic frameworks [34-36], chiral monoliths [37,38], *etc*.

The current contribution describes the state-of-the-art in tuning the macropore structure of polymer-based monolithic materials for liquid chromatography. First, the characteristics of conventional monolithic materials are discussed and shortcomings are debated. Next, general design rules are provided to optimize monolithic support structures towards high-speed separations and very high separation efficiencies. Furthermore, the pros and cons of promising approaches to control the macropore structure of polymer monoliths are discussed.

2. Characteristics of conventional polymer-monolithic stationary phases

The two most frequently used precursor solutions to create polymer-based monolithic stationary phases reported in literature are either composed of methacrylate/dimethacrylate monomers, in the presence of a porogen mixture composed of binary alcoholic mixture (*e.g.* cyclohexanol and dodecanol or 1,4 butanediol and propanol), yielding a methacrylate-ester-based monolithic stationary phase or of styrene and divinylbenzene monomers dissolved in a porogen of THF and decanol, resulting in a poly(styrene-*co*-divinylbenzene) monolithic entity. Following the original recipe developed by Svec and Fréchet [18,21,39,40], most monoliths reported in literature are based on a polymerization mixture with a monomer to porogen ratio of 40/60 wt%. Typically, 1 to 2 wt% initiator, *i.e.*, azobisisobutyronitrile (AIBN), is added with respect to the total monomer content. AIBN decomposes upon application of heat or UV light forming radicals triggering the free-radical polymerization reaction. In the early stage of the polymerization reaction cyclization, wherein the formation of intramolecular crosslinks is favored over reacting with growing chains, leads to the formation of microgel particles (nuclei). As the microgel particles become more crosslinked, reactions with fresh monomers will mainly take place at the surface of the growing microgel particles. During the course of the polymerization reaction, the microgel particles start to crosslink with other nearby microgel particles and coalesce into microglobules. During this transition stage the molecular weight increases significantly, resulting in a phase separation creating domains richer in polymer (and monomers) and domains richer in porogen (and monomers). This eventually leads to the formation of a pregel and when these pregel clusters extend throughout the column macrogelation will take place and the co-continuous monolithic structure is formed.

Figure 1 shows scanning electron microscopy images of the cross section (Fig. 1A) and a zoom-in (Fig. 1B) of a poly(styrene-*co*-divinylbenzene) monolithic stationary phase prepared *in-situ* in a 200 μm i.d. capillary column. The size of the polymer globules is estimated to have a mode diameter of 400 nm. The corresponding macropore distribution measured with mercury-intrusion porosimetry depicted in Fig. 1C shows that the majority of macropores range between 1.2 and 2.2 μm

(with a mode macropore size of 1.5 μm). The total surface area of the monolith was determined with multi-point Brunauer-Emmett-Teller (BET) analysis to be only 7.8 m^2/g. The meso- (2-50 nm) and micropore (< 2 nm) distribution determined via argon-adsorption isotherms displayed in Fig. 1D shows that the monolith contains a small fraction of pores < 10 nm [41]. It has been demonstrated that capillary column formats (≤ 200 μm i.d.) are pressure stable at UHPLC conditions due to the establishment of covalent bonds between the monolith and the column wall after surface modification [42].

In scientific literature it is frequently reported that mass transfer in monolithic structures is accelerated by convection, as the large macropores allow for high velocities in the flow-through pores. Often, this is linked to an 'apparent' better chromatographic performance. Making this statement, authors clearly refer to the mass transfer occurring inside the flow-through pores, which in general is referred to as the mobile zone mass transfer or C_m-term contribution [43]. At this point, it should be remarked that the only mechanism for mobile-phase mass transfer in a porous chromatographic support structure operated in a laminar flow regime is diffusion and not convection. Figure 2 shows the result of a flow simulation using computational fluid dynamics (CFD) [44], demonstrating that the main flow direction of the propelled fluid occurs in parallel with the surface. To realize interaction with the stationary-phase surface, analytes need to diffuse perpendicular to the main flow direction and this process is independent of the actual velocity inside the pore under the prevailing laminar conditions. As a consequence, having larger through-pores is in fact counterproductive as it enlarges the distance across which diffusion has to occur. The drawback of large through-pores can also be directly noted from Eq. (55) in [45], where the C_m-contribution to mass transfer is explicitly written as a function of the hydraulic through-pore diameter, revealing a quadratic dependency of the C_m-term on the through-pore diameter. Another consideration to understand the negative effect of a large through-pore diameter on the mass-transfer band broadening is by noting that the physical meaning of the reduced velocity (ν) is that it is proportional to the ratio of the time needed for the mass transfer

by diffusion over the time needed for mass transfer by convection [46]. Since $\nu = u_0 \cdot d / D_{mol}$, implying that ν increases linearly with d = macropore diameter, and since it is well known that the mass-transfer band broadening increases with increasing ν, the undesirable effect of large through-pores again becomes apparent. Modeling of convective flow and diffusion of molecules adsorbing in monoliths providing insights in the design of monoliths was also performed by Liapis *et al* [47,48].

Next to the C_m-contribution, there is also a contribution arising from the mass transfer of analytes by diffusion in the stagnant mobile phase inside the mesopores of the chromatographic support structure (C_s- contribution). Argon-adsorption experiments have shown that the number of micro- and mesopores in conventional polymer-monolithic supports is very low. Hence, stationary-phase mass transfer in the stagnant mobile phase in the pores is intrinsically limited [41]. Moreover, the dimensions of these pores are too small for large biomolecules (peptides, oligonucleotides, and proteins) to enter. In the case of small-molecule separation, Huo *et al.* demonstrated using methacrylate-based monolithic columns that surface diffusion effects of small molecular-weight analytes significantly degrade the separation performance [49]. This observation is also known as "gel-porosity effect" [50]. It should be noted however, that high-resolution separations are obtained with similar monolithic support structures in electro-driven mode, where the electro-osmotic flow propels the mobile phase [51,52]. It appears that gel porosity effects (C_s-contribution) are merely absent in CEC, suggesting that the dispersion phenomenon observed may possibly be explained by the effects of eddy dispersion and mobile-phase mass transfer as a function of flow velocity. These seemingly contradictory results demonstrate that there is a need to enhance the understanding of chromatographic dispersion of small molecules with respect to the morphology and also material properties, such a monomer composition, crosslinking content, *etc.*

The formation of the macropore structure is an effect of a complex interplay of thermodynamic and kinetic effects, see also '*Section 4*'. The research groups of Svec and Irgum (among others) have readily demonstrated that one way to control the diameter of the macropores is

by altering the porogen ratio [19,21,24,25,37,53]. The porogen is typically composed of a binary solvent composition, *i.e.*, a 'good' and a 'poor' solvent. By increasing the amount of good solvent in the porogen the solubility is enhanced and generally the phase separation is postponed resulting in monolithic structures with smaller globules sizes and macropores, provided that reaction kinetics is not slowed-down. When monoliths with smaller polymer globules are formed, microglobules tend to agglomerate forming cavities yielding a low number of mesopores ranging between 20 and 40 nm. To compare the width of the macropore size distributions of different polymer monolithic materials, Wouters *et al.* normalized the ordinate to the mode pore diameter [41]. In this way it became apparent that the monolith with smaller characteristic sizes (smaller globules and mode pore size) is characterized by a relatively narrow pore-size distribution but also exhibits a low number of larger macropores ranging between 500 and 1500 nm. When comparing the separation efficiencies by means of reduced plate height (independent on the normalization approach, *i.e.*, mode macropore size, globule size, or domain size) it became apparent that the eddy dispersion (*A*-term) contribution to band broadening becomes significant. This is despite the fact that the interconnected macroporous monolithic structure allows for trans-channel coupling, relieving the polydispersity to some extent. The structure inhomogeneity was also confirmed by Müllner *et al.*, who used serial block-face scanning electron microscopy to reconstruct the 3D structure of polymer monoliths [54,55]. Fig. 3 shows the volume-rendered corner-cut 3D reconstruction of a poly(styrene-*co*-divinylbenzene) monolithic entity. Distinct segments can be distinguished where small macropores (< 1 μm) percolate the polymer structure and segments containing very large macropores of several micrometers in size can be identified.

3. Column design and kinetic performance limits

The kinetic performance limit represents a threshold value for the chromatographic performance of a column operated at the maximum allowable operating pressure yielding the highest

separation efficiency within the shortest analysis time. For microparticulate columns, it has been demonstrated that high-speed separations are realized using columns packed with small diameter particles, whereas longer columns packed with larger diameter particles are required to realize high-efficiency separations. For a support structure with a fixed characteristic size (such as particle diameter), the best kinetic performance is obtained when the column length is tuned such that the column is operated at its optimum velocity matching the minimum in the plate-height curve. In this case, the optimal (and maximum) number of plates (N_{opt}) is defined as:

$$N_{opt} = \frac{\Delta P_{max}}{\eta} \cdot \frac{K_{v,0}}{H_{min} \cdot u_{0,opt}} \quad (1)$$

where ΔP_{max} is the maximum operating pressure, η the mobile-phase viscosity, $K_{v,0}$ the column permeability, and H_{min} is the achieved plate height value when operating a column at the optimum flow velocity ($u_{0,opt}$). Eq. 1 provides general design rules for monolithic column support structures, which include:

- Column stability at ultra-high pressures is desired.
- The chromatographic support should provide the highest possible $K_{v,0}$, which corresponds to high external porosity, defined as the fraction of column volume occupied by the macropores, which can be tuned.
- To minimize H_{min}, the A- and C_s- contributions to band broadening should be reduced by using the smallest possible characteristic features while maximizing structure homogeneity.

Considering the typical interconnected globular morphology of polymer-monolithic columns, two 3D monolith support structures are proposed in Figure 4 aimed towards high-speed separations (Fig. 4A) and to maximize separation efficiency (Fig. 4B). To minimize the eddy-dispersion contribution (A-term) to band broadening the monolithic support structure contains small diameter globules. This is independent of the application of the column aiming to speed or efficiency. Note that small interconnected globules are typically also preferred to reduce the stationary-phase mass transfer

contribution (C_s-term). However, due to the low number of mesopores, the C_s-term contribution induced by diffusion of analytes in stagnant mobile phase is virtually absent. Since the peak variance increases proportional to the square of the pore size, it is evident that to realize high-speed separations the development of short columns with monolithic stationary phases characterized by small macropores (with a diameter in the 100 - 500 nm range) is required to minimize mobile-phase mass transfer (C_m-term). To accommodate high plate numbers, long columns need to be employed, taking into account that N is proportional to column length. This can only be achieved when the macropore diameter is increased. This will result in a monolith support structure that has a higher external porosity (and hence higher $K_{v,0}$) compared to the 3D model depicted in Fig. 4A. Note that increasing $K_{v,0}$ by creating monoliths with larger macropores leads to a significant increase in the mobile-phase mass-transfer contribution (C_m-term) to band broadening. Hence, the increase in macropore diameter compared to monoliths optimized for throughput is only modest and should be in the range of 500 nm - 1 μm to reach a good compromise between performance and analysis time.

4. Promising approaches to control the macropore structure

As discussed in '*Section 2*', the formation of the macropore structure is a complex interplay of thermodynamic and kinetic effects. The most proficient approach to tune the macropore and globule size is by selecting the appropriate porogenic solvents and tuning their respective ratio. Porogens must have preferably a high boiling point and be chemically inert. Furthermore, a homogeneous solution should be obtained when dissolving the monomers. The polarity and solubility parameters of monomers, crosslinker agent, and the polymer attribute to the property of a porogen, *i.e.*, a solvating / 'good' solvent or a non-solvating / 'poor' solvent. Figure 5 shows the effect of porogen ratio (THF/decanol) on resulting macropore structure. From the Hildebrand solution theory and the solubility parameters of poly(S-*co*-DVB) with respect to that of THF and decanol, it is apparent that THF is more proficient in solubilizing the growing polymer network compared to

decanol [56]. Varying the solvent ratio thus alters the overall thermodynamic quality of the porogen and consequently the timing of the phase separation. In a polymerization mixture containing less 'good' solvent, the pregel prior to the phase separation will be swollen with monomer and large globules will be formed. It should be noted that a small change in the content of porogen can lead to a rather abrupt change in pore and globule size, especially in the target range to create macropore diameters ranging between 100-500 nm (for high speed separations) and 500 nm - 1 μm to create long columns with high permeability for high-efficiency separations. It should be noted however, that monolithic entities have been also created, in which the globule and macropore diameter decreased when increasing the amount of 'poor' solvent in the porogen. In this case it appears that the reaction kinetics are accelerated, and a higher number of smaller globules was formed leading to a structure with smaller mode macropore size. Reaction kinetics is in turn influenced by the type and amount of initiator used [39,57,58] and also by the polymerization conditions, such as temperature or irradiation intensity [39,58-60]. During a thermal polymerization, excess heat is generated during the polymerization reaction, and the heat flux can better be controlled in small i.d. reaction vessels, leading to more homogeneous structures. In general, the polymerization is brought to completion more rapidly with UV light (10-20 min) compared to a thermally-initiated system, in which conversion is typically proceeded for 20-24 h. Hence, gravity related phenomena inducing structure inhomogeneity should be less apparent in case of a fast photopolymerization. Note that due to the limited penetration depth of UV-light capillary columns formats < 200 μm must be used to prevent radial inhomogeneity.

Most polymer-monolithic stationary phases reported in scientific literature are typically synthesized using a 40/60 wt% monomer to porogen ratio. However, to advance the kinetic performance, monolithic columns with significantly higher porosities and hence higher permeability need to be developed, taking into account that high mechanical strength is required to operate at the highest possible operating pressure. In our group we developed monolithic support structures with

porosity exceeding 70% and systematically optimized the content of initiator, crosslinker, and porogen to create monoliths containing nanoglobule features and macropores in the 100-500 nm scale allowing for high-speed (sub-minute) peptide separations at ultra-high pressure (800 bar) [57]. With respect to kinetic performance, one of the most promising poly(S-*co*-DVB) stationary phases for biomolecule separations was developed by the research group of C.G. Huber [61,62]. Still, better monolithic support structures can likely be developed surpassing the performance limits currently established.

Instead of completing the polymerization reactions at the end of the conversion curve, different studies have been conducted in which the polymerization reaction is terminated at the course of the reaction in an attempt to optimize the macropore (and mesopore) structure [56,63-66]. As expected, this approach leads to a monolithic structure with smaller globules and hence monoliths characterized by larger surface areas, and also likely by a higher total porosity since monomer conversion is not completed. While in some cases good separation performances have been demonstrated, it is likely that trying to stop a polymerization reaction in the steepest part of the conversion curve leads to issues with the repeatability in morphology. We speculate that similar structures can be realized by carefully optimizing the monomer to porogen and porogens' ratios, while completing the conversion curve. An alternative initiation method is the use of γ-ray, as was explored by the Gasparrini research group [67]. Using this approach, monolithic capillary columns based on lauryl methacrylate and hexanediol dimethacrylate operated in pressure-driven LC yielded high-efficiency separations (N > 100,000 and H_{min} ~ 10 μm) of small-molecule mixtures [67]. It should be noted that excessive peak-broadening phenomena (as function of retention factor), as frequently observed using conventional monolithic columns, were not observed. Moreover, very high-peak capacity separations were achieved for gradient biomolecule separations, including peptides and intact proteins [68-70].

It has been speculated that polymerization in confined spaces results in different macropore structures than when conducting the polymerization in large i.d. conduits. Bystrom *et al.* synthesized monolithic structures via thermally-initiated polymerization reactions in 250 μm i.d. capillary columns, and in (micro-)vials with an i.d. of 4.4 mm and 9.7 mm, respectively [71]. While SEM images did not reveal significant differences, difference in mode pore diameter and macropore size distribution were observed applying mercury-intrusion porosimetry. With decreasing column diameter, the macropore size was found to increase. He *et al.* investigated confinement effects on morphology in cylindrical column formats of different i.d., *i.e.*, 50, 20, 10, and 5 μm [72]. The extent of deformation from the bulk porous structure under confinement was found to depend on the ratio of characteristic length of the confined space to the monolith pore size. For a confinement dimension to pore size ratio < 10, the morphology is affected and at the extreme limit a polymer layer is formed at the column wall. Experiments conducted by Nischang *et al.* reconfirmed that confinement from 75 μm to 5 μm of i.d. led to monolithic structures with distinctly different morphologies and observed a decrease in peak width recorded in gradient mode when downscaling column dimensions [73]. To prevent the formation of a dense polymer layer at the column wall it was proposed to optimize the surface modification procedure (prior to polymerization) and decrease the number of vinyl groups at the wall or increase the polymerization temperature. The results presented by different groups to date suggest that the morphology is affected when changing the column diameter. The extent to which the pore-size distribution is affected remains unclear and requires a comprehensive performance characterization in isocratic mode to obtain insights in eddy-dispersion characteristics as function of flow velocity.

In pursuit of controlling the macropore structure and to increase the mesoporosity, different research groups have experimented with controlled/living polymerization methods. Living polymerization is based on establishing an equilibrium between active and inactive chains, via the addition of an agent that can deactivate them reversibly. In this way, the contribution of chain transfer

and termination is minimized, and it is possible to achieve better control over the molecular weight (minimizing polydispersity) and the composition and chain architecture is improved [74]. Ideally this should lead to better control over the macropore and globule size and possibly also structure homogeneity. Different living polymerization approaches have been investigated for the synthesis of polymer monolithic support structures, including living radical nitroxide-mediated polymerization [20,23,75,76], atom-transfer radical polymerization (ATRP) [77,78], reversible addition-fragmentation chain transfer (RAFT) [79,80], organotellurium-mediated living radical polymerization (TERP) [81-84], ring-opening metathesis polymerization (ROMP) [85-88], *etc*. Cumulative pore-size distributions measured with mercury-intrusion porosimetry did not reveal difference in porosity when comparing the porous properties of monolithic materials prepared via a free-radical polymerization or in the presence of the stable nitroxide radical 2,2,6,6-tetramethylpiperidinyl-1-oxy (TEMPO)-mediated polymerization approach [20]. During the course of a free-radical polymerization, the Trommsdorff effect leads to temperature distribution effects inducing radial inhomogeneity. In the case of a living polymerization reaction, no differences in radial homogeneity were observed, even in large i.d. conduits (5 cm in diameter), due to the mere absence of the Trommsdorff effect [20,89]. Results from different studies are to some extent inconclusive and strongly depend on the polymerization conditions selected. Also, structure homogeneity affecting the magnitude of the *A*-term contribution should be studied while being independent of the globule size and preferably also macropore size. Alternatively, the development of hybrid polymer materials as developed by the research group of Zou may be attractive. Liu *et al* reported on the development of a polymer monolithic columns via photo-initiated thiol-yne click polymerization yielding good structure homogeneity (low *A*-term) and excellent separation efficiency for retained small molecules (low *C*-term) [90,91].

A promising approach to create hierarchically ordered monolithic materials is by the use of structure-directing agents, such as (nano)particles, micelles, ice templates, *etc* [92-96]. Freeze casting

is arguably the most straightforward approach and has been used since the early 1960s to form lamellar ice crystals that expel dissolved molecules as they grow. The polymerization mixture is then carried out in the cavities between unidirectional-aligned ice crystals allowing to create macroporous layered monoliths [97,98]. Following similar methodology, Arrua and Hilder created ordered monolithic entities in capillary columns by directional freezing of the polymerization mixture in liquid nitrogen and photoinitiation in the frozen state, yielding structures as depicted in the SEM displayed in Fig. 6A and B (for the zoom in) [99]. It appeared that freezing of the solvent crystals occurs in the direction of the temperature gradient from the surface to the reactor to the center. Zuo *et al.* described the construction of ordered microporous π-conjugated polymer monoliths using naphthalene crystals as template [100]. The macroporous structure, depicted in Fig. 6C, was formed along the unidirectional freezing direction inside the template crystals. It should be noted however, that downscaling of the pore diameter is required to minimize the C_m-term contribution to band broadening and realize sufficiently high separation efficiencies. Alternatively, advances made in 3D printing techniques may open the route to create highly ordered 3D structures having independent control over macropore dimensions and size of the skeletons [101,102]. A promising 3D microfabrication method to create nanoscale structures is based on two-photon polymerization in which two photons need to be simultaneously absorbed to initiate radical polymerization. By using a 2D translational stage, any 3D computer structure can be created via direct laser writing into a photosensitive material. A disadvantage currently encountered is the long writing time to create nanostructures in high volume, such as chromatographic bed with comparable dimensions to a conventional capillary column.

5. Concluding remarks

The value of rigid polymer-monolithic materials, as pioneered by Svec and coworkers, have been demonstrated for biomolecule separations in gradient mode by different research groups. To

significantly advance the kinetic performance limits currently achieved, monolithic support structures with higher external porosity need to be created, and the globule sizes and macropore diameter need to be downscaled significantly. An intrinsic property of monolithic materials is the structure inhomogeneity, negatively affecting eddy-dispersion contribution. Eddy dispersion induced by structure inhomogeneity is only relieved to a small extent by the interconnected macropore structure providing flow-through channel intermixing points. In addition of creating high-porosity monolithic support structures with globules and macropores on the 100-500 nm scale, a key parameter to increase performance limits up to one order of magnitude is the structure order and homogeneity.

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7 References

[1] Neue, U. D., HPLC Columns: Theory, Technology, and Practice, Wiley-VCH, New York 1997.

[2] Snyder, L. R., HPLC: past and present. *Anal. Chem.* 2000, *72*, 412a-420a.

[3] Gritti, F., Guiochon, G., The current revolution in column technology: how it began, where is it going? *J. Chromatogr. A* 2012, *1228*, 2-19.

[4] Tanaka, N., Kobayashi, H., Nakanishi, K., Minakuchi, H., Ishizuka, N., Peer reviewed: monolithic LC columns. *Anal. Chem.* 2001, *73*, 420a-429a.

[5] Eeltink, S., Wouters, S., Dores-Sousa, J. L., Svec, F., Advances in organic polymer-based monolithic column technology for high-resolution liquid chromatography-mass spectrometry

profiling of antibodies, intact proteins, oligonucleotides, and peptides. *J. Chromatogr. A* 2017, *1498*, 8-21.

[6] Unger, K. K., Skudas, R., Schulte, M. M., Particle packed columns and monolithic columns in high-performance liquid chromatography-comparison and critical appraisal. *J. Chromatogr. A* 2008, *1184*, 393-415.

[7] Svec, F., Huber, C. G., Monolithic materials: promises, challenges, achievements. *Anal. Chem.* 2006, *78*, 2100-2107.

[8] Mould, D. L., Synge, R. L. M., Electrokinetic ultrafiltration analysis of polysaccharides. A new approach to the chromatography of large molecules. *Analyst* 1952, *77*, 964-969.

[9] Kubín, M., Špaček, P., Chromeček, R., Gel permeation chromatography on porous poly(ethylene glycol methacrylate). *Coll. Czechoslov. Chem. Commun.* 1967, *32*, 3881-3887.

[10] Végvári, Á., Földesi, A., Hetényi, C., Kocnegarova, O., Schmid, M.G., Kudirkaite, V., Hjertén, S., A new easy-to-prepare homogeneous continuous electrochromatographic bed for enantiomer recognition. *Electrophoresis* 2000, *21*, 3116-3125.

[11] Ross, W. D., Jefferson, R. T., In situ-formed open-pore polyurethane as chromatography supports. *J. Chromatogr. Sci.* 1970, *8*, 386-389.

[12] Schnecko, H., Bieber, O., Foam filled columns in gas chromatography. *Chromatographia* 1971, *4*, 109–112.

[13] Lynn, T. R., Rushneck, D. R., Cooper, A. R., High resolution-low pressure liquid chromatography. *J. Chromatogr. Sci.* 1974, *12*, 76-79.

[14] Hjertén, S., Liao, J.-L., Zhang, R., High-performance liquid chromatography on continuous polymer beds. *J. Chromatogr. A* 1989, *473*, 273-275.

[15] Svec, F., Stellan Hjerten's contribution to the development of monolithic stationary phases. *Electrophoresis* 2008, *29*, 1593-1603.

[16] Svec, F., Monolithic columns: A historical overview. *Electrophoresis* 2017, *38*, 2810-2820.

[17] Tennikova, T. B., Svec, F., Belenkii, B. G., High-performance membrane chromatography. a novel method of protein separation. *J. Liq. Chromatogr.* 1990, *13*, 63-70.

[18] Wang, Q. C., Svec, F., Fréchet, J. M., Macroporous polymeric stationary-phase rod as continuous separation medium for reversed-phase chromatography. *Anal. Chem.* 1993, *65*, 2243-2248.

[19] Nordborg, A., Svec, F., Fréchet, J. M., Irgum, K., Extending the array of crosslinkers suitable for the preparation of polymethacrylate-based monoliths. *J. Sep. Sci.* 2005, *28*, 2401-2406.

[20] Peters, E. C., Svec, F., Fréchet, J. M. J., Viklund, C., Irgum, K., Control of porous properties and surface chemistry in "molded" porous polymer monoliths prepared by polymerization in the presence of TEMPO. *Macromolecules* 1999, *32*, 6377-6379.

[21] Viklund, C., Svec, F., Fréchet, J. M. J., Irgum, K., Monolithic, "molded", porous materials with high flow characteristics for separations, catalysis, or solid-phase chemistry: control of porous properties during polymerization. *Chem. Mater.* 1996, *8*, 744-750.

[22] Viklund, C., Pontén, E., Glad, B., Irgum, K., Hörstedt, P., Svec, F., "Molded" macroporous poly(glycidyl methacrylate-co-trimethylolpropane trimethacrylate) materials with fine controlled porous properties: preparation of monoliths using photoinitiated polymerization. *Chem. Mater.* 1997, *9*, 463-471.

[23] Viklund, C., Nordström, A., Irgum, K., Svec, F., Fréchet, J. M. J., Preparation of porous poly(styrene-co-divinylbenzene) monoliths with controlled pore size distributions initiated by stable free radicals and their pore surface functionalization by grafting. *Macromolecules* 2001, *34*, 4361-4369.

[24] Viklund, C., Svec, F., Fréchet, J. M., Irgum, K., Fast ion-exchange HPLC of proteins using porous poly(glycidyl methacrylate-co-ethylene dimethacrylate) monoliths grafted with poly(2-acrylamido-2-methyl-1-propanesulfonic acid). *Biotechnol. Prog.* 1997, *13*, 597-600.

[25] Peters, E. C., Petro, M., Svec, F., Fréchet, J. M. J., Molded rigid polymer monoliths as separation media for capillary electrochromatography. 1. Fine control of porous properties and surface chemistry. *Anal. Chem.* 1998, *70*, 2288-2295.

[26] Hilder, E. F., Svec, F., Fréchet, J. M. J., Latex-functionalized monolithic columns for the separation of carbohydrates by micro anion-exchange chromatography. *J. Chromatogr. A* 2004, *1053*, 101-106.

[27] Lv, Y., Alejandro, F. M., Fréchet, J. M. J., Svec, F., Preparation of porous polymer monoliths featuring enhanced surface coverage with gold nanoparticles. *J. Chromatogr. A* 2012, *1261*, 121-128.

[28] Terborg, L., Masini, J. C., Lin, M., Lipponen, K., Riekolla, M. L., Svec, F., Porous polymer monolithic columns with gold nanoparticles as an intermediate ligand for the separation of proteins in reverse phase-ion exchange mixed mode. *J. Adv. Res.* 2015, *6*, 441-448.

[29] Lv, Y., Lin, Z., Svec, F., Hypercrosslinked large surface area porous polymer monoliths for hydrophilic interaction liquid chromatography of small molecules featuring zwitterionic functionalities attached to gold nanoparticles held in layered structure. *Anal. Chem.* 2012, *84*, 8457-8460.

[30] Cao, Q., Xu, Y., Liu, F., Svec, F., Fréchet, J. M. J., Polymer monoliths with exchangeable chemistries: use of gold nanoparticles as intermediate ligands for capillary columns with varying surface functionalities. *Anal. Chem.* 2010, *82*, 7416-7421.

[31] Geiser, L., Eeltink, S., Svec, F., Fréchet, J. M. J., In-line system containing porous polymer monoliths for protein digestion with immobilized pepsin, peptide preconcentration and nano-liquid chromatography separation coupled to electrospray ionization mass spectroscopy. *J. Chromatogr. A* 2008, *1188*, 88-96.

[32] Krenkova, J., Lacher, N. A., Svec, F., Highly efficient enzyme reactors containing trypsin and endoproteinase LysC immobilized on porous polymer monolith coupled to MS suitable for analysis of antibodies. *Anal. Chem.* 2009, *81*, 2004-2012.

[33] Lv, Y., Cao, Y., Svec, F., Tan, T., Porous polymer-based monolithic layers enabling pH triggered switching between superhydrophobic and superhydrophilic properties. *Chem. Commun.* 2014, *50*, 13809-13812.

[34] Wang, X., Lamprou, A., Svec, F., Bai, Y., Liu, H., Polymer-based monolithic column with incorporated chiral metal-organic framework for enantioseparation of methyl phenyl sulfoxide using nano-liquid chromatography. *J. Sep. Sci.* 2016, *39*, 4544-4548.

[35] Wen, L., Gao, A., Cao, Y., Svec, F., Tan, T., Lv, Y., Layer-by-layer assembly of metal–organic frameworks in macroporous polymer monolith and their use for enzyme immobilization. *Macromol. Rapid Commun.* 2016, *37*, 551-557.

[36] Carrasco-Correa, E. J., Martínez-Vilata, A., Herrero-Martínez, J. M., Parra, J. B., Maya, F., Cerdà, V., Cabello, C. P., Palomino, G. T., Svec, F., Incorporation of zeolitic imidazolate framework (ZIF-8)-derived nanoporous carbons in methacrylate polymeric monoliths for capillary electrochromatography. *Talanta* 2017, *164*, 348-354.

[37] Lämmerhofer, M., Peters, E. C., Yu, C., Svec, F., Fréchet, J. M., Chiral monolithic columns for enantioselective capillary electrochromatography prepared by copolymerization of a monomer with quinidine functionality. 1. Optimization of polymerization conditions, porous properties, and chemistry of the stationary phase. *Anal. Chem.* 2000, *72*, 4614-4622.

[38] Lämmerhofer, M., Tobler, E., Zarbl, E., Lindner, W., Svec, F., Fréchet, J. M., Macroporous monolithic chiral stationary phases for capillary electrochromatography: New chiral monomer derived from cinchona alkaloid with enhanced enantioselectivity. *Electrophoresis* 2003, *24*, 2986-2999.

[39] Svec, F., Fréchet, J. M. J., Kinetic control of pore formation in macroporous polymers. formation of "molded" porous materials with high flow characteristics for separations or catalysis. *Chem. Mater.* 1995, *7*, 707-715.

[40] Svec, F., Fréchet, J. M. J., Continuous rods of macroporous polymer as high-performance liquid chromatography separation media. *Anal. Chem.* 1992, *64*, 820-822.

[41] Wouters, S., Hauffman, T., Mittelmeijer-Hazeleger, M. C., Rothenberg, G., Desmet, G., Baron, G. V., Eeltink, S., Comprehensive study of the macropore and mesopore size distributions in polymer monoliths using complementary physical characterization techniques and liquid chromatography. *J. Sep. Sci.* 2016, *39*, 4492-4501.

[42] Vaast, A., Nováková, L., Desmet, G., de Haan, B., Swart, R., Eeltink, S., High-speed gradient separations of peptides and proteins using polymer-monolithic poly(styrene-co-divinylbenzene) capillary columns at ultra-high pressure. *J. Chromatogr. A* 2013, *1304*, 177-182.

[43] Knox, J. H., Band dispersion in chromatography – a new view of A-term dispersion. *J. Chromatogr. A* 1999, *831*, 3-15.

[44] Vervoort, N., Gzil, P., Baron, G. V., Desmet, G., Model column structure for the analysis of the flow and band-broadening characteristics of silica monoliths. *J. Chromatogr. A* 2004, *1030*, 177-186.

[45] Desmet, G., Broeckhoven, K., Equivalence of the different C_m- and C_s-term expressions used in liquid chromatography and a geometrical model uniting them. *Anal. Chem.* 2008, *80*, 8076-8088.

[46] Meyers, J. J., Liapis, A. I., Network modeling of the convective flow and diffusion of molecules adsorbing in monoliths and in porous particles packed in a chromatographic column. *J. Chromatogr. A* 1999, *852*, 3-23.

[47] Liapis, A. I., Meyers, J. J., Crosser, O. K., Modeling and simulation of the dynamic behavior of monoliths: Effects of pore structure from pore network model analysis and comparison with columns packed with porous spherical particles. *J. Chromatogr. A* 1999, *865*, 13-25.

[48] Giddings, J. C., Dynamics of Chromatography: Principles and Theory, Marcel Dekker Inc, New York 1965.

[49] Huo, Y., Schoenmakers, P. J., Kok, W. T., Efficiency of methacrylate monolithic columns in reversed-phase liquid chromatographic separations. *J. Chromatogr. A* 2007, *1175*, 81-88.

[50] Nishang, I, Bruggemann, O., On the separation of small molecules by means of nano-liquid chromatography with methacrylate-based macroporous polymer monoliths. *J. Chromatogr. A* 2010, *1217*, 5389-5397.

[51] Svec, F., Peters, E. C., Sýkora, D., Yu, C., Fréchet, J. M. J., Monolithic stationary phases for capillary electrochromatography based on synthetic polymers: designs and applications. *J. High Resol. Chromatogr.* 2000, *23*, 3-18.

[52] Eeltink, S., Hilder, E. F., Geiser, L., Svec, F., Fréchet, J. M., Rozing, G. P., Schoenmakers, P.J., Kok, W.T., Controlling the surface chemistry and chromatographic properties of methacrylate-ester-based monolithic capillary columns via photografting. *J. Sep. Sci.* 2007, *30*, 407-413.

[53] Lämmerhofer, M., Svec, F., Fréchet, J. M. J., Lindner, W., Capillary electrochromatography in anion-exchange and normal-phase mode using monolithic stationary phases. *J. Chromatogr. A* 2001, *925*, 265-277.

[54] Müllner, T., Zankel, A., Mayrhofer, C., Reingruber, H., Höltzel, A., Lv, Y., Svec, F., Tallarek, U., Reconstruction and characterization of a polymer-based monolithic stationary phase using serial block-face scanning electron microscopy. *Langmuir* 2012, *28*, 16733-16737.

[55] Müllner, T., Zankel, A., Svec, F., Tallarek, U., Finite-size effects in the 3D reconstruction and morphological analysis of porous polymers. *Mat. Today* 2014, *17*, 404-411.

[56] Wouters, S., Wouters, B., Vaast, A., Terryn, H., Van Assche, G., Eeltink, S., Monitoring the morphology development of polymer-monolithic stationary phases by thermal analysis. *J. Sep. Sci.* 2014, *37*, 179-186.

[57] Vaast, A., Terryn, H., Svec, F., Eeltink, S., Nanostructured porous polymer monolithic columns for capillary liquid chromatography of peptides. *J. Chromatogr. A* 2014, *1374*, 171-179.

[58] Rohr, T., Yu, C., Davey, M. H., Svec, F., Fréchet, J. M. J., Porous polymer monoliths: Simple and efficient mixers prepared by direct polymerization in the channels of microfluidic chips. *Electrophoresis* 2001, *22*, 3959-3967.

[59] Svec, F., Fréchet, J. M. J., Temperature, a simple and efficient tool for the control of pore size distribution in macroporous polymers. *Macromolecules* 1995, *28*, 7580-7582.

[60] Bernabé-Zafón, V., Cantó-Mirapeix, A., Simó-Alfonso, E. F., Ramis-Ramos, G., Herrero-Martínez, J. M., Comparison of thermal- and photo-polymerization of lauryl methacrylate monolithic columns for CEC. *Electrophoresis* 2009, *30*, 1929-1936.

[61] Oberacher, H., Huber, C. G., Capillary monoliths for the analysis of nucleic acids by high-performance liquid chromatography–electrospray ionization mass spectrometry. *TrAC-Trends Anal. Chem.* 2002, *21*, 166-174.

[62] Premstaller, A., Oberacher, H., Walcher, W., Timperio, A. M., Zolla, L., Chervet, J.-P., Cavusoglu, N., van Dorsselaer, A., Huber, C. G., High-performance liquid chromatography−electrospray ionization mass spectrometry using monolithic capillary columns for proteomic studies. *Anal. Chem.* 2001, *73*, 2390-2396.

[63] Maya, F., Svec, F., A new approach to the preparation of large surface area poly(styrene-co-divinylbenzene) monoliths via knitting of loose chains using external crosslinkers and application of these monolithic columns for separation of small molecules. *Polymer* 2014, *55*, 340-346.

[64] Greiderer, A., Trojer, L., Huck, C. W., Bonn, G. K., Influence of the polymerisation time on the porous and chromatographic properties of monolithic poly(1,2-bis(p-vinylphenyl))ethane capillary columns. *J. Chromatogr. A* 2009, *1216*, 7747-7754.

[65] Nischang, I., Teasdale, I., Brüggemann, O., Towards porous polymer monoliths for the efficient, retention-independent performance in the isocratic separation of small molecules by means of nano-liquid chromatography. *J. Chromatogr. A* 2010, *1217*, 7514-7522.

[66] Trojer, L., Bisjak, C. P., Wieder, W., Bonn, G. K., High capacity organic monoliths for the simultaneous application to biopolymer chromatography and the separation of small molecules. *J. Chromatogr. A* 2009, *1216*, 6303-6309.

[67] Simone, P., Pierri, G., Capitani, D., Ciogli, A., Angelini, G., Ursini, O., Gentile, G., Cavazzini, A., Villani, C., Gasparrini, F., Capillary methacrylate-based monoliths by grafting from/to γ-ray polymerization on a tentacle-type reactive surface for the liquid chromatographic separations of small molecules and intact proteins. *J. Chromatogr. A* 2017, *1498*, 46-55.

[68] Badaloni, E., Barbarino, M., Cabri, W., D'Acquarica, I., Forte, M., Gasparrini, F., Giorgi, F., Pierini, M., Simone, P., Ursini, O., Villani, C., Efficient organic monoliths prepared by γ-radiation induced polymerization in the evaluation of histone deacetylase inhibitors by capillary(nano)-high performance liquid chromatography and ion trap mass spectrometry. *J. Chromatogr. A* 2011, *1218*, 3862-3875.

[69] Pierri, G., Kotoni, D., Simone, P., Villani, C., Pepe, G., Campiglia, P., Dugo, P., Gasparrini, F., Analysis of bovine milk caseins on organic monolithic columns: an integrated capillary liquid chromatography–high resolution mass spectrometry approach for the study of time-dependent casein degradation. *J. Chromatogr. A* 2013, *1313*, 259-269.

[70] Simone, P., Pierri, G., Foglia, P., Gasparrini, F., Mazzoccanti, G., Capriotti, A. L., Ursini, O., Ciogli, A., Laganà, A., Separation of intact proteins on γ-ray-induced polymethacrylate monolithic columns: A highly permeable stationary phase with high peak capacity for capillary high-performance liquid chromatography with high-resolution mass spectrometry. *J. Sep. Sci.* 2016, *39*, 264-271.

[71] Byström, E., Viklund, C., Irgum, K., Differences in porous characteristics of styrenic monoliths prepared by controlled thermal polymerization in molds of varying dimensions. *J. Sep. Sci.* 2010, *33*, 191-199.

[72] He, M., Zeng, Y., Sun, X., Harrison, D. J., Confinement effects on the morphology of photopatterned porous polymer monoliths for capillary and microchip electrophoresis of proteins. *Electrophoresis* 2008, *29*, 2980-2986.

[73] Nischang, I., Svec, F., Fréchet, J. M. J., Downscaling limits and confinement effects in the miniaturization of porous polymer monoliths in narrow bore capillaries. *Anal. Chem.* 2009, *81*, 7390-7396.

[74] Svec, F., Porous polymer monoliths: Amazingly wide variety of techniques enabling their preparation. *J. Chromatogr. A* 2010, *1217*, 902-924.

[75] Meyer, U., Svec, F., Fréchet, J. M. J., Hawker, C. J., Irgum, K., Use of stable free radicals for the sequential preparation and surface grafting of functionalized macroporous monoliths. *Macromolecules* 2000, *33*, 7769-7775.

[76] Kanamori, K., Nakanishi, K., Hanada, T., Rigid macroporous poly(divinylbenzene) monoliths with a well-defined bicontinuous morphology prepared by living radical polymerization. *Adv. Mater.* 2006, *18*, 2407-2411.

[77] Lei, H., Bai, L., Zhang, X., Yang, G., Preparation of a tetrazolyl monolithic column via the combination of ATRP and click chemistry for the separation of proteins. *J. Chromatogr. Sci.* 2014, *52*, 1211-1216.

[78] Wang, H.-S., Feng, X.-Y., Wei, J.-P., Biocompatible chiral monolithic stationary phase synthesized via atom transfer radical polymerization for high performance liquid chromatographic analysis. *J. Chromatogr. A* 2015, *1409*, 132-137.

[79] Moad, G., RAFT (Reversible addition–fragmentation chain transfer) crosslinking (co)polymerization of multi-olefinic monomers to form polymer networks. *Polym. Int.* 2015, *64*, 15-24.

[80] Bai, L., Yang, G., Lei, H., Wang, Y., Bai, L., Preparation of porous functional polymer by a simple method and its application in high performance liquid chromatography. *Anal. Methods* 2012, *4*, 2948-2952.

[81] Hasegawa, J., Kanamori, K., Nakanishi, K., Hanada, T., Yamago, S., Pore formation in poly(divinylbenzene) networks derived from organotellurium-mediated living radical polymerization. *Macromolecules* 2009, *42*, 1270-1277.

[82] Hasegawa, G., Kanamori, K., Nakanishi, K., Yamago, S., Fabrication of highly crosslinked methacrylate-based polymer monoliths with well-defined macropores via living radical polymerization. *Polymer* 2011, *52*, 4644-4647.

[83] Liu, K., Aggarwal, P., Tolley, H. D., Lawson, J. S., Lee, M. L., Fabrication of highly cross-linked reversed-phase monolithic columns via living radical polymerization. *J. Chromatogr. A* 2014, *1367*, 90-98.

[84] Hasegawa, J., Kanamori, K., Nakanishi, K., Hanada, T., Yamago, S., Rigid crosslinked polyacrylamide monoliths with well-defined macropores synthesized by living polymerization. *Macromol. Rapid Commun.* 2009, *30*, 986-990.

[85] Sinner, F., Buchmeiser, M. R., A new class of continuous polymer supports prepared by ring-opening metathesis polymerization: a straightforward route to functionalized monoliths. *Macromolecules* 2000, *33*, 5777-5786.

[86] Sinner, F. M., Buchmeiser, M. R., Ring-opening metathesis polymerization: access to a new class of functionalized, monolithic stationary phases for liquid chromatography. *Angew. Chem. Int. Ed. Engl.* 2000, *39*, 1433-1436.

[87] Sinner, F. M., Gatschelhofer, C., Mautner, A., Magnes, C., Buchmeiser, M. R., Pieber, T. R., Ring-opening metathesis polymerization-derived monolithic capillary columns for high-performance liquid chromatography: downscaling and application in medical research. *J. Chromatogr. A* 2008, *1191*, 274-281.

[88] Mayr, B., Hölzl, G., Eder, K., Buchmeiser, M. R., Huber, C. G., Hydrophobic, pellicular, monolithic capillary columns based on cross-linked polynorbornene for biopolymer separations. *Anal. Chem.* 2002, *74*, 6080-6087.

[89] Peters, E. C., Svec, F., Fréchet, J. M. J., Preparation of large-diameter "molded" porous polymer monoliths and the control of pore structure homogeneity. *Chem. Mater.* 1997, *9*, 1898-1902.

[90] Liu, Z., Ou, J., Lin, H., Wang, H., Liu, Z., Dong, J., Zou, H., Preparation of monolithic polymer columns with homogeneous structure via photoinitiated thiol-yne click polymerization and their application in separation of small molecules. *Anal. Chem*. 2014, *86*, 12334-12340.

[91] Liu, Z., Ou, J., Lin, H., Liu, Z., Wang, H., Dong, J., Zou, H., Photoinduced thio-ene polymerization reaction for fast preparation of macroporous hybrid monoliths and their application in capillary liquid chromatography. *Chem. Commun.*, 2014, *50*, 9288-9290.

[92] Kumaraswamy, G., Biswas, B., Choudhury, C. K., Colloidal assembly by ice templating. *Faraday Discuss.* 2016, *186*, 61-76.

[93] Nishihara, H., Mukai, S. R., Fujii, Y., Tago, T., Masuda, T., Tamon, H., Preparation of monolithic SiO_2–Al_2O_3 cryogels with inter-connected macropores through ice templating. *J. Mater. Chem.* 2006, *16*, 3231-3236.

[94] Nakanishi, K., Kobayashi, Y., Amatani, T., Hirao, K., Kodaira, T., Spontaneous formation of hierarchical macro−mesoporous ethane−silica Monolith. *Chem. Mater.* 2004, *16*, 3652-3658.

[95] Amatani, T., Nakanishi, K., Hirao, K., Kodaira, T., Monolithic periodic mesoporous silica with well-defined macropores. *Chem. Mater.* 2005, *17*, 2114-2119.

[96] Innocenzi, P., Malfatti, L., Soler-Illia, G. J. A. A., Hierarchical mesoporous films: from self-assembly to porosity with different length scales. *Chem. Mater.* 2011, *23*, 2501-2509.

[97] Zhang, H., Hussain, I., Brust, M., Butler, M. F., Rannard, S. P., Cooper, A. I., Aligned two- and three-dimensional structures by directional freezing of polymers and nanoparticles. *Nat. Mater.* 2005, *4*, 787.

[98] Qian, L., Zhang, H., Controlled freezing and freeze drying: a versatile route for porous and micro-/nano-structured materials. *J. Chem. Technol. Biotechnol.* 2011, *86*, 172-184.

[99] Dario Arrua, R., Hilder, E. F., Highly ordered monolithic structures by directional freezing and UV-initiated cryopolymerisation. Evaluation as stationary phases in high performance liquid chromatography. *RSC Adv.* 2015, *5*, 71131-71138.

[100] Zuo, Z., Guo, Y., Li, Y., Lv, J., Liu, H., Xu, J., Li, Y., Construction of large-scale highly ordered macroporous monoliths of π-conjugated polymers. *Macromol. Rapid Commun.* 2009, *30*, 1940-1944.

[101] Broeckhoven, K., Cabooter, D., Eeltink, S., De Malsche, W., Matheuse, F., Desmet, G., Current and Future Chromatographic Columns: Is One Column Enough to Rule Them All? *LC GC North America* 2018, *36*, 9-17

[102] Fee, C., Nawada, S., Dimartino, S., 3D printed porous media columns with fine control of column packing morphology. *J. Chromatogr. A* 2014, *1333*, 18-24.

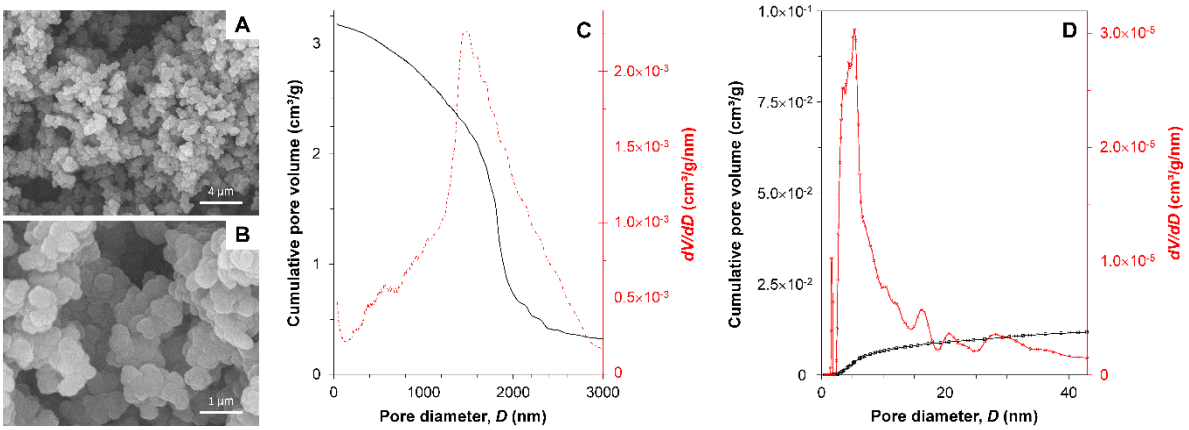

Figure 1. Size characteristics of a conventional polymer-monolithic support structure visualized via scanning electron microscopy (A and B), mercury-intrusion porosimetry (C), and argon-adsorption measurement (D). Adapted from [41], with permission.

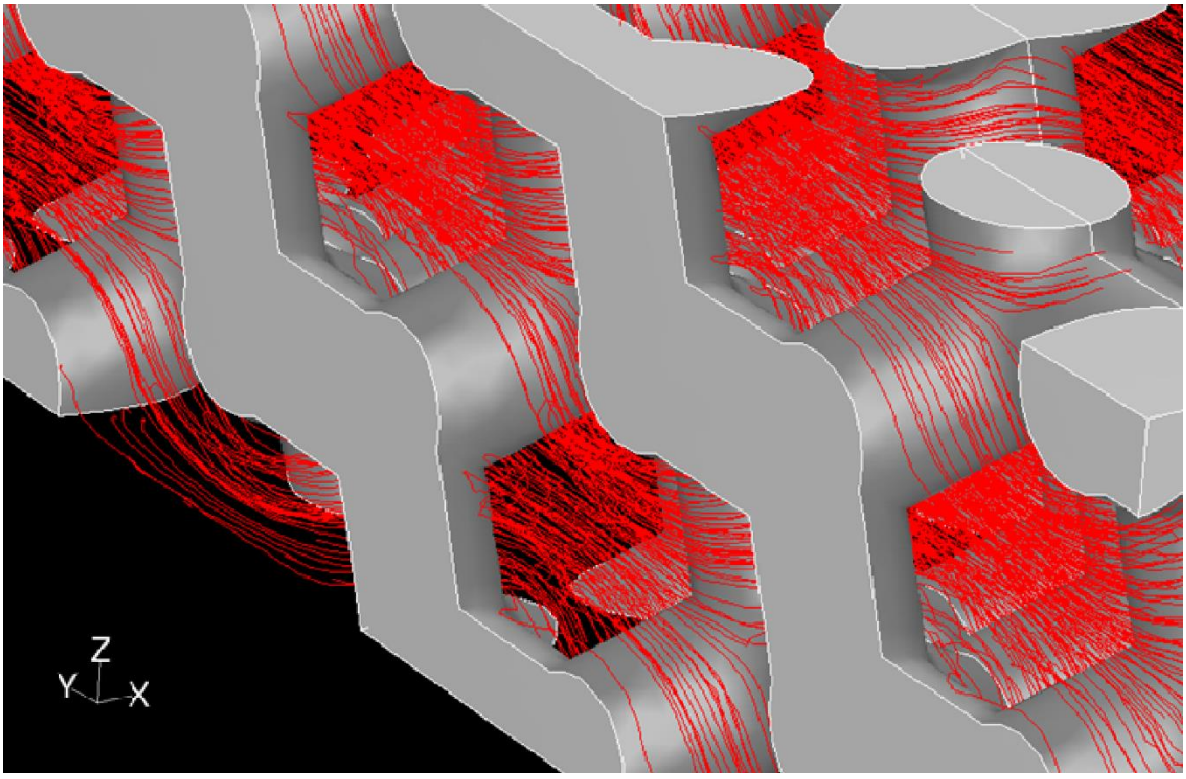

Figure 2. Computational fluid dynamics (CFD) simulation demonstrating that convective mass transfer distributes analytes in parallel with the surface of the stationary phase. Adapted from [44], with permission.

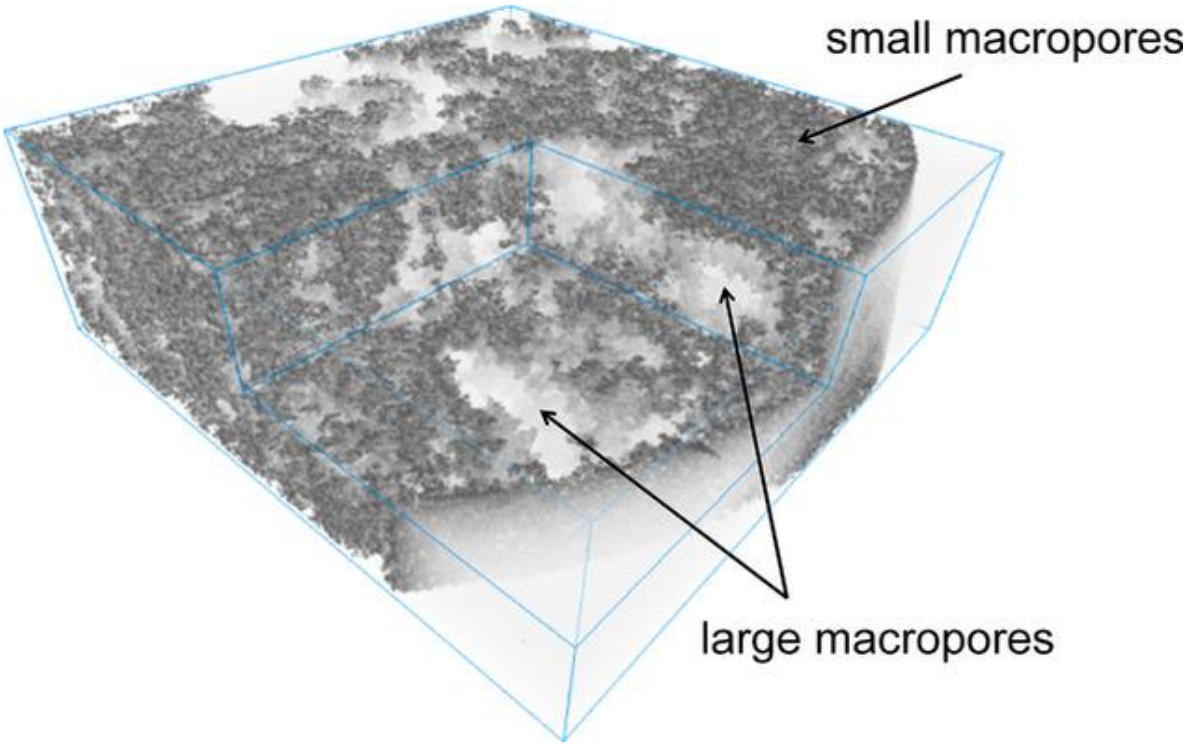

Figure 3. Reconstruction of the 3D structure of a poly(styrene-*co*-divinylbenzene) monolithic columns from serial block-face scanning electron microscopy showing the broad heterogeneity in macropores. Adapted from [54], with permission.

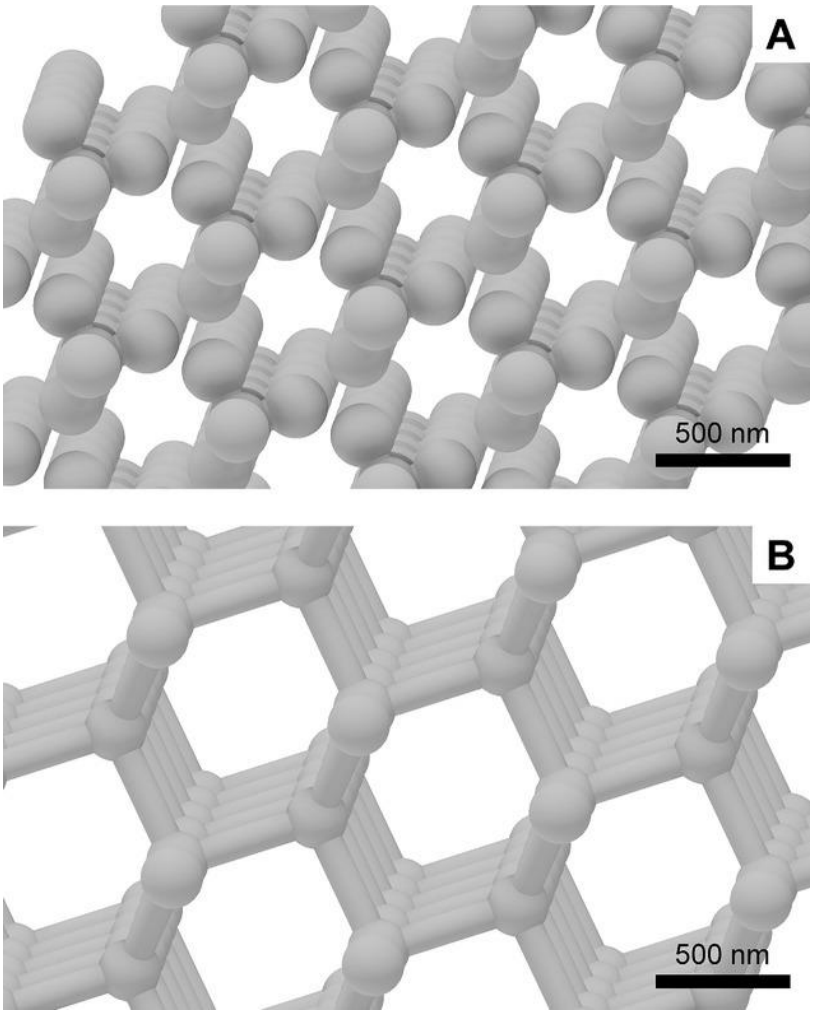

Figure 4. Three dimensional macropore structures with two different porosities aiming at realizing high speed-separations (A) and high-efficiency separations (B), respectively. The globule size is kept constant to minimize eddy dispersion and the macropore size is adjusted to tune either mobile-phase mass transfer and column length.

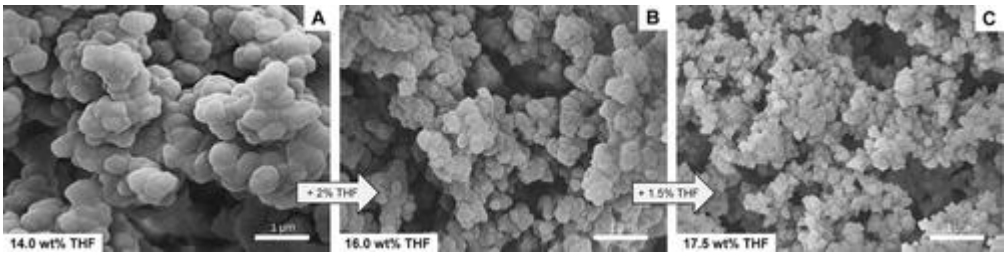

Figure 5. Scanning electron micrographs (SEM) of monolithic poly(styrene-*co*-divinylbenzene) columns showing the effect of the porogen ratio on resulting macropore structure. The polymerization mixtures consist of 15 wt% styrene and 15 wt% divinylbenzene, with a porogen composition of (A) 14.0 wt% THF and 56.0 wt% 1-decanol, (B) 16.0 wt% THF and 54.0 wt% 1-decanol, and (C) 17.5 wt% THF and 52.5 wt% 1-decanol, using 3 wt% AIBN with respect to the monomer content of 30 wt% in total. Adapted from [57], with permission.

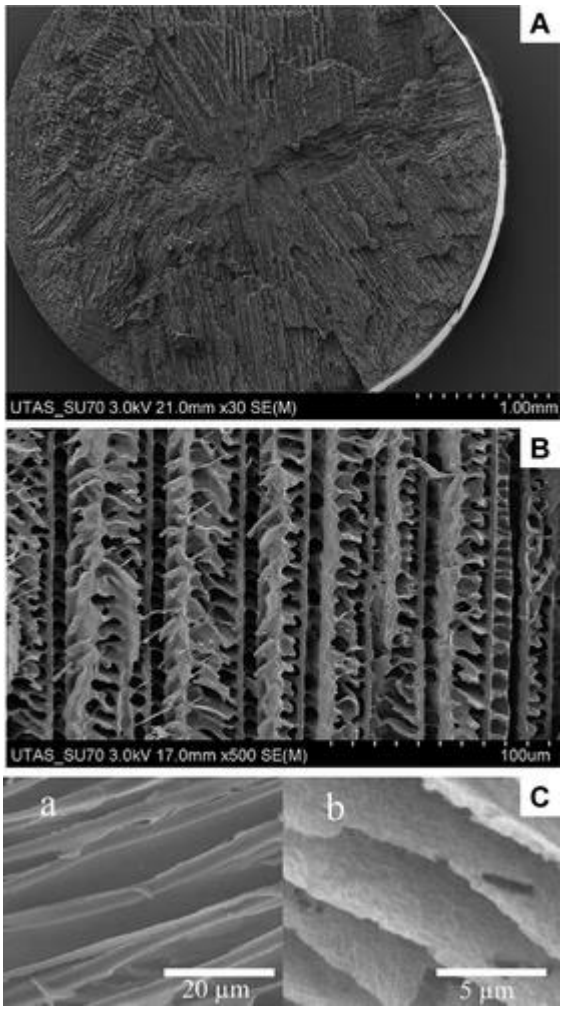

Figure 6. SEM images of a cross-section of poly[poly(ethyleneglycol diacrylate] (PEGDA) monolith prepared by directional freezing in liquid nitrogen and UV-initiated cryopolymerization (A), and the respective zoom-in showing a dendritic porous structure (B) [99] - Published by The Royal Society of Chemistry, and of a poly(p-phenylenevinylene) (PPV) well-ordered monolith using two different freezing temperatures (left: T_f = 10 °C, and right: T_f = -78 °C) (C) [100]. Adapted with permission.