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Molecular imprinting: a tool of modern chemistry for the preparation of highly selective monolithic sorbents

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Characteristic features of the synthesis of molecularly imprinted polymer systems used as sorbents for separation of complex mixtures into components are discussed. The data about the molecules imprinted in monoliths of various natures and shapes are integrated. Examples of application of new-generation separating media are discussed. Data on the utilization of the molecular imprinting principle for the fabrication of supermacroporous monolithic cryogels specific to particular molecules, which is important for the design of smart biomaterials, are analyzed.
The bibliography includes 293 references.

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I. Introduction

Studies of the mechanisms of specific interaction at a liquid/solid interface are highly important for the selection or development of specific receptors capable of recognition and selective binding of diverse biological target molecules. The molecular recognition behaviour is manifested in the processes that occur in living organisms, for example, in enzymatic reactions, receptor-mediated endocytosis and intracellular transport, cell activity regulation, antigen – antibody interaction, hormone – receptor interaction and so on. The molecular recognition is known to be based on the spatial matching of the structures and on non-covalent interactions, particularly, electrostatic, hydrophobic, van

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der Waals, π–π and cation–π interactions and hydrogen bonds.[1] Biological systems typically combine the selectivity of specific complex formation with the strength and ability to undergo fast reversible changes.

In practice, selective receptors are, most often, represented by biomolecules of high-purity natural compounds (which are, hence, very expensive) attached to a solid support surface by physical or covalent bonds. Alternatives to natural molecules are specific segments of the molecules, recognition sites of biocomplementary partners, which are prepared by synthetic or genetic engineering techniques. However, natural receptors exist only for some of the biomolecules; therefore, the design of systems with artificial receptors possessing high selectivity towards target biomolecules is a topical problem. Owing to the advances of synthetic chemistry, a large number of chemical structures capable of molecular recognition that do not exist in nature appeared in the 20th century.[2,3] Examples of such compounds are crown ethers, cryptands and calixarenes capable of selective cation binding,[4–6] as well as cyclophanes,[7] dendrimers[8] and cyclodextrin derivatives,[9] which can selectively form complexes with some organic molecules.

The molecular imprints of various compounds incorporated in polymer matrices obtained by molecular imprinting techniques also refer to synthetic receptors. This approach was used for the first time in 1972 independently by Wulff and Sarhan[10] and Klotz and Takagishi.[11] The extensive development of the molecular imprinting technique dates back to the early 1990s. The essence of preparation of molecularly imprinted polymers (MIPs) is in the formation of a prepolymerization complex between the template molecule and the functional monomer. This is followed by polymerization in the presence of a cross-linking agent during which the prepolymerization complex is rigidly secured in the polymer network. After the end of the process, the template molecules are removed from the cross-linked polymer matrix. This results in the formation of imprints, or imprint sites in the polymer, which represent cavities complementary in size, shape and positions of functional groups to the template molecule. Thus, a MIP system is actually a solid matrix with artificial receptors for the template molecule capable of repeated highly specific interaction with this molecule or its analogue.[12]

Obviously, the attraction of imprinted polymer systems for practical applications is due to relative simplicity of the preparation and high stability combined with selectivity and specificity, which are often comparable with those of natural ligand–receptor pairs.[13,14] Being synthetic structures, MIP systems withstand drastic conditions of application, in particular, elevated pressure and temperature,[15,16] extreme pH values[16] and the use of organic solvents.[17] In addition, obvious advantages are the possibility of obtaining imprints of various molecules and the ease of adjustment to various conditions.[18]

Molecularly imprinted polymers are widely used in solid-phase extraction,[19,20] high performance liquid chromatography,[21–23] thin layer chromatography,[24] capillary electrochromatography,[25,26] as biosensor elements[27] and catalyst systems[28,29] and for screening of potential drugs.[30] Currently, MIP-based products of several successfully functioning companies are supplied to the world market, *e.g.*, MIP Technologies (Sweden), POLYIntel (France), MIP Solutions (USA) and Semorex (USA).

In most cases, MIP systems are used as the stationary phases in dynamic flow type processes. Therefore, most of the currently synthesized MIP systems are dispersed particles packed in columns of different size.[31,32] When dynamic interfacial processes are carried out using these columns, as in the case of other dispersed sorbents, the mass transfer between the liquid and solid phases is considerably restricted by diffusion of the substance to the sorbent pores. In the dynamic flow, diffusion proves to be the principal limiting stage, which dictates the time of the whole process. Most of the mobile phase in the columns packed by any dispersed particles flows through the interparticle space ('empty space'), which additionally decreases the efficiency of compound mass transfer under dynamic conditions. The introduction of separating stationary phases as macroporous monolithic materials into the chromatographic practice minimized the undesirable 'empty space'.[33]

The first attempts to obtain porous continuous stationary phases were made in the early 1970s.[34] Macroporous monolithic sorbents in the modern sense were proposed and widely discussed in the late 1980s–early 1990s by several independent groups of researchers. In particular, monolithic columns based on polyacrylamide have been developed.[35] Highly cross-linked polymethacrylate matrices shaped like discs[37] and rods[38] have been synthesized. Historically, these sorbents are the first in the series of monolithic macroporous polymers meant for the use as stationary phases in the interaction types of liquid chromatography. The morphological features of these supports (in particular, the structure of pore space) are suitable for separation processes with high flow rates of the mobile phase. The sharp decrease in the separation time became possible due to virtually complete absence of diffusion restrictions to the normal interfacial transfer of the substance.[33,39]

The first paper reporting the preparation of molecularly imprinted monoliths was published in 1993 by Matrui *et al.*,[40] *i.e.*, this was almost simultaneously with the first publications dealing with the synthesis of monolithic sorbents for high performance liquid chromatography (HPLC).[35,37] Unfortunately, this paper has not attracted due attention of researchers and for a long period of time there were no papers dealing with the production of monolithic MIP systems. The next publications of Matrui *et al.*[41,42] in this field appeared in the late 1990s, and only in the beginning of the new century, this field was explored by other researchers.[43–45] This situation is caused, first of all, by the fact that monolithic sorbents themselves started to be actively investigated as stationary phases only in the second half of the 1990s.[46–50] A noticeable interest in imprinted macroporous monoliths was aroused only in the mid-2000s.[51–55] Thus, despite the fact that the key principles of molecular imprinting have been known for relatively long time, the use of these principles to produce macroporous monoliths containing molecular imprints is a relatively new branch in the field of synthesis and applications of MIP systems.

Lately, researchers' attention has also been attracted by supermacroporous monolithic stationary phases obtained by cryostructuring.[56–58] This type of materials possesses all advantages of usual monoliths indicated above. The cryogel features usually listed as advantages include supermacroporosity, which is fairly important for separation of large

particles and whole cells, and also the ease of preparation.

The first studies devoted to the design of molecularly imprinted cryogels appeared in the middle of the past decade and were devoted to extraction of low-molecular-mass contaminants.[59] Lately, the number of publications in this field has considerably grown [58] and it has become necessary to systematize the obtained results and to outline the prospects for further research.

The first literature review on the use of MIP monoliths for HPLC [60] was published in 2005. In essence, it outlined a new trend in the preparation of MIP materials. Recently, two more reviews were published devoted to macroporous MIP monoliths for HPLC, capillary electrochromatography [61] and solid-phase extraction.[62]

This review is an attempt to summarize the data published mainly from 2003 to 2014. Attention is given not only to the application of these materials but also to details of their preparation. The results of papers dealing with the preparation of cryogel supermacroporous MIP monoliths, which are still few in number, are also analyzed.

II. Traditional methods for the synthesis of molecularly imprinted polymer systems

The MIP systems are fabricated using three methods of imprinting of the target molecules and several methods for the synthesis of polymer matrices.

Covalent imprinting [63–65] is based on the formation of covalent bonds between the monomers and the template. After polymerization, the template is detached and removed from the imprint area. Advantages of this approach include the formation of uniform imprints and, hence, high selectivity. Most often, the covalent imprinting involves the preparation of conjugates between the monomer and the template to give boronic or carboxylic acid esters,[66, 67] ketals [68] or imines (Schiff bases).[69] The imprint site thus formed is able to bind the template molecule *via* the same covalent bonds. The choice of suitable monomer–template bonds is markedly restricted by the necessity of subsequent cleavage of these bonds to remove the template. As a rule, in the case of covalent imprinting, the efficiency of recovery of the template does not exceed 90%.

Drawbacks of this method include slow binding and release of the target molecule during the application of this MIP system, which is related to the rate of formation and cleavage of the covalent bonds.[18, 70]

Non-covalent imprinting [71] implies non-covalent binding between the monomers and the template. In this case, the prepolymerization complex is formed upon self-association of the monomers and the template *via* hydrogen bonding, hydrophobic, electrostatic or π–π interactions, or van der Waals forces. The template is removed by multiple extraction with a solvent or a solvent mixture. The residual content of the template in the matrix for non-covalent imprinting reaches 5% of the initial amount. The repeated template binding to the resulting MIP system occurs due to the same type of interactions that were used to form the prepolymerization complex.

Due to a number of advantages such as simple preparation of the monomer–template complex, easy removal of the template from the polymer matrix, fast binding kinetics of the template by the MIP system and broad selection of templates, this method has found wider use than the above-considered covalent imprinting. As a shortcoming of the non-covalent approach, one can note non-uniformity of the molecular recognition sites † caused by the fact that orientations of the template and monomer molecules relative to each other in the prepolymerization complex are not always unambiguous.[70, 72]

Semicovalent imprinting, which combines the advantages of the covalent and non-covalent approaches, was proposed by Sellergren and Andersson.[73] In this method, imprinting is carried out *via* polymerization of functional monomers as covalent complexes with the template, and the subsequent recognition of the templates by MIP systems occurs *via* non-covalent interactions and is not subject to any kinetic restrictions except for diffusion. As in the case of covalent imprinting, semicovalent approach tends to produce more uniform binding sites for target molecules than the non-covalent method.

The types of interactions and stages that underlie the preparation of molecularly imprinted materials are depicted in Scheme 1. The formation of a prepolymerization complex may involve both a single type of interaction (non-covalent, covalent or based on metal ion coordination) and various combinations of the interaction types.

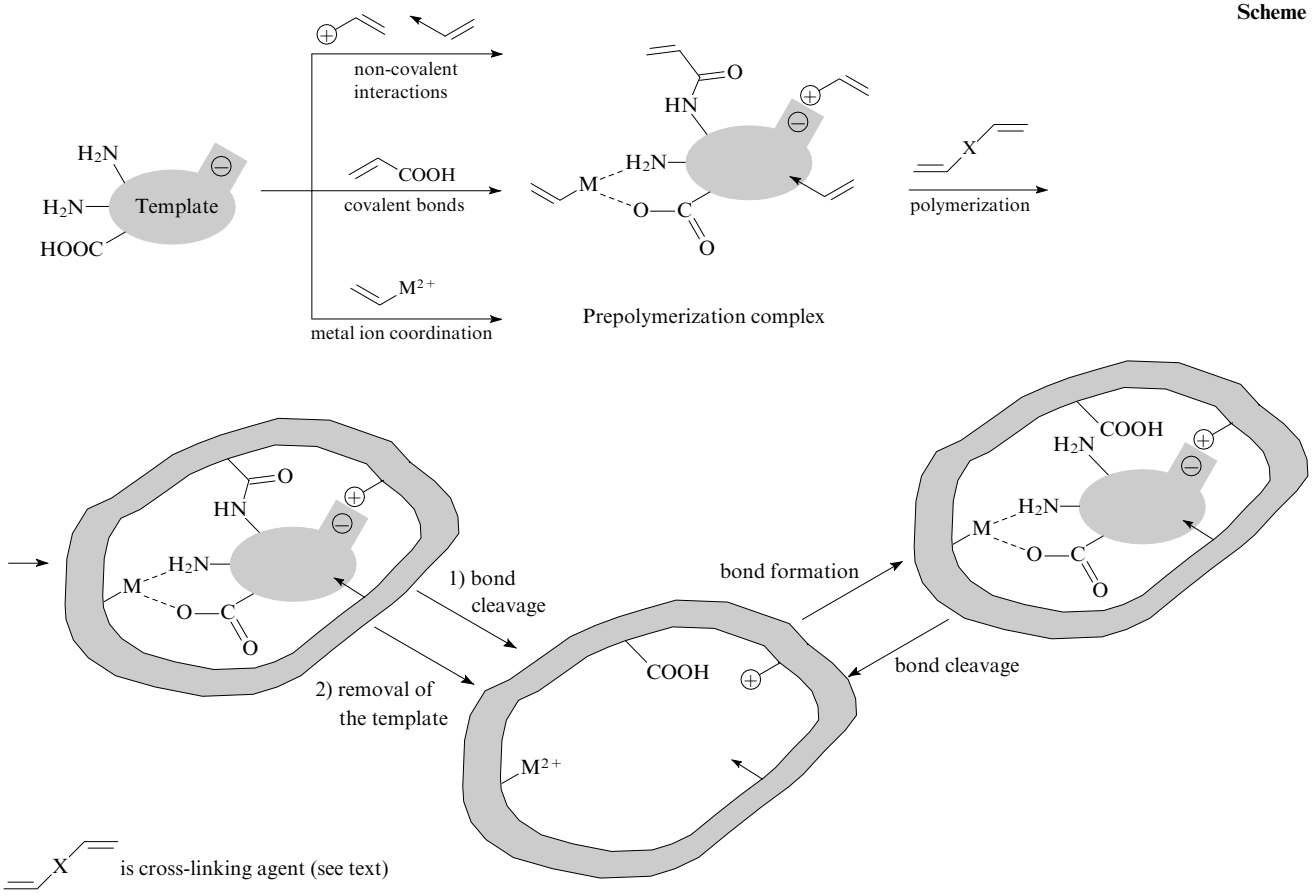
Most often, MIP systems are synthesized by free-radical polymerization. This choice is caused, first, by the possibility of conversion of a wide range of vinyl monomers having various functional groups, which are often commercially available. Second, radical polymerization proceeds under relatively mild conditions and does not require a high degree of reactant purity. Detailed information on the functional and cross-linking monomers used to synthesize MIP systems can be found in several reviews devoted to molecular imprinting (see, for example [12, 33, 74]). The polymerization mixture is prepared from one or several functional monomers, a cross-linking agent, a polymerization initiator, the solvent needed for pore formation and the template. Depending on the initiator used, polymerization can be initiated by either heating the reaction mixture to the temperature of initiator decay or by UV irradiation.

As noted above, most MIP materials are currently obtained as polymer particles, which are packed into columns and capillaries of various sizes and are used for solid-phase extraction, liquid chromatography and capillary electrochromatography. The MIP particles mixed with a binding agent can also be deposited on a solid substrate to form thin layer chromatography plates. Usually, vacuum-dried MIP particles of diameter 25–50 μm [75] are used; they can be stored for long without the loss of functional properties.

Bulk polymerization is a traditional method for the preparation of MIP materials.[76–78] According to this method, the polymerization mixture containing functional monomers, cross-linking agent, template, initiator and the pore-forming solvent is freed from oxygen by purging with inert gas. After polymerization, the resulting monolith is ground and sieved to obtain uniform particle fractions. This method is fairly simple and does not require complicated equipment; however, the particles obtained by mechanical grinding are non-uniform both in shape and size; therefore, the efficiency of the columns packed with such MIP sorb-

† Implies the formation of sorbent areas formed with the participation of different amounts of the monomers; hence, these areas can differ in the specificity toward the target molecules.

Scheme 1



ents in adsorption-based separation processes is relatively low. Other drawbacks of this method are excessive consumption of monomers and the template caused by the fact that as a result of mechanical grinding of the obtained polymer material and particle sieving, only a small portion of the product can be used as the stationary phase. The loss of the MIP material during these procedures can reach 50%.[79]

Suspension, dispersion and precipitation polymerization techniques are widely used for direct preparation of monodispersed grains. Suspension polymerization is a simple and frequently used method for the synthesis of MIPs. In this case, polymerization occurs in microdrops of the mixture dispersed in water or perfluorinated hydrocarbons. In both media, highly uniform MIP particles with 5 to 50 μm size are formed. However, in the case of non-covalent imprinting, the use of water as the dispersion medium is undesirable. Less polar solvents such as benzene, toluene or acetonitrile, which ensure the stability of non-covalent bonds, are preferred.[80] Polar solvents that can dissociate with release of a proton (water or methanol) hamper polymerization and destabilize hydrogen bonds between the template and the functional monomer. Numerous studies demonstrated the efficiency of MIP particles obtained by suspension polymerization in chromatographic processes. Examples of the preparation of MIP particles by this method were considered in a number of publications (see, for example,[60,81–83]).

Dispersion polymerization [83] is similar to the suspension synthesis. In this case, polymerization starts in the homogeneous medium containing all components needed for molecular imprinting (monomers, template, solvents and initiator) and surfactants. The dispersion polymerization technique gives particles measuring from a few to ten micrometres.

An alternative approach to the synthesis of MIP particles is precipitation polymerization.[84,85] This method is based on high dilution of the polymerization mixture with a solvent that is a poor solvent for the polymer and can provide monodisperse microspheres of the submicrometre size (~5 μm). An advantage of this method is relative simplicity and the absence of a surfactant.[86]

A two-stage emulsion polymerization procedure proposed [87] in the early 1990s is based on the preparation of MIP beads without internal cross-linking of the polymer chains in the first stage followed by swelling in a mixture of porogens, a template and functional and cross-linking monomers and polymerization in the second stage. This method produces narrow-dispersed MIP particles with a controlled size in the 5–100 μm range, which have proved to be useful as chromatographic sorbents.[88,89] However, the necessity to carry out the process in water, as in the case of suspension polymerization, restricts the possibility of imprinting for some target molecules.

Inside of MIP particles prepared by the described methods, there are imprint sites the access to which for target molecules is limited by the diffusion to the polymer network, which leads to slow mass transfer between the phases. In order to increase the accessibility of the binding sites for target molecules, by analogy with pellicular sorb-

ents, it was proposed to fabricate MIP systems by grafting MIP layers on the surface of inorganic or polymer particles.[90,91] For this purpose, the particle surface is first modified by the initiator and then the particles are introduced into the polymerization mixture containing the template. As a result, the particle surface is coated by a MIP layer with a controlled thickness, which would ensure higher accessibility of imprint sites and, consequently, intensify the mass transfer.

Detailed information about the preparation methods and applications of fine-particle MIP systems can be found in several recent reviews.[18,32,92,93]

III. Macroporous monolithic sorbents

III.1. Synthesis of macroporous monolithic sorbents

The first macroporous monoliths based on synthetic polymers were obtained by thermally initiated free radical polymerization *in situ* and were used as stationary phases for HPLC.[35,94,95] A necessary condition for the formation of a pore structure is the presence of an inert solvent functioning as a pore forming agent (porogen). The key criterion for using particular compounds as porogens is their thermodynamic incompatibility with the polymer formed. The mechanism of formation of the pore structure of monolithic supports is well studied and has been considered in detail in the literature (see, for example[33]).

The pore formation process can be briefly described in the following way. After decomposition of the initiator at the initial polymerization step, gel-like oligomer particles (nuclei) are formed and start to precipitate from the organic phase due to low solubility in the porogens. Under these conditions, the monomeric part of the organic phase is a better solvent for the originating polymer chains than the porogen phase. Hence, monomers penetrate into the precipitating insoluble nuclei and continue to polymerize inside the nuclei, which gradually reach the size of microglobules. The growing polymer globules are joined into clusters being held by the polymer chains piercing the neighbouring particles. At the final polymerization step, the clusters become large enough to contact one another, resulting in the formation of a continuous block within the polymerizing system. The matrix thus formed is gradually strengthened by interglobule cross-links and progressing polymerization. This leads to the formation of the final porous polymeric material. In this stage, the porogenic solvents occur as a separate organic phase which fills the voids of the porous polymer bulk. The fraction of voids (or macropores) in the final polymer is close to the volume fraction of porogenic solvents in the initial polymerization mixture.[33]

The properties of macroporous monoliths used as stationary phases differ crucially from the properties of columns packed with sorbent particles. A key difference is that monolithic sorbents represent a continuous polymer matrix pierced by interconnected flow-through channels or macropores and, hence, they have no dead (empty) interparticle space, whereas in packed columns, this space often amounts up to 40% of the sorbent volume. The difference between the morphologies of these materials accounts for different hydrodynamics: in packed columns, the mass transfer between the phases is controlled by substance diffusion into the sorbent pores, whereas in monoliths, convective mass transfer predominates. The permeability of macroporous monoliths is usually 1–2 orders of magnitude higher than the permeability of packed columns, while the pressure in the system is an order of magnitude lower owing to the absence of diffusion restrictions to normal phase transfer of the substance. In practice, this markedly decreases the time needed for mass transfer and, hence, for the whole interfacial dynamic process. These and other features of macroporous monolithic sorbents were described in detail in some original papers and reviews.[36,39,96–100] Additional information concerning the theory of high performance liquid chromatography with macroporous monolithic sorbents can be found in reviews[101,102] and in a monograph.[33]

The range of modern macroporous sorbents based on synthetic polymers is fairly extensive, most of them being polymethacrylates.[103,104] Obvious advantages of the monolithic sorbents based on synthetic polymers include the simplicity of the synthesis and the possibility of varying functional monomers depending on the objective of the study. A polymer matrix obtained under optimized conditions has a more uniform structure than a packed column. Since the macroporous polymeric monoliths are obtained by *in situ* polymerization in a mould of a desired size, for example, in a stainless-steel column or in a quartz capillary, the separation cell thus formed can be immediately built-in into a high-pressure or low-pressure chromatographic system or into a capillary electrophoresis system. Usually, the fraction of the cross-linking agent in the polymerization mixture is 30 mol.% to 50 mol.%, while the fraction of porogens is from 50 vol.% to 75 vol.%. As in other cases of radical polymerization, initiation is performed by thermally or photo sensitive initiators.[103]

Apart from monoliths based on synthetic polymers, widely used are inorganic (silica) or organic-inorganic systems obtained either by sintering of silicate particles or by a sol–gel process comprising hydrolysis and condensation of alkoxysilanes in aqueous and aqueous organic media.[33,105,106] Enhanced mass transfer for silica matrices, as in the case of polymeric monoliths, is attained *via* the formation of flow-through cannels (pores), the diameter of which depends on the preparation conditions and can reach 8 μm.

The surface morphology of macroporous monoliths is usually studied by scanning electron microscopy (SEM) and atomic force microscopy. The pore characteristics, namely average pore size, pore size distribution and the total porosity, are determined by mercury intrusion porosimetry. This method is widely used for quantitative characterization of pores in the 0.02 to 15 μm range of their size. The average pore size and the total porosity can also be determined from the data on hydrodynamic permeability of monolithic columns, which can be calculated based on the dependence of the column pressure on the liquid flow rate.[107,108]

Currently, both organic and inorganic monoliths are widely used not only in the liquid but also in gas chromatography,[109] capillary electrochromatography,[110] solid-phase extraction,[111] as supports in the solid-phase organic synthesis,[112] and as stationary phases for flow enzyme reactors.[113] The successful use of monolithic sorbents functioning in the dynamic regime gave impetus to the idea of the design of monolithic thin layers of macroporous materials for thin layer chromatography[114,115] and the base (platform) for biochips.[116,117] The geometric design of macroporous supports is diversified: they can represent membranes, rods, discs or tubes.[33]

III.2. Synthesis of macroporous monolithic molecularly imprinted systems

The synthesis of macroporous MIPs is a relatively new branch in the synthesis of MIP systems. Along with the advantages inherent in the preparation of monolithic sorbents in general, noteworthy is the economic feasibility of the *in situ* synthesis of monolithic MIP matrices associated with the absence of excess expenditure of templates, which are often expensive. Furthermore, the possibility of using the monolithic column immediately after the synthesis substantially facilitates the procedure of template removal from the pores of the resulting matrix by the flow of a mobile phase. The considerable difference between the monolithic MIP systems from dispersed particles is in the position of imprint sites, which are mainly concentrated in the surface layer of the flow-through macropores, which is evidently beneficial for the kinetics of sorption of target molecules.

Meanwhile, despite the publications on the synthesis of macroporous monolithic MIP supports available in current periodicals, the preparation of these systems is not a trivial task. The reason is the following: in the case of synthesis of monolithic polymeric systems containing molecular imprints, the polymerization mixture should contain not only monomers, initiator, template and (in some cases) initiator solvent but also poor solvents for the resulting polymer required for the formation of the macroporous structure. However, often the templates are solids and for being introduced into the polymerization mixture, they should be dissolved. In the ideal case, the template can be dissolved in one of the functional monomers provided that it is a liquid. However, in practice, it is far from being always possible to compose a system such that, first, the functional monomer is a liquid that can dissolve the template and, second, the non-covalent imprinting gives a monomer–template prepolymerization complex. If this possibility cannot be implemented, the template is dissolved in the porogen or in a good solvent. However, any introduction of a good solvent into the system influences the phase separation during polymerization and, hence, the formation of the pore structure. Therefore, the most complicated task in the synthesis of macroporous monolithic MIP matrices is to select a system of porogens, the ratio between them, the ratio of the monomers to the system of porogens taking into account the nature and the number of template molecules and the added amounts of the good solvent.

Thus, the synthesis of macroporous monolithic MIP sorbents should solve simultaneously two problems: the preparation of a highly permeable macroporous matrix and efficient imprinting of the target template molecules. Furthermore, if the imprinted molecules are labile biomolecules such as proteins, the selected polymerization conditions should also be suitable for preserving the activity of the imprinted biological substance; otherwise, there is high probability of the loss of specific capacity by the imprinted matrix.

III.2.a. Monomers and cross-linking agents

The selection of a functional monomer is very important for the formation of a large number of stable prepolymerization complexes and subsequent formation of molecular imprints in the polymer matrix. While choosing one or several functional monomers for fabricating an artificial receptor for one or another molecule, it is important to bear in mind that the interaction of the template with monomer(s) *via* several functional groups gives rise to a larger number of high-affinity and specific molecular recognition sites as compared with artificial receptors obtained with a single monomer functional group and a single template group. The maximum number of such interactions can be implemented if the templates containing acidic and basic groups are imprinted with the participation of basic and acidic monomers, respectively.

Despite a rather extensive list of monomers suitable for the synthesis of macroporous monolithic sorbents,[103, 118] examination of the literature demonstrated that the most popular acid-type monomers used for the synthesis of MIP monoliths comprise methacrylic (MAA) and 2-trifluoromethylacrylic (TFMAA) acids,[40, 43, 119] while the most popular base-type monomers are 2- and 4-vinylpyridines (VP) [120–122] and 1-vinylimidazole (1-VI).[55] As comonomers, neutral hydrophilic compounds are used most often such as acrylamide (AAm) and 2-hydroxyethyl methacrylate (HEMA).[123, 124] There are studies in which hydrophobic comonomers such as butyl methacrylate (BMA) [125, 126] and styrene (ST) were used together with MAA as the functional monomer.[45]

The association constants of monomers with the templates are insufficiently high for the formation of stable complexes; therefore, to shift the equilibrium towards the formation of complexes, functional monomers are taken in excess. A study of the effect of the template to functional monomer ratio on the properties of the obtained macroporous MIP monoliths demonstrated that better selectivity and adsorption capacity can be achieved when the template to functional monomer ratio is 1 : 4.[45, 122, 127] A change in this ratio, for example, to 1 : 3 or 1 : 2 (higher percentage of the template) decreases the retention parameters for target molecules and, hence, reduces the adsorption capacity of the MIP sorbent; a change in the template to functional monomer ratio to 1 : 6 or 1 : 8 (lower percentage of the template) markedly increases the adsorption capacity and sharply deteriorates the selectivity of isolation of target compounds, which is manifested as binding of molecules structurally related to the template by the MIP matrix.

When choosing the cross-linking agent, the authors of the vast majority of publications gave preference to ethylene glycol dimethacrylate (EDMA).[40, 53, 121, 123, 128] Nevertheless, some publications are known in which *N*,*N*′-methylenebis(acrylamide) (MBAAm),[129] di(ethylene glycol) dimethacrylate (DEGDMA),[130] divinylbenzene (DVB) [45] and trifunctional trimethylolpropane trimethacrylate (TRIM) served as the bifunctional monomers in the synthesis of macroporous MIP monoliths.[131, 132]

In most cases, in the synthesis of macroporous monolithic sorbents, good properties of the polymer could be attained using 30 mol.%–50 mol.% of the cross-linking monomer.[107, 133–136] However, according to some studies devoted to the preparation of macroporous MIP materials and covering the period from 2003 to 2014, the use of $>75\%$ of a cross-linking agent with respect to the total amount of monomers may give systems possessing high selectivity towards target molecules. Meanwhile, if the amount of the cross-linking agent is $<75\%$, the MIP materials either do not possess or possess very low capability for molecular recognition. Indeed, in a study [44] devoted to the synthesis of monolithic MIP columns for separation of enantiomers and diastereomers, the best

selectivity of MIPs was observed when the cross-linking agent (EDMA) was taken in a proportion of 90% with respect to the functional monomer–template complex, which was 4-VP–*N*-Fmoc-L-Trp or 4-VP–*N*-Cbz-L-Trp (Fmoc is fluoren-9-ylmethoxycarbonyl, Trp is tryptophane, Cbz is benzyloxycarbonyl). When the proportion of EDMA was $< 70\%$, no molecular recognition was present. A similar result was confirmed by other authors[51,122,127] who demonstrated that high selectivity of the formed MIP monoliths was attained for 80%–90% of the cross-linking agent.

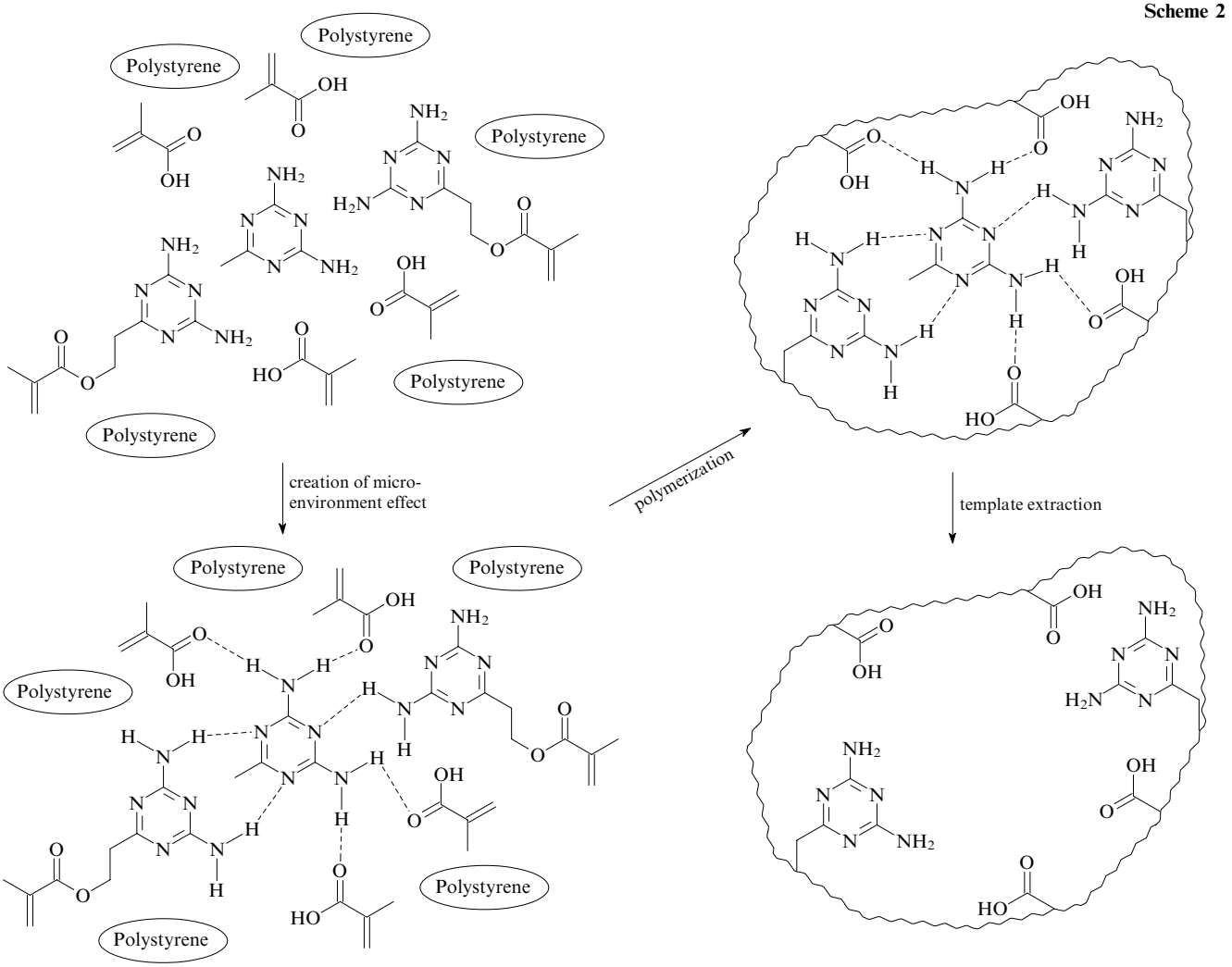
Recently,[128] a method for the preparation of low-cross-linked MIP monoliths has been proposed (Scheme 2). The proportion of the cross-linking agent (EDMA) was reduced to 9%–20% by adding polystyrene dissolved in one of the porogenic solvents (tetrahydrofuran) into the polymerization mixture. The polystyrene molecules were added in order to generate the effect of a crowded microenvironment for the functional monomer–template complex, which enhaces the stability of the highly ordered structure being formed. The increase in the viscosity of the polymerization mixture upon the addition of the linear polymer gave rise to a greater number of imprint sites in the low-cross-linked polymer matrix, and, as a consequence, high retention factors of the target component were achieved. The beneficial effect of introduction of polymers (polymethyl methacrylate and polystyrene) for the formation of a crowded microenvironment during imprinting was also demonstrated by Matsui *et al.*[137]

III.2.b. Porogens

Most often, dodecanol and toluene are used as porogens in the polymerization of the above monomers. Dodecanol is a solvent thermodynamically incompatible with most of polymethacrylate polymers and responsible for the formation of macropores. Toluene, being a non-polar aprotic solvent, is preferred for the formation of non-covalent bonds between the monomer and the template.

As a rule, an increase in the proportion of toluene in the toluene–dodecanol porogen mixture results in a decrease in the average pore size and simultaneously in an increase in the specific surface area. It was shown[138] that in the synthesis of theophylline- and caffeine-imprinted monolithic columns based on the MAA–EDMA copolymer, an increase in the toluene proportion to 40% resulted in the formation of a monolithic matrix with very small pores and, as a consequence, poor hydrodynamic properties. Satisfactory flow properties were achieved by using 15% toluene in the mixture of porogens. In this case, for an acetonitrile flow rate of 1 ml min^{-1}, pressure in a 100×3.2 mm column

Scheme 2



was 1 MPa. This result was supported in another study,[127] which demonstrated that the optimal proportion of toluene in the same system of monomers and porogens but with a different template (sinomenine) was 20%. The matrix thus formed possessed high efficiency, that is, the maximum values of both retention and separation factors in the chromatographic analysis of a mixture containing the template and structurally related molecules.

A similar outcome was achieved for the synthesis of columns based on 4-VP–EDMA copolymers using quinine and quinidine as templates.[43] In this case, the optimal content of toluene was 18% of the total volume of porogens. A monolithic column (150×4 mm) with an extensive specific surface area (111 $m^2\ g^{-1}$) was obtained; the column developed a pressure of 0.78 MPa at a mobile phase flow rate of 1 ml min^{-1}.

Apparently, the introduction of a template into the polymerization mixture affects the phase separation process during polymerization and, hence, the pore characteristics of the resulting material. The effect of the template addition can be both negative and positive, which undoubtedly depends on the properties of molecules introduced into the polymerization mixture.

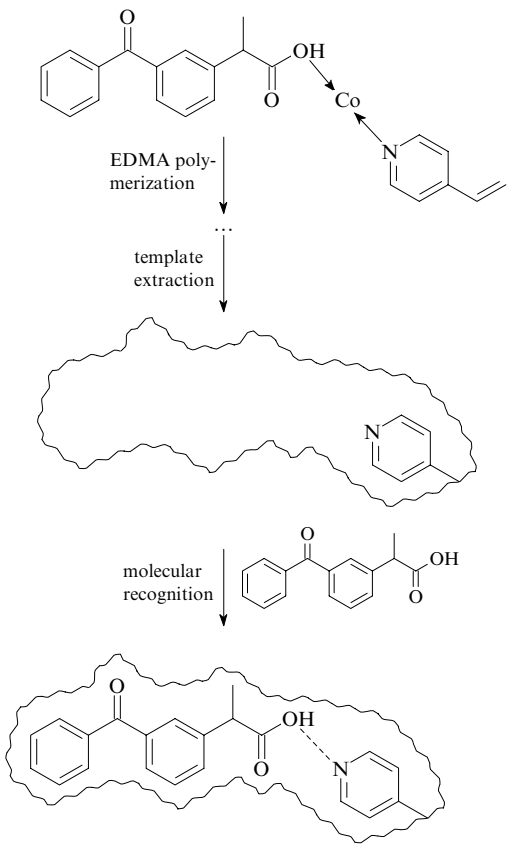
Often, the average pore size in non-imprinted polymer (NIP) matrices is larger than in imprinted polymers, the other synthesis conditions being the same. For example, Zhang *et al.*[45] obtained ceramide III-imprinted and non-imprinted polymer columns (100×4.6 mm) based on the glycidyl methacrylate–MAA–ST–DVB–tri(allyl) isocyanurate copolymer. A mixture of toluene (64 mass %) and heptane (36 mass %) was used as a pore-forming agent. According to the mercury porosimetry data, pore size distribution in NIP sorbents was in the range of 150–5000 nm with a maximum at 2200 nm. The pore distribution range in the MIP matrix containing ceramide III imprints was 100–600 nm with a clear maximum at 300 nm.

The same result was indirectly confirmed [122] considering the pressure levels, which directly depend on the pore characteristics of the sorbent, in the MIP and NIP columns at equal mobile phase flow rates. Evidently, the larger the average pore size, the lower the pressure in the column. At a mobile phase flow rate of 1 ml min^{-1} in a 70×5 mm monolithic column prepared from the 4-VP–EDMA copolymer using a 1 : 5 dimethylformamide–dodecanol mixture of porogens and imprinted with the antimicrobial drug sulfamethoxazole, the pressure was 0.67 MPa. Meanwhile, for an analogous NIP column, this value was only 0.15 MPa.

In recently published papers,[136, 139] high specificity of imprinting was achieved using metal ions as bridges between the functional monomer and the template (Scheme 3). The introduction of Co^{2+}, Zn^{2+} or Ni^{2+} into a mixture consisting of the functional monomer and the template provides a self-assembling system: the arrangement of monomers around the template is ordered due to the coordination metal pivot involving the metal ion. This type of bridge promotes stronger binding of the monomer (and the oligomer formed) and the template in the complex and, hence, the formation of a larger number of uniform imprint sites.

Zhao *et al.*[139] obtained macroporous monolithic matrices based on the 4-VP–EDMA copolymer imprinted with the anti-inflammatory drug ketoprofen. Since metal compounds (acetates) are insoluble in the traditionally used porogen systems, dimethyl sulfoxide (DMSO) was chosen as the solvent in this case. Using the toluene–DMSO binary mixture, it is impossible to obtain the macroporous structure but when isooctane is added as the third porogenic component, highly permeable monolithic sorbent is formed. As in the above-considered publications, an increase in the toluene proportion to 35% resulted in a decrease in the retention factor of ketoprofen on the synthesized MIP sorbent almost to zero. Also, it was shown that introduction of both the template and the metal ions into the polymerization mixture was favourable for increasing the average pore size as compared with the pores of the NIP matrix.

Scheme 3



Similarly to metal ions, polar templates are insoluble in most of porogen systems used. Ionic liquids are unique solvents with excellent solvating properties and chemical and thermal stability. Ionic liquids were tested as porogens in the manufacture of MIP sorbents.[136, 140] Zhong *et al.*[136] reported the preparation of the macroporous monolith based on the 4-VP–EDMA copolymer having imprints of a water-soluble methyl gallate. This template was introduced in the polymerization mixture using an ionic liquid, 1-n-butyl-3-methylimidazolium tetrafluoroborate ([Bmim]BF_4), as the solvent and for achieving a better solubility of the matrix, the above-described approach comprising the addition of metal ions (Co^{2+}) was used. For the formation of the macroporous structure of the monolithic material, a ternary system of porogens consisting of DMSO, DMF and [Bmim]BF_4 was chosen. An increase in the DMSO content above 30% was found to substantially decrease the monolith permeability, which was undoubtedly related to the decrease in the average pore size.

The proportion of DMSO needed to dissolve the cobalt acetate was found to be 30%. Upon varying the proportion of the ionic liquid from 50% to 70%, the optimal value providing both high permeability and good adsorption characteristics and selectivity was found to be 63%.

In a study[141] devoted to the preparation of macroporous monolithic columns based on the 4-VP–EDMA copolymers imprinted with the hypotensive drug (+)-nilvadipine, it was proposed to use *N*-Cbz-L-Trp as the accompanying template in the polymerization mixture to improve the properties of the MIP matrix being formed. The addition of this template promoted better phase separation during the polymerization, which, in turn, resulted in the formation of a more permeable sorbent. The pressure in the column synthesized without *N*-Cbz-L-Trp reached 5 MPs for 0.15 ml min^{-1} flow rate of the mobile phase, whereas in the column obtained in its presence, the pressure remained rather low even at 1.5 ml min^{-1} flow rate. In a study of the effect of the *N*-Cbz-L-Trp to (+)-nilvadipine ratio, the best resolution of chromatographic peaks in the separation of nilvadipine enantiomers was achieved for the ratio of 0.5 : 1.

Apart from the nature and the ratio of the porogens, the ratio between the monomers and porogens is also very important for the formation of a macroporous structure. As a rule, the higher the proportion of the cross-linking agent in the polymerization mixture, the higher the proportion of porogens required to obtain a macroporous material. At high content of the cross-linking agent and low content of porogens, phase separation does not take place, which results in the formation of a slightly solvated polymer looking like a semitransparent glass-like material rather than a macroporous matrix. Usually, with an appropriately selected system of porogens, if the proportion of the cross-linking agent is ~40 mol.%, the macroporous material can be obtained if the polymerization mixture contains 60 vol.%–70 vol.% of porogens.[142–145] If the proportion of the cross-linking monomer is 80 mol.%–90 mol.% as in the synthesis of MIP monoliths, the content of porogens is also increased to 75 vol.%–85 vol.%.[125, 127, 136]

III.2.c. Polymerization conditions

The key method for the preparation of macroporous MIP monoliths is polymerization using the 2,2′-azobis(isobutyronitrile) (AIBN) initiator, which decays on heating above 45 °C. Polymerization conditions, that is, reaction temperature and time, undoubtedly influence the properties of the obtained material. A change in the polymerization temperature is a very convenient method for the control of the material properties without changing the composition of the polymerization mixture. In particular, temperature variation can affect the rate of initiator decay (generation of free radicals), the formation of gel-like nuclei, phase separation, *etc*.

The synthesis of macroporous monolithic columns initiated by AIBN using 40% of a cross-linking agent with respect to the total monomer weight gives sorbents with good hydrodynamic and adsorption properties in 4–8 h at 70 °C.[107] As the amount of the cross-linking agent increases to 80% at the same temperature, polymerization time is usually ~4 h. However at elevated temperature, the initiator decays rapidly and, as a consequence, the decay rate is more difficult to control, which, in turn, has an adverse effect on the reproducibility of the structure of MIP materials. Moreover, the elevated temperature also unfavourably influences the stability of the functional monomer–template complex. For the synthesis of macroporous MIP materials, the preferred temperature range is 45–60 °C. In view of the fact that the lower polymerization temperature, the longer the process, most researchers choose values at about 50–60 °C as the optimal conditions, which, moreover, afford materials with good separating properties; in this case, polymerization time is usually 12–20 h.[44, 51, 55, 127, 128, 138]

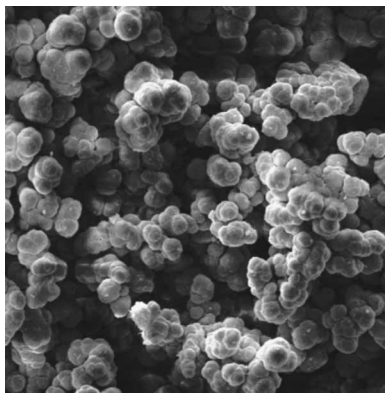


Figure 1. SEM image of the surface of macroporous matrine-imprinted monolithic sorbent based on the MAA–EDMA copolymer (magnification 1000).[146]

In conclusion, it is important to note that MIP monoliths have the same microglobular morphology as conventional monolithic sorbents (Fig. 1).

III.3. Application of macroporous molecularly imprinted polymeric monoliths

Currently, macroporous MIP monoliths are used as stationary phases in three key areas, namely, HPLC, solid-phase extraction and capillary electrochromatography. The published data on the application of MIP materials are summarized in Table 1. The most interesting examples are considered below.

III.3.a. High performance liquid chromatography

The application of fine-particle MIP sorbents in liquid chromatography started rather long ago.[200] Molecularly imprinted sorbents proved to be a competitive alternative to affinity chromatography for the separation of enantiomers, diastereomers, compounds containing several chiral centres and various analogues.[79, 201–203] Apart from packed columns, macroporous MIP monoliths can also be utilized to solve this type of problems (see Table 1).

The chromatographic efficiency of MIP monoliths is evaluated using a number of parameters, one of the most important among them being the retention factor (k) — a dimensionless value defined as the ratio of the time the compound resides in the stationary phase to the time it resides in the mobile phase,[138, 156]

$$k = \frac{t_r - t_0}{t_0} \quad (1)$$

where t_r is the retention time of the compound in the column, t_0 is the time an unretained component passing through the column.

Table 1. Application of macroporous MIP monoliths as sorbents in HPLC, capillary electrochromatography and solid-phase extraction.

Template	Functional monomer	Cross-linking monomer	Scope of application	Size of monolithic columns/mm	Ref.
High performance liquid chromatography					
Diaminonapthalene isomers	AAm, TFMAA	EDMA	study of specific complex formation	50 × 7.6	40
Nicotine	MAA	EDMA	study of the effect of the nature of the functional monomer on the strength of specific complex formation	50 × 4.6	41
Cinchonine, cinchonidine, quinine, quinidine	AAm, TFMAA	EDMA	the same	50 × 4.6	42
Cinchonine	MAA, 4-VP	EDMA	separation of diastereomer mixture on MIP columns of different size	150 × 4, 100 × 5, 38 × 8, 10 × 16	147
Nitrophenol isomers, salicylic and 4-hydroxy-benzoic acids	4-VP, AAm	EDMA	separation of nitrophenol isomers and hydroxybenzoic acid isomers	150 × 4	123
(*S*)-(−)-1,1′-Bi-2-naphthol	4-VP	EDMA	separation of enantiomers	50 × 4	121
Atrazine	MAA	EDMA	study of the selectivity of atrazine binding in comparison with other triazine herbicides	50 × 4.6	137
2,4-Diamino-6-(meth-acryloyloxy)ethyl-1,3,5-triazine	MAA + BMA	EDMA	study of the effect of chromatographic conditions on the molecular recognition of 2,4-diamino-6-methyl-1,3,5-triazine and its analogues	100 × 4.6	128
L-Tetrahydropalmatine	MAA	EDMA	quantification of enantiomers; combination of MIP column with a reversed phase column for analysis of the extract from *Corydalis yanhusuo*	50 × 4.0	148
Methyl gallate	4-VP	EDMA	study of the thermodynamics of separation of methyl gallate and its structural analogues	100 × 4.6	136
Bupivacaine	MAA	TRIM, EDMA	comparison of the chromatographic efficiency with non-monolithic MIP sorbents imprinted with bupivacaine	70 × 0.3	149
	MAA	EDMA	study of the bupivacaine interactions with the MIP sorbent	35 × 3	150
Theophylline	MAA	EDMA	separation of caffeine and theophylline	100 × 3.2	138
	AAm	EDMA	study of the effect of chromatographic conditions on the molecular recognition	100 × 3.2	151
	AAm	EDMA	quantification of theophylline and caffeine in green tea	150 × 4.0	152
Sulfamethoxazole	AAm, 4-VP, 1-VI	EDMA	separation of a sulfanilamide mixture contained in tablets	70 × 5.0	122
Ketoprofen	4-VP	EDMA	separation of a mixture of ketoprofen and its structural analogues	100 × 4.6	139
Sinomenine	MAA, TFMAA	EDMA	separation of a mixture of sinomenine and its analogues	100 × 4.6	127
Nateglinide	AAm	EDMA	separation of a mixture of nateglinide and its L-enantiomer	150 × 4.6	51
Pefloxacin	MAA	DEGDMA	analysis of fluoroquinolone mixtures in milk samples	150 × 2	130
(+)-Nilvadipine	4-VP	EDMA	separation of enantiomers	50 × 4.0	141
(*S*)-Naproxen	MAA + BMA	EDMA	separation of a mixture of chiral compounds	100 × 4.6	125, 126
N-Cbz-L-Tryptophane, *N*-Fmoc-L-Tryptophane	MAA, 4-VP	EDMA	separation of *N*-Cbz-DL-Trp and *N*-Fmoc-DL-Trp enantiomers	150 × 4	44, 147
N-Cbz-L-Phenylalanine	AAm	EDMA	separation of enantiomers of *N*-Cbz-phenylalanine, Trp and *N*-Cbz-phenylalanine	100 × 3.2	124
N-Cbz-L-Phenylalanine	AAm, 4-VP, 1-VI	TRIM	study of the adsorption of *N*-Cbz-L-phenylalanine	250 × 4.6	55
Methyl ester of *N*-Fmoc-phosphotyrosine	MAA	TRIM, EDMA	study of the selectivity of binding of Fmoc derivatives of phosphoserine, tyrosine, glutamic acid and phosphotyrosine	70 × 0.25	153
N-Cbz-Thr-Ala-Ala-OMe	MAA	TRIM, PETA	study of binding selectivity in relation to the tripeptide *Z*-Thr-Ala-Ala-OMe and its analogue *N*-Cbz-Thr-Ile-Leu-OMe	glass tube with a volume of 20 ml	154

Table 1 (continued).

Template	Functional monomer	Cross-linking monomer	Scope of application	Size of monolithic columns/mm	Ref.
Lysozyme, BSA	AAm	MBAAm	separation of a mixture of competing proteins	150 × 4.6	155
Cytochrome *c*	MAAm, MAA	PAAm	separation of cytochrome *c* and lysozyme	250 × 4.6	156
Capillary electrochromatography					
(*R*)-Propranolol	MAA	TRIM	separation of propranolol enantiomers	350 × 0.075	157
(*S*)-Propranolol	MAA	TRIM	the same	85 × 0.1	158
(*S*)-Propranolol, (*S*)- metoprolol, (*S*)-atenolol	MAA, MMA, BMA, GMA	TRIM	separation of metoprolol and atenolol enantiomers	265 × 0.075	159
(*S*)-Ornidazole	MAA + 4-VP	EDMA	separation of ornidazole enantiomers	300 × 0.1, 430 × 0.1	160
(−)-Norepinephrine	MAA, IA	EDMA	separation of a mixture of structural analogues: norepinephrine, dopamine, octopamine, synephrine, epinephrine and isoproterenol	500 × 0.075	161
(*S*)-Naproxen	MAA	EDMA	separation of naproxen enantiomers	200 × 0.1	162
(*S*)-Ornidazole	HEMA, DEAEM	EDMA	separation of ornidazole enantiomers	300 × 0.1	163
17β-Estradiol	MAA	EDMA, TRIM	separation of α- and β-estradiols	250 × 0.075	164
4-Aminopyridine	MAA	EDMA	separation of aminopyridine isomers	100 × 0.1	165
4-Hydroxybenzoic acid	MAA	EDMA	separation of a mixture of thiourea and hydroxybenzoic acid isomers	200 × 0.1	166
	MAA	EDMA	separation of hydroxybenzoic acid isomers	245 × 0.1	167
(*R*)-1,1′-Bi-2,2′-naphthol	MAA	EDMA	separation of binaphthol enantiomers	200 × 0.1	168
1,1′-Binaphthyl-2,2′-diamine	MAA	EDMA	separation of a mixture of structural analogues: 1,1′-binaphthyl-2,2′-diamine and 1,1′-bi-2,2′-naphthol	200 × 0.1	53
(*S*)-Ibuprofen	4-VP, MAA	EDMA, TRIM	separation of ibuprofen enantiomers	265 × 0.1, 415 × 0.1	169
	MAA, 4-VP	EDMA	the same	200 × 0.1	170
L-Tetrahydropalmatine	MAA	EDMA	separation of DL-tetrahydropalmatine enantiomers	245 × 0.075	54, 171
Sulfamethazine	MAA	EDMA	separation of a mixture of ten sulfanilamides	250 × 0.1	172
Vanillin	MAA	EDMA	separation of isomers and structural analogues of vanillin	250 × 0.1	173
L-Tyrosine, L-alanine, L-phenylalanine, L-tryptophane, *N*-Cbz-L-tyrosine	MAA + 4-VP	EDMA	separation of enantiomers of these amino acids	diameter 0.1, the length is not indicated	174
Tyr-Pro-Leu-Gly	MAA	EDMA	separation of peptides Tyr-Pro-Leu-Gly and Tyr-Pro-Gly-Leu	200 × 0.1	175, 176
Thiabendazole	MAA	EDMA	analysis of thiabendazole in crude citrus extract	85 × 0.1	177
Solid-phase extraction and microextraction					
Ceramide III	ST + GMA + MAA	DVB, TAIC	isolation of ceramide III from yeast	150 × 4.6	45
Strychnine	MAA	EDMA	selective extraction of strychnine from a mixture of indole, quinine, strychnine and brucine	150 × 4.6	178
Caffeine	AAm or MAA	EDMA, MBAAm	determination of caffeine in chilren's milk	50 × 2.5	179
Caffeic acid	AAm	EDMA	extraction of caffeic, ferulic and protocatechuic acid from *Salicornia herbacea*	45 × 9	180
Matrine	MAA	EDMA	extraction of matrine from the *Sophorae flavescentis* root	150 × 4.6	146
Protocatechuic acid	AAm	TRIM	extraction of protocatechuic acid from *Cirsium segetum Bunge*	250 × 4.6	132

Table 1 (continued).

Template	Functional monomer	Cross-linking monomer	Scope of application	Size of monolithic columns/mm	Ref.
Berberine	AAm	EDMA	analysis of berberine in blood plasma and urine	micropipette extension	181
Norfloxacin	MAA	EDMA	determination of norfloxacin and other quinolones in pork	250 × 4.6	140
	HEMA	EDMA	analysis of enrofloxacin and ciprofloxacin in urine	15 × 3.2	182
Ciprofloxacin	MAA + AAm	EDMA	determination of ciprofloxacin in urine	100 × 3.2	183
Tetracycline	MAA	EDMA	determination of tetracycline and its structural analogues in milk	100 × 4.6	184
3,4-Methylenedioxy-methamphetamine	MAA	EDMA	determination of traces of methamphetamine in saliva	40 × 0.3	185
Diacetylmorphine	MAA	EDMA	microextraction of diacetylmorphin and its analogues	40 × 0.3	186
Mepivacaine, (*S*)-ropivacaine and/or bupivacaine	MAA	EDMA	study of the extraction mechanism, selectivity and cross interactions	70 × 0.1	187
Horseradish peroxidase	dopamine, VPBA	PETA	extraction of the peroxidaze from blood	250 × 0.1	188
Guanosine	AAm + 4-VP	MBAAm	determination of 8-hydroxy-2′-deoxyguanosine in urine	50 × 0.53	129, 189, 190
2,4-Diamino-6-undecyl-1,3,5-triazine	MAA	EDMA	determination of melamine and cyromazine in milk	50 × 4.6	191
Metribuzin	MAA	EDMA	analysis of metribuzin in soil samples	50 × 4.6, 15 × 4.6	119
Bisphenol A	4-VP	EDMA	extraction of bisphenol A and its analogue from river water	200 × 4.6	120
Diphenoconazole	MAA	EDMA	determination of diphenoconazole in tap water and grape juice	micropipette extension	192
2,4-Dichlorophenyl-acetic acid	AAm	EDMA	determination of 2,4-dichlorophenylacetic acid in rice	the same	193
Pirimicarb	MAA	EDMA	determination of pirimicarb in tomatoes and pears	"	194
Isoprocarb	MAA, MTMS, MPTMS	EDMA	analysis of isoprocarb in rice	"	195
Carbaryl + naproxen	MAA	EDMA	analysis of carbaryl and naproxen in salad leaves and river water	"	196
Nickel nitrate	HEMA, MAH	EDMA	determination of Ni^{2+} in aqueous media	50 × 10	197
Iron(II) nitrate	HEMA, MAC	EDMA	determination of Fe^{2+} in blood plasma	100 × 10	198
Cadmium nitrate	methyl ester of MAC, HEMA	EDMA	removal of Cd^{2+} from blood plasma	20 × 10	199

Notes. The following designations are used: 1-VI is 1-vinylimidazole, MAAm is methacrylamide, MAH is *N*-methacryloyl-L-histidine, MAC is *N*-methacryloyl-L-cysteine, IA is itaconic acid, VPBA is 4-vinylphenylboronic acid, TAIC is triallylisocyanurate, PETA is pentaerythritol triacrylate, PAAm is piperazine diacrylamide, DEAEM is diethylaminoethyl methacrylate, MTMS is methyltri(methoxy)silane, MPTMS is methacryloxy(propyl)trimethoxysilane, GMA is glycidyl methacrylate.

The retention factors are needed to calculate the so-called imprinting factor (*IF*), which is a measure of interaction of imprint sites with target molecules

$$IF = \frac{k_{MIP}}{k_{NIP}} \quad (2)$$

where k_{MIP} is the retention factor of the target compound by the MIP sorbet, k_{NIP} is the retention factor of the target compound by an unimprinted sorbent. The imprinting factors of $\geqslant 2$ are considered to be satisfactory.[62]

The selectivity of an imprinted column is estimated by means of the separation factor (or selectivity factor) α, which reflects the selectivity of binding of the target molecule by the MIP sorbent with respect to the selectivity of binding of structural analogues of the template used to form the imprint sites. The separation factor is calculated from the equation [156]

$$\alpha = \frac{k_1}{k_2} \quad (3)$$

where k_1 is the retention factor of the target component, k_2 is the retention factor of analogue molecules.

The chromatographic separation efficiency is generally evaluated from the resolution of the peaks of two compounds (R_s)[156]

$$R_s = \frac{2(t_{r_1} - t_{r_2})}{W_1 - W_2} \quad (4)$$

where t_{r_1} and t_{r_2} are the retention times of the target compound and a structural analogue, respectively; W_1 and W_2 are the baseline peak widths for the elution of the target compound and its structural analogue.

An interesting study devoted to comparison of the efficiency of traditional fine-particle and monolithic MIP matrices was reported by Fu *et al.*[146] The authors compared columns packed with synthesized MIP microspheres and particles obtained by grinding of the MIP sorbent synthesized in block with capillary-shaped monolithic columns (70×0.32 mm). The packed columns had dimensions of 50×4.6 mm and 100×4.6 mm, respectively. All of the sorbents were synthesized based on the MAA–EDMA copolymer using the anesthetic drug bupivacaine as the template. According to the results, the maximum imprinted factor ($IF = 5.1$) was found for a packed column containing ground particles, whereas for columns packed with microspheres, this factor was minimum ($IF = 1.4$); in the case of a macroporous monolithic sorbent, $IF = 2.5$. The minimum non-specific retention detected under the optimal elution conditions was observed for the monolithic sorbent, while the maximum value was found for a column packed with microspheres.

Effect of the composition of the mobile phase and pH. The composition of the mobile phase has a substantial influence on the separation efficiency of the target component and its analogues.[204, 205] First of all, this is related to the mechanism of specific interaction between the imprint sites and the target molecules. In non-polar media, these interactions are usually based on hydrogen bonds, while in aqueous organic solutions with a high content of water, these are hydrophobic and/or ionic interactions.

The influence of the amount of the polar solvent in the mobile phase on the separation selectivity of structural analogues was demonstrated in a number of publications (see, for example,[44, 121, 136, 138, 151, 152]). Ou *et al.*[121] reported the separation of chiral compounds by means of a monolithic sorbent based on the 4-VP–EDMA copolymer molecularly imprinted with (*S*)-(−)-1,1′-bi-2-naphthol. Upon the addition of 2% of acetic acid to the mobile phase (acetonitrile), the enantioseparation factor decreased from 4.3 to 3.1, *i.e.*, by 28%. An increase in the proportion of acetic acid to 5% (and above) resulted in complete termination of the separation of enantiomers. The same trend was induced by addition of water to acetonitrile. In this case, with 4% water content in the mobile phase, the enantioseparation factor decreased by 35% (from 4.3 to 2.8), and as the water content was raised to 10%, no adsorption of the mixture components took place. Further increase in the water content induced again the adsorption of the target component. In particular, as the 40% content of water in the mobile phase was achieved, the retention of (*S*)-(−)-1,1′-bi-2-naphthol was too strong for desorption of the analyte under isocratic conditions. This behaviour of the system is attributable to the change in the molecular recognition mechanism. The introduction of minor amounts (up to 10%) of polar components to the mobile phase diminishes the hydrogen bond formation between the analyte and the sorbent due to competition for the adsorption sites between the target component and the polar agent added. At higher contents (> 30%) of the polar agent, hydrophobic interactions rather than hydrogen bonds start to play the key role in the specific binding.

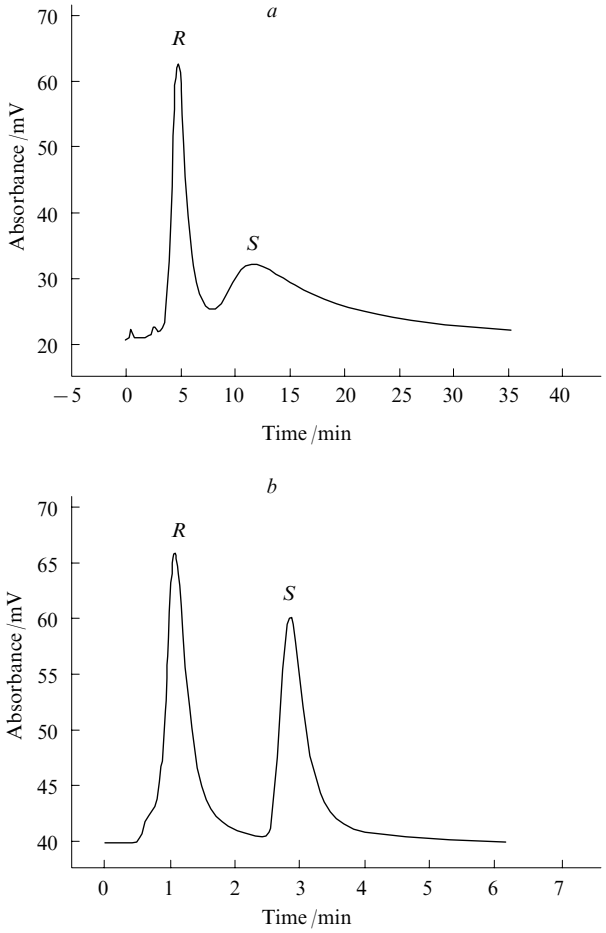


Figure 2. Chromatograms for the separation of 1,1′-bi-2-naphthol enantiomers on a monolithic MIP column.[121]
Conditions: (*S*)-(−)-1,1′-bi-2-naphthol-imprinted sorbent based on the 4-VP–EDMA copolymer as the stationary phase, a 50×64 mm column, temperature of 20 °C, detection at 254 nm.
(*a*) isocratic elution: acetonitrile as the mobile phase, mobile phase flow rate of 0.3 ml min^{-1}; (*b*) gradient elution: from 0 to 2.5 min, with acetonitrile; from 2.5 to 6 min, with a 2% solution of acetic acid in acetonitrile; mobile phase flow rate of 1.0 ml min^{-1}.

In most cases, enantiomers can be separated in the isocratic mode. Drawbacks of isocratic elution are long retention times and, hence, considerable broadening of the eluted zones. It is possible to considerably reduce the time of elution of mixture components and the chromatographic peak width by using gradient elution and/or by increasing the mobile phase flow rate.[147] This is illustrated in Fig. 2, which presents the results of separation of (*R*)-(+)- and (*S*)-(−)-1,1′-bi-2-naphthols by isocratic and gradient elution.[121] Whereas in the former case, effective separation of

enantiomers was attained at a mobile phase flow rate of 0.3 ml min^{-1} in 30 min, in the latter case, the same efficiency was attained in 4 min at a mobile phase flow rate of 1 ml min^{-1}.

A study of the influence of mobile phase composition on the separation of theophylline and caffeine using theophylline-imprinted macroporous monolithic columns based on MAA–EDMA and AAm–EDMA copolymers has been undertaken.[138, 151, 152] As the mobile phases, methanol, water and acetonitrile were tested. As in the previous case, better separation was achieved with acetonitrile. The addition of acetic acid to acetonitrile led to lower retention factors of the components. The addition of only 4% of acetic acid into the mobile phase decreased the retention factor of theophylline from 3.52 to 1.34, *i.e.*, by almost 60%. A similar trend was initiated by adding methanol or water to the mobile phase.

The outcome of separation of *N*-benzyloxycarbonylphenylalanine enantiomers on a monolithic column based on the AAm–EDMA copolymer imprinted with *N*-Cbz-L-phenylalanine was similar. Similarly to the previous cases, the addition of 5% of acetic acid to acetonitrile resulted in low enantioselectivity and low resolution.[124]

The influence of pH on the chromatographic behaviour of structurally related compounds is the subject of several publications.[44, 121, 156, 194] In particular, Huang *et al.*[44] performed separation of *N*-Cbz-Trp enantiomers using columns based on the 4-VP–EDMA copolymer imprinted with the L-isomer. Initially, the optimal content of the buffer solution in the mobile phase was determined. While the proportion of the buffer solution (pH 4.0) was increased from 20% to 100%, the best separation was achieved for 60% content. When the proportion of the buffer solution was increased to 80%, the retention time of the target component considerably increased. As the pH of the buffer solution changed from 3.0 to 5.5, the separation factor remained almost invariable being in the range of 3.0–4.5, and after that it slightly decreased (Fig. 3). The resolution factor did not change in the pH range of 3.0–4.0, and on going to pH 5.0–5.5, it decreased to zero. This may be indicative of a close relationship between the hydrophobic and ionic mechanisms of interaction involved in the enantioselective separation in aqueous media.

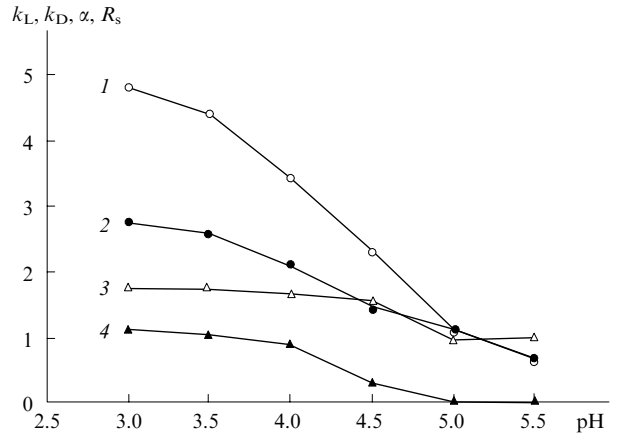


Figure 3. Effect of the pH of the mobile phase on the retention factors (*1*, *2*), separation factor (*3*) and peak resolution factor (*4*) for the separation of Cbz-Trp enantiomers.[44]
Conditions: Cbz-L-Trp-imprinted sorbent based on the MAA–EDMA copolymer as the stationary phase; 50% of 10 mM acetate buffer in acetonitrile as the mobile phase; mobile phase flow rate of 0.5 ml min^{-1}; k_L, k_D are retention factors.

Zhong *et al.*[136] studied the effect of pH on the binding selectivity of methyl gallate and related compounds using a macroporous monolithic sorbent based on 4-VP–EDMA and imprinted with methyl gallate. Since methyl gallate is readily soluble in polar solvents, an aqueous organic medium, namely, a mixture of acetonitrile and acetate buffer solution, was chosen as the optimal mobile phase for chromatography. The highest selectivity of the MIP sorbent towards methyl gallate was observed at pH 4.2 with 60% of acetonitrile present in the mobile phase. Since pK_a of methyl gallate is 4.3, the retention is most likely due to both hydrophobic and ionic forces. If the proportion of acetonitrile increased to 90% or decreased to 50%, this selectivity substantially decreased, as well as on going to lower or higher pH. A decrease in the pH to 3.0 resulted in almost zero selectivity of binding due to the lack of electrostatic interactions between the target molecule and the functional monomer. Upon increase in the pH to 5.6, the retention factor decreased to zero due to the decrease in the site selectivity; in the case of structurally related molecules, the separation factor did not follow so clear-cut pH dependence. Detailed information on the effect of the mobile phase composition on the separation of chiral compounds on MIP sorbents is presented in a review[205] where the molecular recognition mechanism is also discussed.

Effect of the mobile phase flow rate. The flow rate of the mobile phase is a very important parameter of any dynamic process. In the case of macroporous monolithic sorbents, the efficiency of chromatographic separation of both low- and high-molecular-mass compounds does not depend on the flow rate of the mobile phase over a rather broad range (up to 10 ml min^{-1}).[36, 49, 50, 206]

The behaviour of analytes in the chromatographic process with MIP monoliths at various flow rates of the mobile phase has been studied.[44, 121, 138, 141, 151, 152] In most cases, the results demonstrate insignificant changes in the efficiency of separation of structural analogues with a considerable increase in the process velocity, which is consistent with the behaviour of monolithic columns in general. Yan and Row[151] detected only a slight decrease in the efficiency of separation of theophylline and caffeine on a theophylline-imprinted AAm–EDMA sorbent following increase in the mobile phase flow rate from 0.5 to 6.0 ml min^{-1}, despite the fact that the number of contacts between the compound and the surface markedly decreased. In other experiments with the same system of components but using MIP columns based on the MAA–EDMA copolymer, the increase in the mobile phase flow rate in the range from 0.5 to 3.0 ml min^{-1} decreased the separation factor by only 7% (from 4.35 to 4.07).[138] Thus, despite the high flow rate of the mobile phase, the separation level was virtually the same. A similar outcome was attained in the separation of *N*-Cbz-Trp enantiomers in the 0.25–2.0 ml min^{-1} range of mobile phase flow rates using a 4-VP–EDMA monolithic column imprinted with the L-isomer.[44]

However, Ou *et al.*,[121] who performed separation of chiral compounds using a 4-VP–EDMA monolithic sorb-

ent imprinted with (*S*)-(−)-1,1′-bi-2-naphthol, found that an increase in the flow rate from 0.1 to 1.0 ml min^{-1} resulted in a 1.7-fold decrease in the separation factor (from 3.05 to 1.80) and a 2-fold decrease in the resolution (from 1.16 to 0.58). A similar result was reported by another publication of the same authors [148] but for the separation of tetrahydropalmatine enantiomers on a monolithic column based on the MAA–EDMA copolymer imprinted with the L-enantiomer. In this case, an increase in the flow rate from 0.1 to 1.0 ml min^{-1} was accompanied by a 1.3-fold decrease in the separation factor (from 6.44 to 4.97) and a 1.7-fold decrease in the resolution (from 1.65 to 0.96). Note that this range of flow rates is not typical of macroporous monolithic sorbents.

One advantage of this type of stationary phases related first of all to enhanced mass transfer due to convection mechanism can be achieved at high flow rates of elution, which increase the permeability of the separation medium.[36] In the region of low rates typical of packed columns, longitudinal diffusion of the substance deposited on the column starts to make a substantial contribution to the mass transfer. In addition, since the authors did not report the pore characteristics of the obtained sorbents, it is difficult to evaluate objectively the obtained results. The presented dependence of the pressure in the system on the flow rate of the mobile phase indicates indirectly that the hydrodynamic properties of these sorbent were poor for this class of materials.

Effect of temperature. In some studies in which the separation was carried out in acetonitrile, increase in the temperature in the chromatographic process was found to result in a lower retention factor for the target compound.[138, 151] In particular, in the separation of theophylline and caffeine on the theophylline-imprinted AAm–EDMA sorbent, a temperature rise from 20 to 50 °C led to a substantial decrease in the retention factor of theophylline (from 4.5 to 2.5) and a smaller change in the retention factor of caffeine.[151] When the same compounds are separated on a monolithic sorbent prepared using MAA as a functional monomer, temperature rise from 24 to 60 °C induced a less pronounced decrease in the retention factor (from 5.7 to 5.0). The decrease in the retention factor at elevated temperature is attributable to weakening of hydrogen bonds, which play a key role in chromatography with an organic mobile phase, which, in turn, diminishes the adsorption level of the target compound.[138]

The effect of temperature for mobile phases represented by aqueous organic systems, in which the analyte and the polymer matrix interact mainly by a hydrophobic mechanism, was examined in a number of other publications.[44, 121, 123, 136, 146, 152] For example, Fu *et al.*[146] found that a temperature rise from 30 to 60 °C is accompanied by a more than twofold increase in the retention factor of matrine on a matrine-imprinted monolithic sorbent and by an almost 1.7-fold increase in the separation factor. As the mobile phase, the authors used a 50 : 50 v/v acetonitrile–20 mM phosphate buffer (pH 5.0) mixture.

In some publications, an increase in the separation factor with temperature rise was also noted but the increment was not so noticeable.[44, 121, 123] Yan and Row,[124] who synthesized a MIP column using acrylamide and *N*-Cbz-L-phenylalanine as the functional monomer and the template, respectively, demonstrated that upon a temperature rise from 20 to 50 °C, the separation factor increased from 1.32 to 1.43.

Effect of the separating layer length. In the adsorption-based types of separation on monoliths in which diffusion limitations are restricted, the column length barely influences the efficiency of separation.[50, 207]

Huang *et al.*[147] considered the effect of the length of the stationary phase layer on the selectivity of separation of the *N*-Cbz-Trp and *N*-Fmoc-Trp enantiomers and separation of diastereomers (cinchonine and cinchonidine) using macroporous monolithic MIP columns. The authors obtained series of columns based on the 4-VP–EDMA and MAA–EDMA copolymers bearing the imprints of either the tryptophane or cinchonine L-isomer, which were of different length but had almost identical volume (150 × 4 mm, 100 × 5 mm and 38 × 8 mm). The selectivity of these monolithic columns virtually did not depend on the length of the separating layer, this result being in full agreement with the behaviour of monolithic columns in other types of adsorption chromatography (Table 2). However, it is known that in short columns, the mobile phase flow resistance is much lower than in long columns. Indeed, it was shown that when the column length was 38 mm, the pressure in the system was 3.6 MPa at a mobile phase flow rate of 7.0 ml min^{-1}, whereas for 150 mm-long columns, the pressure was 6.7 MPa at a 2.5 ml min^{-1} flow rate. Relying on these results, the authors synthesized a 10 mm thick cinchonine-imprinted monolithic disc with a diameter of 16 mm, which served to confirm that the separation performance in a short layer and in longer columns is the same. By optimizing the conditions of chromatographic procedure, they achieved an efficient separation of cinchonine and cinchonidine on monolithic MIP disks at a mobile

Table 2. Dependence of the separation factor on the length of the separating layer.[144]

Compounds to be separated	Separation factor for columns with the size (mm)		
	150 × 4 (1.88 ml column volume)	100 × 5 (1.96 ml column volume)	38 × 8 (1.91 ml column volume)
Cinchonine and cinchonidine [a]	5.88	5.27	5.22
N-Cbz-DL-tryptophane [b]	1.85	2.08	1.87
N-Fmoc-DL-tryptophane [c]	2.21	2.28	2.21

Note. Conditions: isocratic elution, mobile phase flow rate of 1.0 ml min^{-1}, detection at 280 nm.
[a] Mobile phase: 3% solution of acetic acid in acetonitrile, stationary phase: cinchonine-imprinted sorbent.
[b] Mobile phase: 0.3% solution of acetic acid in acetonitrile, stationary phase: *N*-Cbz-L-Trp-imprinted sorbent.
[c] Mobile phase: 0.2% solution of acetic acid in acetonitrile, stationary phase: *N*-Fmoc-L-Trp-imprinted sorbent.

phase flow rate of 9.0 ml min^{-1} over a very short period of time (2.5 min).

III.3.b. Capillary electrochromatography

Capillary electrochromatography combines the principles of capillary electrophoresis and high performance liquid chromatography, *i.e.*, separation occurs due to both difference between their electrophoretic mobility and different interaction with the stationary phase. This method combines the advantages of capillary electrophoresis (high separation efficiency) and HPLC (high selectivity). Moreover, substantial advantages of capillary electrochromatography include low solvent consumption and small volume of the sample.

The first method for preparing MIP monoliths for capillary electrochromatography was proposed by Schweitz *et al.*[157] The authors synthesized macroporous MIP monolith by photoinitiated polymerization in a capillary (350 mm length and 75 μm diameter) and successfully used it to separate a racemic mixture of the β-adrenergic antagonist propranolol.

The electroosmotic flow is a highly important parameter in electrochromatography, which, in combination with the intrinsic electrophoretic mobility of the analyte, substantially influences the retention time, peak resolution factor and actually the separation performance. In some studies concerned with the synthesis and properties of MIP monoliths in the capillary format, it was demonstrated that the electroosmotic flow (*EOF*) depends on the composition, pH and ionic strength of the mobile phase.[53, 54, 160, 166, 174] In particular, for monolithic copolymer-based capillaries with methacrylic acid as the functional monomer, an increase in the acetonitrile proportion in the mobile phase from 60% to 90% results in an increase in the *EOF* value; as the pH of the solution changes from 3.0 to 7.0, the *EOF* value also increases. Meanwhile, an increase in the solution ionic strength is accompanied by a more complicated dependence with a maximum salt concentration (in most cases, NaCl is used) normally in the 20–50 mmol $litre^{-1}$ range.

The same factors affect the chromatographic parameters. Several research groups studied the effect of the acetonitrile concentration in the mobile phase considering cases of separation of structural analogues. However, the results were contradictory. For example, in the electrochromatographic separation of 1,1′-binaphthyl-2,2′-diamine (template) and its analogue, 1,1′-binaphthol, using the MIP sorbent and separation of tetrahydropalmatine enantiomers on the MIP sorbent imprinted with the L-isomer, the separation and retention factors were reported[53, 54] to decrease with an increase in the acetonitrile concentration. This was attributed to the predominance of the electrophoretic mechanism of substance migration in the protonated state over the substance interaction with the solid phase surface. However, hydrogen bonding is known to be more pronounced in a hydrophobic microenvironment, *i.e.*, an increase in the acetonitrile content should be favourable for molecular recognition — the retention and separation factors should increase. Particularly this trend was detected by another research group[160, 174] in a study of the behaviour of tyrosine and (*S*)-ornidazole enantiomers.

Some researchers ascertained that an increase in the pH from 2.0 to 6.0 induces a decrease in the retention factors of 4-hydroxybenzoic acid, L-tetrahydropalmatine and 1,1′-binaphthyl-2,2′-diamine on the MIP sorbents containing methacrylic acid as the functional monomer.[53, 54, 166] A separation of tyrosine enantiomers also showed a decrease in the separation factor and resolution of the chromatographic peaks following increase in the pH from 3.0 to 6.0.[174] A study of the effect of temperature on the separation of the structural analogues of 1,1′-binaphthyl-2,2′-diamine and 1,1′-binaphthol in the temperature range of 25–45 °C did not reveal substantial changes in the separation factors.[53]

Ou *et al.*[54, 171] investigated the dependence of the selectivity on the electric voltage for electrochromatographic analysis of the tetrahydropalmatine enantiomer. As the voltage increased from 5 to 25 kV, the resolution factor gradually decreased from 1.32 to 1.08. For the same voltage but different sample deposition times, the R_s value was higher for faster sample deposition. For the voltage of 5 kV and sample loading for 6 s, the resolution of the peaks was 1.12. When the sample was injected during 3 s at the same voltage, the R_s value increased to 1.25.

As an example of successful application of capillary electrochromatography, note the publication of Cacho *et al.*,[177] who proposed a method for analysis of the fungicide thiabendazole in the samples of orange and lemon pulp and peel using a MIP monolith. According to the results, the efficiency of recovery of the analyte with the thiabendazole-imprinted sorbent based on MAA–EDMA was 85%–100%. Thus, capillary electrochromatography with macroporous MIP monoliths is obviously promising for quantification of various compounds.

III.3.c. Solid-phase extraction and microextraction

Solid-phase extraction based on adsorption of the components of interest on the solid support is one of the most popular methods for the removal of particular components from solution. As compared with the liquid extraction, the solid-phase alternative ensures a higher degree of purity of extracts for smaller volumes of solvents and samples. Solid-phase extraction techniques including microextraction based on macroporous molecularly imprinted monoliths have now been developed and are demanded in various fields (see Table 1).

The evaluation of the extraction efficiency and optimization of the experimental process conditions are performed, most often, by means of two key parameters, namely, the enrichment factor (*EF*) and extraction recovery (*ER*).[179, 194] The first parameter is defined as the ratio of the analyte concentration in the eluent after extraction (c_{elu}) to its initial concentration (c_0)

$$EF = \frac{c_{elu}}{c_0} \tag{5}$$

The second parameter represents the amount of the recovered compound (m_{elu}) divided by the total amount (m_0). The extraction recovery (%) can be calculated as follows:

$$ER = \frac{m_{elu}}{m_0} \times 100 = \frac{c_{elu} V_{elu}}{c_0 V_0} \times 100 = EF \times \frac{V_{elu}}{V_0} \times 100 \tag{6}$$

where V_{elu} is the volume of the analyte solution after extraction, V_0 is the volume of the initial solution.

The efficient use of the solid-phase extraction with MIP monoliths for medical analysis can be illustrated by a number of studies devoted to determination of 8-hydroxy-

2′-deoxyguanosine (8-OHdG), which is a biomarker of the oxidative DNA damage, inducing mutagenesis and carcinogenesis in the body.[129, 189, 190] As the solid-phase extraction device, a guanosine-imprinted capillary-shaped monolith based on the AAm–4-VP–MBAAm copolymer was used. The recovery of the target compound was 80%. In addition, a quantitative method for determining urinary 8-OHdG marker for diagnosis of some types of cancer was developed.

Zhang and Chen[181] demonstrated the applicability of MIP monoliths for the extraction of berberine from urine and blood plasma. Berberine is an alkaloid present in some medicinal plants and used in the therapy of bacterial diarrhea, parasitic intestinal infections and eye trachoma. The recovery of berberine from both urine and blood plasma exceeded 91%.

A method for online analysis of the antibiotics ciprofloxacin and enrofloxacin in urine using a system comprising a monolithic MIP sorbent and an analytical column for reversed phase chromatography (C_{18}) has been developed.[181, 182] The recovery of enrofloxacin was 90.2%–94.8%, while that for ciprofloxacin was 87.5%–96.1% depending on the concentration of the loaded compound.

Some publications are devoted to the use of macroporous monolithic MIP columns for determining antibiotics in foodstuffs. For example, a method for determination of norfloxacin and other quinolone antibiotics in pork has been developed[140] and a method for analysis of tetracycline antibiotics in milk and honey has been proposed.[184] In the latter publication, the effect of the concentration of the target compound loaded on the column on the recovery is considered. In particular, for six antibiotics it was shown that, as their concentration increased from 0.1 to 5.0 mg ml^{-1}, the recovery increased by 6%–15% depending on the particular analyte. For the concentration of 5.0 mg ml^{-1}, the recovery of antibiotics was in the range of 81.7%–90.6% for extraction from milk and in the range of 76.1%–82.3% for extraction from honey.

A similar dependence of the recovery on the concentration of the loaded analyte was elucidated[191] for the extraction of melamine from milk. Melanin present as a component of many plastics is a potent carcinogen. A 16-fold increase in the concentration of the loaded sample resulted in an increase in the recovery by 74.4% to 88.5%.

A microextraction technique using a molecularly imprinted monolith formed in plastic micropipette tips was proposed[194] (Fig. 4). Three sorts of monolithic materials were synthesized based on different copolymers — MAA–EDMA, AAm–EDMA and MAA–4-VP–EDMA molecularly imprinted with the pesticide pirimicarb. A comparison of the efficiency of imprinting and extraction of pirimicarb from model solutions showed the best performance for the sorbent based on MAA–EDMA. Extraction of pirimicarb from tomatoes and pears was carried out; the subsequent analysis of the extract by reversed phase chromatography showed high recovery of the pesticide close to 100%.

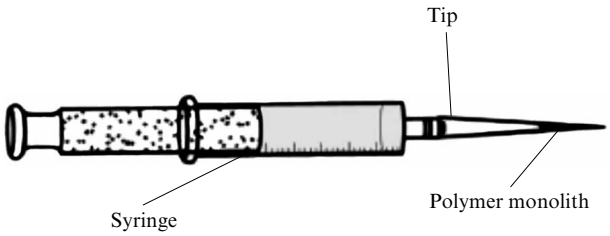


Figure 4. Scheme of the solid-phase MIP microextraction monolith manufactured by synthesis of the MIP sorbent in the micropipette tip.[194]

This approach to the fabrication of stationary phases for microextraction has been further developed in a number of studies in environmental analysis. Du *et al.*[192] reported the fabrication of a MIP sorbent based on the MAA–EDMA copolymer for microextraction of the fungicide difenoconazole used to suppress fungal infections and, as a consequence, to increase the shelf life of fruit and vegetables and plant seeds. The manufactured solid-phase extractors were employed for efficient determination of difenoconazole in pears and tap water.

Along with publications reporting organic monoliths, there are papers[193, 195, 196] dealing with the development of the synthesis of hybrid organic-inorganic monolithic MIP sorbents for microextraction. For example, Liu *et al.*[193] used this approach to analyze 2,4-dichlorophenylacetic acid, which is a highly popular herbicide. The recovery of this compound from rice was 80%–94% depending on the initial content in the sample. Zhang *et al.*[196] made the system more complicated by creating an organic-inorganic MIP sorbent selective towards two agents — carbaryl and naproxen. The resulting dual-action sorbent was successfully utilized to quantify these compounds in salad leaves and river water.

Thus, the as yet few examples presented above convincingly demonstrate good prospects of using macroporous MIP monoliths as high-performance sorbents for solid-phase extraction not inferior and sometimes even superior to other systems for solid-phase extraction.

III.4. Dissociation constants of artificial receptor–ligand complexes

Dissociation constant is a key parameter for estimating the strength of specific complexes in the studies of interactions involving natural molecules.[208–210] The lower this parameter, the stronger the complex formed between molecules and, hence, the higher the affinity of these molecules to each other. In essence, MIP systems are synthetic receptors capable of molecular recognition; therefore, the dissociation constants of complexes with the ligands for which these receptors were obtained are often determined.

Most suitable and most often used for the calculation of this value is the Scatchard equation[62, 191, 208]

$$\frac{Q}{c} = \frac{Q_{max} - Q}{K_d} \qquad (7)$$

where Q is the amount of the target compound attached to the sorbent in equilibrium, c is the concentration of the target compound in the solution, Q_{max} is the maximum adsorption capacity of the sorbent, K_d is the dissociation constant of the specific complex.

In practice, Q_{max} and K_d are usually determined from the experimental data on the equilibrium adsorption by plotting the isotherm reflecting the equilibrium capacity of

the adsorbent (Q) at various analyte concentrations in the solution (c).[208] As a rule, this is done using the frontal elution method based on passing solutions of the target compound with definite concentrations through an affine sorbent up to complete saturation of all adsorption sites followed by quantitative desorption of the component to be isolated. Linearization of the adsorption isotherm in the Q/c *vs.* Q or reciprocal ($1/Q$ *vs.* $1/c$) coordinates can serve to calculate Q_{max} and K_d.

The dissociation constants of the monolithic MIP sorbents are in the $10^{-3}-10^{5}$ mol litre^{-1} range.[51, 122, 127, 182, 183] Indeed, in a study of the binding of the hypoglycemic agent nateglinide with a monolithic MIP sorbent imprinted with this compound,[51] the K_d value was found to be 7.40×10^{-3} mol litre^{-1}. It was shown[127] that K_d of the antimicrobial drug sulfametoxazol and its imprint sites generated in the MIP monolith is 1.88×10^{-5} mole litre^{-1}. In some cases MIP monoliths are formed with two types of imprint sites having different specificity. For example, a MIP monolith imprinted with the analgesic agent sinomenine was found[122] to have highly specific ($K_d = 7.3\times10^{-5}$ mol litre^{-1}) and less specific ($K_d = 3.8\times10^{-3}$ mol litre^{-1}) imprint sites. A similar result was demonstrated[182, 183] in analysis of the antibiotic ciprofloxacin; the dissociation constants were 1.64×10^{-4} and 2.92×10^{-3} mol litre^{-1}, respectively.

The specificity of synthetic receptors undoubtedly depends on the preparation conditions and investigation methods, in particular, on how properly the functional monomer–template, monomer–cross-linking agent and monomer–porogens systems were selected and on the conditions of elution, which should be maximally favourable for the formation of a specific complex.

III.5. Reproducibility of the results and sorbent stability

Reproducibility of the results is an important issue in the solution of any analytical problem. One more fairly important condition for successful implementation of solid-phase processes is the sorbent stability. Macroporous monolithic matrices used as stationary phases for dynamic processes are known to have high mechanical, chemical and antimicrobial stability. Since these sorbents are highly cross-linked and, hence, virtually do not swell, the porous structure they possess is very stable, which, in turn, ensures a high level of reproducibility of chromatographic results. Moreover, conditions of synthesis of these materials are relatively simple and easily reproducible, which generally ensures the reproducibility of chromatographic results for comparison of stationary phases from different batches.

In the separation of (*R*)- and (*S*)-naproxen using the (*S*)-naproxen-imprinted monolithic column,[125] the standard deviation of the peak resolution is 4.09%. In a study of a series of MIP columns obtained under identical optimized conditions, the standard deviation of R_s was 7.44%.

Lin *et al.*[155] estimated the reproducibility of the retention factor for the proteins lysozyme and bovine serum albumin (BSA). The standard deviation for one and the same column based on the AAm copolymer with MBAAm was 5.2% for lysozyme-imprinted and 4.7% for BSA-imprinted stationary phases. For a series of sorbents, this value was 8.6% for lysozyme-selective and 7.8% for BSA-selective matrices. Study of the stationary phase stability demonstrated that the column performance remained unchanged during 15 days (more than 100 chromatographic cycles).

High stability of monolithic MIP sorbents in repeated cycles was also confirmed by Djozan *et al.*[185] In particular, for a methamphetamine-imprinted MIP monolith based on MAA–EDMA in 100 methamphetamine adsorption–desorption cycles during a month, the standard deviation of the results was 7.7%. Thus, in all of the described cases, the standard deviation did not exceed 10%, which obviously attests to a high reproducibility of chromatographic results and stability of flow-through MIP monoliths for a larger number of operation cycles.

IV. Supermacroporous cryogels

IV.1. Preparation methods of supermacroporous cryogels

Monolithic stationary phases may represent, apart from conventional polymethacrylate sorbents, also cryostructured supermacroporous gels known under the general name 'cryogels'. The fabrication of these systems and the mechanism of their formation are the subjects of numerous studies reported by research groups headed by Lozinsky[211] and Mattiasson.[57]

The synthesis of cryogels includes freezing of the solution or preformed gel, cross-linking reaction and removal of the solvent (Fig. 5). As the solution is frozen, the growth of the solvent crystals takes place until they get in contact with other crystals. Thus, the solvent crystals form a common extended structure. The phase containing the

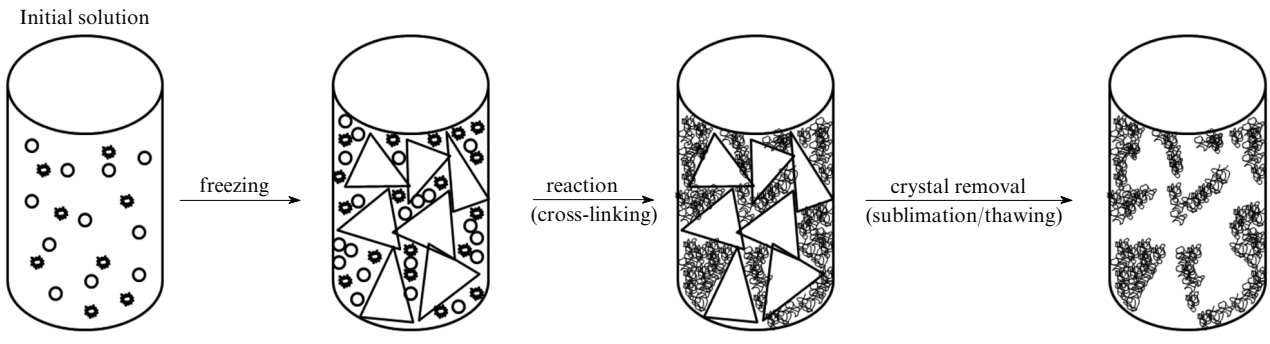


Figure 5. Scheme of formation of supermacroporous monolithic cryogel.

solutes or the gel and concentrated around the crystals is called unfrozen liquid microphase (ULMP), which emphasizes its minor fraction with respect to the total system volume.[212] This cryoconcentration promotes low-temperature reactions, resulting in the formation of the monolithic structure. The last stage of the synthesis is removal of the solvent crystals *via* drying (usually, sublimation)[213] or thawing.[214] Thus, the solvent crystals function as pore-forming agents and also determine the pore morphology for the resulting monolith (Fig. 6).

The matrix structure is considerably affected by the process temperature and the solvent nature.[211,213] On cooling, the solvent should crystallize but not be vitrified because this would preclude cryoconcentration and cryostructuring. The process temperature should be such as to maintain the ULMP and, hence, the mobility of molecules it contains, which is needed for chemical reactions to proceed. The effect of the solution freezing and keeping the resulting frozen system at a definite temperature on the morphology of the cryogels thus formed has been described.[215]

In most cases, water served as the solvent;[57,214] this is the solvent of choice as regards the biocompatibility and biosafety of the fabricated stationary phases. However, this restricts the range of suitable initial compounds to only hydrophilic molecules, which should be cross-linked to retain the monolithic structure. In some studies, it was shown that organic solvents such as dimethyl sulfoxide, dioxane, formamide, nitrobenzene, benzene and cyclohexane can be used to prepare hydrophobic cryogels.[216–223]

During the preparation of monolithic cryogels, both copolymerization of mono- and bifunctional monomers (cryopolymerization) and cross-linking of the existing polymer precursors can take place in the ULMP. The functional monomers used in monolith preparation by cryopolymerization include acrylamide,[224–227] *N*,*N*′-dimethylacrylamide,[227,228] 2-hydroxyethyl methacrylate,[229,230] *N*-isopropylacrylamide[231] and so on. Bifunctional cross-linking agents include MBAAm,[225] EDMA[232] and polyethylene glycol dimethacrylate.[233] For the preparation of monoliths based on finished polymers, polyvinyl alcohol,[234,235] agarose and alginate[236] and other natural and synthetic polymers have been successfully used.[237] Furthermore, cryostructuring of suspensions of monodispersed particles to give a monolithic phase has been reported.[238,239]

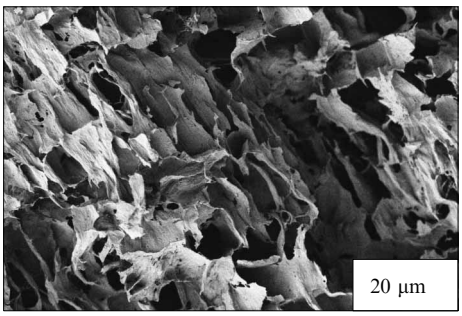


Figure 6. SEM image of a macroporous cryogel obtained by cryostructuring of polyvinyl alcohol followed by cross-linking with glutaraldehyde.
Preparation procedure: 50 μl of a solution of glutaraldehyde (25 mass %) was added to 2 ml of an aqueous solution of polyvinyl alcohol (5 mass %), the mixture was frozen at −12 °C and kept at this temperature for 10 h and then the sample was freeze-dried.

Of interest is the fact that during cryostructuring of a gel under conditions of pseudosteady state crystal growth,[240] the crystal grow along the freezing direction. As a result, cryogels obtained upon unidirectional freezing followed by freeze-drying possess macropores as parallel channels extended along the freezing direction.[241] Thus, the freezing anisotropy is important for the morphology of the monolithic phases formed.

Apart from the fabrication of supermacroporous polymeric materials, there have been reported methods for the preparation of inorganic monoliths with the macroporous structure formed during freezing of the freshly prepared gels. The latter are formed with the participation of both inorganic and polymeric precursors. For example, in the synthesis of monoliths based on silica[241] and its mixtures with alumina[213] and titania,[242] gelation of a sodium silicate solution was induced by a decrease in the pH, while for the formation of supermacroporous materials based on carbon nanotubes,[243] silk fibroin was used as the gel-forming component. In many cases, the removal of the frozen solvent was followed by high-temperature treatment of the obtained materials in which the polymeric component burned out and the inorganic structure of the matrix was stabilized due to sintering.

The key advantages of the cryogels obtained as the monolithic phase include the supermacroporous structure and good interpore connectivity. For example, the presence of 10–100 μm pores allows one to use the cryogels not only for the chromatography of the proteins[244,245] but also for the separation of large particles including whole cells.[246,247] The advantages of cryogels include relatively simple preparation methods and the possibility of varying the chemical composition and pore morphology. The key disadvantage of the cryogels used for the chromatographic separation of proteins and low-molecular-mass compounds is the low adsorption capacity related to the small specific surface areas of supermacroporous stationary phases. Moreover, owing to the cryoconcentration of the polymer in the ULMP, its density in the pore walls is rather high and there are no micropores accessible for the penetration of proteins and low-molecular-mass compounds. In order to increase the effective surface area, attempts are made to create smaller pores in the macropore walls by introducing salts (NaCl, $CaCl_2$) or solvents (acetone, methanol) into the polymerization mixture.[248] An increase in the adsorption capacity of the cryogel monoliths can also be achieved by grafting polymer molecules on their surface.[225,249,250]

Functional groups can be introduced into cryogels by either modification of the finished material or addition of functional monomers to a cryopolymerization mixture.[216,244,251] Processes for the preparation of composite cryogels suitable for modification and containing nanoparticles bearing functional groups, which have been introduced at the cryostructuring stage, have been reported.[252]

The presence of large interconnected pores in cryogels promotes their wide use not only for the separation of biological molecules and particles but also for biotechnological purposes,[214,253] for example, for immobilization of enzymes[254–256] and bacteria[257] in order to obtain biocatalytic and bioproducing systems, respectively.

Mention should also be made of the increasing interest in the application of monolithic supermacroporous cryogels

as the scaffolds for cell immobilization and living tissue growth in the tissue engineering.[257, 258] Most publications along this line are related to cryostructuring and cross-linking of natural polymers — gelatine,[259] chitosan,[260] agarose [261] and other.

IV.2. Characteristic features of the preparation of molecularly imprinted polymeric cryogels

Two key methods for the preparation of monolithic MIP cryogels can be distinguished:

— formation of polymeric MIP particles, which are subsequently embedded into the composite supermacroporous monolithic matrix during cryogelation (Fig. 7, path *a*);

— imprinting directly into the cryogel structure during cryopolymerization (Fig. 7, path *b*).

In the former case, exactly the particles with voids that have remained after the template removal determine the cryogel capacity for the specific adsorption of the analyte. In most cases, the MIP particles incorporated in the composite cryogel were obtained by block polymerization and then the product was ground and sieved to isolate a requited fraction.[59, 262, 263] It is also possible to obtain MIP particles by suspension [264] and precipitation [265] polymerization. Also, imprinting of bromate anions into chitosan-based microspheres cross-linked with epichlorohydrin was described.[265, 266]

The composite monolithic matrix with embedded MIP particles is formed at the stage of gelation, which may occur due to both cryocopolymerization of mono- and bifunctional monomers [262, 267] and cross-linking of the existing synthetic or natural macromolecules.[59] Of special interest is the publication [265] in which the propranolol-imprinted particles containing amino groups on the surface are covalently trapped in the monolithic matrix based on polyvinyl alcohol upon the addition of glutaraldehyde. The latter is required for both cross-linking of polyvinyl alcohol and binding of the particles.

A drawback of the composite cryogels is the substantial decrease in the flow rate upon inclusion of MIP particles into the initial supermacroporous material. Indeed, it has been shown [262] that an increase in the concentration of 38–90 and 15–38 μm MIP particles embedded into a monolithic matrix based on AAm–MBAAm with a pore diameter of 10–100 μm from 50 to 200 mg ml^{-1} results in the decrease in the flow rate from 2.5 to 0.45 and from 5 to 0.9 ml min^{-1}, respectively. This is evidently due to similar sizes of the MIP particles and pores of the monolithic phase, which is confirmed by the fact that the highest flow rate (13 ml min^{-1}) is achieved when the <15 μm fraction of particles is used and the particle concentration is 50 mg ml^{-1}.

In the latter case, namely, imprinting into the cryogel (see Fig. 7, path *b*), the template is added directly to the mixture for cryogelation, which occurs in this case as cryopolymerization. Note that most studies dealing with the preparation of MIP cryogels by this method imply the

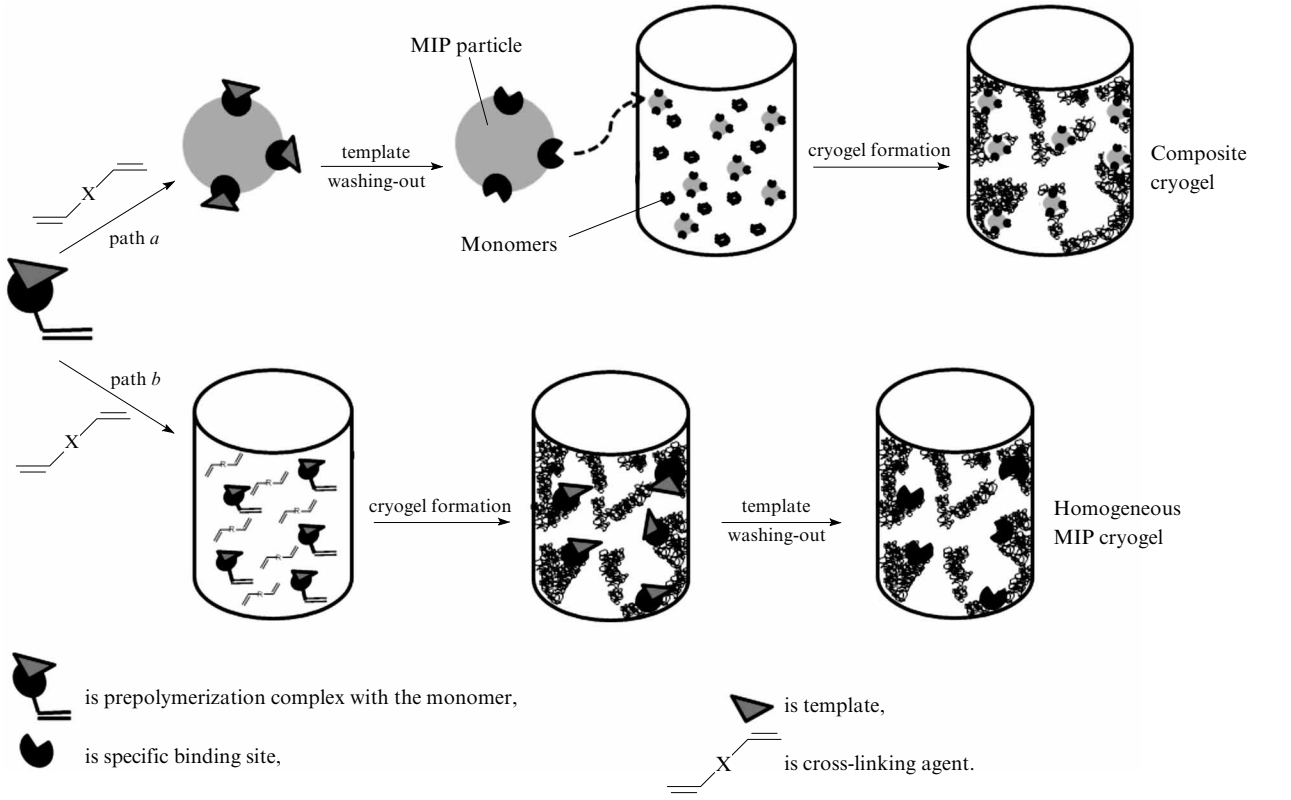


Figure 7. Preparation paths of supermacroporous monolithic MIP cryogel.
Path *a*: formation of polymeric MIP particles, which are then embedded into a composite supermacroporous monolith during cryogel formation; path *b*: imprinting directly into the cryogel structure during cryopolymerization.

use of proteins [266, 268–273] or rather large biomolecules [274] as the templates. Apparently, this is due to drawbacks of imprinting of small molecules and ions, which requires fairly rigid highly cross-linked systems such as the above-described MIP particles. The high cross-linking of the matrix in the case of imprinting of biomacromolecules gives rise to certain restrictions such as mass transfer by the diffusion mechanism, incomplete removal of the template, slow binding and low adsorption capacity. Therefore, the imprinting of protein macromolecules should better be accomplished using a less cross-linked and more mobile hydrogel structure. Indeed, the functional monomer : cross-linking monomer ratios (5–10) used for protein imprinting are, most often, higher than those used to prepare MIP particles (1–4).

Nevertheless, studies devoted to the imprinting of small ions directly into the cryogel structure are in progress. For example, formation of the cryogel based on 2-hydroxyethyl methacrylate and MBAAm imprinted with iron ions has been reported.[275] The authors rationalized the advantage of using particularly this approach to the preparation of supermacroporous cryogels by the possibility to remove the excess of iron directly from the blood of β-thalassemia patients without special preparation. Large pore size and high interconnectivity of the pore structure ensure in this case free migration of blood cells through the stationary phase.

The flow rate of the mobile phase in homogeneous MIP cryogels is mainly determined by the pore size ad interconnectivity. The pore size is usually 10–150 μm. The pore interconnectivity typical of cryogel-based monoliths accounts for the possibility of using high flow rates of the mobile phase. It was shown [263] that the flow rate is also affected by the ratio of the functional to cross-linking monomers. An increase in the AAm : MBAAm molar ratio from 3 to 12 increases the flow rate from 30 to 55 ml min^{-1}.

It is important that the molecular imprinting principles are the same for both methods for the preparation of cryogels. Both for preparation of MIP particles and for imprinting directly into the cryogel structure, functional and cross-linking monomers are used. In some studies, imprinting was accomplished merely by polymerization in the presence of the template,[263, 266] whereas in other works, polymerization was preceded by the formation of a prepolymerization complex with a special monomer; most often, methacryloyl amino acid derivatives were used for this purpose.[265, 269, 270] Unlike the mentioned studies, Bereli *et al.*[269] carried out the template imprinting during cross-linking of chitosan microspheres with epichlorohydrin.

IV.3. Applications of molecularly imprinted polymeric cryogels

Cryogels are fairly convenient materials for molecular imprinting; almost all authors note the relative ease of obtaining stationary phases and their advantages typical of monoliths. Nevertheless, the number of publications on this issue is modest compared with the number of papers on imprinting using classical monoliths. The principal studies in this area were accomplished by Swedish and Turkish research groups. Data on the application of both composite and homogeneous MIP cryogels of various compositions are summarized in Tables 3 and 4, respectively.

The vast majority of studies dealing with the fabrication of MIP cryogels are devoted to the solid-phase extraction of ions, pharmaceuticals, metabolites, proteins and so on. The discussed stationary phases can be classified most easily in terms of applications. Almost all publications fall into one of the following three categories: water purification for environmental protection, removal of toxic metabolites from the blood flow and isolation and purification of biotechnology products.

IV.3.a. Application of molecularly imprinted polymeric cryogels for environmental protection

An important issue in the environmental protection technologies is the removal of potentially hazardous pollutants present in low concentrations (micro- or nanogrammes per millilitre), for example, in waste waters. In this case, the use of bacteria for the removal of these substances is inefficient as bacteria cannot utilize these pollutants to gain energy or as building materials. Therefore, of particular interest is the development of adsorption processes for the removal of pollutants.[58]

The use of MIP cryogels in the environmental protection is beneficial because it is possible to produce adsorbents stable against biodegradation by bacteria. The use of traditional molecularly imprinted polymer particles for solving this problem is restricted by unsatisfactory mechanical properties of columns packed with these particles and slow diffusion mass transfer. The fabrication of the composite monolithic stationary phases by embedding particles into cryogels triggered the development of adsorption processes for water treatment. The enclosing of these adsorbents into plastic housing provided a facility for high-performance waste water treatment.[58, 278, 279]

In recent years, increasing attention has been given to water purification from so-called endocrine disrupting contaminants, that is, chemicals, including hormones, that may affect the endocrine systems of animals and humans. Molecular imprinting was used [59, 280] to obtain composite cryogels bearing 4-vinylpyridine- and ethylene glycol dimethacrylate-based particles with cavities functioning as 17β-estradiol (E2) receptors. High efficiency of hormone trapping was demonstrated. The amount of analyte recovered after passing two litres of waste water at a 50 ml min^{-1} flow rate through the monolith was 2 μg, which corresponds to virtually complete retrieval over a period of 40 min. For comparison, with a beaded MIP sorbent, the maximal flow rate was 1 ml min^{-1}, which implied process duration of 6 h. In this case, only 10% of E2 was recovered from the waste water.

Along with the efficiency, Le Noir *et al.*[59] demonstrated high selectivity of the obtained MIP cryogel. They also studied the effect of the preparation method on the ability of the MIP cryogel to specifically absorb E2 as compared with the composite cryogel in the absence of a template (NIP cryogel). The MIP particles were embedded into the cryogel by the following procedures:

— AAm–MBAAm cryopolymerization;

— cross-linking of polyvinyl alcohol with glutaraldehyde;

— cross-linking of chitosan with glutaraldehyde.

In the case of cross-linking of polymers, the binding of E2 to the MIP sorbent was much higher than to the NIP sorbent. Conversely, in the case of cryopolymerization, the difference between the amounts of hormone recovered after sorption were approximately equal for both sorbents. The authors attributed this to blockage of the

Table 3. Preparation conditions and applications of composite MIP cryogels.

Template	MIP particles			Monolithic MIP cryogel		Application	Ref.
	monomer for complex formation	functional monomer	cross-linking monomer	functional monomer	cross-linking monomer		
$AsCl_3$	2-VP + 4-hydroxybiphenyl	2-VP	EDMA	AAm	MBAAm	water purification from arsenic ions	263
4,4′-Dihydroxy-2,2-diphenyl-1,1,1,3,3,3-hexafluoropropane	–	4-VP	TRIM	AAm	MBAAm	extraction of BPA[a] from water and wine	262
Propranolol	–	MAA	TRIM	PVA particles	glutaraldehyde	separation of drug mixtures	265
Glucuronic acid	*N*-methacryloyl-L-histidine – Cu^{2+}	–	EDMA	AAm	MBAAm	isolation and purification of hyaluronic acid	264
Bilirubin	*N*-methacryloyl-L-tyrosine	methyl ester of HEMA	EDMA	HEMA	MBAAm	removal of bilirubin from blood	267
17β-Estradiol	–	4-VP	EDMA	AAm	MBAAm	water purification from endocrine disruptor	59
$NaBrO_3$	–	chitosan microspheres	epichlorohydrin	AAm	MBAAm	potable water purification from bromate anions	266

[a] Bisphenol A.

Table 4. Preparation conditions and applications of homogeneous MIP cryogels.

Template	Monomer for complex formation	Functional monomer	Cross-linking monomer	Application	Ref.
Fab	*N*-methacryloyl-L-histidine – Cu^{2+}	HEMA	MBAAm	isolation and purification of IgG	270
Lysozyme	*N*-methacryloyl-L-histidine	HEMA	MBAAm	isolation and purification of lysozyme	269
Bilirubin	*N*-methacryloyl-L-tyrosine	HEMA	MBAAm	removal of bilirubin from blood	274
BSA	–	AAm	MBAAm	purification of BSA	273
Alpha-interferon	*N*-methacryloyl-L-histidine – Cu^{2+}	HEMA	MBAAm	isolation and purification of α-interferon	276
Myoglobin	*N*-methacryloyl-L-tryptophane	HEMA	MBAAm	isolation and purification of myoglobin	268
Cytochrome *c*	*N*-methacryloyl-L-histidine – Cu^{2+}	HEMA	MBAAm	isolation and purification of cytochrome *c*	271
Histidine	*N*-methacryloyl-L-histidine	HEMA	MBAAm	Isolation and purification of lysozyme	277
$Fe(NO_3)_2 \cdot 9\,H_2O$	*N*-methacryloyl-L-cysteine	HEMA	MBAAm	removal of iron ions from blood	275

Note. The following designations are used: Fab is the antigen-binding fragment of immunoglobulin molecule; IgG is immunoglobulin G.

specific binding sites of E2 upon polymerization during cryoconcentration.

Yet another interesting example of application of the composite MIP cryogel for the solid-phase extraction of the hazardous endocrine disrupting contaminant, bisphenol A (BPA), which can function in the body similarly to estrogens, is based on imprinting of the template mimetic rather than the template itself. The replacement of the native template by the mimetic is due to the fact that during polymerization in the presence of BPA, the imprinted binding sites are formed deep within the bulk of the polymer matrix, which restricts the quantitative extraction of the template. As a result, the sorbent capacity decreases and the probability of contamination of the samples being treated increases. Baggiani *et al.*[262] used the fluorinated bisphenol A derivative (FBPA) — 4,4′-dihydroxy-2,2-diphenyl-1,1,1,3,3,3-hexafluoropropane as the template. The FBPA molecule mimics well the target analyte, as it has a similar structure and rigidity but differs by higher hydrophobicity. The latter circumstance facilitates the removal of FBPA from the resulting composite MIP cryogels. A study of the selectivity of sorbents using BPA and FBPA and other compounds structurally similar to BPA demonstrated that the maximal retention is observed for FBPA. The retention factor for BPA is only 1.5-fold lower than that for FBPA, whereas for other analogous compounds this ratio was 3–10. High efficiency of BPA extraction was shown. The recovery of the contaminant at concentrations from 1 to 100 μg ml^{-1} in the samples was 94% to 102%, respectively.

Apart from the removal of endocrine disrupting contaminants, of high value for environmental protection is the extraction of minor quantities of pharmaceutical drugs. Using the composite cryogel imprinted with the β-blocker propranolol, Unluer *et al.*[264] successfully recovered this agent from complex mixtures, in particular, from bovine plasma containing β-blockers of various structures. The possibility of extracting not only one compound but a group of structurally related compounds was also demonstrated. The design of a multitemplate MIP cryogel for the extraction of a number of acid-based pharmaceuticals (ibuprofen, naproxen, ketoprofen, diclofenac) from contaminated water was reported.[281] For this purpose, all of the listed compounds were imprinted simultaneously. The authors ascertained a high capacity of the MIP sorbent towards all of the above-mentioned drugs.

One more important example of application of MIP cryogels for increasing the quality of water resources is extraction of hazardous ions. Onnby *et al.*[263] compared the adsorption of highly toxic arsenic ions on composite cryogels containing alumina nanoparticles or MIP particles. It is of interest that despite the lower adsorption capacity of the composite cryogels (5–7 mg of As per g of the adsorbent) containing MIP particles compared with the matrices bearing nanoparticles (17–20 mg of As per g of the adsorbent), the former were considerably more selective. It is obvious that the high adsorption capacity is caused by the great surface area of the nanoparticle-bearing sorbent, while the selectivity of the composite MIP cryogel is dictated by the formation of cavities capable of selective trapping of arsenic ions. A similar effect was observed by Hajizadeh *et al.*,[266] who studied selective removal of bromate anions from potable water: the adsorption capacity and the process speed were higher for the inorganic adsorbent but the selective binding of bromate anions was observed particularly for MIP adsorbents.

IV.3.b. Application of molecularly imprinted polymeric cryogels for removal of toxic metabolites from the blood flow

A serious problem encountered in some diseases and dysfunctions is the formation of toxic metabolites, which are accumulated in the blood. Therefore, it is a challenge to extract these compounds from blood plasma by, *e.g.*, plasmapheresis. Blood and plasma are multicomponent systems that contain, apart from toxic metabolites, also substances needed for living. This fact enhances researchers' interest in the use of MIP sorbents, including MIP cryogels, for blood purification. The use of stationary phases of the latter type is especially beneficial due to the presence of supermacropores and highly developed pore connectivity, which ensures free migration of not only large macromolecules but also colloid-sized particles through the sorbent.

Bilirubin extraction from the human blood plasma using both composite[262] and homogeneous[274] MIP cryogels has been studied. In the case of composite stationary phases, higher adsorption capacity was observed (10.0 mg g^{-1} of the polymer) compared to homogeneous supports (3.7 mg g^{-1} of the polymer), which is probably attributable to the greater effective surface area, and, hence, a greater number of bilirubin-binding sites in the former case. By varying the amount of particles introduced into the composite, it is possible to control the adsorption capacity of the sorbent. As the amount of introduced particles increases from 10 to 300 mg, the amount of adsorbed bilirubin increases from 3.5 to 10 mg g^{-1}. Further increase in the particle content no longer results in an increase in the adsorption capacity.

For both sorbents, an increase in the flow rate of the mobile phase from 1 to 4 ml min^{-1} resulted in a 5-fold decrease in the amount of retained analyte. Also, both adsorbent displayed similar high selectivities towards recovery of the target analyte from a bilirubin + testosterone + cholesterol mixture, satisfactory hemocompatibility and almost invariable adsorption capacity after ten adsorption–desorption cycles.

The major benefit of homogeneous MIP cryogels is the possibility of bilirubin adsorption directly from blood without removal of its cell components. This is due to suitable pore size and morphology: blood cells of 2 to 20 μm size can pass through the stationary phase.[274]

In view of this possibility, a homogeneous MIP cryogel for extraction of excess iron ions accumulated in the blood of β-thalassemia patients has been developed.[275] The formation of specific cavities for binding iron ions took place during cryopolymerization. The prepolymerization complex *N*-methacryloyl-L-cysteine–Fe^{3+} was used. After formation of the cryogel, the iron ions were desorbed with a 0.1 M solution of ethylenediaminetetraacetic acid. Good hemocompatibility of the adsorbent and the presence of numerous macropores (73% of the total number of pores) were demonstrated. The adsorption capacity of the MIP cryogel in the extraction of iron ions from the blood plasma was 60 μg g^{-1}. The capacity towards Cd^{2+} and Ni^{2+} ions did not exceed 10 μg g^{-1}, which is indicative of high selectivity of the obtained adsorbent.

IV.3.c. Application of molecularly imprinted polymeric cryogels for purification of biotechnology products

The molecularly imprinted monolithic stationary phases prepared by cryopolymerization are also of high practical value for the isolation and purification of various biotechnological products, most often, proteins. It has been noted above (see Section IV.2) that for the imprinting of biomacromolecules, lower degrees of cross-linking should be used than for small ions and molecules. Hence, for the preparation of MIP proteins, imprinting directly into the cryogel structure is used. As in the case of classical monoliths, almost in all cases, a prepolymerization complex involving methacryloyl amino acid derivatives is formed prior to cryopolymerization (see Table 4).

V. Conclusion

Macroporous monolithic sorbents proposed in the late 1980s and still remaining at the cutting edge of research and development are characterized by predominance of the convective mass transfer mechanism over traditional diffusion. The accelerated interfacial exchange inside the pore space that is highly permeable for a liquid flow enables separation within several minutes or even seconds, which is beneficial from the standpoints of both the process productivity and the economical cost for isolation of valuable biological compounds, in particular, genetic engineering products, pharmaceuticals and so on. The introduction of highly specific adsorption sites (ligands) complementary to the analyte into the surface structure by covalent attachment *via* reactive groups of the support has a number of limitations. They include high cost of biological ligands, stringent immobilization conditions minimizing the impact on the active conformation of the biological adsorption site and so on. An alternative approach often successfully competing with the traditional ligand immobilization is specific surface functionalization during the sorbent synthesis based on the molecular imprinting principle. The imprinting, *i.e.*, introduction of molecular templates — compounds to be subsequently isolated or related compounds — into the monomer reaction mixture during the synthesis, aroused considerable interest for the preparation of small-particle (grained) sorbents finely tuned for isolation of one component from a complex mixture. This review considers the results of studies of the preparation processes and applications of this type of supports behaving as artificial receptors but based on monoliths.

Analysis of the modern literature, on the one hand, showed a substantial growth of the interest in the macroporous MIP monoliths in the last decade and, on the other hand, served to evaluate the prospects of further development of this research and practical area. For example, unlike the molecular imprinting of low-molecular-mass compounds, the imprinting of proteins in macroporous monolithic materials is a virtually unexplored area: about a hundred publications dealing with the synthesis and study of macroporous polymeric monoliths molecularly imprinted with various low-molecular-mass compounds are now available but only three publications on the protein imprinting were found (see Table 1). Furthermore, there are no publications in the current periodicals that would illustrate the possibility of designing artificial receptors based on macroporous monolithic MIP materials for catalytic processes, although they have been described long ago for dispersed MIP systems.[281]

Recently, it was reported that macroporous monolithic layers are suitable as effective platforms for 3D biochips based on the biological recognition principle.[282, 283] In this respect, it appears pertinent to utilize molecular imprinting for the design of non-flow-through macroporous monolithic layers for parallel microanalysis implemented as a biochip bearing artificial receptors instead of the immobilized bioprobes.

The review covers the major applications of the monolithic stationary phases based on MIP cryogels: water treatment to protect the environment, removal of toxic metabolites from the blood of patients with various diseases and isolation and purification of biomacromolecules for biotechnology (most often, proteins). In the first two cases, the problems of elaboration of effective adsorbents have been virtually solved; the imprinting of proteins and other biomacromolecules is still a scientific challenge (of interest in this respect are studies that approach the specific recognition of biomacromolecules *via* imprinting of molecular fragments — epitopes).

The possibility of recognition of biomolecules by the imprinted receptors and the supermacroporous nature and high degree of porosity of cryogels open up broad prospects for the use in biotechnology and biomedicine. Indeed, studies dealing with the separation of whole cells on cryogels utilizing ion exchange,[283] hydrophobic[284] and chelating[285] interactions have already been published. The use of contacts between cell membrane components and imprints of their fragments can give rise to novel stationary phases for the separation of large biological objects — bacteria, viruses and eukaryotic cells.

Yet another application of molecularly imprinted cryogels is tissue engineering. The formation of scaffolds — supermacroporous media providing the three-dimensional cell growth for the formation of a new living tissue — often proceeds *via* cryogel formation in organic[286, 287] or aqueous[288] media. It is obvious that successful growth of a living tissue requires that scaffolds should mimic the properties of a natural intercellular matrix, in particular, provide for the specific interactions of the cellular receptors with the material surface for the control of cell adhesion, growth and differentiation processes.[289] These interactions can be controlled by introducing special bioligands into the scaffolds.[290, 291] There are publications devoted to modification of the surface of biomaterials using the molecular imprinting technique.[292, 293] Thus, molecular imprinting appears a fairly promising technique for the design of efficient biorecognition systems by imprinting the ligands able to specifically interact with cell receptors, thus inducing the desired biochemical processes in the cells.

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References

1. M Delaage, in *Molecular Recognition Mechanisms* (Ed. M Delaage) (Weinheim: VCH, 1991) p. 1
2. G Wulff, P A Kollman, J M Lehn *J. Mol. Struct. (THEOCHEM)* **93** 153 (1983)
3. Y Masaki, N Hideyuki *Anal. Lett.* **15** 1197 (1982)

4. R Franski, B Gierczyk, G Schroeder *J. Am. Soc. Mass Spectrom.* **20** 257 (2009)
5. X X Zhang, R M Izatt, J S Bradshaw, K E Krakowiak *Coord. Chem. Rev.* **174** 179 (1998)
6. R Ludwig, N T K Dzung *Sensors* **2** 397 (2002)
7. R A Bartsch, P Kus, N K Dalleyc, X Kou *Tetrahedron Lett.* **43** 5017 (2002)
8. M Pittelkow, C B Nielsen, A Kadziola, J B Christensen *J. Inclusion Phenomena Macrocycl. Chem.* **63** 257 (2009)
9. L F B Malta, Y Cordeiro, L W Tinoco, C C Campos, M E Medeiros, O A C Antunes *Tetrahedron: Asymmetry* **19** 1182 (2008)
10. G Wulff, A Sarhan *Angew. Chem., Int. Ed.* **11** 341 (1972)
11. T Takagishi, I M Klotz *Biopolymers* **11** 483 (1972)
12. C Alexander, H S Andersson, L I Andersson, R J Ansell, N Kirsch, I A Nicholls, J O'Mahony, M J Whitcombe *J. Mol. Recogn.* **19** 106 (2006)
13. I Chianella, M Lotierzo, S A Piletsky, I E Tothill, B Chen, K Karim, A P F Turner *Anal. Chem.* **74** 1288 (2002)
14. Y Hoshino, K J Shea *J. Mater. Chem.* **21** 3517 (2011)
15. C Baggiani, G Giraudi, F Trotta, C Giovannoli, A Vanni *Talanta* **51** 71 (2000)
16. J Svenson, I A Nicholls *Anal. Chim. Acta* **435** 19 (2001)
17. A Bossi, F Bonini, A P F Turner, S A Piletsky *Biosens. Bio Electron.* **22** 1131 (2007)
18. C He, Y Long, J Pan, K Li, F Liu *J. Biochem. Biophys. Methods* **70** 133 (2007)
19. X-M Jiang, W Tian, C D Zhao *Talanta* **72** 119 (2007)
20. R Garcia, M Cabrita, A M Costa Freitas *Am. J. Anal. Chem.* **2** 16 (2011)
21. H Zhang, L Ye, K Mosbach *J. Mol. Recogn.* **19** 248 (2006)
22. K I Kitahara, I Yoshihama, T Hanada, H Kokuba, S Arai *J. Chromatogr., A* **1217** 7249 (2010)
23. A P Leshchinskaya, A R Groshikova, I V Polyakova, O A Pisarev, E F Panarin *J. Mol. Imprint.* **1** 17 (2013)
24. R Suedee, C Sonogkram, A Petmoreekul, S Sangkunakup, S Sankasa, N Kongyarit *J. Pharm. Biomed. Anal.* **19** 519 (1999)
25. C Giovanolli, C Baggiani, L Anfossi, G Giraudi *Electrophoresis* **29** 3349 (2008)
26. Y P Huang, Z S Liu, C Zheng, R Y Gao *Electrophoresis* **30** 155 (2009)
27. C J Tan, Y W Tong *Anal. Chem.* **79** 299 (2007)
28. G Wulff *Chem. Rev.* **102** 1 (2002)
29. N Kirsch, J Hedin-Dahlstrom, H Henschel, M J Whitcombe, S Wikman, I A Nicholls *J. Mol. Catal., B* **58** 110 (2009)
30. L Ye, K Mosbach *J. Am. Chem. Soc.* **123** 2901 (2001)
31. X Ding, P Heiden *Macromol. Mater. Eng.* **299** 268 (2014)
32. K Yang, L Zhang, Z Liang, Y Zhang *Anal. Bioanal. Chem.* **403** 2173 (2012)
33. *Monolithic Materials: Preparation, Properties and Applications* (Eds F Svec, T Tennikova, Z Deyl) (Amsterdam: Elsevier, 2003)
34. H Schnecko, O Bieber *Chromatographia* **4** 109 (1971)
35. S Hjerten, J L Liao, R Zhang *J. Chromatogr.* **473** 273 (1989)
36. T B Tennikova, B G Belenkii, F Svec *J. Liq. Chromatogr.* **13** 63 (1990)
37. T B Tennikova, F Svec *J. Chromatogr.* **646** 279 (1993)
38. F Svec, J M J Frechet *Anal. Chem.* **64** 820 (1992)
39. N D Ostryanina, O V Il'ina, T B Tennikova *J. Chromatogr. B* **770** 35 (2002)
40. J Matsui, T Kato, T Takeuchi, M Suzuki, K Yokoyama, E Tamiya, I Karube *Anal. Chem.* **65** 2223 (1993)
41. J Matsui, T Takeuchi *Anal. Commun.* **34** 199 (1997)
42. J Matsui, I A Nicholls, T Takeuchi *Anal. Chim. Acta* **365** 89 (1998)
43. X D Huang, H F Zou, X Q Mao, Q Z Luo, X M Chen, X Z Xiao *Sepu* **20** 436 (2002)
44. X D Huang, H F Zou, X M Chen, Q Z Luo, L Kong *J. Chromatogr., A* **984** 273 (2003)
45. M Zhang, J Xie, Q Zhou, G Chen, Z Liu *J. Chromatogr., A* **984** 173 (2003)
46. A Štrancar, M Barut, A Podgornik, P Koselj, H Schwinn, P Raspor, D Josic *J. Chromatogr., A* **760** 117 (1997)
47. C Viklund, F Svec, J M J Fréchet *Biotechnol. Prog.* **13** 597 (1997)
48. C Kasper, L Meringova, R Freitag, T Tennikova *J. Chromatogr., B* **65** 65 (1998)
49. M B Tennikov, N V Gazdina, T B Tennikova, F Svec *J. Chromatogr., A* **798** 55 (1998)
50. A Podgornik, M Barut, J Jančar, A Štrancar, T B Tennikova *Anal. Chem.* **71** 2986 (1999)
51. J Yin, G Yang, Y Chen *J. Chromatogr., A* **1090** 68 (2005)
52. H Li, Y Liu, Z Zhang, H Liao, L Nie, S Yao *J. Chromatogr., A* **1098** 66 (2005)
53. Z S Liu, Y L Xu, C Yan, R Y Gao *J. Chromatogr., A* **1087** 20 (2005)
54. J Ou, J Dong, T Tian, J Hu, M Ye, H Zou *J. Biochem. Biophys. Methods* **70** 71 (2007)
55. A Seebach, A Seidel-Morgenstern *Anal. Chim. Acta* **591** 57 (2007)
56. V I Lozinsky, F M Plieva, I Y Galaev, B Mattiasson *Bioseparation* **10** 163 (2001)
57. F M Plieva, H Kirsebom, B Mattiasson *J. Sep. Sci.* **34** 2164 (2011)
58. G Ertürk, B Mattiasson *J. Chromatogr., A* **1357** 24 (2014)
59. M Le Noir, F Plieva, T Hey, B Guieysse, B Mattiasson *J. Chromatogr., A* **1154** 158 (2007)
60. H Liu, K H Row, G Yang *Chromatographia* **61** 429 (2005)
61. C Zheng, Y P Huang, Z S Liu *J. Sep. Sci.* **34** 1988 (2011)
62. J Tan, Z T Jiang, X P Yan *Trends Anal. Chem.* **39** 207 (2012)
63. G Wulff, A Sharhan, K Zabrocki *Tetrahedron Lett.* **14** 4329 (1973)
64. G Wulff, R Vesper, E Grobe, A Sarhan *Makromol. Chem.* **178** 2799 (1977)
65. G Wulff *Angew. Chem., Int. Ed.* **34** 1812 (1995)
66. K J Shea, E A Thompson *J. Org. Chem.* **43** 4253 (1978)
67. K J Shea, E A Thompson, S D Pandey, P S Beauchamp *J. Am. Chem. Soc.* **102** 3149 (1980)
68. K J Shea, D Y Sasaki *J. Am. Chem. Soc.* **111** 3442 (1989)
69. G Wulff, J Vietmeier *Makromol. Chem.* **190** 1727 (1989)
70. F Puoci, G Cirillo, M Curcio, F Iemma, O I Parisi, U G Spizzirri, N Picci, in *Biotechnology of Biopolymers* (Ed. M Elnashar) (Rijeka; Sciyo, 2010) p. 547
71. R Arshady, K Mosbach *Macromol. Chem. Phys.* **182** 687 (1981)
72. M Komiyama, T Takeuchi, T Mukawa, H Asanuma *Molecular Imprinting: from Fundamentals to Applications* (Weinheim: Wiley-VCH, 2003)
73. B Sellergren, L Andersson *J. Org. Chem.* **55** 3381 (1990)
74. O D Gendrikson, A V Zherdev, B B Dzantiev *Usp. Biol. Khim.* **46** 149 (2006) [a]
75. B Sellergren, K J Shea *J. Chromatogr., A* **635** 31 (1993)
76. A Martin-Esteban *Fresenius J. Anal. Chem.* **370** 795 (2001)
77. N M Ezhova, I S Garkushina, O A Pisarev *Appl. Biochem. Microbiol.* **47** 698 (2011)

78. N M Ezhova, I V Polyakova, O A Pisarev *Appl. Biochem. Microbiol.* **45** 221 (2009)
79. O Brüggemann, K Haupt, L Ye, E Yilmaz, K Mosbach *J. Chromatogr., A* **889** 15 (2000)
80. P A G Cormack, A Z Elorza *J. Chromatogr., B* **804** 173 (2004)
81. L Y Zhang, G X Cheng, C Fu *React. Funct. Polym.* **56** 167 (2003)
82. L Y Zhang, G X Cheng, C Fu, X H Liu, X S Pang *Adsorption Sci. Technol.* **21** 775 (2003)
83. K E J Barrett *Dispersion Polymerization in Organic Media* (London: Wiley, 1975)
84. L Ye, P A G Cormack, K Mosbach *Anal. Commun.* **36** 35 (1999)
85. L Ye, K Mosbach *React. Funct. Polym.* **48** 149 (2001)
86. J Wang, P A Cormack, D C Sherrington, E Khoshdel *Angew. Chem., Int. Ed.* **42** 5336 (2003)
87. K Hosoya, K Yoshizako, N Tanaka, K Kimata, T Araki, J Haginaka *Chem. Lett.* **23** 1437 (1994)
88. G Masci, F Aulenta, V Crescenzi *J. Appl. Polym. Sci.* **83** 2660 (2002)
89. L Piscopo, C Prandi, M Coppa, K Sparnacci, M Laus, A Lagana, R Curini, G D'Ascenzo *Macromol. Chem. Phys.* **203** 1532 (2002)
90. C Sulitzky, B Ruckert, A J Hall, F Lanza, K Unger, B Sellergren *Macromolecules* **35** 79 (2002)
91. N W Turner, C W Jeans, K R Brain, C J Allender, V Hlady, D Britt *Biotechnol. Prog.* **22** 1474 (2006)
92. Y Lv, T Tan, F Svec *Biotechnol. Adv.* **31** 1172 (2013)
93. O A Pisarev, I V Polyakova *Trends Chromatogr.* **7** 85 (2012)
94. F Svec, T B Tennikova *J. Bioact. Compat. Polym.* **6** 393 (1991)
95. T B Tennikova, M Bleha, F Svec, T V Almazova, B G Belenkii *J. Chromatogr.* **555** 97 (1991)
96. N I Dubinina, O I Kurenbin, T B Tennikova *J. Chromatogr., A* **753** 217 (1996)
97. F Svec, J M J Frechet *J. Chromatogr., A* **702** 89 (1995)
98. F Svec *J. Sep. Sci.* **28** 729 (2005)
99. K Stulik, V Pacakova, J Suchankova, P Coufal *J. Chromatogr., B* **841** 79 (2006)
100. E Vlakh, T B Tennikova *J. Chromatogr., A* **1216** 2637 (2009)
101. G Guiochon *J. Chromatogr., A* **1168** 101 (2007)
102. A-M Siouffi *J. Chromatogr., A* **1126** 86 (2006)
103. E Vlakh, T Tennikova *J. Sep. Sci.* **30** 2801 (2007)
104. E Calleri, S Ambrosini, C Temporini, G Massolini *J. Pharm. Biomed. Anal.* **69** 64 (2012)
105. T Bamba, E Fukusaki *J. Sep. Sci.* **32** 2699 (2009)
106. A Galarneau, J Iapichella, D Brunel, F Fajula, Z Bayram-Hahn, K Unger, G Puy, C Demesmay, J-L Rocca *J. Sep. Sci.* **29** 844 (2006)
107. E G Vlakh, E F Maksimova, T B Tennikova *Polym. Sci., Ser. B* **55** 55 (2013) [*Vysokomol. Soedin., Ser. B* **55** 209 (2013)]
108. E A Ponomareva, M V Volokitina, D O Vinokhodov, E G Vlakh, T B Tennikova *Anal. Bioanal. Chem.* **405** 2195 (2013)
109. A Kurganov *Anal. Chim. Acta* **775** 25 (2013)
110. S Eeltink, F Svec *Electrophoresis* **28** 137 (2007)
111. O G Potter, E Hilder *J. Sep. Sci.* **31** 1881 (2008)
112. E Vlakh, A Novikov, G Vlasov, T Tennikova *J. Pept. Sci.* **10** 719 (2004)
113. M V Volokitina, E G Vlakh, G A Platonova, D O Vinokhodov, T B Tennikova *J. Sep. Sci.* **36** 2793 (2013)
114. B Bakry, G K Bonn, D A Mair, F Svec *Anal. Chem.* **79** 486 (2007)
115. E F Maksimova, E G Vlakh, T B Tennikova *J. Chromatogr., A* **1218** 2425 (2011)
116. E S Sinitsyna, E G Vlakh, M Y Rober, T B Tennikova *Polymer* **52** 2132 (2011)
117. M Rober, J Walter, E Vlakh, F Stahl, C Kasper, T Tennikova *Anal. Chim. Acta* **644** 95 (2009)
118. E G Vlakh, T B Tennikova *J. Sep. Sci.* **36** 1149 (2013)
119. S J Zhang, G L Yang, Z S Zheng, Y Chen *Chromatographia* **69** 615 (2009)
120. J J Ou, L H Hu, L G Hu, X Li, H F Zou *Talanta* **69** 1001 (2006)
121. J J Ou, S W Tang, H F Zou *J. Sep. Sci.* **28** 2282 (2005)
122. X J Liu, C B Ouyang, R Zhao, D H Shangguan, Y Chen, G Q Liu *Anal. Chim. Acta* **571** 235 (2006)
123. X D Huang, L Kong, X Li, C J Zheng, H F Zou *J. Mol. Recogn.* **16** 406 (2003)
124. H Yan, K H Row *Biotechnol. Bioproc. Eng.* **11** 357 (2006)
125. Y P Huang, S J Zhang, X Wu, Q W Zhang, Z S Liu *Chromatographia* **70** 691 (2009)
126. Z Y Li, Z S Liu, Q W Zhang, H Q Duan *Chin. Chem. Lett.* **18** 322 (2007)
127. L Q Lin, Y C Li, Q Fu, L C He, J Zhang, Q Q Zhang *Polymer* **47** 3792 (2006)
128. L-N Mu, X-H Wang, L Zhao, Y-P Huang, Z-S Liu *J. Chromatogr., A* **1218** 9236 (2011)
129. S W Zhang, J Xing, L S Cai, C Y Wu *Anal. Bioanal. Chem.* **395** 479 (2009)
130. M M Zheng, R Gong, X Zhao, Y Q Feng *J. Chromatogr., A* **1217** 2075 (2010)
131. Y L Hu, F Zhu, J W Li, G K Li *Chin. J. Anal. Chem.* **37** 466 (2009)
132. H Li, L Nie, Y Li, Z Zhang, H Shi, W Hu, Y Zhang *Sep. Sci. Technol.* **44** 370 (2009)
133. U Meyer, F Svec, J M J Frechet, C J Hawker, K Irgum *Macromolecules* **33** 7769 (2000)
134. M Guerrouache, M C Millot, B Carbonnier *Macromol. Rapid Commun.* **30** 109 (2009)
135. R Bandari, C Elsner, W Knolle, C Kuhnel, U Decker, M R Buchmeiser *J. Sep. Sci.* **30** 2821 (2007)
136. D D Zhong, Y P Huang, X L Xin, Z S Liu *J. Chromatogr., B* **934** 109 (2013)
137. J Matsui, S Goji, T Murashima, D Miyoshi, S Komai, A Shigeyasu, T Kushida, T Miyazawa, T Yamada, K Tamaki, N Sugimoto *Anal. Chem.* **79** 1749 (2007)
138. H Yan, K H Row *Sep. Sci. Technol.* **41** 1841 (2006)
139. L Zhao, L Ban, Q W Zhang, Y P Huang, Z S Liu *J. Chromatogr., A* **1218** 9071 (2011)
140. X L Sun, J He, G R Cai, A Q Lin, W J Zheng, X Liu, L X Chen, X W He, Y K Zhang *J. Sep. Sci.* **33** 3786 (2010)
141. J Haginaka, A Futagami *J. Chromatogr., A* **1185** 258 (2008)
142. M K Danquah, J Ho, G M Forde *J. Sep. Sci.* **30** 2843 (2007)
143. C Viklund, K Irgum *Macromolecules* **33** 2539 (2000)
144. X Wei, J Yin, G Yang, C He, Y Chen *J. Sep. Sci.* **30** 2851 (2007)
145. E Maksimova, E Vlakh, E Sinitsyna, T Tennikova *J. Sep. Sci.* **36** 3741 (2013)
146. Q Fu, Q Fang, B Feng, S Sun, W Du, E Amut, A Xiao, C Chang *J. Chromatogr., B* **879** 894 (2011)
147. X Huang, F Qin, X Chen, Y Liu, H Zou *J. Chromatogr., B* **804** 13 (2004)
148. J Ou, L Kong, C Pan, X Su, X Lei, H Zou *J. Chromatogr., A* **1117** 163 (2006)

149. J Oxelbark, C Legido-Quigley, C S A Aureliano, M M Titirici, E Schillinger, B Sellergren, J Courtois, K Irgum, L Dambies, P A G Cormack, D C Sherrington, E De Lorenzi *J. Chromatogr., A* **1160** 215 (2007)
150. J Courtois, G Fischer, S Schauff, K Albert, K Irgum *Anal. Chem.* **78** 580 (2006)
151. H Yan, K H Row *J. Liq. Chromatogr. Rel. Technol.* **29** 1393 (2006)
152. H W Sun, F X Qiao, G Y Liu *J. Chromatogr., A* **1134** 194 (2006)
153. M Emgenbroich, C Borrelli, S Shinde, I Lazraq, F Vilela, A J Hall, J Oxelbark, E De Lorenzi, J Courtois, A Simanova, J Verhage, K Karim, B Sellergren *Chem.–Eur. J.* **14** 9516 (2008)
154. A Rechichi, C Cristallini, U Vitae, G Ciardelli, N Barbani, G Vozzi, P Guisti *J. Cell Mol. Med.* **11** 1367 (2007)
155. Z Lin, F Yang, X He, X Zhao, Y Zhang *J. Chromatogr., A* **1216** 8612 (2009)
156. J Liu, Q Deng, K Yang, L Zhang, Z Liang, Y Zhang *J. Sep. Sci.* **33** 2757 (2010)
157. L Schweitz, L I Andersson, S Nilsson *Anal. Chem.* **69** 1179 (1997)
158. L Schweitz, L I Andersson, S Nilsson *Anal. Chim. Acta* **435** 43 (2001)
159. L Schweitz, L I Andersson, S Nilsson *Analyst* **12** 22 (2002)
160. S Liao, X Wang, X Lin, X Wu, Z Xie *J. Sep. Sci.* **33** 2123 (2010)
161. B Y Huang, Y C Chen, G R Wang, C Y Liu *J. Chromatogr., A* **1218** 849 (2011)
162. Y L Xu, Z S Liu, H F Wang, C Yan, R Y Gao *Electrophoresis* **26** 804 (2005)
163. S Liao, X Wang, X Lin, Z Xie *Electrophoresis* **31** 2822 (2010)
164. M Szumski, B Buszewski *J. Sep. Sci.* **27** 837 (2004)
165. W Y Yan, R Y Gao, Z C Zhang, Q S Wang, C V Jiang, C Yan *J. Sep. Sci.* **26** 555 (2003)
166. Z S Liu, Y L Xu, C Yan, R Y Gao *Anal. Chim. Acta* **523** 243 (2004)
167. Y P Zhang, G Q Zuo, W J Gong, Y E Deng, Q M Li *Chin. Chem. Lett.* **18** 734 (2007)
168. Z S Liu, Y L Xu, H Wang, C Yan, R Y Gao *Anal. Sci.* **20** 673 (2004)
169. P Spegel, L Schweitz, L I Andersson, S Nilsson *Chromatographia* **69** 277 (2009)
170. Q L Deng, Z H Lun, R Y Gao, L H Zhang, W B Zhang, Y K Zhang *Electrophoresis* **27** 4351 (2006)
171. J Ou, X Li, S Feng, J Dong, X Dong, L Kong, M Ye, H Zou *Anal. Chem.* **79** 639 (2007)
172. J X He, G Z Fang, Y C Yao, S Wang *J. Sep. Sci.* **33** 3263 (2010)
173. Z H Lun, Q L Deng, C Yan, R Y Gao *Chin. J. Chem.* **24** 442 (2006)
174. M Li, X Lin, Z Xie *J. Chromatogr., A* **1216** 5320 (2009)
175. C Zheng, Z S Liu, R Y Gao, Y K Zhang *Chin. J. Chem.* **26** 1857 (2008)
176. C Zheng, Z Liu, R Gao, L Zhang, Y Zhang *Anal. Bioanal. Chem.* **388** 1137 (2007)
177. S Sacho, L Schweitz, E Turiel, C Perez-Conde *J. Chromatogr., A* **1179** 216 (2008)
178. J Zhang, C He, Q Fu *Chromatographia* **62** 319 (2005)
179. X Liu, N Sun, Q Zhu, M Wu, Y Ye, H Chen *J. Chromatogr., A* **1304** 10 (2013)
180. T Zhu, S Li, K H Row *J. Appl. Polym. Sci.* **121** 1691 (2011)
181. W Zhang, Z Chen *Talanta* **103** 103 (2013)
182. H Yan, F Qiao, K H Row *Chromatographia* **70** 1087 (2009)
183. H Yan, K H Row *Biomed. Chromatogr.* **22** 487 (2008)
184. X Sun, X He, Y Zhang, L Chen *Talanta* **79** 926 (2009)
185. D Djozan, M A Farajzadeh, S M Sorouraddin, T Baheri *Chromatographia* **73** 975 (2011)
186. D Djozan, T Baheri *J. Chromatogr., A* **1166** 16 (2007)
187. J Courtois, G Fischer, B Sellergren, K Irgum *J. Chromatogr., A* **1109** 92 (2006)
188. Z Lin, J Wang, X Tan, L Sun, R Yu, H Yang, G Chen *J. Chromatogr., A* **1319** 141 (2013)
189. S-W Zhang, C J Zou, N Luo, Q F Weng, L-S Cai, C-Y Wu, J Xing *Chin. Chem. Lett.* **21** 85 (2010)
190. S Zhang, X Song, W Zhang, N Luo, L Cai *Sci. Total Environ.* **450–451** 266 (2013)
191. S Wang, D Li, Z Hua, M Zhao *Analyst* **136** 3672 (2011)
192. T Du, J Cheng, M Wu, X Wang, H Zhou, M Cheng *J. Chromatogr., B* **951–952** 104 (2014)
193. X Liu, Q Zhu, H Chen, L Zhou, X Dang, J Huang *J. Chromatogr., B* **951–952** 32 (2014)
194. J Zhou, C Ma, S Zhou, P Ma, F Chen, Y Qi, H Chen *J. Chromatogr., A* **1217** 7478 (2010)
195. T Yang, C Ma, H Chen, Y Zhang, X Dang, J Huang *J. Sep. Sci.* **37** 587 (2014)
196. T Zhang, C Ma, M Wu, Y Ye, H Chen, J Huang *Microchim. Acta* **180** 695 (2013)
197. E C Demiralay, M Andac, R Say, G Alsancak, A Denizli *J. Apll. Polym. Sci.* **117** 3704 (2010)
198. S Ozkara, R Say, C Andac, A Denizli *Ind. Eng. Chem. Res.* **47** 7849 (2008)
199. S Asir, L Uzun, D Turkmen, R Say, A Denizli *Sep. Sci. Technol.* **40** 2167 (2005)
200. I A Nicholls, L I Andersson, K Mosbach, B Ekberg *Trends Biotechnol.* **13** 47 (1995)
201. J Matsui, I A Nicholls, T Takeuchi *Tetrahedron: Asymmetry* **7** 1357 (1996)
202. M Kempe, K Mosbach *Tetrahedron Lett.* **36** 3563 (1995)
203. K G I Nilsson, K Sakguchi, P Gemeiner, K Mosbach *J. Chromatogr., A* **707** 199 (1995)
204. I A Nicholls, O Ramstrom, K Mosbach *J. Chromatogr., A* **691** 349 (1995)
205. B Sellergren *J. Chromatogr., A* **906** 227 (2001)
206. N D Ostryanina, G P Vlasov, T B Tennikova *J. Chromatogr., A* **949** 163 (2002)
207. M Vodopivec, A Podgornik, M Berovic, A Strancar *J. Chromatogr. Sci.* **38** 489 (2000)
208. G A Platonova, T B Tennikova *J. Chromatogr., A* **1065** 19 (2005)
209. K Kasai, Y Oda, S Nishikawa, S Ishii *J. Chromatogr.* **376** 33 (1986)
210. D E Epps, R A Poorman, F Mandel, H J Schostarez *J. Med. Chem.* **34** 2107 (1991)
211. V I Lozinsky *Russ. Chem. Bull., Int. Ed.* **57** 1015 (2008) [*Izv. Akad. Nauk, Ser. Khim.* 996 (2008)]
212. G B Sergeev, V A Batyuk *Russ. Chem. Rev.* **45** 391 (1976)
213. H Nishihara, S R Mukai, Y Fujii, T Tago, T Masudas, N Tamon *J. Mater. Chem.* **16** 3231 (2006)
214. V I Lozinsky, I Yu Galaev, F M Plieva, I N Savina, H Jungvid, B Mattiasson *Trends Biotechnol.* **21** 445 (2003)
215. V I Lozinsky, O Okay *Adv. Polym. Sci.* **263** 49 (2010)
216. V I Lozinsky, E S Vainerman, G F Korotaeva, S V Rogozhin *Colloid Polym. Sci.* **262** 617 (1984)
217. X Z Zhang, C C Chu *J. Mater. Chem.* **13** 2457 (2003)
218. F Plieva, H T Xiao, I Y Galaev, B Bergenstahl, B Mattiasson *J. Mater. Chem.* **16** 4065 (2006)
219. D Ceylan, O Okay *Macromolecules* **40** 8742 (2007)

220. Q Zhao, J Z Sun, Q C Ling, Q Y Zhou *J. Polym. Sci., Part A* **46** 6594 (2008)
221. S Dogu, O Okay *Polymer* **49** 4626 (2008)
222. O Okay, D Ceylan *Langmuir* **26** 7574 (2010)
223. X L Sun, W D He, T T Pan, Z L Ding, Y J Zhang *Polymer* **51** 110 (2010)
224. F M Plieva, I N Savina, S Deraz, J Andersson, I Y Galaev, B Mattiasson *J. Chromatogr., B* **807** 129 (2004)
225. J Yun, S Shen, F Chen, K Yao *J. Chromatogr., B* **860** 57 (2007)
226. M C Cheeks, N Kamal, A Sorrell, D Darling, F Farzaneh, N K H Slater *J. Chromatogr., A* **1216** 2705 (2009)
227. P Persson, O Baybak, F M Plieva, I Y Galaev, B Mattiasson, N Nilsson, A Axelsson *Biotechnol. Bioeng.* **88** 224 (2004)
228. H Kirsebom, G Rata, D Topgaard, B Mattiasson, I Y Galaev *Polymer* **49** 3855 (2008)
229. I N Savina, V Cnudde, S D'Hollander, L V Hoorebeke, B Mattiasson, I Y Galaev, F Du Prez *Soft Matter* **3** 1176 (2007)
230. M Odabasi, G Baydemir, M Karatas, A Derazshamshir *J. Appl. Polym. Sci.* **116** 1306 (2010)
231. P Perez, F Plieva, A Gallardo, J S Roman, M Aguilar, I Morfin, F Ehrburger-Dolle, F Bley, S Mikhalovsky, I Y Galaev *Biomacromolecules* **9** 66 (2008)
232. M Bakhshpour, N Bereli, S Senel *Colloids Surf., B* **113** 261 (2014)
233. Z Chen, L Xu, Y Liang, J Wang, M Zhao, Y Li *J. Chromatogr., A* **1182** 128 (2008)
234. V I Lozinsky *Russ. Chem. Rev.* **67** 573 (1998)
235. F M Plieva, M Karlsson, M-R Aguilar, D Gomez, S Mikhalovsky, I Yu Galaev, B Mattiasson *J. Appl. Polym. Sci.* **100** 1057 (2006)
236. A Tripathi, A Kumar *Macromol. Biosci.* **11** 22 (2011)
237. V I Lozinsky *Russ. Chem. Rev.* **71** 489 (2002)
238. H Kirsebom, B Mattiasson, I Yu Galaev *Macromol. Rapid Commun.* **31** 1095 (2010)
239. H F Zhang, I Hussain, M Brust, M F Butler, S P Rannard, A I Cooper *Nat. Mater.* **4** 787 (2005)
240. S R Mukai, H Nishihara, H Tamon *Chem. Commun.* 874 (2004)
241. H Nishihara, S R Mukai, D Yamashita, H Tamon *Chem. Mater.* **17** 683 (2005)
242. H Nishihara, S R Mukai, S Shichi, H Tamon *Mater. Lett.* **64** 959 (2010)
243. S M Kwon, H S Kim, H J Jin *Polymer* **50** 2786 (2009)
244. F Yilmaz, N Bereli, H Yavuz A Denizli *Biochem. Eng. J.* **43** 272 (2009)
245. L Wang, S Shen, X He, J Yun, K Yao, S J Yao *Biochem. Eng. J.* **42** 237 (2008)
246. M B Dainiak, A Kumar, I Yu Galaev, B Mattiasson *Proc. Natl. Acad. Sci. USA* **103** 849 (2006)
247. M B Dainiak, I Yu Galaev, B Mattiasson *Enzymol. Microb. Technol.* **40** 688 (2007)
248. H Kirsebom, D Topgaard, I Galaev, B Mattiasson *Langmuir* **26** 16129 (2010)
249. I N Savina, I Y Galaev, B Mattiasson *Polymer* **46** 9596 (2005)
250. K Yao, J Yun, S Shen, F Chen *J. Chromatogr., A* **1157** 246 (2007)
251. I Yu Galaev, M B Dainiak, F Plieva, B Mattiasson *Langmuir* **23** 35 (2007)
252. K Yao, J Yun, S Shen, L Wang, X He, X Yu *J. Chromatogr., A* **1109** 103 (2006)
253. J Yun, H Wu, J Liu, S Shen, S Zhang, L Xu, K Yao, S Yao *Ind. Eng.Chem. Res.* **54** 2564 (2015)
254. Yu N Belokon, K A Kochetkov, F M Plieva, N S Ikonnikov, V I Maleev, V S Parmar, R Kumar, V I Lozinsky *Appl. Biochem. Biotechnol.* **88** 97 (2000)
255. F M Plieva, K A Kochetkov, I Singh, V S Parmar, Yu N Belokon', V I Lozinsky *Biotechnol. Lett.* **22** 551 (2000)
256. A V Bacheva, F M Plieva, E N Lysogorskaya, I Y Filippova, V I Lozinsky *Bioorg. Med. Chem. Lett.* **11** 1005 (2001)
257. M Szczesna-Antczak, T Antczak, S Bielecki *Enzymol. Microb. Technol.* **34** 168 (2004)
258. D W Hutmacher *Biomaterials* **21** 2529 (2000)
259. M B Dainiak, I U Allan, I N Savina, L Cornelio, E S James, S L James, S V Mikhalovsky, H Jungvid, I Yu Galaev *Biomaterials* **31** 67 (2010)
260. H Kirsebom, M R Aguilar, J S Roman, M Fernandez, M A Prieto, B Bondar *J. Bioact. Compat. Polym.* **22** 621 (2007)
261. K Bloch, A Vanichkin, L G Damshkaln, V I Lozinsky, P Vardi *Acta Biomater.* **6** 1200 (2010)
262. C Baggiani, P Baravalle, C Giovannoli, L Anfossi, G Giraudi *Anal. Bioanal. Chem.* **397** 815 (2010)
263. L Onnby, V Pakade, B Mattiasson, H Kirsebom *Water Res.* **46** 4111 (2012)
264. O B Unluer, A Ersoz, A Denizli, R Demirel, R Say *J. Chromatogr., B* **934** 46 (2013)
265. S Hajizadeh, C Xu, H Kirsebom, L Ye, B Mattiasson *J. Chromatogr., A* **1274** 6 (2013)
266. S Hajizadeh, H Kirsebom, I Y Galaev, B Mattiasson *J. Sep. Sci.* **33** 1752 (2010)
267. G Baydemir, N Bereli, M Andaç, R Say, I Yu Galaev, A Denizli *React. Funct. Polym.* **69** 36 (2009)
268. G Ertürk, N Bereli, P W Ramteke, A Denizli *Appl. Biochem. Biotechnol.* **173** 1250 (2014)
269. N Bereli, M Andaz, G Baydemir, R Say, I Y Galaev, A Denizli *J. Chromatogr., A* **1190** 18 (2008)
270. S Asliyuce, L Uzun, R Say, A Denizli *React. Funct. Polym.* **73** 813 (2013)
271. E Tamahkar, N Bereli, R Say, A Denizli *J. Sep. Sci.* **34** 3433 (2011)
272. M Uygun, A A Karaglzler, A Denizli *Art. Cells Nanomed. Biotechnol.* **42** 128 (2014)
273. Q L Deng, Y L Li, L H Zhang, Y K Zhang *Chin. Chem. Lett.* **22** 1351 (2011)
274. G Baydemir, N Bereli, M Andaç, R Say, I Y Galaev, A Denizli *Colloids Surf., B* **68** 33 (2009)
275. S Aslíyüce, N Bereli, L Uzun, M A Onur, R Say, A Denizli *Sep. Purif. Technol.* **73** 243 (2010)
276. G Ertürk, N Bereli, M A Tümer, R Say, A Denizli *J. Mol. Recogn.* **26** 633 (2013)
277. N Bereli, Y Saylan, L Uzun, R Say, A Denizli *Sep. Purif. Technol.* **82** 28 (2011)
278. F M Plieva, B Mattiasson *Ind. Eng. Chem. Res.* **47** 4131 (2008)
279. M L Noir, F Plieva, B Mattiasson *ACS Symp. Ser.* **1022** 25 (2010)
280. C M Dai, J Zhang, Y L Zhang, X F Zhou, Y P Duan, S G Liu *Chem. Eng. J.* **211–212** 302 (2012)
281. C Alexander, L Davidson, W Hayes *Tetrahedron* **59** 2025 (2003)
282. E S Sinitsyna, J-G Walter, E G Vlakh, F Stahl, C Kasper, T B Tennikova *Talanta* **93** 139 (2012)

283. P Arvidsson, F M Plieva, I N Savina, V I Lozinsky, S Fexby, L Bulow, I Y Galaev, B Mattiasson *J. Chromatogr., A* **977** 27 (2002)
284. M S Kuyukina, E V Rubtsova, I B Ivshina, R V Ivanov, V I Lozinsky *J. Microbiol. Methods* **79** 76 (2009)
285. M B Dainiak, F M Plieva, I Yu Galaev, R Hatti-Kaul, B Mattiasson *Biotechnol. Prog.* **21** 644 (2005)
286. S N Yoon, G P Tae *J. Biomed. Mater. Res.* **47** 8 (1999)
287. V A Korzhikov, K V Gusevskaya, E N Litvinchuk, E G Vlakh, T B Tennikova *Int. J. Polym. Sci.* **2013** Article ID 476748 (2013)
288. N Kathuria, A Tripathi, K K Kar, A Kumar *Acta Biomater.* **5** 406 (2009)
289. M S Shoichet *Macromolecules* **43** 581 (2010)
290. V Korzhikov, S Roeker, E Vlakh, C Kasper, T Tennikova *Bioconjug. Shem.* **19** 617 (2008)
291. S Roeker, S Böhm, S Diederichs, F Bode, A Quade, V Korzhikov, M van Griensven, T Tennikova, C Kasper *J. Biomed. Mater. Res., B* **91** 153 (2009)
292. P Bures, Y Huang, E Oral, N A Peppas *J. Controll. Rel.* **72** 25 (2001)
293. J Z Hilt, M E Byrne *Drug Deliv. Rev.* **56** 1599 (2004)

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