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REVIEWS

Molecularly imprinted polymers as synthetic mimics of biorteceptors. 1. General principles of molecular imprinting.

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The review is devoted to analysis of the publications in the area of synthesis of artificial mimics of biological receptors using the method of molecular imprinting. General principles of molecular imprinting as well as main types of polymers being used in molecular imprinting are described. The special attention is paid to the polymers-biomimics synthesized using the method of non-covalent molecular imprinting.

Keywords: molecular imprinting, molecularly imprinted polymer, polymers-biomimics.

The ability of living cells to obtain information from external environment is based on molecular recognition phenomenon. Regulation of virtually all biochemical processes in living organisms is associated with molecular recognition based on complementarity of biomolecules: enzymatic catalysis, intracellular transport, interaction of hormones and other mediators with their receptors, and antigen-antibody interactions. In other words, molecular recognition is a fundamental principle of life.

Unique selectivity of biomolecules provides their wide practical applications – for the development elaboration of analytical methods and biotechnological processes as well as in medical diagnostics. Normally natural receptors interact with the corresponding ligands with high affinity, however, under non-physiological conditions they are very unstable. Unfortunately, all biomolecules are extremely sensitive to changes of temperature, pH, [the] presence of organic solvents, toxins, heavy metals, etc. The general draw-

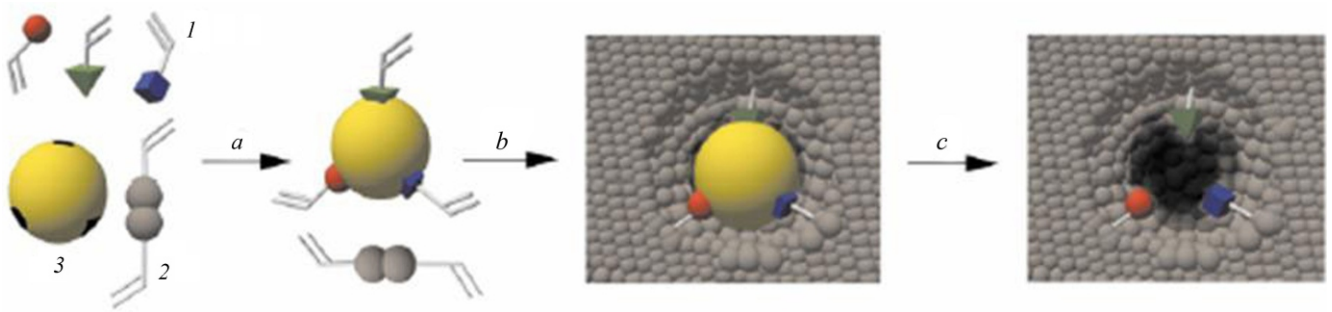

Fig. 1. The main principle of molecular imprinting. *a*. A template molecule (*3*) forms a complex with functional monomers (*1*) in a solutioni. *b*. Polymerization in the presence of a cross-linker (*2*). *c*. Extraction of the template and formation of synthetic binding sites complementary to the template in their shape and arrangement of functional groups.

backs of all biomolecules, limiting their wide practical application, are complicated procedures of their isolation and purification as well as high cost. Possibilities of getting natural receptors in preparatory quantities are often limited. Moreover, not all the molecules of interest have their natural receptors.

Synthesis of artificial receptors able to recognize and bind different target molecules with high affinity and specificity is a topical problem of current biotechnology, analytical biochemistry, and medicine. Ideally, these materials are to combine the ability of bioreceptors to recognize corresponding analytes selectively with stability, easy synthetic procedure, and low cost.

During recent years molecularly-imprinted polymers (MIPs or so-called polymers-biomimics), mimicking active sites of antibodies and biological receptors, have attracted significant attention [1]. They can provide high selectivity [2-4], while the method of their synthesis is quite simple. Normally, they demonstrate sufficient thermal and mechanical stability, as well as stability in aggressive media [5]. Therefore, polymers-biomimics combine high selectivity of biomolecules with the stability of synthetic polymers in harsh environments.

The method of molecular imprinting [1] is widely used for the synthesis of polymers-biomimics. It assumes formation of highly cross-linked polymers around so-called template molecules. The synthesis takes place due to co-polymerization of functional and cross-linking monomers in the presence of template molecule (the template molecule is at the same time an analyte of interest). Extraction of the template molecules from the fully-formed polymeric network results in formation of cavities, which [are complementary to the template] in their size, shape, and spatial arrangement of functional groups are complementary to the template. The molecularly imprinted polymers synthesized according to this principle are able of further selective binding the template molecules (Fig. 1).

The complex between functional monomers and templates can be formed under participation of both reversible covalent and noncovalent (hydrogen, ionic, hydrophobic, van der Waals) interactions. The method of covalent imprinting, proposed by Wulff and co-authors [6-13], assumes synthesis of the template molecule derivative, capable of polymerization. After the synthesis of the covalent molecularly-imprinted polymer, the template molecule is to be removed through cleavage of the covalent bonds between the template and functional monomers. Application of this method has significant limitations in selection of potential template molecules, while kinetics of their binding by the polymers is quite slow. However, a significant advantage of this approach as compared to the non-covalent one is formation of more homogeneous (in terms of affinity) population of binding sites.

More universal approach to the synthesis of molecularly-imprinted polymers was proposed by Mosbach and co-authors [14-18]. According to the non-covalent approach, formation of the complex template-functional monomer takes place due to non-covalent interactions. This approach is more flexible as compared to the covalent one, since the choice

of templates and functional monomers is virtually unlimited, while the template molecules can be easily extracted from the polymer by the corresponding organic solvent. It is widely recognized that the non-covalent molecularly-imprinted polymers contain heterogeneous (in terms of affinity towards the template molecule) population of synthetic binding sites, which is often compared to populations of polyclonal antibodies [19, 20].

Vulfson et al. demonstrated a possibility of synthesis of hybrid materials, where interaction of a template with functional monomers takes place due to both covalent and non-covalent interactions [21].

Since the approach based on non-covalent imprinting is more universal for the synthesis of artificial analogues of biomolecules, the main attention in the present review is focused on the molecularly-imprinted polymers, synthesized according to this principle.

Template molecules. The special feature of molecularly-imprinted polymers is the possibility of their synthesis towards virtually unlimited number of substances. Most of papers in the area of molecular imprinting describe synthesis of polymers for selective recognition of small organic molecules (medicines [22-24], drugs [25-28], herbicides [29-36], toxins [37-40], enzyme co-factors [41, 42], amino acids [43-45], nucleotides [46-49], hormones [50-54], sugars [55, 56], dyes [57-59], aromatic substances [60-64], etc.). A number of papers describe imprinting of metal ions [65-72]. During recent years the publications reporting [on] synthesis of molecularly-imprinted polymers for selective recognition of peptides [73-78], proteins [79-84], and even cells [85-88] as well as mineral crystals [89-90] have appeared. However, imprinting of high-molecular weight substances is still problematic.

To be successfully used as a template, a substance is to be stable under polymerization conditions (t=60-80°C, UV-irradiation), and it is not supposed to contain groups inhibiting/able to take part in polymerization.

Types of the polymers used in molecular imprinting. The structure of a polymeric matrix is crucial in molecular imprinting, since it determines selectivity of synthetic binding sites in resulting polymers. The latter are to fulfill the following requirements [1]:

1) the polymer is to be highly cross-linked so that selective sites retain their shapes after removal of the template molecules,

2) certain flexibility of polymer chains, which for the first site contradicts high degree of cross-linking, is necessary for fast kinetics under [the] binding of template molecules,

3) as many as possible synthetic binding sites are to be accessible for the interactions with template molecules,

4) the polymer is to be mechanically stable, which is crucial requirement in the case of its application in harsh environments (i.e. organic solvents), HPLC or SPE under increased pressures, in industrial reactors under constant stirring, etc.

5) thermal stability is essential in the case of MIPs application under increased temperatures, that are favorable for better kinetics.

The main types of the polymers that are being used in molecular imprinting are organic polymers, composite materials consisting of thin layers of organic polymers on/in inorganic carriers, silica gels, and biopolymers.

Organic polymers. Most of molecularly-imprinted polymers are macroporous acrylate or vinyl polymers. That is determined by the wide spectrum and availability of the monomers for their synthesis. Macroporous organic polymers are synthesized by radical co-polymerization of functional and cross-linking monomers in the presence of template molecules and inert solvents (porogens). Macroporous polymers are formed in the case of a high content (up to 90-95%) of cross-linker in the initial monomer composition. Further polymerization leads to phase separation resulting in formation of permanent pore structure. The most widely used functional monomers for the synthesis of molecularly-imprinted polymers are summarized in Fig. 2.

Formation of stable template-functional monomer complexes is crucial in the technique of molecular imprinting. Normally, formation of stable complexes with template molecules in non-covalent MIPs is

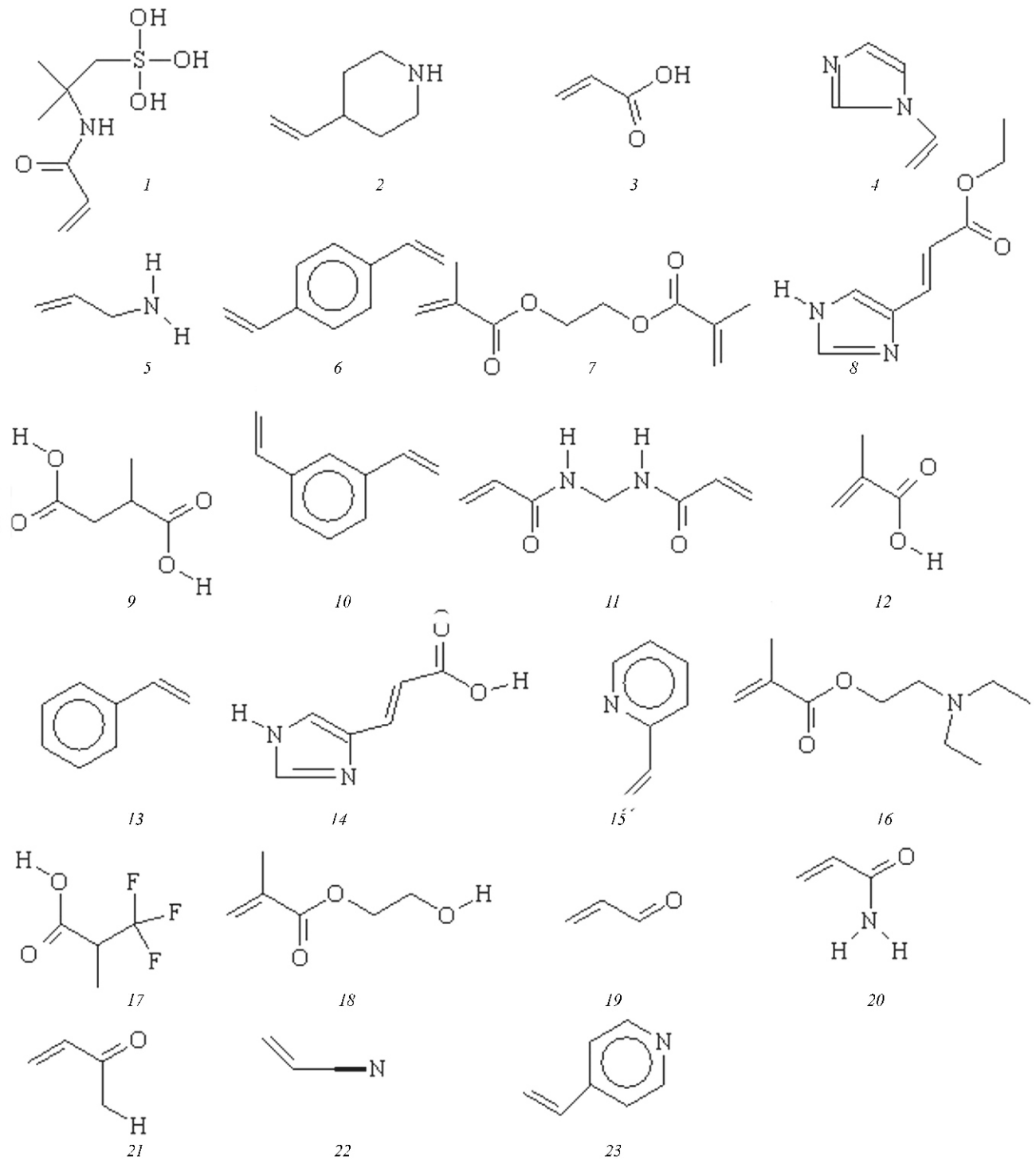

Fig. 2. Functional monomers used in molecular imprinting: *1* - (2-acrylamido-2-methyl-1-propanesulfonic acid), *2* - 4-vinylpyridine, *3* - acrylic acid, *4* - vinylimidazole, *5* - allylamine, *6* - p-divinylbenzene, *7* - ethyleneglycoldimethacrylate, *8* - urocanic acid ethyl ester, *9* - itaconic acid, *10* - m-divinylbenzene, *11* - N,N'-methylenebisacrylamide, *12* - methacrylic acid, *13* - styrene, *14* - urocanic acid, *15* - 2-vinylpyridine, *16* - diethylaminoethylmethacrylate, *17* - 2-(triftormethyl)acrylic acid, *18* - hydroxyethylmethacrylate, *19* - acroleine, *20* - acrylamide, *21* - acrylic acid, *22* - acrylonitrile, *23* - 4- vinylpyridine.

achieved under excess of functional monomers in the initial monomer mixture. This shifts the equilibrium to formation of the complexes. It is widely known that due to exothermic nature of the polymerization reaction as well as possible structural changes of both template and functional monomers, the structure of a part of these complexes will be changed or destroyed. The number of "defect" sites can decrease in the case of formation of strong complexes between templates and functional monomers. Therefore, selection of functional monomer is of great importance, since it influences directly the affinity and selectivity of a molecularly-imprinted polymer.

Most of scientists chose functional monomer taking into account just general considerations [90-93]. Some papers describe application of combinatorial approach to MIP synthesis and functional monomer selection [94-96]. The authors synthesized MIP libraries, where the type of functional monomer and a ratio template:functional monomer were varied in the initial monomer mixture. That was followed by [the] screening of the resulting MIPs as for their ability to recognize template molecules selectively. Using this approach, compositions of the polymers able to recognize selectively triazine herbicide terbutylazine [95], phenytoin, and nifedipine [92] were optimized. Despite the fact that every MIP from the library was synthesized in small quantity, the approach itself is laborious and time-consuming, which significantly limits its application.

Significantly more effective approach to optimization of MIP compositions (selection of functional monomers able to form strong complexes with template molecules) was proposed by the other authors [38, 40, 97-99]. The method provides a possibility of fast preliminary screening [of] a large number of potential functional monomers, while [the] results of the screening of a virtual library of functional monomers correlate with [the] experimental data on binding template molecules with computational MIPs. Moreover, [an] analysis of [the] computational modeling data as for formation of complexes template-functional monomer gives a possibility to synthesize materials capable of both highly-selective recognition of individual substances and recognition of groups of substances with similar structures [39, 40]. Up to now, this approach is the most promising, since significantly decreases time losses for optimization of MIP composition. At the same time, it gives information as for possible structure of template-selective binding sites in polymers-biomimics.

The effect of molecular imprinting is based on rigid fixation of template-functional monomer complexes in a polymeric network, which provides a desired spatial arrangement of functional groups of the monomers and, as a result, of a whole synthetic binding site. As it was mentioned, that is achieved by addition of 90-95% of bi/three-functional cross-linkers in the initial monomer composition. The most widely used cross-linkers in non-covalent molecular imprinting are ethyleneglycol dimethacrylate, trimethylolpropane trimethacrylate, n-divinylbenzene, N,N'-bisacrylamide (Fig. 3).

The numerous investigations demonstrate that ethyleneglycol dimethacrylate is the cheapest cross-linker, resulting in synthesis of MIPs with optimal properties. It provides high selectivity under separation of enantiomers and structural analogues [100-102], while chromatographic columns based on these polymers don't loose their selectivity under constant use at 80°C and pressure 6-10 MPa for months [103]. Therefore, most of research groups use ethyleneglycol dimethacrylate-based polymers for the purposes of selective binding [104-108]. In recent years synthesis of MIPs based on polyphenol [109], polyaminophenylboronic acid [110], co-polymer of poly(phenylenediamine) with aniline [111], polyuretanes [63], and oxidized polypyrroles [112, 113] was reported.

Organic polymers on/in inorganic carriers. Thin (5-10 nm) layers of macroporous polymers having the structure similar to those described in the section "*Organic polymers*" can be synthesized on the surface of macroporous silica-gels. That is achieved by covalent attachment of methacrylate groups to the silica surface through reaction with 3-(trimethoxysilyl)propylmethacrylate. That is followed by initiation of the radical polymerization of monomers traditionally used in molecular imprinting on the surface of silica-gels. Using this approach one can obtain polymeric adsorbents, which don't swell in organic solvents and aqueous solutions. These materi-

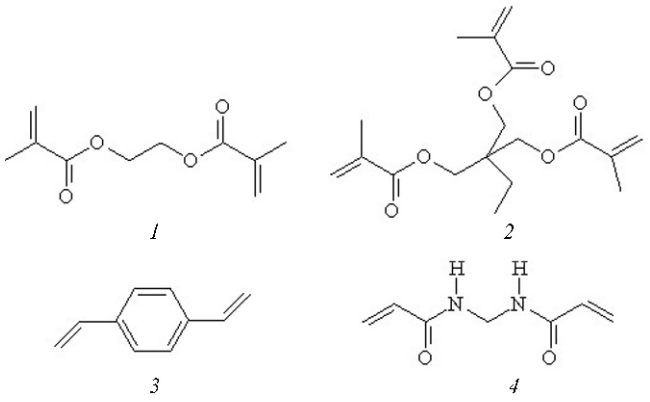

Fig. 3. Cross-linkers used in noncovalent molecular imprinting: *1* - ethyleneglycoldimethacrylate, *2* - 1,1,1,-trimethylolpropanetrimethacrylate, *3* - p-divinylbenzene, 4 - N,N'-methylenebisacrylamide.

als are mainly used for chromatographic separation of enantiomers [17, 114, 115] and close structural analogues [29], as well as in solid-phase extraction [116].

Since surface modification of inorganic materials is of great importance from the point of view of sensor technology, that is of special interest. A number of papers report surface modification of glass, gold, and tin dioxide [98, 117-126]. The paper [117] describes modification of glass surfaces with self-assembled MIP layers. Monolayers of trichloro-*n*-octadecylsilane were formed on glass surfaces in the presence of a detergent-modified dye as a template molecule. Extraction of the template molecules resulted in appearance of cavities, formed by poly-condensed silane molecules. The MIP monolayers were capable of predominant adsorption of the template as compared to its structural analogues. Unfortunately, the recognition process was too slow, since mass transfer took place through the layer of long-chain alkylsilanes. Amphiphylity of molecules is of great importance in this approach. The re-adsorption process was investigated electrochemically [119], using Raman spectroscopy [118], and ellipsometry [120]. Tin dioxide as well as gold can be also modified using this method [121, 122].

Interesting data on modification of gold surfaces by molecularly-imprinted polymers are presented in [123]. The authors described formation of hydrophobic self-assembled molecularly-imprinted monolayers of hexadecanthiol and developed a sensor system for cholesterol detection on their basis. The developed amperometric sensor system was found to be selective to cholesterol as compared to its close structural analogues (cholic and deoxycholic acids), while kinetics of the sensor responses was quite fast (the time of the sensor response comprised 5 min only). Unfortunately, the sensor demonstrated poor storage stability and lost 60% of its initial sensitivity after 10 days of storage. That was a limitation for its wide practical application in medical diagnostics.

Significantly much more effective approach based on modification of golden electrodes by thin layers of molecularly-imprinted polymers using the method of grafting polymerization was developed recently [98, 124-126]. The highly cross-linked structure of molecularly-imprinted polymers provided highly-selective recognition of analytes (triazine herbicides), while sensor responses remained stable for a long time (approximately 1 year).

Imprinted silica gels. Application of silica-gels in molecular imprinting was pioneered by Dickey, the first who synthesized substrate-selective adsorbents capable of selective recognition of dyes [127-129]. The imprinted material with the increased affinity to the template molecule was obtained by precipitation of silica-gels in the presence of methyl orange. Silica gels were also imprinted by other template molecules and used for separation of enantiomers [130-132], pesticides [133, 134], and drugs [135]. The materials synthesized in this way were not stable and quickly lost their selectivity. Therefore, this direction was not further proceeded. Moreover, a significant disadvantage of this approach is impossibility of selective silica gel synthesis towards water-insoluble substances, while Partikeev's method of synthesis of silica gels from organogels was found to be ineffective [133].

Molecularly-imprinted composite inorganic materials. This approach is a continuation of Dikey's papers [127-128]. It is based on synthesis [of] highly-cross-linked polysiloxanes on the surface of silica gel particles. The polycondensation reaction takes place in the presence of silanes having functional groups able to interact with template molecules. This

approach is similar to the above-mentioned one, where polymerizable double-bonds are replaced with silane groups capable of polycondensation. A number of works on application of cross-linked polysiloxanes for selective recognition of dyes [136], glycoproteins [114], and NAD [137] were published.

Bioimprinting. The method of molecular imprinting is applicable not only for synthetic polymers, but also for biopolymers [1, 138]. So-called bioimprinting was developed for producing enzymatic activity/substrate selectivity/change of enzymatic activity in proteins [139, 140], for producing enantioselectivity [141], as well as for retention of protein receptor properties in organic solvents [141, 142]. Usually, partly denaturated protein undergoes interaction with a template molecule, while its structure is stabilized by bi-functional cross-linkers. Using this approach enzymatic activity can be produced in proteins (albumin, concavanin A), enzymatic activity of ribonuclease, glucoseoxidase, urease, -amylase can be changed, while their stability can be increased [143-145].

Another approach to bioimprinting proteins is based on their ability to retain "structural memory" after the transfer from aqueous to anhydrous environment. In the case of precipitation or liophilization of a the protein from aqueous environment in the presence of a template molecule, the latter can further demonstrate predictable changes of its properties in organic solvents [146-157], and (under condition of additional stabilization) in aqueous solutions [158]. Synthetic receptor sites can be also formed using the method of molecular imprinting in cross-linked carbohydrates – starch [159] and amylase [160, 161]. Using this approach, polymers capable of selective recognition of methylene blue [159], and glucose [160, 161] were obtained.

All the methods described in the present review give a possibility to obtain synthetic mimics of biological receptors with different selectivity, since all of them under certain conditions provide formation of complexes between templates and functional groups on the surface of polymers as well as accessibility of the selective sites due to formation of porous structure.

In author's opinion, molecularly imprinted polymers due to their selectivity under recognition of template molecules and stability in harsh environments are much more promising material as to their practical application in current analytical chemistry and biotechnology (including sensor technology) as compared to than traditional biomolecules. One of the attractive features of these materials is a possibility of synthesis of inexpensive artificial receptors to virtually unlimited number of substances. Synthesis of MIPs towards substances which have no natural receptors or synthesis of natural receptors to which is problematic (low-molecular weight compounds, mycotoxins, bacterial toxins, etc.) is of special interest. From this point of view application of the methods of computational modeling, providing fast optimization of artificial receptors' composition able to bind effectively target analytes is of special interest. Highly cross-linked organic polymers with stable physicochemical characteristics obtained in the form of both polymeric particles and thin films seem to be the most promising to be used in current biotechnology. The analysis of literature data demonstrates effectiveness of application of polymers-biomimics for both fundamental investigations of molecular recognition processes and practical applications.

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Т. А. Сергеєва

Молекулярно-імпринтовані полімери як штучні аналоги біологічних рецепторів. 1. Загальні принципи молекулярного імпринтингу

Резюме

Огляд присвячено аналізу робіт у галузі отримання штучних аналогів біологічних рецепторів із застосуванням методу молекулярного імпринтингу. Розглянуто загальні принципи зазначеного методу та типи полімерів, які одержують із його використанням. Основну увагу приділено полімерам- біоміметикам, синтезованим методом нековалентного молекулярного імпринтингу.

Ключові слова: молекулярний імпринтинг, молекулярно-імпринтовані полімери, полімери-біоміметики.

Т. А. Сергеева

Молекулярно-импринтированные полимеры как искусственные аналоги биологических рецепторов. 1. Принципы молекулярного импринтинга

Резюме

Обзор посвящен анализу работ в области получения синтетических аналогов биологических рецепторов с использованием метода молекулярного импринтинга. Рассмотрены общие принципы указанного метода и типы полимеров, получаемые при его использовании. Особое внимание уделено полимерам-биомиметикам, синтезированным методом нековалентного молекулярного импринтинга.

Ключевые слова: молекулярный импринтинг, молекулярно-импринтированные полимеры, полимеры-биомиметики.

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