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Molecularly imprinted materials: synthesis, properties, applications

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Abstract. This review is devoted to the method of molecular imprinting. The physicochemical fundamentals and mechanisms of covalent and non-covalent molecular imprinting aimed at the development of organic polymeric sorbents capable of molecular recognition are considered. Attention is focused on the preparation of molecular imprints on mineral supports. The mechanisms of molecular recognition in adsorption are discussed. Application fields of materials with molecular imprints are briefly surveyed. The bibliography includes 112 references.

I. Introduction

The molecular imprinting method consists of the synthesis of organic or inorganic polymers capable of recognising molecules that were used as templates during their synthesis.[1–10] The recognition becomes possible because the structure of such polymers includes areas (the so-called imprints) that can specifically interact (complementary interactions) with the template molecules or compounds with similar structures. The concept of complementarity includes the shape and size matching of an imprint to the template as well as the presence in the imprint of auxiliary functional groups capable of interacting with functional groups in the template.

The procedure of preparation of polymeric materials with molecular imprints includes three stages. In the first stage, a mixture of functional monomers and a template molecule is prepared. The so-called pre-assembling, *i.e.*, the formation of a molecular pre-polymerisation complex between the monomer and the template molecule takes place in this stage (Fig. 1). These complexes can be formed as a result of either the covalent bonding (covalent molecular imprinting) or non-covalent intermolecular interactions (hydrogen bonding, ion – ion and ion – dipole interactions, *etc.*). In the latter case, we deal with non-covalent molecular imprinting. In the second stage, polymerisation (polycondensation) of pre-polymerisation complexes with a large excess of a cross-linking agent results in the formation of a polymeric matrix that contains areas with functional groups arranged in positions determined by the template molecules. The third stage consists of either the removal of template molecules from the polymeric matrix by liquid extraction methods (for non-covalent imprinting) or the cleavage of chemical bonds between the template molecule and the polymer (for covalent imprinting). As a result, the material acquires cavities that, in ideal, should match the template molecule in their size, shape and the arrangement of functional groups.

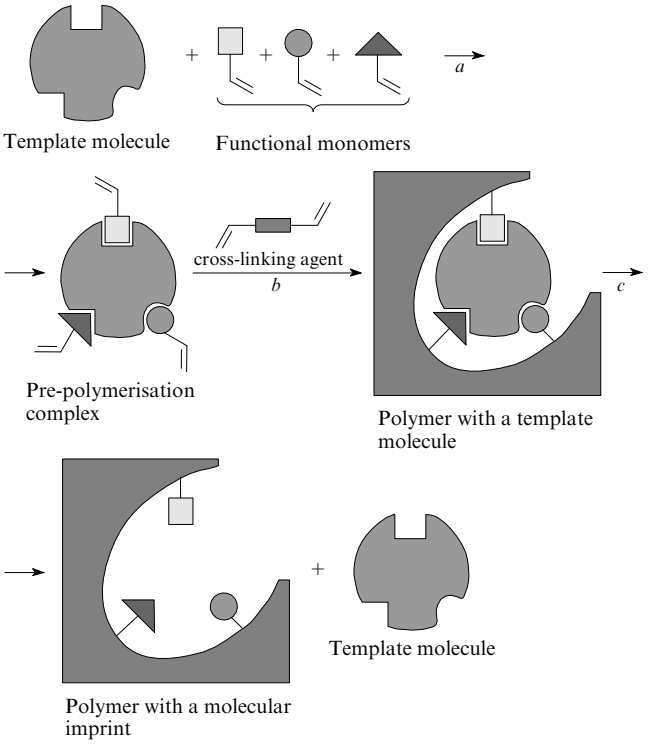


Figure 1. Synthesis of polymeric materials with imprints. (*a*) Formation of a pre-polymerisation complex of functional monomers with a template; (*b*) formation of a polymeric matrix incorporating the template molecules; (*c*) removal of the template molecules from the polymeric matrix and the formation of cavities complementary to the template molecule.

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It can be expected that the synthesised material will exhibit high specificity with respect to template molecules or molecules of compounds with similar structures. In nature, such specificity is typical of antibodies; this is why the materials prepared by molecular imprinting are often referred to as 'antibody mimics'.

II. Classification of molecular imprinting methods

The methods of synthesis of polymeric materials capable of molecular recognition can be classified according to the following criteria: (1) the nature of the polymeric matrix, (2) the method of preparation of a complex between the functional monomer and the template molecule, (3) the structure of imprints obtained.

Depending on the nature of functional monomers used in the synthesis of polymeric matrices, all materials capable of molecular recognition can be divided into organic and inorganic. The synthesis of organic matrices with molecular imprints involves the use of organic functional monomers and cross-linking agents containing C=C bonds. Inorganic matrices are usually prepared by polycondensation. Most often, functionalised silanes serve as the monomers and tetraethoxysilane as the cross-linking agent.

Depending on the method of preparation of pre-polymerisation complexes between a functional monomer and a template molecule, the methods of synthesis of materials capable of molecular recognition are conventionally divided into the imprinting in pre-assembled systems (the pre-polymerisation complex is synthesised beforehand) and the imprinting in self-assembling systems (the pre-polymerisation complex is synthesised immediately during the polymerisation). Imprinting in pre-assembled systems is referred to as covalent imprinting, because the complex is formed as a result of covalent interactions. Molecular imprinting in self-assembling systems is called non-covalent. In the latter case, the pre-polymerisation complex is formed as a result of non-covalent intermolecular interactions between template molecules and functional monomers.

Finally, molecular imprints can be formed either on the surface (2D-imprinting) or in the bulk (3D-imprinting) in the presence of appropriate solvents. In addition, the polymeric layers capable of molecular recognition can be applied onto pre-fabricated supports (the latter usually represent inorganic oxide supports with hydroxyl groups uniformly distributed over the surface †). Hence, the third classification is based on the structure of obtained imprints.

1. Non-covalent molecular imprinting

In the method of non-covalent imprinting, the polymerisation of functional monomers with a great amount of a cross-linking reagent in the presence of template molecules affords a highly cross-linked polymer incorporating template molecules. Template molecules are fixed within the polymeric matrix by non-covalent interactions (hydrogen bonding, electrostatic or $\pi-\pi$-interactions) with the functional groups of the monomers. Following removal of the template, which is usually carried out by solvent extraction, regions emerge in which functional groups are arranged complementary with respect to the functional groups in the template molecule. Being relatively simple and universal, the non-covalent approach is used to date most widely among the methods of molecular imprinting.

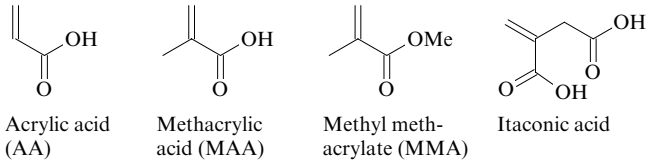
The first adsorbents with enhanced specificities with respect to molecular templates were synthesised by Polyakov [11] in the early 1930s. He demonstrated that silica gels formed in the presence of various alkylbenzenes exhibited enhanced (by approximately 15%) selectivity with respect to the adsorption of those particular hydrocarbons that have been used in their synthesis. Later, these experiments were continued with involvement of a wider range of template molecules.[12, 13] In 1960, Patrikeev, Balandin *et al.* published a paper [14] in which they showed that the pore size and adsorption properties of silica gels depend on the type of the substance introduced as a template at the stage of their synthesis. Silica gels thus obtained play the role of molecular sieves with respect to the template molecules.

The idea of changing the adsorption properties of solid adsorbents by the introduction of template substances at the stage of their synthesis was independently advanced by Pauling. He entrusted its experimental verification to his disciple Dickey. By allowing the solid structure of a sorbent to form in the presence of a compound for which this specific sorbent was intended, Dickey showed [15] that the material synthesised can indeed selectively retain the substance that has been used as the molecular template in the synthesis of this material. In the synthesis of an adsorbent for Methyl Orange (**1**) and Ethyl Orange (**2**) dyes, in one of experiments the solution of sodium silicate was acidified in the presence of Methyl Orange (**1**) to afford a hydrogel, which contained molecules of this dye in its structure (Scheme 1). The material formed upon drying of the hydrogel and removal of the dye by extraction turned out to adsorb a double amount of Methyl Orange (**1**) as compared with Ethyl Orange (**2**).

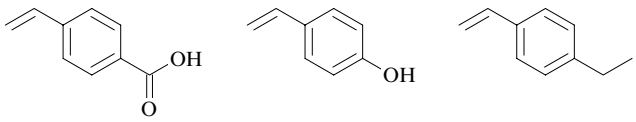
Later, Dickey's concepts on the mechanism of molecular recognition were corrected by Wulff *et al.*[16, 17] who have shown that it is the complementarity between functional groups in the template molecule and in the recognition site that is the driving force of molecular recognition rather than the shape and size matching of the polymeric-matrix cavity to the template.

Below, we show examples of functional monomers and cross-linking agents that are used in the synthesis of selective sorbents by the non-covalent molecular imprinting method.[18]

A. Functional monomers



Acrylic acid (AA) | Methacrylic acid (MAA) | Methyl methacrylate (MMA) | Itaconic acid

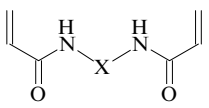


4-Vinylbenzoic acid | 4-Vinylphenol | 4-Ethylstyrene

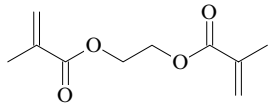


1-Vinylimidazole | 2-Vinylpyridine (VPy)

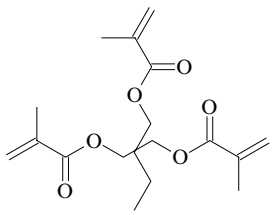
B. Cross-linking reagents



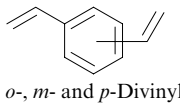
X = CH_2 [*N*,*N*′-methylenedi(acrylamide)], 1,4-C_6H_4 [*N*,*N*′-1,4-phenylenedi(acrylamide)], 3,5-C_6H_3COOH [3,5-di(acrylamido)benzoic acid].



Ethylene glycol dimethacrylate (EDMA)



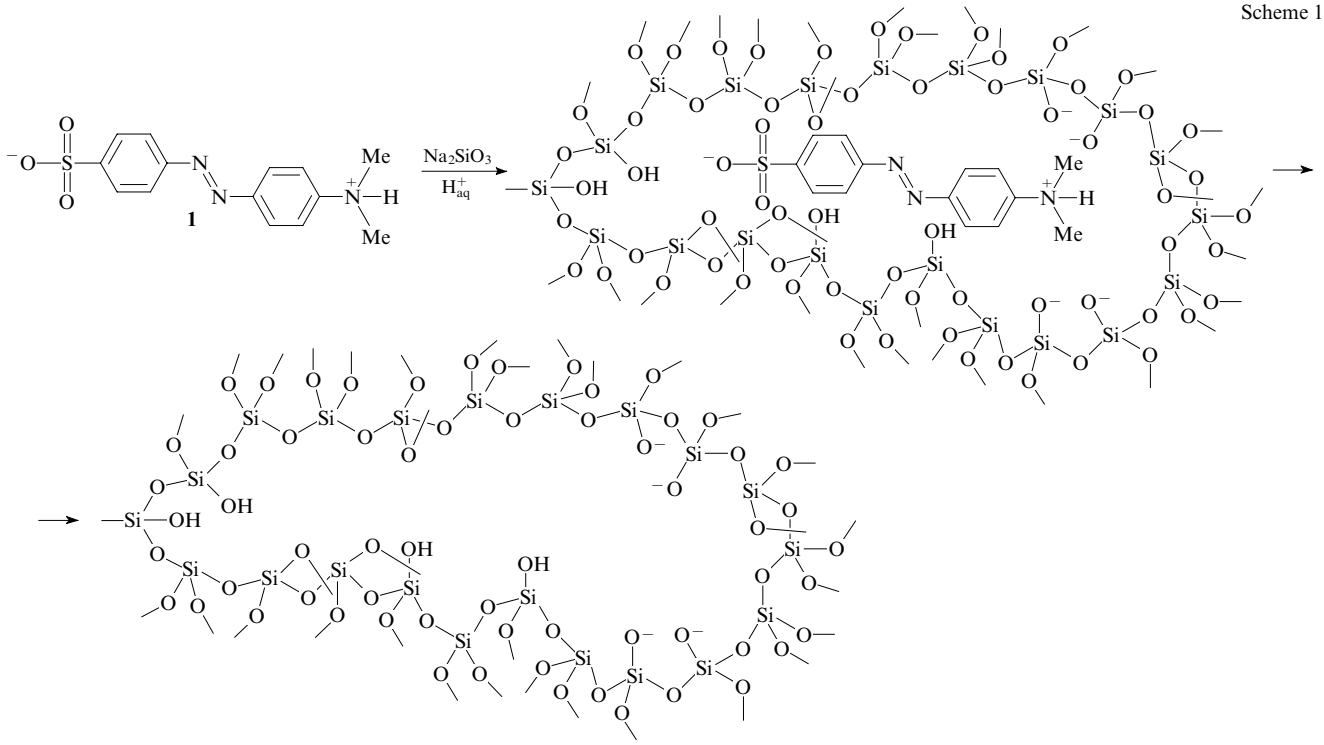
1,1,1-Trimethylolpropane trimethacrylate (TRIM)



o-, *m*- and *p*-Divinylbenzenes

† For silica, the surface concentration of hydroxyl groups is 4.6 nm^{-2}. On the metal surface, hydroxyl groups are formed as a result of oxidation followed by hydroxylation.

Scheme 1

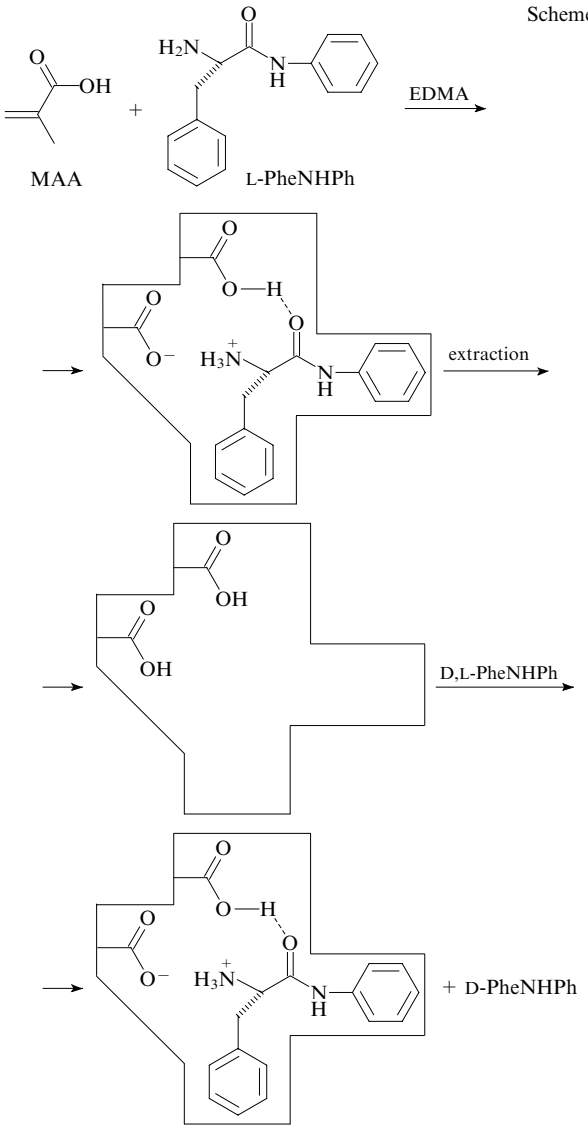


Functional monomers are selected in such a way as to provide their interaction with the functional groups of the template molecule at the stage of synthesis of the polymer. Methacrylic acid is used most often as the functional polymer because it can form complexes with many template molecules, *viz*., with amines by forming an ionic bond and with amides, carbamates, alcohols, *etc*. by forming a hydrogen bond. It is known that electrostatic interactions are stronger than van der Waals interactions (particularly, hydrogen bonds); hence, the polymers that form ionic bonds with template molecules exhibit higher selectivity than those that form only hydrogen bonds. This was taken into account in the preparation of chiral stationary phases for the separation of racemates by HPLC (High-Performance Liquid Chromatography) (*e.g*., for the separation of anilides of D- and L-phenylalanine, Scheme 2).[19–21]

Studying the nature of molecular recognition of template molecules by polymers synthesised with involvement of non-covalent imprinting is a complex task. The literature contains relatively few publications devoted to the mechanism of such recognition.

The formation of non-covalent intermolecular monomer–template complexes in pre-polymerisation systems was proved by UV and NMR spectroscopy[19,22] as well as by chromatographic methods. In particular, the effect of the functional monomer : template molar ratio in the pre-polymerisation mixture on the chromatographic characteristics of the resulting sorbents was investigated.[23] A series of polymers based on methacrylic acid (functional monomer) and ethylene glycol dimethacrylate (cross-linking agent) were prepared in the absence (control sample **P0**) and in the presence of a molecular template (template samples **P1**–**P6**). (−)-Nicotine served as the molecular template. The functional polymer : template molar ratio was varied from 4 : 1 to 1 : 10. The methacrylic acid : nicotine molar ratio was shown to have a substantial effect on the amount and quality of specific adsorption sites formed during the polymerisation, which followed from the results of chromatographic separation of a mixture of (−)-nicotine and 4,4′-bipyridyl on these polymers.

Scheme 2



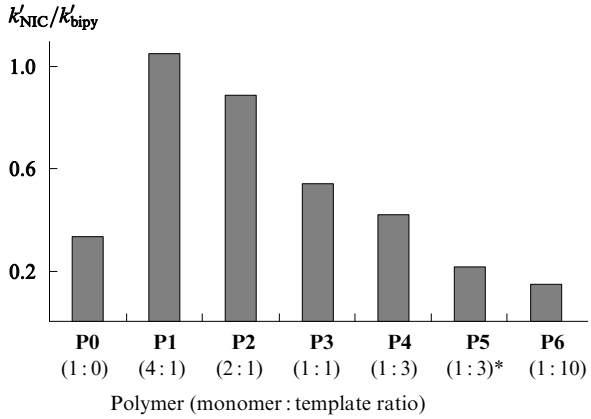
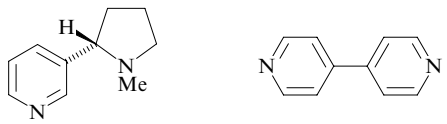
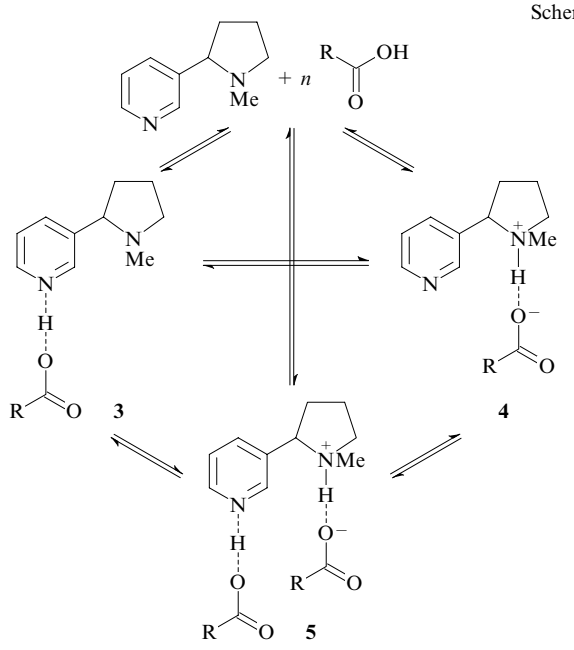


Figure 2. The ratio of selectivity factors in chromatographic separation of (−)-nicotine and 4,4′-bipyridyl on sorbents **P0**–**P6**.[23]
For the description of polymers **P0**–**P6**, see text.



It was shown that the excess of (−)-nicotine in the pre-polymerisation mixture has an adverse effect on the selectivity of the resulting sorbent with respect to nicotine (Fig. 2, polymers **P4**–**P6**), which followed from the low selectivity ratios of the separation of nicotine and 4,4′-bipyridyl (k'_{NIC}/k'_{bipy}) on polymers **P4**–**P6** (see Fig. 2) and also from the fact that the addition of acetic acid to the reaction mixture during the synthesis of polymer **P5** decreased the selectivity of separation of the above compounds on this polymer as compared with **P4** due to the competition between methacrylic and acetic acids for the binding of nicotine. This can be explained by the shift of equilibrium between pre-polymerisation complexes **3**–**5** to the formation of complexes **3** and **4** (Scheme 3). On the other hand, a small excess of the monomer favours the increase in the selectivity of sorbents (polymers **P1** and **P2**), which is associated with the preferential formation of complex **5**. However, a large excess of methacrylic acid gives rise to a great number of non-specific binding sites in the polymeric sorbent, which decreases its selectivity.

Scheme 3

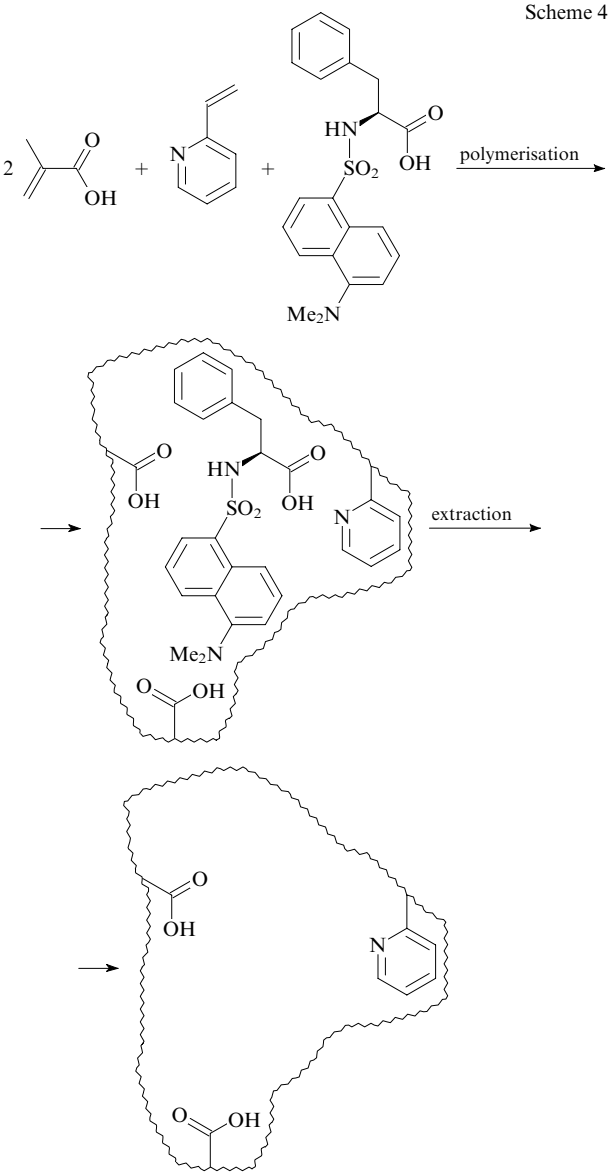


Insofar as selectivity of obtained sorbens is the result of pre-assembling of monomer molecules with template molecules, it can be assumed that the formation of complex **5** is most favourable for the formation of highly selective adsorption sites in the polymeric network. Polymerisation of complexes **3** and **4** obviously results in the formation of regions with lower affinity to the template molecule, although it should be borne in mind that weak interactions of template molecules with the cross-linking agent (ethylene glycol dimethacrylate) can partly compensate the deficiency of methacrylic acid.

The idea of using vinylpyridines as the functional basic monomers was first forwarded in 1993 (Ref. 24). Three types of sorbents were prepared, namely, from 2-vinylpyridine (sorbent **A**), from methacrylic acid (sorbent **B**) and from both monomers taken in the molar ratio of 1 : 1 (sorbent **C**). Synthesis of these sorbents in the presence of Boc-L-tryptophan (as the molecular template) and their use in the chromatographic separation of Boc-L,D-tryptophan racemate have shown that the most efficient separation was achieved on sorbent **C**. Sorbent **A** was inferior, and sorbent **B** demonstrated the lowest separation ability.

Similar results were obtained with the use of dansyl-L-phenylalanine as the template (Scheme 4). Presumably, the formation of a pre-polymerisation complex, which would favour the formation

Scheme 4



of selective sites, requires the simultaneous presence of both monomers in the reaction mixture, *viz*., methacrylic acid for binding amino groups and 2-vinylpyridine for binding COOH groups in the amino acid.

Chromatographic properties of sorbents obtained in the presence of a non-polymerisable analogue of 2-vinylpyridine or methacrylic acid, namely, either pyridine or acetic acid, were analysed in order to confirm this hypothesis. In both cases, these sorbents proved inefficient in the separation of racemates as compared with sorbents prepared in the presence of two functional monomers simultaneously. This suggests that the formation of the pre-polymerisation complex involves the molecules of both monomers.

2. Covalent molecular imprinting

In covalent imprinting, the complex between a template molecule and a functional monomer, which can undergo co-polymerisation with a cross-linking agent in an appropriate solvent, is synthesised beforehand. Polymerisation of such a complex with an excess of the cross-linking agent affords a polymer incorporating chemically bound template molecules. In the final stage, under the action of the corresponding chemical reagents, the chemical bonds of template molecules with the matrix are broken and they are liberated. The cavities formed upon the template removal are characterised by a more definite (complementary to the template molecule) position of the functional group, which took part in the complex formation, than it can be achieved in non-covalent imprinting.

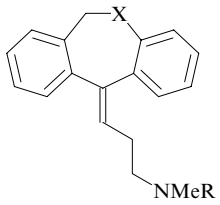
As compared with non-covalent imprinting, the imprinting in pre-assembled systems is much more laborious as regards both the choice of the template molecule and the experimental procedure. Hence, it is not surprising that the literature contains much fewer examples of the implementation of this approach as compared with the non-covalent imprinting.

The covalent imprinting method was first applied by Wulff *et al*.[25] They used phenyl α-D-mannopyranoside functionalised with 4-vinylphenylboronic acid as the template molecule. This compound was polymerised. The removal of the glycoside from the polymer afforded cavities that contained boronate functional groups.

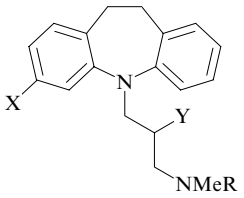
The use of covalent imprinting in the synthesis of sorbent **P4** (Table 1), which is highly sensitive to nortriptyline and other tricyclic antidepressants, was reported.[26]

Table 1. Methods of synthesis of polymeric sorbents studied in Ref. 26.

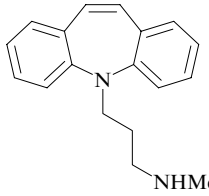
Polymeric sorbents (abbreviation)	Template (mol.%)	Cross-linking agent TRIM (mol.%)	Functional monomer (mol.%)
Sorbents without NOR molecular imprints			
P1	–	96	4-vinylphenol (4)
P2	–	64	methacrylic acid (24) + styrene (12)
P3	–	63	4-vinylphenol (12) + styrene (12) + methacrylic acid (13)
Sorbents containing NOR molecular imprints			
P4	NOR-alkene (4)	96	none
P5	NOR (4)	60	4-vinylphenol (12) + styrene (12) + methacrylic acid (12)
P6	NOR (2)	98	none
P7	NOR (2)	62	methacrylic acid (24) + styrene (12)



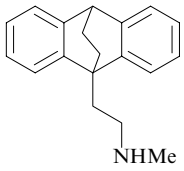
Nortriptyline (NOR): X = CH_2, R = H;
Amitriptyline (AMI): X = CH_2, R = Me;
Doxepin (DOX): X = O, R = Me.



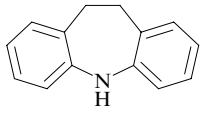
Desipramine (DES): X = Y = R = H;
Imipramine (IMI): X = Y = H, R = Me;
Trimipramine (TRI): X = H, Y = R = Me;
Clomipramine (CLO): X = Cl, Y = H, R = Me.



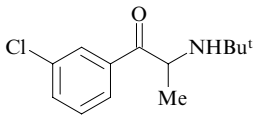
Protriptyline (PRO)



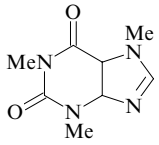
Maprotiline (MAP)



Iminodibenzyl (IDB)



Bupropion (BUP)



Caffeine (CAF)

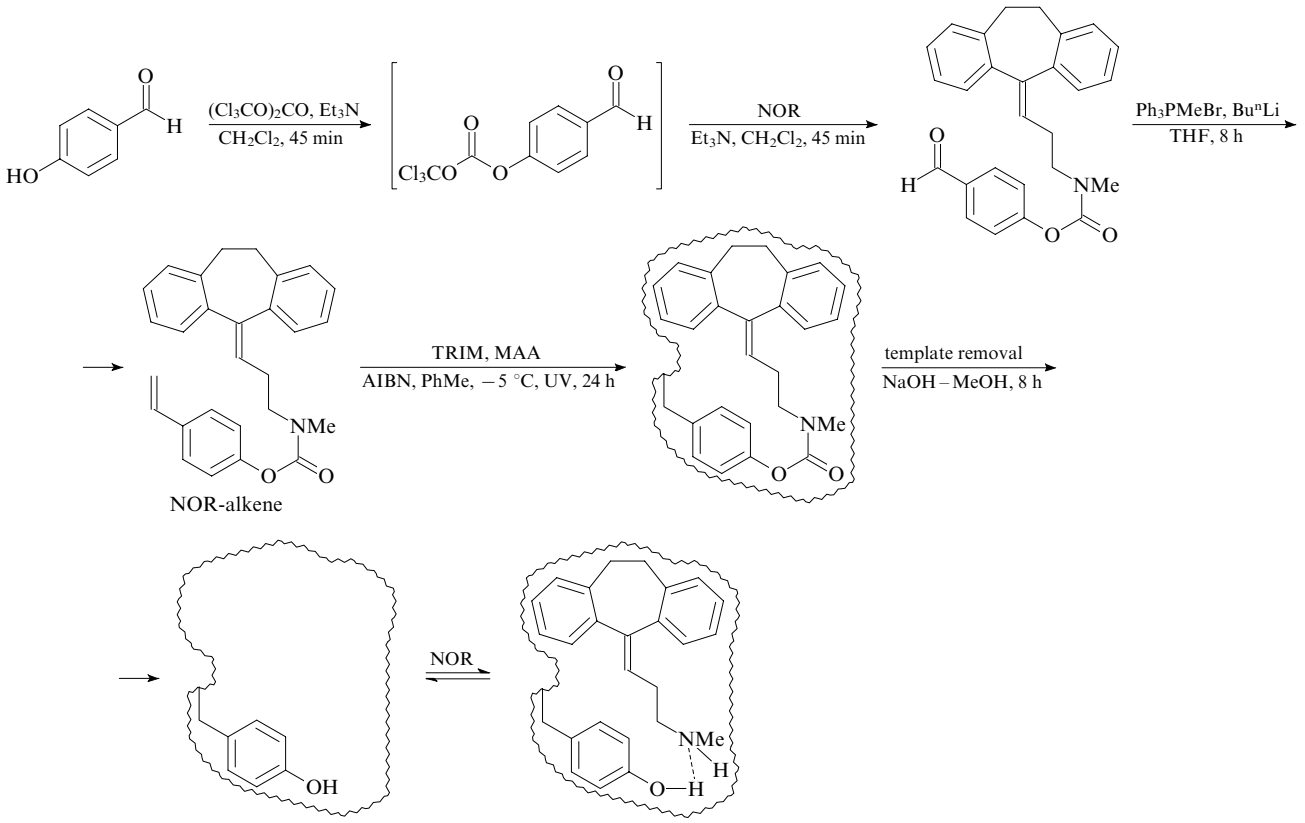
Functionalisation of nortriptyline and preparation of specific sorbent **P4** were carried out as shown in Scheme 5. Sorption properties of sorbent **P4** synthesised by covalent imprinting were compared with the properties of other polymeric sorbents used for sorption of these tricyclic compounds. To this end, a series of sorbents based on methacrylic acid and trimethylolpropane trimethacrylate were synthesised. Sorbents **P1** – **P3** were prepared by conventional polymerisation in the absence of a template, sorbents **P5** – **P7** were obtained by non-covalent imprinting in the presence of NOR (see Table 1).

The chromatographic properties of the obtained sorbents were studied. Several tricyclic antidepressants and compounds with similar physiological properties were screened. Table 2 shows the retention factors (k) for these compounds on sorbents **P4** – **P7**, which contained imprints of NOR molecules. As expected, the retention factor of nortriptyline on sorbents **P4**, **P5** and **P7** was substantially larger than those of other compounds

Table 2. Retention factors of compounds on different sorbents.

Compound	Retention factors (k) of compounds on sorbents			
	P4	**P5**	**P6**	**P7**
NOR	2.83	4.55	0.16	2.68
DES	2.59	3.53	0.20	2.48
PRO	2.35	3.58	0.18	2.24
MAP	2.49	3.96	0.20	2.44
DOX	1.43	0.75	0.14	1.13
TRI	1.33	0.53	0.15	1.03
AMI	1.57	0.85	0.14	1.11
IMI	1.52	0.88	0.14	1.12
CLO	1.61	0.97	0.16	1.09
BUP	1.42	0.58	0.20	0.88
IDB	0.17	0.53	0.27	0.37
CAF	0.19	0.40	0.13	0.68

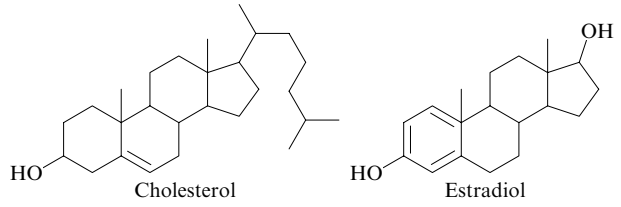
Scheme 5



studied. It should be noted that sorbent **P6** the synthesis of which involved no functional monomers exhibits smaller k values. This confirms the important role of functional groups in molecular recognition. Sorption of compounds containing a secondary nitrogen atom (PRO, DES, MAP) on polymers **P4**, **P5** and **P7** was less efficient than that of nortriptyline but more efficient as compared with compounds containing a tertiary nitrogen atom (AMI, IMI, CLO, TRI). This can be explained by the fact that alkyl substituents at the nitrogen atom hinder the formation of the hydrogen bond N···HO–**P**. Despite being secondary amines, BUP and IDM exhibit smaller retention factors as compared with NOR, which suggests that the shape and size of a molecular imprint affect the molecular recognition.

Usually, polymeric sorbents prepared by non-covalent molecular imprinting are less efficient in the chromatographic isolation of the analyte that has been used as the molecular template in comparison with sorbents obtained by covalent imprinting. This is manifested in pronounced broadening of the elution peak of the substance in question.[27,28] The shape of the chromatographic peak of NOR on polymer **P4** prepared by covalent molecular imprinting could be substantially improved (Fig. 3 *a*) as compared with the NOR peaks on sorbents **P5** and **P7** synthesised by non-covalent imprinting (Fig. 3 *b*,*c*).[26]

Covalent imprinting was also employed for the preparation of a sorbent that selectively adsorbs cholesterol[29] based on cholesteryl 4-vinylphenyl carbonate and ethylene glycol dimethacrylate as the cross-linking agent. The sorption properties of this sorbent were compared with those of sorbents obtained by non-covalent imprinting with cholesterol as the template and methacrylic acid or 4-vinylpyridine as functional monomers. Chromatographic separation of a mixture of cholesterol with β-estradiol was carried out.



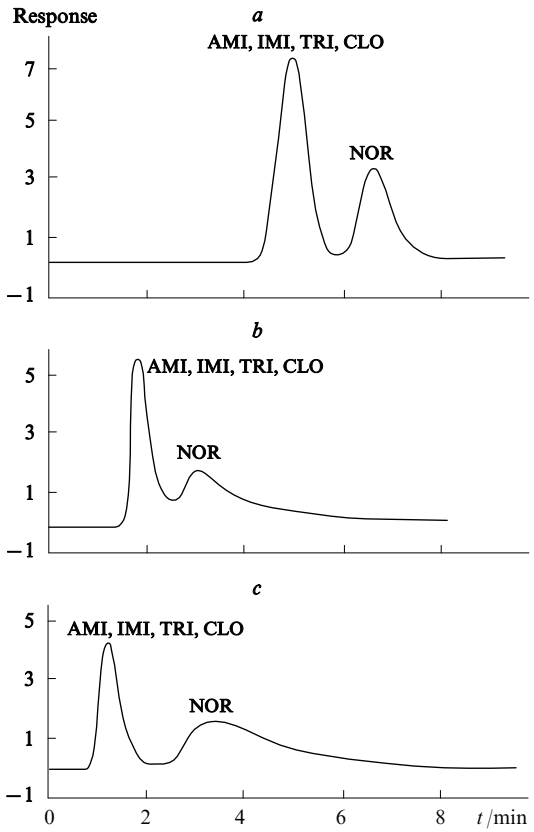
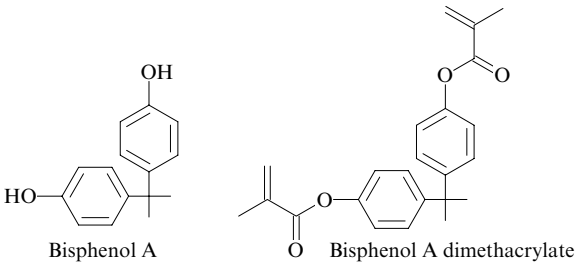


Figure 3. Chromatograms illustrating the efficiency of sorbents **P4** (*a*), **P5** (*b*) and **P7** (*c*) in the separation of a mixture of tricyclic antidepressants.

The separation was observed on all sorbents; however, the polymer prepared by covalent imprinting had a higher adsorption capacity with respect to cholesterol and exhibited five-fold higher chromatographic efficiency as compared with polymers prepared by non-covalent imprinting.

A polymer that selectively adsorbs bisphenol A [2,2-bis(4-hydroxyphenyl)propane] was prepared [30] by co-polymerisation of bisphenol A dimethacrylate with triethylene glycol dimethacrylate or trimethylolpropane trimethacrylate.‡



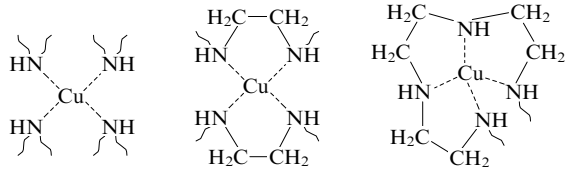
Bisphenol A Bisphenol A dimethacrylate

The retention of bisphenol A, its analogues and certain hormones on the polymeric sorbent prepared was studied. It was shown that the sorbents retain best the compounds that contain two 4,4′-hydroxyl groups and have rigid structures and molecular sizes close to that of bisphenol A.

In the 1970s, Kabanov *et al.* [31–33] developed an original method of synthesis of polymeric materials with three-dimensional networks 'tuned' to the sorption of definite ions. The complementarity of the 3D-regions of polymeric networks and a given type of metal ions was achieved by the preliminary conformational tuning § of macromolecules of non-cross-linked polymers with respect to these ions, which was followed by the fixation of such a conformation by intermolecular cross-linking and removal of metal ions (templates) from the system. It should be emphasised that complementarity does not exist on its own but arises in the process of adsorption of a given ion and manifests itself in the decrease in the energy and entropy consumed in the conformational rearrangement of segments of macromolecules during the formation of their complexes with the metal.

In general features, the procedure of preparation of such 'tuned' sorbents is as follows:[34] an aqueous-ethanolic solution of a transition metal salt (*e.g.*, 0.2 M $CuCl_2$) is added to a dilute solution of a non-cross-linked polymer in an organic solvent [*e.g.*, 5% solution of partly quaternised poly(4-vinylpyridine) in ethanol] together with aqueous ammonia for precipitation of the metal–polymer complex. Following drying, the metal–polymer complex is cross-linked by a cross-linking agent. The resulting cross-linked polymer is washed with dilute HCl to remove the metal ions. Naturally, upon desorption of the metal ions the conformation of chain segments of the polymeric network that incorporated these ions changes; however, due to cross-linking, the polymeric matrix retains by and large its topological characteristics, which favours the resorption of the same metal ions.

The relationship between the structure of complexes 'polymeric matrix–metal ion' and the recognition efficiency was studied systematically [35] using oligomers of poly(ethyleneimine) (PEI) cross-linked with a reaction product of epichlorohydrin with ammonia. Before cross-linking, PEI was treated with a solution of copper(II) and nickel(II) salts (the concentration of ions varied from 1 to 3 mmol g^{-1} PEI). A preliminary EPR study has shown that the PEI solution contained several complexes of Cu^{2+} with PEI, which differed in the degree of deformation of the square-planar coordination of the Cu^{2+} ion and in the number of ethylenediamine fragments.



A **B** **C**

Complex **A** is formed as a result of interaction of a Cu^{2+} ion with amino groups that belong to four different chains, whereas complex **B** involves its interaction with ethylenediamine fragments that belong to two chains. The existence of complex **C** all amino groups in which belong to the same PEI chain is also possible. Obviously, complexes with the same composition can exist in the sorbent as well. At low degrees of protonation of amino groups, the sorbents preferentially contained complexes of the **B** and **C** types, whereas at high degrees of protonation, they contained complexes **A**. Sorbents non-tuned with respect to Cu^{2+} ions produced complexes **A** and even triamine complexes. In contrast, tuned sorbents preferentially produced complexes of the type **C**. EPR spectroscopic studies proved that for protonated sorbents, the increase in the content of copper leads to the rearrangement of interchain complexes into intrachain complexes. Thus, the introduction of a great amount of copper favours the fixation of such molecular conformations that are best suited for the formation of complexes and that are observed in solutions where the molecules exhibit the highest mobility. Indeed, studies of the adsorption properties of a sorbent prepared in the presence of a great amount of copper demonstrated that this sorbent exhibited the maximum sorption capacity and sorption rate. This favours the formation of uniform active sites, which is essential for the synthesis of selective catalysts.

Synthesis of catalysts tuned with respect to ethylbenzene, namely, cobalt complexes with a co-polymer of diethyl vinylphosphonate and acrylic acid, is decribed.[36] Kinetic studies of liquid-phase oxidation of ethylbenzene with molecular oxygen have shown that the oxidation rate on a catalyst the structure of which was formed in the presence of ethylbenzene substantially exceeds that on the same catalyst prepared by conventional procedures without tuning of its structure.

III. Physicochemical fundamentals of molecular imprinting

Despite the seeming transparency and relative simplicity of the scheme of synthesis of polymeric sorbents capable of molecular recognition and the clarity of the model of the recognition process, many unsolved problems still exist. Particularly, the physical nature of non-covalent interactions responsible for the formation of template–monomer complexes is unclear; there is no versatile model that would allow one to describe the distribution of recognition sites according to the energy.

Relatively few studies are devoted to the physicochemical properties of molecular imprinting. The factors that govern the formation of polymeric systems by the molecular imprinting method are considered in a review.[37] The first stage in the formation of polymeric systems capable of molecular recognition is the formation of a pre-polymerisation complex of the template molecule with functional monomers due to non-covalent intermolecular interactions. The stability of this complex (which ultimately determines the ability of the synthesised material to recognise molecules) depends on many factors, particularly, thermodynamic. The change in the Gibbs free energy (ΔG_{bind}) due to the formation of a template–monomer pre-polymerisation complex is described by equation

‡ Certain complications were associated with the chemical removal of biphenol A from the polymer under the action of a sodium hydroxide solution.

§ By changing the polymeric chain conformation due to the formation of complexes with metal ions.

$$\Delta G_{\text{bind}} = \Delta G_{\text{t+r}} + \Delta G_{\text{r}} + \Delta G_{\text{h}} + \Delta G_{\text{vib}} + \Sigma\Delta G_{\text{p}} + \\ + \Delta G_{\text{conf}} + \Delta G_{\text{vdW}}.$$

Here, the terms G_i reflect the changes in the Gibbs free energy due to translation and rotation ($\Delta G_{\text{t+r}}$), restriction of rotors as a result of the formation of complexes (ΔG_{r}), hydrophobic interactions (ΔG_{h}), residual vibrational modes (ΔG_{vib}), the sum of contributions of polar interactions template – monomer ($\Sigma\Delta G_{\text{p}}$), adverse conformational changes (ΔG_{conf}) and unfavourable van der Waals interactions (ΔG_{vdW}).

Ultimately, the value of ΔG_{bind} determines the number of molecular recognition sites and their distribution according to the energy (the degree of heterogeneity). Obviously, the more stable the pre-polymerisation complex the higher its concentration in the equilibrium pre-polymerisation mixture and, hence, the larger the number of recognition sites in the polymer formed and the more efficient the recognition of the original template molecules. The probability of the formation of template – monomer complexes and their stability depend on the nature of all components (template, monomers, solvent, *etc*.) present in the pre-polymerisation mixture and also on the external conditions (pressure, temperature, *etc*.). The strength of interactions such as monomer – monomer, template – template, solvent – monomer and solvent – template affects the equilibrium of the formation of a complex of the template molecule with the functional monomer and, hence, the number and 'quality' of the resulting molecular recognition sites.

The second stage is the polymerisation (polycondensation) of the template – monomer complex in the presence of the cross-linking agent and other components of the mixture. It is evident that the optimum conditions of polymerisation can differ from the conditions that favour the formation of a pre-polymerisation complex. For example, the polymerisation process often requires elevated temperatures to be maintained; however, bearing in mind that the functional monomer – template complex is formed only due to electrostatic interactions, the increase in the temperature is undesirable due to weakening of these interactions.

In the third stage, the template is removed, which is accompanied by the liberation of molecular recognition sites. It should be noted that it is sometimes difficult to remove the template from highly cross-linked polymers; hence, a certain amount of template molecules remain incorporated into the polymeric matrix. This reduces the analyte detection limit. When such a polymer is used in analytical studies, the template is gradually eluted from it, which decreases the accuracy of analyte detection.

Different procedures for the efficient removal of template molecules from the polymeric matrix are described.[38] These include, for example, sonication or microwave irradiation during the extraction, extraction with supercritical CO_2 containing an organic solvent as an additive, the use of anhydrous formic, acetic or trifluoroacetic acids for washing the polymer. It was found that, in the general case, the extraction should preferably be carried out with the solvent that has been used in the synthesis of this polymer.

Yet another problem, which one inevitably faces in the synthesis of polymers capable of molecular recognition (especially when preparing sorbents by non-covalent molecular imprinting), is the energy heterogeneity ¶ of the adsorption sites formed. The synthesised polymeric sorbent usually contains a certain number of recognition sites with high affinity to the original template molecules [according to some data, the fraction of highly specific sites of molecular recognition may vary from 0% to 35% (Refs 39 and 40)], a certain number of adsorption sites with a low degree of affinity and also a fraction of adsorption sites with intermediate energy characteristics. Thus, there is always a certain distribution of recognition sites according to the energy.

¶ Heterogeneity of adsorption sites limits the application of these polymers as the stationary phases for HPLC.

It is known that the efficiency and selectivity of chromatographic separation on polymers prepared by molecular imprinting strongly depend on the load of the mixture to be separated, the separation being highly efficient and selective for low load and *vice versa*.[41]

There are several general experimental approaches to prove that the materials prepared by molecular imprinting recognise better the template molecules than other components in the mixture. In any case, a correct comparison requires a sorbent prepared under identical conditions and with the same reagents but in the absence of template molecules. To prove the existence of the molecular imprinting effect, it is necessary to compare the adsorption of template molecules on the material prepared in its presence (the so-called molecularly imprinted sample) and a material synthesised in the absence of template molecules (the so-called control sample). The comparison is carried out under either dynamic or static conditions. In the dynamic mode (chromatographic conditions), the synthesised polymers (including the control sample) play the role of a stationary phase, and the imprinting effect is judged from chromatographic parameters (retention time and retention factors, separation factors and indices) measured for different components in a mixture that also contains the molecular template. The static mode includes, as a rule, measurements of either the equilibrium adsorption of template molecules on the molecularly imprinted and control samples or the adsorption of different substances and, separately, the adsorption of template molecules on the imprinted sample.

As was noted above, the synthesis of polymeric sorbents using the molecular imprinting method is virtually always characterised by the distribution of recognition sites according to the energy. To assess the fraction of sites highly specific with respect to template molecules, one has to study the energy distribution of adsorption sites.

The simplest model that assumes the presence of only two (less often, three) types of recognition sites corresponds to the bi-Langmuir (or tri-Langmuir) isotherm

$$a = \frac{a_{01}K_1C}{1 + K_1C} + \frac{a_{02}K_2C}{1 + K_2C}$$

$$\left(\text{or } a = \frac{a_{01}K_1C}{1 + K_1C} + \frac{a_{02}K_2C}{1 + K_2C} + \frac{a_{03}K_3C}{1 + K_3C}\right),$$

where a is the equilibrium adsorption /mol g^{-1}; C is the equilibrium concentration of adsorbate in solution /mol $litre^{-1}$; a_{01}, a_{02} (a_{03}) are the numbers of recognition sites of the first and second (third) types, respectively /mol g^{-1}; K_1, K_2 (K_3) are the adsorption constants on these sites /litre mol^{-1}.

This model was used for the approximation of experimentally obtained [41–43] adsorption isotherms. The adsorption isotherm was plotted in coordinates $a/C - a$ and the resulting curve was approximated with two crossed lines to find the parameters a_{01}, a_{02}, K_1, K_2. Based on the slopes of these lines and the segments they intercept on the ordinate, the values of a_{01}, K_1 and a_{02}, K_2 were found for the recognition sites of the first and second types, respectively. However, it should be admitted that the model that assumes the presence of only two types of recognition sites is rather limited; hence, it was proposed to use other models.

In one of such models, the Freundlich isotherm was used in the approximation of a set of experimental points [44]

$$a = KC^m,$$

where m is a constant ($0 < m < 1$). The applicability of the Freundlich equation to the description of experimental adsorption isotherms was checked for 12 different sorbents prepared by non-covalent molecular imprinting. It was found that 11 of 12 isotherms are well ($R > 0.95$) fitted by the Freundlich equation. The main drawback of this equation is that this is inapplicable in the region of high concentrations, because, according to the

Freundlich equation, adsorption cannot infinitely increase with the increase in the concentration.

A more versatile model [45] employs the Langmuir – Freundlich equation

$$a = \frac{a_{\max} K C^m}{1 + K C^m},$$

where $a_{\max}$ is the maximum adsorption /mol g^{-1}; K is the constant related with the average adsorption constant (K_0) by the equation $K_0 = K^{1/m}$; m is the heterogeneity index ($0 < m < 1$). The Langmuir – Freundlich isotherm is transformed into the Langmuir isotherm for $m = 1$ and into the Freundlich isotherm for $C \to 0$ or $K \to 0$.

It was shown [45] that the Langmuir – Freundlich isotherm can be used for the approximation of a set of experimental points for materials synthesised by both non-covalent and covalent imprinting methods. The main advantage of this model is that it allows one to obtain the data on the energy distribution of recognition sites, provided the parameters a_0, K and m are known. A method was proposed [45] that makes it possible to obtain the required distribution. Plotting the distribution of adsorption sites according to the energy makes it possible to compare the efficiency of molecular recognition by diverse materials, irrespective of their nature and the type of template molecules. It is noteworthy that the distribution can be calculated only for a concentration range in which the experimental isotherm was plotted. The limitation of this model is that a large body of experimental data is required to obtain reasonable values of a_0, K and m.

IV. Molecular imprinting on mineral supports

Two approaches to the preparation of molecular imprints on mineral supports were developed, namely, imprinting in the bulk (3D imprinting) and on the surface of a mineral support (2D imprinting).

1. Imprinting in the bulk of a mineral support

Generation of molecular imprints in the bulk of a mineral support assumes that the mineral support structure is formed in the presence of template molecules. An attempt was undertaken [46] to reveal the factors that determine the template recognition. For this purpose, the following six pairs of compounds were chosen: Methyl Red – *p*-Methyl Red, Methyl Orange – Ethyl Orange, histidyl-alanine – histidyl-leucine, Methyl Red – Ethyl Red, 2,7-diamino-9-fluorenone – 3,3′-diaminodiphenylmethane, D,L-histidine – L-histidine.

The effect of imprinting was studied by comparing the adsorption of a molecular template (one compound from a pair chosen) with the adsorption of a structurally related compound (the second in the pair) on a sorbent prepared by molecular imprinting.

According to the results obtained, the imprinting effect was much more pronounced when *p*-Methyl Red was used as the template as compared with Methyl Red. On the whole, the studies of the relative adsorption of the pairs listed above have shown that the effect manifests itself only for the first three pairs of compounds. Based on the results obtained, the following conclusions were drawn:

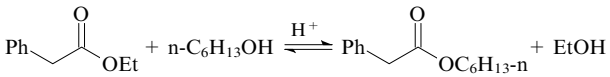
— recognition of template molecules requires the presence of three or more sites of interaction between the molecule to be recognised and the imprint;

— for molecular recognition, the position of a functional group in the template molecule is more important than the size of the latter;

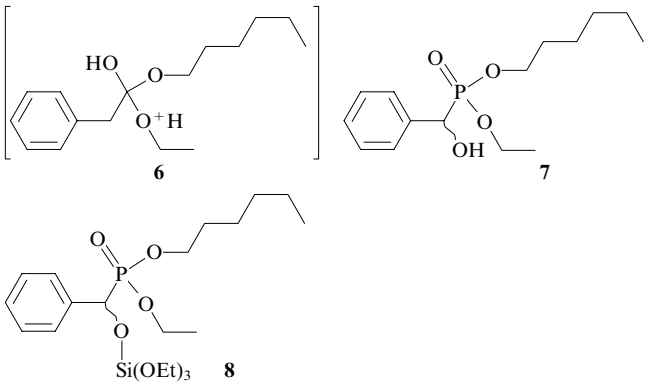
— recognition of chiral molecules requires stronger interactions or larger numbers of interaction sites.

Unfortunately, no data on the diameters of pores and the specific surface areas of the samples prepared were reported in Ref. 46. It cannot be ruled out that these factors can be responsible for the effect observed.

Synthesis of silica gel that specifically catalyses transesterification of ethyl phenylacetate was described.[47]

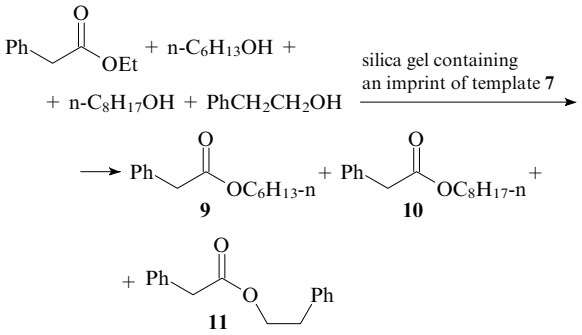


Structure **6** represents the transition state of this reaction; this is why compound **7** was chosen as the template. Derivative **8** was synthesised to incorporate the template into the resulting silica gel. Co-polycondensation with tetraethoxysilane followed by calcination was expected to 'leave behind' the recognition sites matching the transition state of the transesterification reaction.



For comparison, a control silica gel sample was synthesised (in the absence of the template). Silica gel samples prepared both in the presence and in the absence of the template had equal surface areas and the same size distributions of pores.

To check this hypothesis, transesterification of ethyl phenylacetate was carried out using an equimolar mixture of octan-1-ol, hexan-1-ol and 2-phenylethanol on silica gel prepared by covalent imprinting.



The results obtained pointed to the preferential formation of n-hexyl and n-octyl phenylactetates (the ratio **9**:**10**:**11** was 7:8:1), which, at first glance, might indicate the presence of cavities complementary to the analogue of the transition state of this reaction. The transesterification reaction with these three alcohols proceeded at approximately the same rates both in the homogeneous phase and on the control sample.

However, as was found later,[48,49] the results obtained cannot serve as a proof of the imprinting effect on the transesterification reaction. First, comprehensive studies of synthesised materials, which involved solid-state ^{31}P NMR spectroscopy and thermogravimetric analysis, have shown that the template molecule is merely adsorbed by the sorbent rather than covalently bound. Hence, the formation of specific cavities is questionable, *i.e.*, the observed selectivity in the transesterification reaction cannot be unambiguously explained as the manifestation of the molecular imprinting effect. Second, for general reasons, it follows that the

presence of a transition state analogue in the reaction mixture should inhibit the transesterification reaction; however, no such inhibition was observed.[47] Moreover, in their later study,[49] the authors failed to reproduce their own results on the selective transesterification. Apparently, the selectivity observed earlier[47] is explained better as a result of different diffusion rates of alcohols and esters in micropores. Similar results, namely, the dependence of selectivity on the diffusion rate rather than on the presence of specific recognition sites, were obtained[50,51] for materials synthesised by the sol–gel method. These examples revealed the following drawbacks of using the sol–gel synthesis in the technology of molecular imprinting, *viz*., poor reproducibility due to the impossibility of strict control over the pore size during the synthesis and a probability that the observations of diffusion phenomena in micropores would be wrongly interpreted as the imprinting effect. One can avoid some of these drawbacks by employing imprints prepared on the surfaces of mineral supports.

2. Surface modification of mineral supports

As was mentioned above, the presence of a template in the stage of synthesis of sorbents affects the chromatographic properties of the latter[52,53] and also the activity and selectivity of stereospecific catalysts.[54–56] This method is also used in the preparation of materials with definite spatial structures.[57–59]

It is very significant that most of published procedures of the preparation of organised surfaces, which involve the use of template molecules, have a common drawback, namely, the low stability of imprints formed on the surface. For example, operation with modified silica in aqueous media can be accompanied by the surface restructuring, which leads to the blurring and disappearance of imprints formed. This is why attention is focused on the materials with fixed molecular imprints, which represent covalently fixed structures. Such structures can be obtained by chemical modification of the surface.[60,61]

The possibility of construction of ordered structures on the surfaces of inorganic supports, particularly silica, is determined by the fact that its surface is uniformly covered with hydroxyl groups.

The methods of surface modification of oxide supports aimed at the formation of recognising materials can be divided into the following four groups:

(1) preliminary adsorption of template molecules followed by chemical modification of unoccupied surface areas;

(2) multipoint fixation of complex molecules on the surface followed by the removal of the central fixing fragment;

(3) fixation of silanes on the support surface (polycondensation in pores) in the presence of template molecules;

(4) fixation of a polymeric layer capable of molecular recognition on the particles of mineral oxides or in their pores.

A sufficiently comprehensive survey[62] is devoted to the preparation of chemically modified oxide surfaces capable of molecular recognition.

a. Method involving preliminary adsorption of template

This method includes the stages of preliminary adsorption of the template, chemical modification of the remaining unoccupied surface and removal of the template (Fig. 4). As a result of such a modification of the support surface, the latter retains areas that resemble the template in the shape and exhibit chemical properties different from those of the modified areas.

To form ultrathin silica layers, Japanese scientists employed chemical vapour deposition (CVD). For this purpose, tetramethoxysilane (TMOS) was deposited from the gas phase onto oxide supports such as zeolite,[63] alumina,[64–66] titania and zirconia,[66,67] tin oxide[68] or silicalite.[69] The pretreatment of the support surface with an aldehyde (*e.g*., benzaldehyde) results in the formation of surface areas with chemisorbed stable benzoate anions.[70] Further modification of such a surface with TMOS involves only free surface areas. Thus this method was applied for

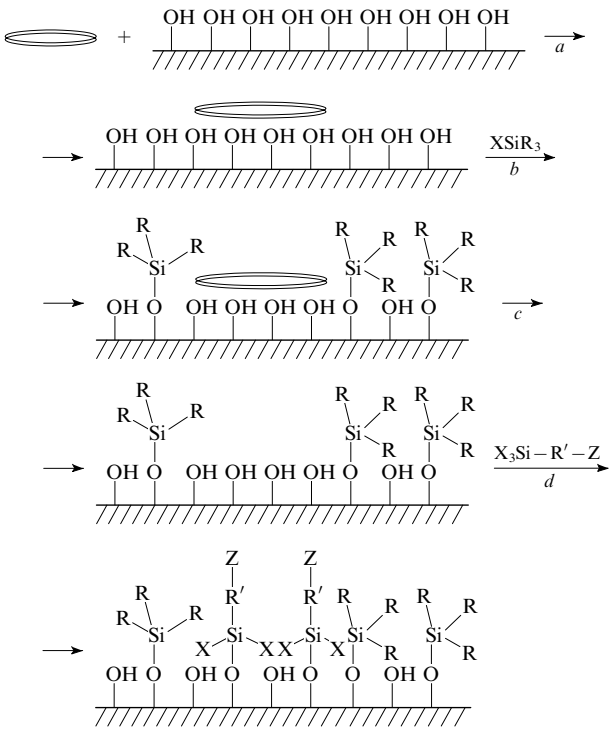


Figure 4. Preparation of molecular imprints in a graft layer. (*a*) template adsorption, (*b*) modification of the unoccupied surface, (*c*) template desorption, (*d*) modification of 'imprints'.

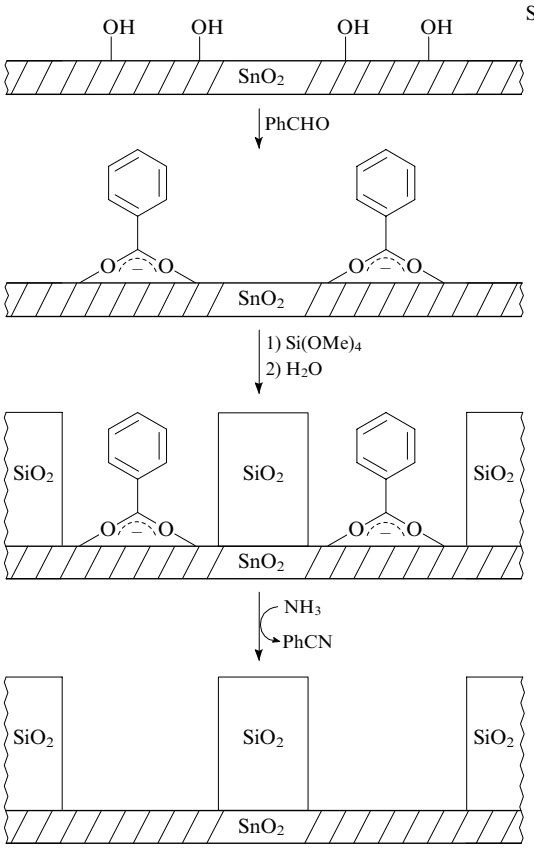
modification of the surface of γ-alumina.[71] Studies of benzaldehyde adsorption on the sorbent samples have shown that they behave as molecular sieves with respect to benzaldehyde. It was found that the formation of a siloxane network in the adsorption of tetramethoxysilane on the surface of benzalehyde-treated γ-Al_2O_3 is prevented due to the presence of template molecules, namely, benzaldehyde, on the surface.

Systems based on tin oxide were also studied.[72,73] Upon adsorption of benzaldehyde (graft density 1.49 nm^{-2}) or α-naphthaldehyde (0.77 nm^{-2}) on the tin oxide surface, the latter was treated with TMOS, which was followed by the hydrolysis. The cycle 'treatment with TMOS–hydrolysis' was repeated several times. As a result, the surface concentration of silicon atoms was 6.2 or 2.9 atoms per nm^2, respectively, which exceeds the monolayer coverage of the free surface. The template was removed with ammonia (Scheme 6).

Studies of the adsorption of these aldehydes and of 2-chloro- and 2,6-dichlorobenzaldehydes have shown that all of them are sorbed better on the unmodified surface of tin oxide. For silica-modified samples studied, the best results were achieved when α-naphthaldehyde was used as the template. However, the samples behaved as molecular sieves only at high silicon contents (4.5 and 6.2 atoms per nm^2).

Electroconductivity and catalytic activity of pure tin oxide (sample *1*) and tin oxide samples with SiO_2 layers deposited by a similar procedure with α-naphthaldehyde (sample *2*), benzaldehyde (sample *3*) and butyraldehyde (sample *4*) as the templates were studied.[74] It was found that the adsorption of C_8 hydrocarbons with different structures first causes a sharp decrease in the resistance of samples and then its increase; this is why the comparison was carried out in the point of a minimum. The ratio between the resistances of the starting sample (R_a) and a sample with adsorbed hydrocarbons (R_g) was used as the criterion of conductivity (Fig. 5).

Scheme 6



The catalytic activity of samples *1–4* was assessed based on the conversion of linear, branched and cyclic hydrocarbons C_6 in the course of their oxidative destruction to CO_2 (Fig. 6). An attempt was undertaken to relate the activity of samples *1–4* in the oxidative cracking of hydrocarbons C_6 to the molecular imprint size and check the correspondence of the latter to the size of the adsorbed hydrocarbon (Table 3).

The idea of using molecular imprints in the preparation of covalently grafted islet structures was put forward by Fadeev [75] in 1986. He noted that chemical modification of a surface that contains pre-adsorbed template molecules allows one to 'fix' the imprints and thus increase their stability. Further modification of the 'imprints' obtained can be carried out by conventional methods of surface chemistry.

'Sharp' imprints can be obtained, if the template is adsorbed unambiguously and sufficiently strongly on the surface, which prevents its washing out during the modification. At the same time, the template adsorption should be reversible to allow its removal from the surface after the chemical modification. It was proposed that metal phthalocyanines, particularly silicon dichloride phthalocyanine ($PcSiCl_2$) and copper naphthalocyanine (NcCu) should be used as the templates.[75]

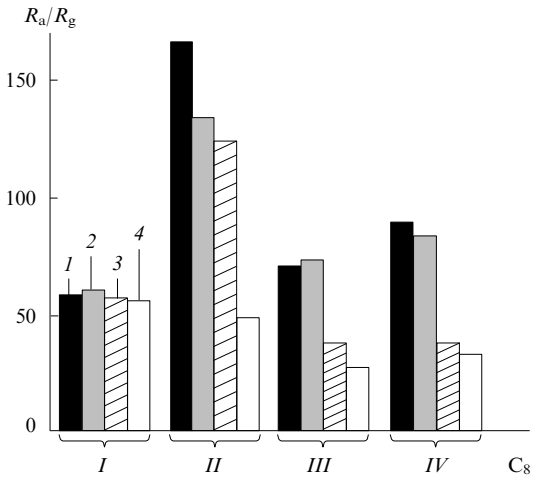


Figure 5. Changes in the conductivity of samples of (*1*) SnO_2 and (*2–4*) SiO_2/SnO_2 as a result of adsorption of different hydrocarbons C_8 (*I–IV*) at 643 K.[74]
(*I*) n-octane, (*II*) 2-methylheptane, (*III*) 2,3-dimethylhexane, (*IV*) cyclooctane. The preparation of samples *2–4* involved preliminary adsorption of the following templates: (*2*) α-naphthaldehyde, (*3*) benzaldehyde and (*4*) butyraldehyde.

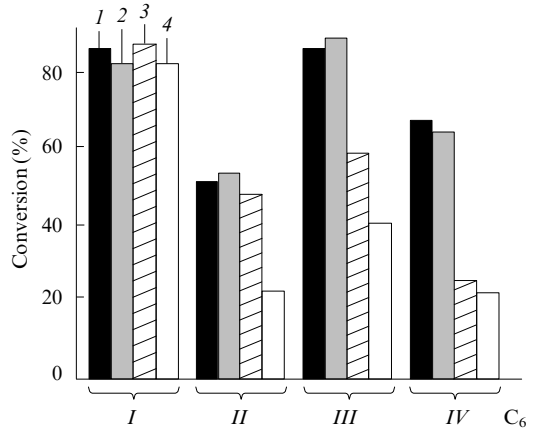
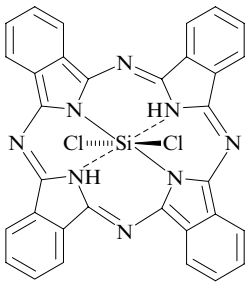


Figure 6. Oxidation of alkanes C_6 at 643 K on (*1*) SnO_2 and (*2–4*) SiO_2/SnO_2 samples.[74]
(*I*) n-hexane, (*II*) 2-methylpentane, (*III*) 2,3-dimethylbutane, (*IV*) cyclohexane. For the description of samples *1–4*, see the caption to Fig. 5.

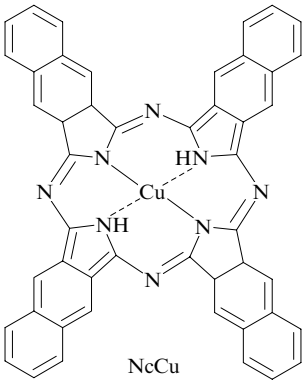
Table 3. The size of template molecules used for the modification of the tin oxide surface and the sensitivity of prepared sorbents with respect to C_6 alkanes.

Template	Sensitivity with respect to				
	template molecule size /nm	n-hexane (0.36 × 0.48)	2-methylpentane (0.37 × 0.60)	2,3-dimethylbutane (0.57 × 0.62)	cyclohexane (0.43 × 0.61)
None	–	h	h	h	h
α-Naphthaldehyde	0.37 × 0.92	h	h	h	h
Benzaldehyde	0.37 × 0.67	h	h	l	l
Butyraldehyde	0.36 × 0.48	h	l	l	l

Notes. The following abbreviations are used: h corresponds to high sensitivity, l corresponds to low sensitivity. Sizes of C_6 molecules are given in nm.



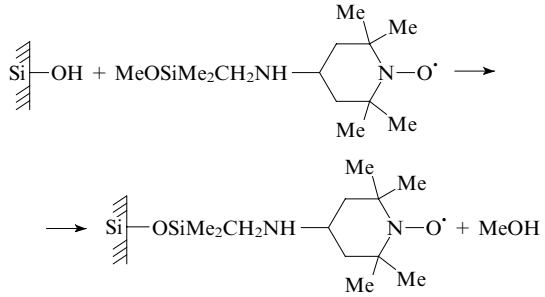
$PcSiCl_2$



NcCu

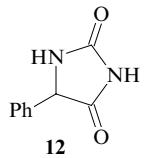
Phthalocyanines are adsorbed sufficiently strongly (but reversibly) on silica, occupy large landing areas per molecule and are easily modified. Moreover, they are excellent chromophores, which makes easier their detection in the adsorbed state. Table 4 shows the conditions of adsorption of copper naphthalocyanine on the surface of silochrome 120 (entry I) and of silicon dichloride phthalocyanine on silochrome 120 modified with 3-(aminopropyl)triethoxysilane (entry II), and also the conditions of surface modification and template removal.

The completeness of the phthalocyanine adsorption and desorption was monitored by spectrophotometric techniques. The formation of imprints in the graft layer upon removal of adsorbed templates was proved using the method of paramagnetic labels, which involved treatment of the sorbent sample with an excess of iminoxyl radicals.[76–78]



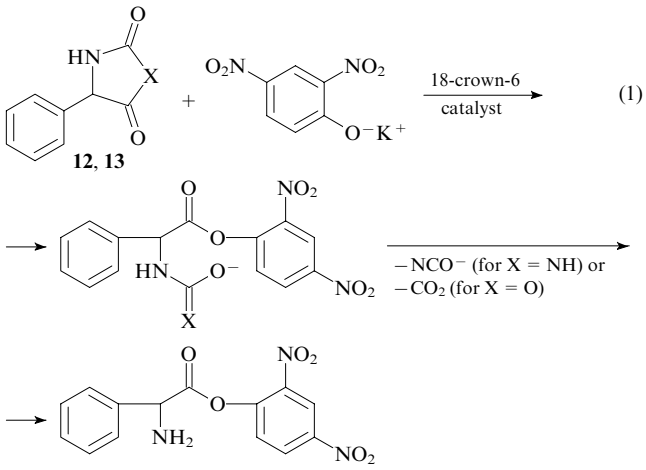
A comparison of EPR spectra of molecularly imprinted sorbent samples, which were prepared in the presence of templates, and the control samples, which had the known uniform distribution of the paramenetic label, has shown that the label molecules in the former samples are located more closely to one another. The EPR data on the distances between the neighbouring labels and the number of labels correspond to the results of calculations based on the silica surface area and the size and number of imprints. The comparison of adsorption properties of molecularly imprinted and the control sorbent samples have shown that the adsorption capacity of the former with respect to the corresponding template molecules increases. These results point to the formation of the corresponding islet nanostructures on the silica surface.

A large series of publications described the development of methods for the synthesis of catalysts capable of molecular recognition. The idea that underlies these methods is that compounds with structures close to that of a catalytic reaction transition state could serve as the templates in the synthesis of catalysts. It was proposed[79–87] to synthesise enantioselective catalysts based on silica gels with *R* or *S* enantioisomers imprinted, in particular, (*R*)- or (*S*)-5-phenylimidazolidine-2,4-dione [5-phenylhydantoin (**12**)].[87]



12

For this purpose, the surface of pure SiO_2 was treated with concentrated HCl to produce silanol groups, washed and then doped with Al^{3+} ions (by adding aluminium chloride and heating to 80 °C for 3 h) to obtain aluminium-substituted active centres (Lewis acid sites). After this treatment, a solution of the template (5-phenylhydantoin) was added to silica gel and the samples were stored for a week at pH 4. Then the template was extracted in the Soxhlet apparatus until its complete removal from the surface. The catalysts obtained were assayed in reactions of *R* and *S* isomers of 5-phenylhydantoin (**12**) and 4-phenyl-1,3-oxazolidine-2,5-dione (**13**) with potassium 2,4-dinitrophenoxide. Table 5 shows the kinetic parameters of reaction (1) for X = O.



12, **13**

X = NH (**12**), O (**13**); catalyst is silica gel with an imprint of (*R*)- or (*S*)-5-phenylhydantoin.

It follows from Table 5 that the reaction rate constants substantially differ depending on the catalyst type used. The highest rate is achieved for a catalyst with the configuration identical to that of the corresponding substrate. The results obtained can be explained by the formation of chiral cavities on the silica gel surface (Fig. 7). It is these cavities that determine the catalyst stereoselectivity. From the temperature dependence of the catalytic reaction rate it was possible to calculate the activation parameters for each catalyst type. The energy of activation of the *S*-substrate on the *S*-catalyst is lower than the energy of activation of the *R*-substrate on the *S*-catalyst. The possibility of the preparation of stereospecific catalysts by the molecular imprinting method was confirmed by the example of other catalytic reactions (see Refs 88 and 89).

b. Multipoint fixation of complex molecules

In essence, the method of multipoint fixation of complex molecules [*e.g.*, $(X_3Si-R'-Z)_mA$] on the surface of oxides consists of the preparation of sorbents in which the functional groups Z are fixed at a certain distance from the sorbent surface that favours subsequent selective binding of fragment A of these molecules.

Table 4. Conditions of template adsorption on the silica surface, subsequent surface modification and removal of template molecules.

Entry	Original surface	Template molecule	Conditions of template adsorption	Amount of adsorbed template /μmol m^{-2}	Conditions of modification of a surface containing adsorbed template	Conditions of removal the template from the surface	'Resulting' surface
I	Silochrome 120	NcCu	Solution in toluene ($\sim 10^{-6}$ M)	0.0017	Treatment with trimethylchlorosilane in gas phase at 60 °C	Treatment with a mixture of trimethylacetyl chloride and morpholine in hexane	'Islets' of original silica on the 'background' of a trimethylsilane monolayer
II	Silochrome 120 modified with aminopropyl groups	$PcSiCl_2$	Solution in toluene ($\sim 10^{-6}$ M)	0.0057	Treatment with ethyl acrylate in propan-2-ol at 60 °C	Extraction with toluene with addition of acetic acid in the Soxhlet apparatus	'Islets' of aminopropyl groups on the 'background' of ethyl acrylamide

Table 5. Kinetic parameters of reaction (1) mediated by a catalyst prepared by the matrix imprinting method.[87]

Configuration of 5-phenylhydantoin imprint on the catalyst surface	Substrate **13** configuration	$10^{-4}\,K_M$ /mol litre^{-1}	$10^{-4}\,k_{max}$ /s^{-1}	$10^{2}\,k_{cat}$ /s^{-1}	$10^{5}\,k_{cat}/K_M$ /mol^{-1} s^{-1} litre	Enantioselectivity [a]
R	*R*	1.93	5.64	4.29	22.2	1.34
	S	2.14	4.68	3.56	16.6	
S	*S*	5.26	7.13	4.87	9.25	2.01
	R	7.16	4.82	3.29	4.60	
RS (see[b])	*R*	5.02	5.45	4.76	9.48	1.0
	S	5.02	5.45	4.76	9.48	

Notes. Designations: K_M is the Michaelis – Menten constant; k_{max} is the maximum rate constant calculated for the first-order reaction mechanism; k_{cat} is the average value of k_{max} normalised with respect to the number of catalytic sites in 50 mg of catalyst. [a] Determined based on the ratio k_{cat}/K_M for (*R*)- and (*S*)-4-phenyl-1,3-oxazolidine-2,5-diones. [b] Catalyst was prepared with the use of a racemate as the template.

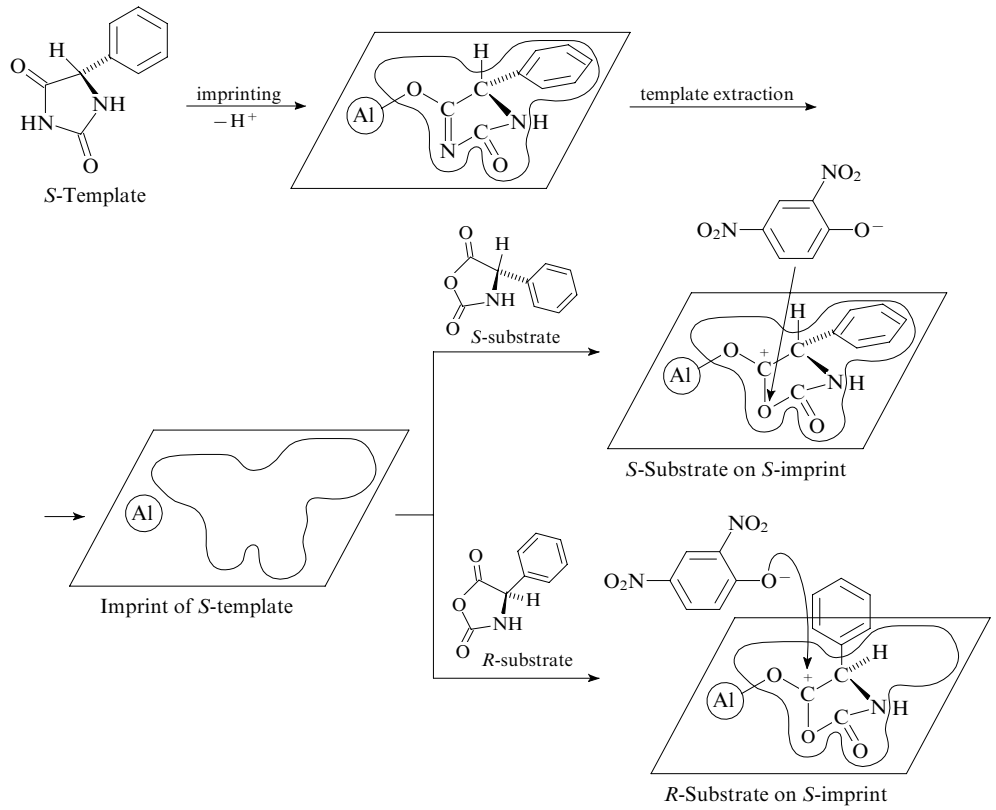
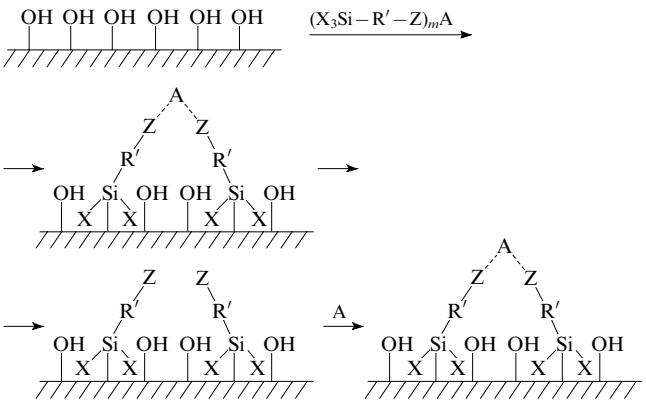
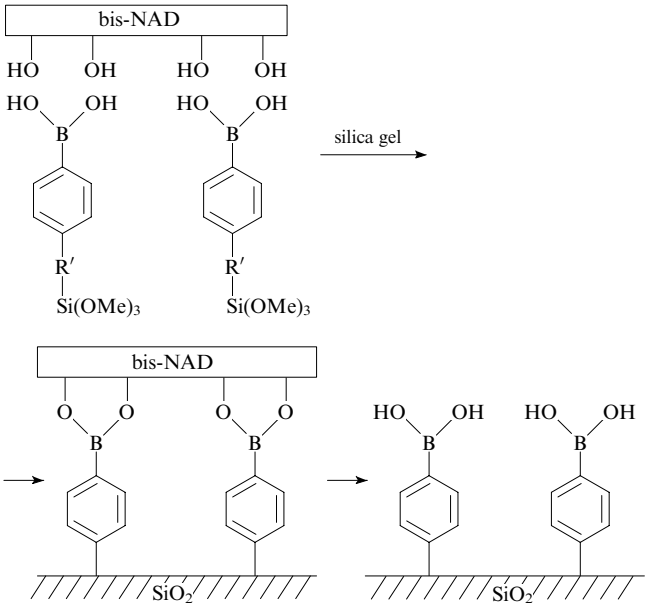


Figure 7. A scheme of enantioselective catalysis on an imprint obtained with the use of *S*-template.[87]



This method was used for the synthesis of a sorbent selective with respect to nicotinamide adenine dinucleotide (NAD) and containing boronate groups at a fixed distance from one another.[90,91] The synthesis is based on the known reaction of phenylboronic acid with the sugar fragments of bis(nicotinamide adenine dinucleotide) (bis-NAD).



The reaction product of 4-[$(MeO)_3SiR'$]$C_6H_4B(OPr^i)_2$ with two types of bis-NAD, which contained spacers of different length (7 and 17 Å) between NAD fragments, was used as the modifier of the silica gel surface.

Following fixation of the modifier, the silica gel surface was additionally silanised with dodecyltriethoxysilane, phenyltriethoxysilane, *N*-(2-aminoethyl)-3-aminopropyltrimethoxysilane and bis(2-hydroxyethyl)aminopropyltriethoxysilane to improve the sorbent properties. The sorbents obtained were used in the chromatographic determination of bis-NAD by HPLC. It was found that the retention of each of bis-NAD types on the sorbent modified with this particular NAD type was twice stronger as compared with sorbents modified with the other NAD types.

A method of modification of the silica gel surface that involved the use of the Ru[bipy-CH=N$(CH_2)_3$Si$(OEt)_3$]$_3$ complex was described.[92] After hydrolytic cleavage of the CH=N bond of this complex, three amino groups fixed at a definite distance from one another remained on the silica gel surface. Such sorbents can be used for selective sorption of ruthenium or as piezoelectric sensors. These amino groups can be modified further with the aim of preparation of sorbents for HPLC.[93]

c. Method of fixation of silanes in the presence of template molecules

The simplest method of preparation of sorbents with molecular imprints is based on the polycondensation of alkoxysilanes in the pores (or on the surface) of silica gel, which is carried out in the presence of a template (Fig. 8).

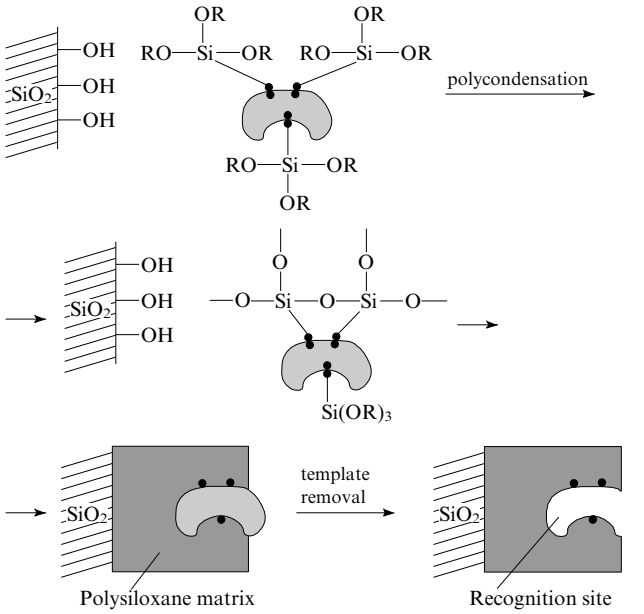


Figure 8. A scheme of hydrolytic polycondensation of alkoxysilanes on the silica gel surface in the presence of a molecular template.

Synthesis of silica gel-based sorbents selective to a given dye was described.[91] A mixture of phenyltriethoxysilane, *N*-(2-aminoethyl)-3-aminopropyltrimethoxysilane and bis(2-hydroxyethyl)-aminopropyltriethoxysilane was added to a porous silica gel impregnated with a dye (Rhodanine Blue or Sapfranine O) and their polycondensation was carried out.

Based on the same silane mixture and a silane with a fragment of 3-aminophenylboronic acid, sorbents containing transferrin and bovine serum albumin imprints were prepared.[91] Of the sorbents obtained, only that synthesised in the presence of transferrin exhibited selectivity.

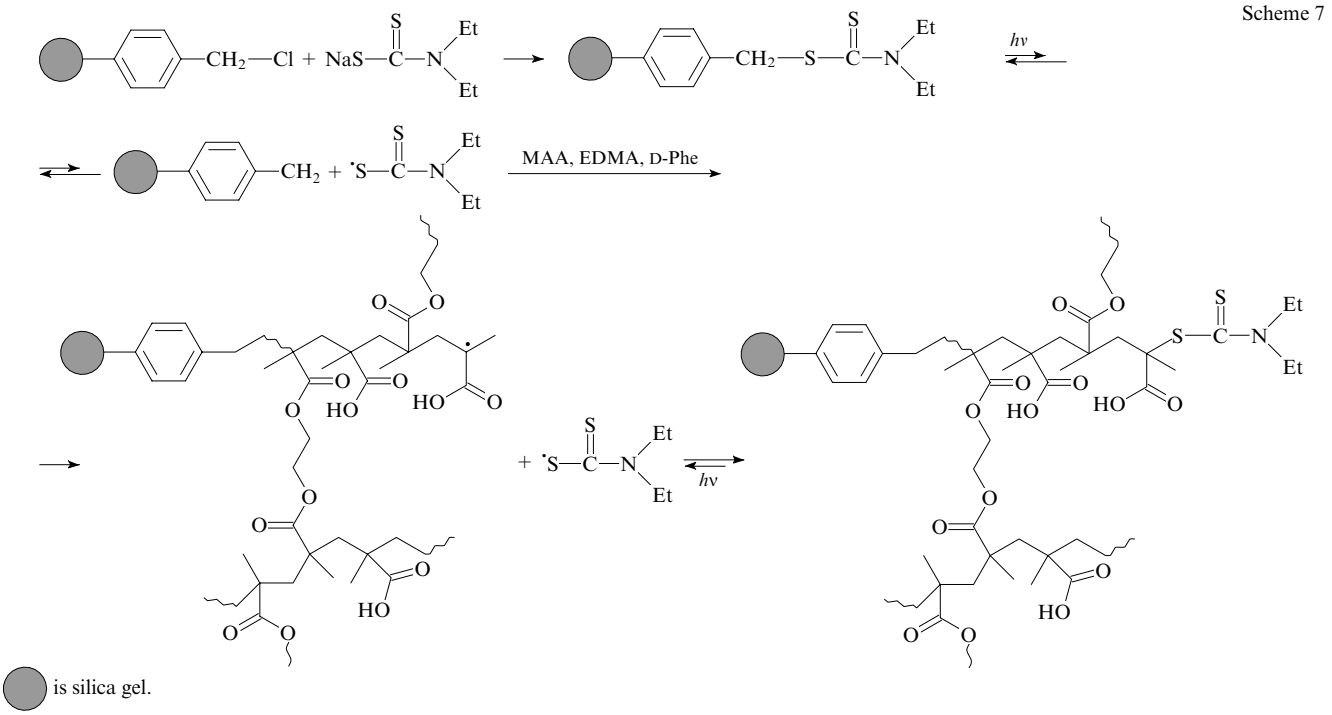
Silica gels with imprints of ricin and its A and B chains were prepared in the pores of silica gels impregnated with these compounds by polymerisation of a mixture of tetraethoxysilane and bis(2-hydroxyethyl)aminopropyltriethoxysilane.[94] Each of three silica gels bound almost exclusively the object that served as the molecular template. Later, a similar method was used in the preparation of silica gels containing imprints of *N*-decyl-D-phenylalanyl-2-aminopyridine.[95]

d. Synthesis of polymers capable of molecular recognition

The methods of synthesis of polymers for molecular recognition are sufficiently well developed. However, swelling, lability and insufficient permeability limit their application. Most of these limitations can be overcome by fixing a thin polymeric layer on the surface of particles of a porous silica gel or in its pores. Strong binding is achieved by preliminary chemical attachment to the silica gel surface of functional groups that can be involved in subsequent polymerisation.

This method was used in the synthesis of sorbents for chromatographic determination of imidazole.[40] First, 3-(trimethoxysilyl)propyl methacrylate was fixed to the silica gel surface. Then, ethylene glycol dimethacrylate, copper *N*-(4-vinylphenyl)-

Scheme 7



iminodiacetate and a molecular template of the imidazole series [1,4- and 1,3-bis(imidazol-1-ylmethyl)benzene or 4,4′-bis(imidazol-1-ylmethyl)biphenyl] were added and polymerisation was induced by azobis(isobutyronitrile). The synthesised material was packed into HPLC columns, copper ions were exchanged for zinc ions, and the sorbent was used in the chromatographic determination of imidazole or template molecules. The preferential binding of molecules that served as the templates during the sample preparation was observed.

Complexes of copper *N*-(4-vinylphenyl)iminodiacetate with L-, D- and L,D-phenylalanine and other amino acids can be used as molecular templates.[96,97] Selectivity of chromatographic resolution of L,D-phenylalanine on a sorbent prepared with a copper–D-phenylalanine complex as the molecular template was 1.65, while the selectivity of separation of L,D-thyrosine on the same sorbent was 1.56. The same column was used for the resolution of racemic α-methylphenylethylamine (selectivity 1.32); however, attempts to resolve a racemate of α-methylcinnamic acid failed.

Treatment of silica gel with surface-bound active chlorobenzyl groups with sodium *N*,*N*′-diethyldithiocarbamate afforded an UV-sensitive initiator of radical polymerisation.[98] This was used in the radical polymerisation of methacrylic acid and ethylene glycol dimethacrylate in the presence of L- or D-phenylalanine (L- or D-Phe) anilides as the templates (Scheme 7). The same materials were prepared in the absence of the template. The separation factors ($\alpha = k'_L/k'_D$, where k'_L and k'_D are the retention factors for L- and D-Phe) of enantiomeric template molecules on HPLC columns packed with sorbents prepared in the presence of L-Phe and D-Phe were 2.4 and 0.35, respectively, and 1 in the absence of amino acid.

Other sorbents intended for the separation of L- and D-phenylalanine anilides were prepared analogously. For this purpose, a radical azo-initiator was fixed on the surface of porous silicas Si-1000, Si-300 and Si-100 and non-porous silica gel; then, MAA and EDMA were polymerised in the presence of L-phenylalanine anilide.[99] The optimum chromatographic separation of L- and D-phenylalanine anilides with the separation factor of up to 7 was achieved for a polymeric film of a thickness of 6–7 nm.

Silica gel with fixed vinyl groups was used in the co-polymerisation of acryloylcyclodextrin and *N*,*N*′-methylenedi(acrylamide) in the presence of molecular templates, namely, L-Phe-L-Phe or D-Phe-D-Phe.[100] Table 6 shows some results obtained on chromatographic columns packed with these sorbents.[101]

Table 6. Retention times of amino acids, peptides and *m*-nitrophenol on chromatographic columns with different sorbents.[101]

Compound	Retention time (min) on sorbents [a]		
	A	B	C
L-Phe-L-Phe	9.61	10.20	10.62
D-Phe-D-Phe	9.68	9.73	11.73
L-Phe	4.21	4.27	4.34
D-Phe	4.17	4.25	4.32
m-Nitrophenol	24.65	21.47	23.65

[a] Sorbents that contain no molecular imprints (A) and contain L-Phe-L-Phe (B) and D-Phe-L-Phe imprints (C).

The surface of capillaries for capillary electrophoresis was modified analogously.[102] First, the capillary surface was treated with (methacryloyloxypropyl)trimethoxysilane. Then, the prepolymerisation mixture that contained monomers, a cross-linking agent (TRIM), a radical initiator [2,2′-azobis(isobutyronitrile)] and template molecules [(*S*)-propranolol, (*S*)-metoprolol or (*S*)-atenolol], was introduced into the capillary columns. After completion of polymerisation, template molecules were removed by extraction with acetonitrile, methanol or a methanol–acetic acid mixture. The columns thus modified were used in the separation of certain organic substances.

V. The applications of materials capable of molecular recognition

Polymers with molecular imprints attract keen attention of scientists and engineers. First of all, this is associated with the active quest for selective sorbents (including sorbents for HPLC), the materials for membranes and sensors and artificial antibodies (Fig. 9).

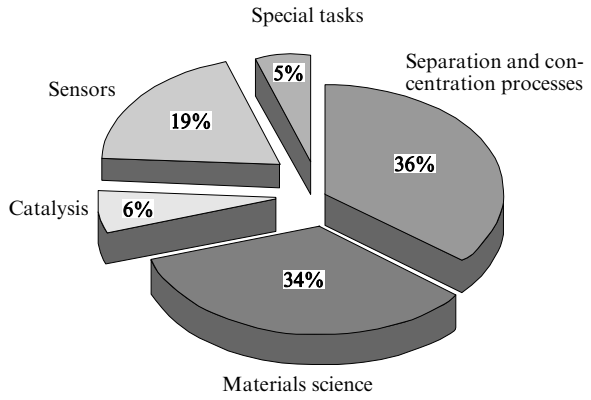


Figure 9. Distribution of application fields of polymers with molecular imprints (according to the data from Ref. 101).

Sorbents based on polymers with molecular imprints exhibit several obvious advantages as compared with conventional sorbents. They are characterised by high chemical affinity and selectivity with respect to target molecules (which in certain cases is comparable with the selectivity of biomolecules), high stability, which exceeds that of biomolecules, relatively easy adaptability to various practical applications and relatively low cost.[101]

Polymers with molecular imprints are employed most often in the separation and concentration (*e.g.*, see a survey [103]). The search for effective sorbents for the exhaustive cleaning of water † and the processing of industrial and domestic wastes is in progress. The USA alone spend more than $ 500 million for the manufacture of sorbents for chromatographic columns, the percentage of polymers with molecular imprints being 1% – 3%.[101] In the pharmaceutical industry, the biopharmaceutic branch (production and purification of recombinant proteins and monoclonal antibodies) is most dynamic. The use of sorbents with molecular imprints in the separation of proteins is described in Ref. 104.

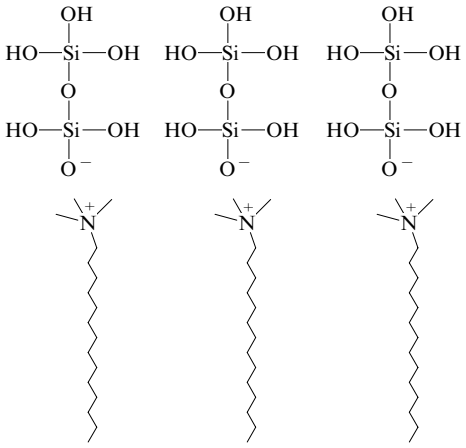
Chemical sensors based on polymers with molecular imprints are being actively developed. They are considered as an alternative to biosensors, which exhibit low stability and are very expensive. To date, sensors based on polymers with molecular imprints have already been developed for the detection of certain herbicides,[105] sugars,[106] medical drugs,[107] nucleic acids,[108] toxins,[109] *etc*.

Porous materials with the template-determined pore sizes closely relate to the subjects considered in the present review. In this case, it is surfactants that serve as templates; however, their sizes exceed those of molecules and are determined by the geometry of surfactant globules. In 1992, synthesis of the first structured mesoporous material, MCM-41 (Mobil Composition of Matter), which is remarkable for its regular pores with a narrow size distribution (a result of hexagonal packing of linear cylindrical pores), was reported.[110]

To explain why mesoporous materials form ordered structures, Beck *et al.*[111] have proposed the so-called LCT (Liquid Crystal Templating) mechanism. They assumed that the formation of an ordered structure is associated with micelles that are formed in surfactant solutions (with a concentration exceeding the critical micelle concentration) and serve as the templates in the synthesis of MCM-41 and related materials. First, spherical micelles are formed and, next, cylindrical. The latter are transformed into the so-called micellar rods upon the reaction with silicate.

† For example, see D M Han, G Z Fang, X P Yan. *J. Chromatogr., A* **1100** 131 (2005).

In the second stage, the micellar rods are ordered hexagonally to afford a sort of a composite, which contains the bound organic and inorganic layers. Calcination of the obtained composite and removal of organic component produce an inorganic structure with hexagonally ordered cylindrical pores of identical diameters.



A recently published review [112] is devoted to the synthesis and physicochemical and catalytic properties of mesoporous titanium silicate materials.

VI. Conclusion

The analysis of data discussed in the present review points to the substantial interest of scientists in the synthesis of materials capable of molecular recognition. The most remarkable results are achieved in the field of synthesis of organic polymeric materials, which is explained by the wide possibilities of varying the chemical nature of monomers and cross-linking agents and also the flexibility of the polymeric structure.

To date, only few studies are devoted to designing mineral matrices with imprints. The approach based on functionalisation of the surface by multipoint fixation of complex molecules can be applied to the solution of only very specific problems. Sorbents prepared by the modification of areas left unoccupied after the template adsorption also find limited application, because the imprints formed on the surface are complementary to the templates only as regards their geometry.

Being versatile, the materials obtained by polycondensation of siloxanes or polymerisation of organic monomers in the support pores in the presence of template molecules show more promise. It is hoped that mineral supports with imprints, first of all, silica will find application in HPLC.

Both the published data and our own experience allow us to suggest that the clarity of the idea of molecular imprinting is rather illusory. The possibility of achieving the desired result is often limited by the ambiguous mechanism of the process under study and, as a consequence, a considerable non-uniformity of generated adsorption sites. Hence, it is not surprising that one can rarely enhance the specificity of a recognising material by more than 30%, whereas biological evolution has developed absolutely specific materials implemented in 'antigene – antibody' interactions. It cannot be ruled out that the use of methods of molecular biology, particularly, the use of aptamers, in the synthesis of recognising materials will prove to be more promising than the attempts to develop chemical models of biological processes. Nonetheless, the synthesis and application of artificial materials with molecular imprints are being progressively developed in scientific studies and occupy the increasingly strong position in many applied fields.

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