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Journal of Chromatography A, 889 (2000) 15–24

JOURNAL OF CHROMATOGRAPHY A

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Review

New configurations and applications of molecularly imprinted polymers

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Abstract

Molecularly imprinted polymers (MIPs) are applicable in a variety of different configurations. For example, bulk polymers imprinted with β-lactam antibiotics are presented to be used as stationary phases for the chromatographic separation of β-lactam antibiotics with both aqueous and organic mobile phases. However, in some analytical applications, monosized spherical beads are preferred over the currently used ground bulk polymers. A precipitation polymerization technique allows preparation of monosized spherical imprinted beads with diameters down to 200 nm having excellent recognition properties for different target molecules. Nevertheless, with current imprinting protocols a substantial amount of template has to be used to prepare the polymer. This can be problematic if the template is poorly soluble, expensive or difficult to obtain. It is shown that for analytical applications, the functional monomer:template ratio can be drastically increased without jeopardizing the polymer's recognition properties. Furthermore, a substantial reduction of the degree of crosslinking is demonstrated, resulting in much more flexible polymers that are useful for example the preparation of thin imprinted films and membranes for sensors. Apart from analysis, MIPs also are applicable in chemical or enzymatic synthesis. For example, MIPs using the product of an enzyme reaction as template are utilized for assisting the synthetic reaction by continuously removing the product from the bulk solution by complexation. This results in an equilibrium shift towards product formation.

Keywords: Molecular imprinting; Stationary phases, LC; Reviews; Lactams; Antibiotics; Penicillins; Aspartam

Contents

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0021-9673/00/$ – see front matter
PII: S0021-9673(00)00350-2

1. Introduction

In the past few years molecular imprinting [1,2] has entered many areas of chemistry, biochemistry and biotechnology. Nowadays polymers imprinted with different templates like drugs, herbicides, sugars, nucleotides, amino acids and proteins are more and more applied in analytics, as well as in catalysis or for synthetic processes. Before generating the molecularly imprinted material, the user has to consider the later purpose, in order to predetermine the adequate format of the polymer. One way to obtain these materials is the production of bulk polymers, which have to be ground and sieved. This process is well established, especially when the polymer is to be utilized in columns for HPLC or for assays. However, the procedure is time consuming and unfortunately implicates a loss of material due to the need of removing fine particles (''fines'') from the usable remains. A polymer yield of useful particles is typically around 20%. To avoid that disadvantage an alternative method was found, namely the generation of microbeads. These materials can be used directly after production, merely the template has to be removed via extraction. It is even more advantageous, because these microspheres can be produced in a uniform manner. For instance chromatographic results are much easier to reproduce, if the molecularly imprinted stationary phase is of a defined structure and reliable quality, which is more difficult to achieve by grinding polymers. Another important configuration of molecularly imprinted polymers (MIPs) are membranes generated by applying different techniques, which are listed in Table 1. A relatively new approach represents the in situ polymerization of molecular imprinting mixtures, e.g., in capillaries [9,10] and columns [11,12] resulting in coatings or macroporous fillings. Fig. 1 demonstrates that a proper format of the MIP has to be selected before applying this imprinted material. For instance for the determination of antibiotics in food samples a robust configuration has to be chosen due to the complexity of the sample composition. Either the sample can be analyzed by HPLC utilizing MIP stationary phases or in case of difficult samples a preconcentration of selected analytes via solid-phase extraction (SPE) using MIPs can be performed. In both cases ground bulk polymers can be selected. In order to achieve very low detection limits due to high sensitivity radioassays should be applied using monodisperse microspheres with higher load capacities than ground particles. In case an amount of template sufficient for generating enough imprints is not available, new and uncommon recipes are required for obtaining MIPs for radioassays. Furthermore, the MIPs can be useful for the acceleration of synthetic reactions. Some examples are presented in this publication.

Table 1
Overview of the techniques used to generate MIP membranes

Method	Ref.
Polymerization on a glass filter surface	[3]
Phase inversion precipitation technique (casting by coagulation in the presence of water)	[4,5]
Evaporation of solvent	[6,7]
Generation of film-like membranes on glass slides	[8]

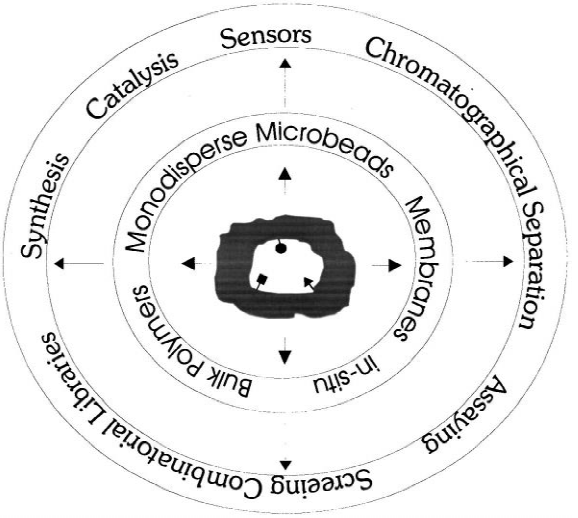

Fig. 1. Configurations and applications of molecularly imprinted polymers.

2. Molecularly imprinted polymers for robust food analysis

Nowadays society is highly sensitive towards contaminants in foodstuff. Such substances may be pesticides, herbicides, hormones and antibiotics. For the detection of contaminants fast analytical tools

have to be developed. MIPs – providing selectivity and durability – can be employed for either extraction of the eligible group of analytes or for the actual separation of the imprinted species from the rest of the components. It has been demonstrated that MIPs are applicable for the determination of food additives, such as carbohydrates [13], peptides [14] or flavor additives. Galactose, fructose and mannose [2,13,15] as well as glucose [16] have been used as templates, or amino acids [17,18] and proteins [19] like ribonuclease A [20] or transferrin [21], and also caffeine [22]. Furthermore vitamins [23] and nucleotides [24,25] can specifically be analyzed utilizing MIPs. A semi-covalent approach of molecular imprinting has been applied to cholesterol [26], where the imprinting took place covalently and the later recognition non-covalently. Another main topic was the imprinting of pesticides and herbicides and their detection in food and water with MIPs. Therefore MIPs have been applied for the determination of, e.g., atrazine with sensors [27,28] and assays [29–31] or for investigating 2,4-dichlorophenoxyacetic acid contamination [32]. Furthermore, drugs [33,34] like local anaesthetics [35] and in particular antibiotics have been employed as template molecules, such as macrolide antibiotics [36], chloramphenicol [37] or clenbuterol [38]. Even surface imprints of bacterial cells have been realized [39].

Here a polymer imprinted with oxacillin, a β-lactam antibiotic (Fig. 2), is presented [40]. The molar ratio of crosslinker:functional monomer:template was 12:4:1 (Table 2). The crosslinker concentration was relatively low due to the consideration that a trifunctional crosslinker (TRIM) was chosen. Fig. 3 shows the separation of a mixture of penicillin V, penicillin G (Fig. 2) and the print molecule on an MIP utilizing an aqueous mobile phase. Baseline separation of the two penicillins from oxacillin was achieved when employing the MIP. On the other hand, when applying the control polymer (CP) the three antibiotics occurred as one major peak with a double shoulder. The separation factors obtained with the CP as well as with the MIP are presented in Table 3. In the case of an organic mobile phase (Fig. 4) no baseline separation of these three β-lactams was obtained with the MIP, and the CP did not show any separation at all. Due to effects non-related to imprinting the group of β-lactams could be separated

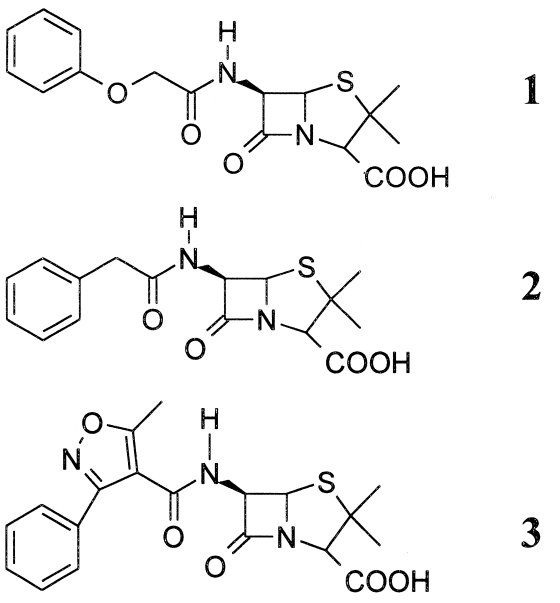

Fig. 2. Structures of penicillin V (1), penicillin G (2) and oxacillin (3).

from a non-β-lactam antibiotic (bacitracin) with both the CP and the MIP. When comparing aqueous and organic mobile phases (Table 3) it became obvious that lower (i.e., better) retention indices could be obtained with the organic phase, although the values of the separation factors were considerably higher in case of the aqueous phase, however, for the MIP and the CP. This can be explained on the one hand by the use of 4-vinylpyridine as functional monomer for all polymers and on the other hand by the fact that penicillin V, penicillin G and oxacillin are composed of the same β-lactam group differing only in the side chains.

When using the organic mobile phase mainly the carboxy residue of the β-lactam group interacts with the basic pyridinium residue of the polymer by transferring the proton, leading to a non-specific retardation by the control polymer. However, the molecularly imprinted polymer led to a separation of penicillin V and penicillin G from the template oxacillin due to additional shape recognition regarding oxacillin. The fact that penicillin V and penicillin G are still to be found as a single peak seems to prove this explanation.

When changing to an aqueous mobile phase the pyridinium residues of the polymer still interacts

Table 2
Recipe of a polymer molecularly imprinted with oxacillin[a]

Component	MIP	Mass (g)	Mol ratio
Template	Oxacillin	1.3	1
Monomer	4-Vinylpyridine	1.35	4
Crosslinker	TRIM	13	12
Initiator	AIBN	0.08	ca. 0.7–1.0% of polymerizable units
Porogen	ACN (6 ml)	–	–

[a] The control polymer was prepared using the same composition but without any template.

with the carboxy function, but as well with the different side chains via hydrogen bonds, leading to a major peak with two shoulders for the mix of the three β-lactam antibiotics when applying the control polymer. Nevertheless, a baseline separation of the template oxacillin was only achieved when changing to the imprinted polymer due to the already described different side chain interactions and an

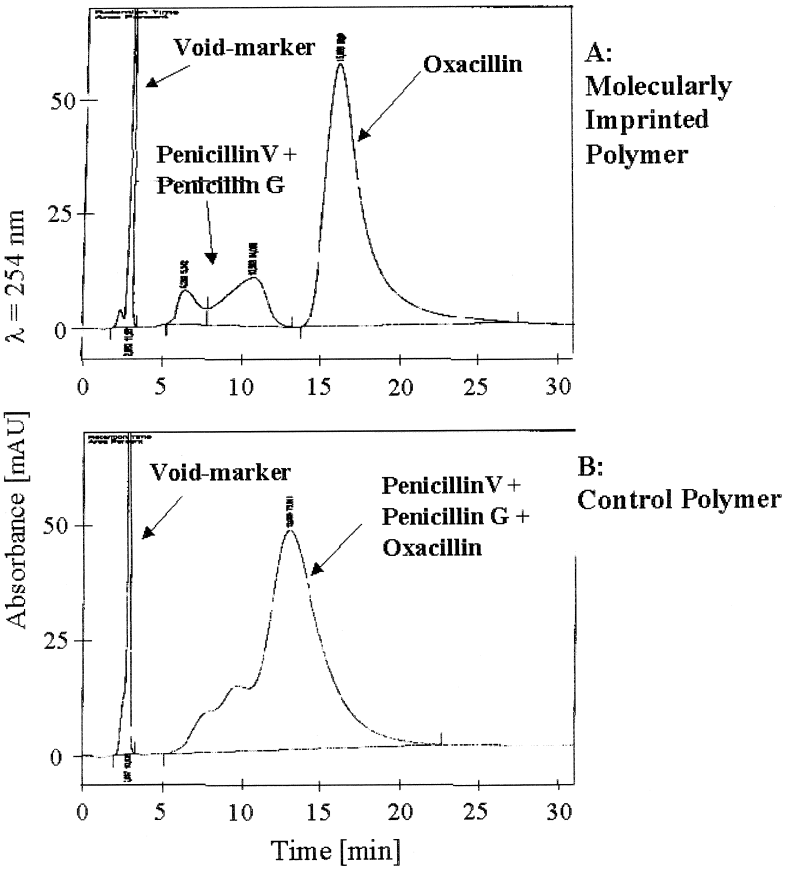

Fig. 3. (A) Chromatogram representing print molecule (oxacillin) and two other β-lactam antibiotics (penicillin G and penicillin V) as test substances from analysis on an oxacillin imprinted MIP containing 4-vinylpyridine residues, crosslinked with TRIM. The analysis was performed in aqueous mobile phase (10 m*M* sodium phosphate buffer, pH 3.5–AcN, 1:1), (B) same conditions but using respective CP.

Table 3
Separation factors and retention indices for an oxacillin imprinted polymer containing 4-vinylpyridine residues, crosslinked with TRIM, when analyzing print molecule and test substances in aqueous [10 m*M* sodium phosphate buffer, pH 3.5)–ACN (1:1, v/v)] and organic mobile phases (ACN–AcOH, 99:1, v/v), (CP= control polymer, MIP=molecularly imprinted polymer)[a]

Test compound	Aqueous phase			Organic phase		
	α_{CP}	α_{MIP}	RI	α_{CP}	α_{MIP}	RI
Oxacillin	1	1	1	1	1	1
Penicillin G	2.02	2.15	0.94	1.14	1.33	0.86
Penicillin V	1.43	1.54	0.93	1.07	1.31	0.82

[a] $\alpha_{CP}=[k'_{PM}(CP)/k'_{TS}(CP)]$; $\alpha_{MIP}=[k'_{PM}(MIP)/k'_{TS}(MIP)]$; $k'=(t_R-t_0)/t_0$; RI=α_{CP}/α_{MIP}; α=separation factor; k'=capacity factor; RI=retention index.

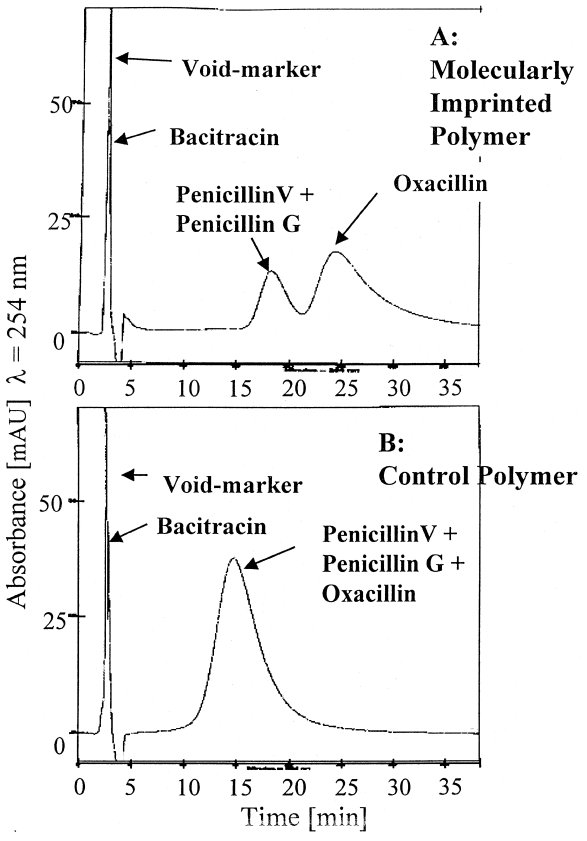

Fig. 4. (A) Chromatogram representing print molecule (oxacillin) and two other β-lactam antibiotics (penicillin G and penicillin V) and a non-β-lactam antibiotic (bacitracin) as test substances from analysis on an oxacillin imprinted MIP containing 4-vinylpyridine residues, crosslinked with TRIM. The analysis was performed in organic mobile phase (ACN–AcOH, 99:1), (B) same conditions but using respective CP. From Ref. [40] with permission.

additional shape recognition. The chromatogram shows the pure oxacillin and a double peak for penicillin V and penicillin G.

3. Molecularly imprinted polymers in high yields with high load capacities

A general problem when generating imprinted bulk polymers is the processing for the later use in chromatography or assays. The polymer has to be ground and then sedimented, to remove fine particles resulting in a loss of material (50% and more). The production of monodisperse microbeads is an alternative and exhibits a controllable, efficient and easy polymerization via precipitation [41] with yields of at least 85%. Table 4 gives a short overview how the microspheres have been generated and detailed informations about the resulting pore sizes and the surface area of the beads. Fig. 5 shows the SEM of particles imprinted against 17β-estradiol. It gives an impression of the narrow range of sizes of the monodisperse microspheres. In binding studies the beads were further evaluated. It seems that morphology of polymers may affect binding behavior. Compared with molecularly imprinted microspheres, the CP particles had slightly higher surface area and smaller size, which should have boosted uptaking of analytes to CP microspheres, had the rebinding force been totally non-specific. However, rebinding of 17β-estradiol by CP microspheres was only one fourth of the amount observed when applying molecularly imprinted beads. In competitive assays these particles were incubated both with a radioligand {[2,4,6,7-^{3}H(N)]estradiol} and a competitor (17β-estradiol, 17α-estradiol or 17-ethynylestradiol). It has been found that the limited amount of (non-

Table 4
Physical characteristics of molecularly imprinted microspheres; polymerization conditions: functional monomer MAA, crosslinker TRIM, initiator AIBN, porogen ACN; UV 350 nm, 20°C, 24 h [41].

17β-Estradiol imprinted microspheres and CP		
Polymer	Surface area ($m^2\ g^{-1}$)	Pore size (Å)
MIP	26.7	298.5
CP, no template	29.3	361.1

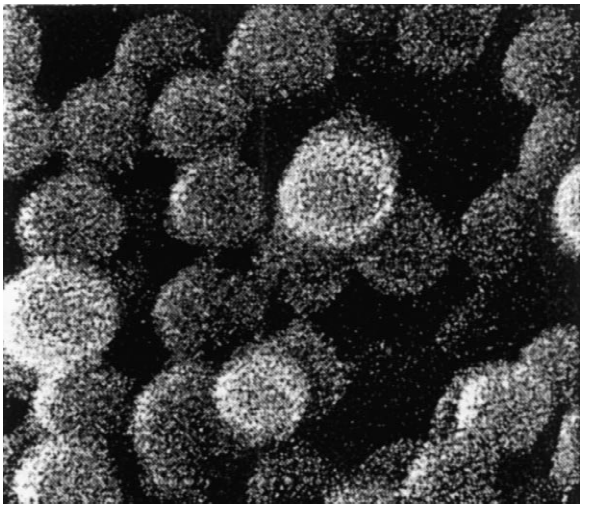

Fig. 5. SEM of molecularly imprinted microspheres imprinted with 17β-estradiol. Magnification 30 000×. From Ref. [41] with permission.

specifically) bound radioligand on CP could not be effectively displaced, while the (specifically) bound radioligand on molecularly imprinted microspheres could be replaced with the same cold (non-radiolabeled) ligand. The displacement of radioligand binding to molecularly imprinted microspheres under equilibrium conditions is demonstrated in Fig. 6. For displacing 30% of the bound radioligand only 2–3 μg ml^{-1} of 17β-estradiol (the print molecule) was required. However, for obtaining the same effect 200–300 μg ml^{-1} 17α-estradiol or 1000 μg ml^{-1} 17-ethynylestradiol were necessary, which demonstrates the much higher affinity of the molecularly imprinted microspheres for the print molecule compared to the structurally differing competitors.

4. Generating molecularly imprinted polymers with low amounts of template

Molecular imprinting usually requires a relatively high amount of template material, so trying to apply this technique as well on expensive print molecules has been a problem since molecular imprinting has been developed. But despite all predictions the concentration of the template in the monomer mixture can be reduced dramatically without major losses of quality or performance of the resulting

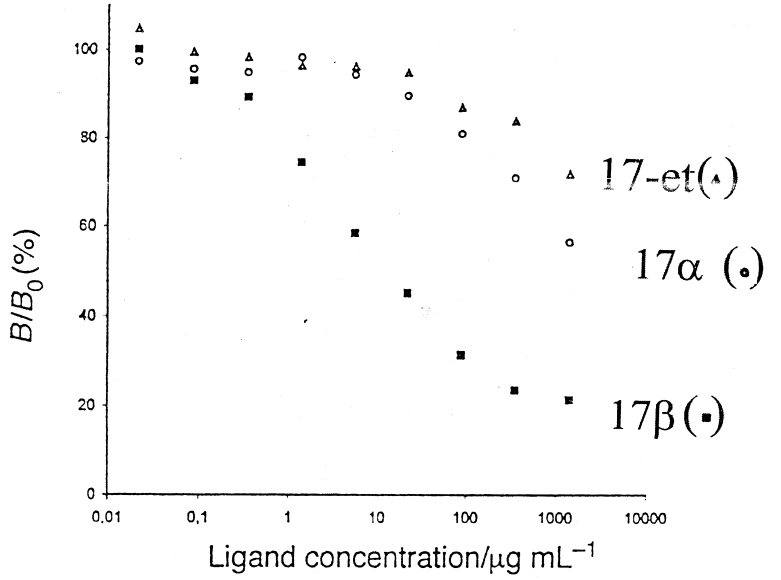

Fig. 6. Displacement of [2,4,6,7-^{3}H(N)]estradiol binding to molecularly imprinted microspheres (imprinted with 17β-estradiol) under equilibrium conditions. Ligands: (■) 17β-estradiol, (○) 17α-estradiol, (△) 17-ethynylestradiol. B/B_0 is the ratio of the amount of radioligand [2,4,6,7-^{3}H(N)]estradiol bound in the presence of displacing ligand, B, to the amount bound in the absence of displacing ligand, B_0. From Ref. [41] with permission.

MIPs. Functional monomer:template (M:T) ratios of 4:1 or even 96:1 commonly are utilized, but surprisingly even with new recipes at ratios of 500:1 or 5000:1 MIPs could be produced, still binding significantly more analyte than the CP [33]. Fig. 7 shows the results of a row of titration experiments with MIPs generated in the presence of different amounts of the template theophylline (M:T 4:1, 12:1, 100:1, 500:1). The quantity of polymer necessary to bind a fixed amount of [^{3}H]theophylline was determined, showing an unexpected high affinity of the 500:1 MIP for the print molecule. Investigations regarding the affinity of the polymer for the template theophylline resulted in binding isotherms (Fig. 8), where the 100:1 polymer possesses the lowest dissociation constants (K_{Dapp}) and the 12:1 MIP the highest number of high affinity binding sites (Q_{Mapp}) (Table 5). It could be demonstrated that the reduction of template content in the imprinting mixture did not lead to a decrease of the performance. The dissociation constant of the 500:1 polymer was found to be in the same order of magnitude as for the other three MIPs (Table 5) and additionally a higher yield was found of the number of high affinity binding sites for the 500:1 MIP (in relation to the amount of template used for imprinting). These findings are similar to those published recently by Mayes and Lowe [42].

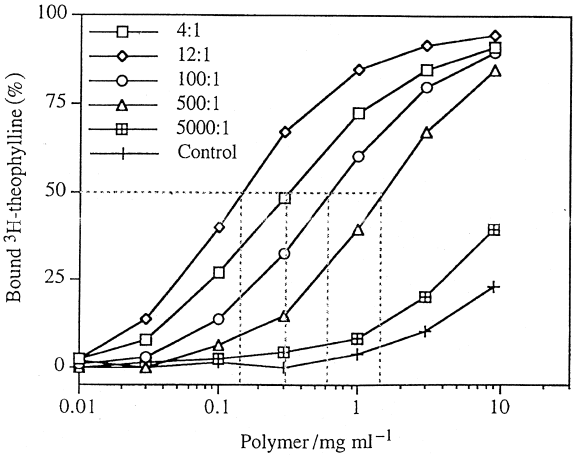

Fig. 7. Binding of [^{3}H]theophylline to imprinted polymers prepared at different M:T ratios, and to a non-imprinted control polymer as a function of polymer concentration. Polymer parameters: crosslinker DVB (20 mol equiv.), functional monomer TFMAA (4 mol equiv.), toluene as porogen. From Ref. [33] with permission.

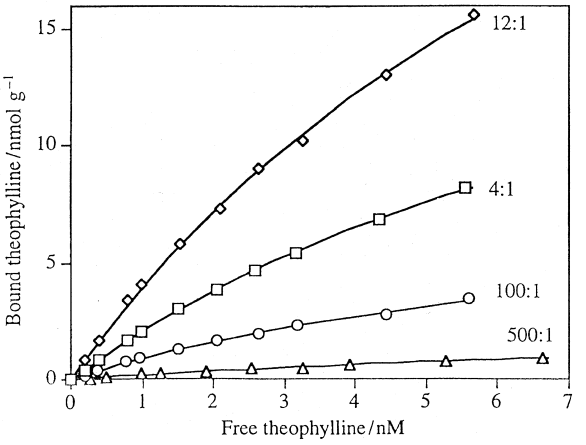

Fig. 8. Binding isotherms of [^{3}H]theophylline binding to imprinted polymers prepared with M:T of 4:1, 12:1, 100:1 and 500:1. From Ref. [33] with permission.

5. Molecularly imprinted polymer assisted synthesis

Another example of the broad applicability of MIPs not only for analytics represents the approach of shifting reaction equilibria towards product formation using MIPs. An adsorbent molecularly imprinted with the product can be added to the substrate mixture and acts as a ''magnet'' fishing out the freshly produced product. Especially when reactions are thermodynamically hindered this kind of technique allows continuous product formation by simply removing it via complexation with the MIP. For

Table 5
Apparent dissociation constants (K_{Dapp}) and apparent number of high affinity binding sites (Q_{Mapp}) for polymers prepared with different M:T ratios, and relative yield of high affinity binding sites[a]

Ratio M:T	K_{Dapp} (nM)	Q_{Mapp} (nmol g^{-1})	Relative yield (%)
4:1	10±0.5	22.8±0.8	0.007
12:1	9.4±0.7	40.9±2.2	0.039
100:1	8.1±0.9	8.2±0.6	0.063
500:1	14.7±2.4	2.9±0.4	0.12

[a] From Ref. [33] with permission.

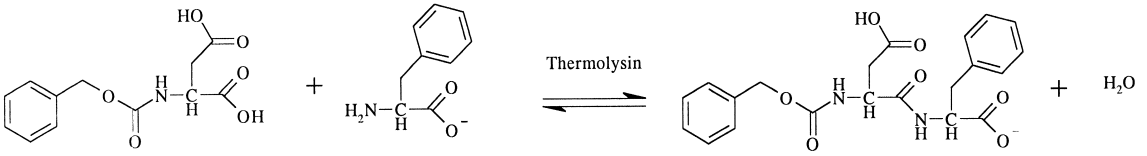

Fig. 9. Enzymatic synthesis of *Z*-aspartame.

instance, a polymer imprinted with *Z*-aspartame (a synthetic precursor of the sweetener aspartame) was applied to the enzymatic synthesis of the precursor starting with the substrates *Z*-L-Asp and L-Phe-OMe (Fig. 9) [43]. After 48 h the product-imprinted polymer provided the highest yield of 63% (Fig. 10, curve 1), compared with a MIP imprinted with the two substrates, which led to a product yield of 39% (Fig. 10, curve 3). For the determination of non-specific adsorption a non-imprinted control polymer has been used resulting in a yield of *Z*-aspartame in the same period of time of 44% (Fig. 10, curve 2). This was more than the value obtained with the substrate-imprinted polymer, probably due to the fact that in the control polymer a larger surface area was available for binding the product non-specifically. When analyzing the polymer-free solution of the substrates and the enzyme after 48 h a product yield of 15.5% was detected (Fig. 10, curve 4). A final control of an enzyme-free substrate solution individually mixed with the three polymers did not result in any detectable product quantities, demonstrating that no print molecule was leaking out of the MIP and that the print molecule itself was not catalyzing product formation.

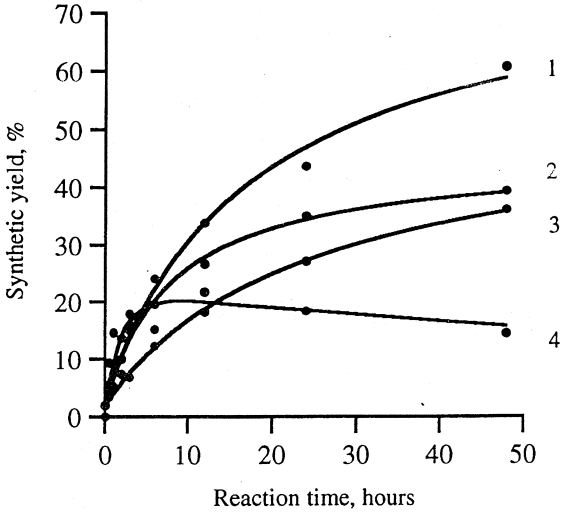

Fig. 10. Synthesis of *Z*-aspartame in the presence and absence of MIPs: curve 1: MIP1, *Z*-aspartame as print molecule; curve 2: polymer reference, no print molecule; curve 3: MIP2, *Z*-L-Asp and L-Phe-OMe as print molecules; curve 4: the polymer-free enzymatic reaction, no polymer added. From Ref. [43] with permission.

6. Conclusion

It has been demonstrated that MIPs can be applied in different areas of chemistry, biotechnology or medicine. The first thing to be considered is the format of the biomimetic material. This has to be chosen wisely, focused on the actual purpose in order to achieve the best performances of the MIPs. Many combinations of formats and applications are imaginable, but for most of the individual goals one solution proofs most advantageous. However, in many cases not every effect is totally predictable, so some parameters still have to be determined experimentally. Nevertheless, for molecular imprinting already a broad range of information has been collected to simplify the right selection of imprinting parameters and configurations for a defined purpose.

7. Nomenclature

ACN	Acetonitrile
AIBN	Azobis(isobutyronitrile)

CP	Control polymer
DVB	Divinylbenzene
HPLC	High-performance liquid chromatography
MAA	Methacrylic acid
MIP	Molecular imprinted polymer
M:T	Ratio of functional monomer to template
PM	Print molecule
SEM	Scanning electron microscopy
SPE	Solid-phase extraction
TFMAA	2-(Trifluoromethyl)acrylic acid
TRIM	Trimethylolpropane trimethacrylate
TS	Test substance

Acknowledgements

Part of the studies (chapter 2) has been achieved within the EC collaborative project FAIR-CT96-1219 (Nov. 1996–Oct. 1999) entitled ''Development of novel and robust molecular imprint-based technology for the real-time analysis of food contaminants and components''. The project partnership comprises Leatherhead Food RA (UK, coordinator), Lund University (Sweden, contractor), University La Sapienza/Polytech (Italy, contractor), UNIR Association (France, associated contractor) and Prolabo (France, contractor). The authors gratefully acknowledge the significant financial support of the European Commission to this project.

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