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Review

Separation and screening of compounds of biological origin using molecularly imprinted polymers

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Abstract

Molecularly imprinted polymers (MIPs) represent a new class of materials possessing high selectivity and affinity for the target molecule. Since their discovery in 1972, molecularly imprinted polymers have attracted considerable interest from bio- and chemical laboratories to pharmaceutical institutes. They have been utilized as sensors, catalysts, sorbents for solid-phase extraction, stationary phase for liquid chromatography, mimics of enzymes, receptors and antibodies. Among which, the application of molecularly imprinted polymers for molecular recognition-based separation and screening of compounds has undergone rapid extension and received much attention in recent years. This article mainly focuses on the separation and screening of certain pharmacophoric compounds of interests from biological origin using molecular imprinting technology. Examples of extraction and recognition of active components as anti-tumors or anti-Hepatitis C virus inhibitors from Chinese traditional herbs using molecularly imprinting technology are particularized in this article. Comparison between the screening effect based on MIPs and that based on antibodies is also represented. Consequently the merits and demerits of these two technologies are highlighted.

Keywords: Reviews; Screening; Molecularly imprinted polymers

Contents

1. Introduction

The technique of molecular imprinting, introduced in 1972 by Wulff and Sarhan [1], and much expanded by the work of the group of Arshady and Mosbach in 1980s [2], has been shown to be capable of producing materials with "antibody-like" selectivity [3]. Molecularly imprinted polymers are extensively cross-linked polymers containing specific recognition sites with a predetermined selectivity for analytes of interest [4–8]. The process of molecular imprinting often consists of the following three steps. (1) The imprint is obtained by arranging polymerisable functional monomers around a template as the target molecule, and complexes are then formed through covalent or non-covalent or semi-covalent molecular interactions between the template and monomer precursors. (2) The complexes are assembled in the liquid phase and fixed by cross-linking polymerization. (3) Removal of the template through extraction or hydrolysis with appropriate solvents

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leaves behind vacant recognition sites exhibiting high affinity for the target molecule. These sites are complementary both sterically and chemically to the template molecule, resembling the "lock and key" paradigm of enzymes postulated by Fischer in 1890s [9]. These recognition sites enable imprinted polymers to be used as the mimics of enzymes, receptors and antibodies for screening various kinds of compounds from a mixture with abundant interferences. Moreover, the functional molecular imprinting has provided the polymers with specific selectivity which can be utilized for chromatographic separations [10], chiral separations [11–15] and more recent biosensors [16–19]. The outstanding advantages of molecularly imprinted polymers include their physical robustness, high strength, resistance to elevated temperatures and pressures, and inertness to acids, bases, metal ions and organic solvents as well as the low cost and ease for preparation, which have enabled them to be used in a large number of systems [20].

Up to now, there have been so many reviews summarizing the development of molecularly imprinted polymers (MIPs) [21–23], which covered widely from the sorbents for sample pre-concentration and stationary phase for separation to bioassays, biosensors and mimics for enzymes, receptors and catalysts. In this article, three main scopes of the applications of MIPs from viewpoint of separation and screening of compounds of interests of biological origin were concentrative discussed.

2. Molecularly imprinted separation and screening

2.1. Solid-phase extraction

Sample pre-concentration is usually carried out by liquid–liquid extraction or solid-phase extraction (SPE) [24]. Compared with liquid–liquid extraction (LLE), SPE seems to provide cleaner extracts although both techniques were equally accurate and precise, and the procedure of SPE is more convenient for automatization. In the last few years, SPE has become the most often used sample preparation technique for trace analysis. But the selectivity of custom SPE sorbents is limited since they differentiate only by some physicochemical property like hydrophobicity. Recently, new types of SPE materials, such as highly cross-linked polymers (MIPs) are therefore currently being developed to allow more attractive extractions [25–31]. The basic concept for the application of MIPs in solid-phase extraction (MISPE) is that the chromatographic parameters are tuned such that the MISPE column traps only the analytes, or a group of structurally related compounds, whereas the other interfering components in the sample matrix were washed without retention. The first molecularly imprinted polymer used for solid-phase extraction was reported by Sellergren in 1994 [32]. The high selectivity of the polymer allowed pentamidine (a drug used to treat AIDS-related pneumonia) to be sufficiently enriched even when present in low concentration in a diluted human urine sample. Up to now, MIPs have been applied as the sorbents for solid-phase extraction for several types of matrices. MISPE has widely been used for the pre-concentration of the template itself, whereas the extraction of other related compounds is still under the minor development of its application. Actually, these two aspects of the application of MISPE discussed as following are both very beneficial for bio- and pharmaceutical analysis.

Table 1
Applications of MISPE for extraction of the template itself in various kinds of samples

Analyte	Sample	Reference
Pentamidine	Human urine	[32]
7-Hydroxycoumarin	Urine	[33]
Propranolol	Dog plasma, rat bile and human urine aqueous solution	[34]
Tamoxifen	Human plasma and urine	[35]
Atrazine	Beef liver extracts	[36]
Nicotine	Chewing gum	[37]
Darifenacin	Human plasma	[38]
Triazines	Urine	[39]
Atenolol	Methanolic or acetonitrile solution	[40]
Bentazone	Aqueous sample	[41]
Chlorotriazines	Sediments and natural waters	[42]
Quercetin	Hydrolyzate of gingko leaves, rat plasma	[43,44]
Matrine	Extract of Chinese medicinal plant	[45]

2.1.1. The template itself

In most analysis of biological, pharmaceutical and environmental samples, MIPs have been usually adopted as SPE materials for the pre-concentration of the template itself. Table 1 shows some efficient applications for pre-treatment of the template molecule itself in various kinds of matrices.

On the imprinted polymer prepared with quercetin [43], a typical active compound of the flavonoid family, the template and one of its analogues, kaempferol, could be directly trapped from the hydrolyzate of gingko leaves by the SPE process, which was displayed in Figs. 1 and 2. The chemical structures of these two compounds were shown in Fig. 3.

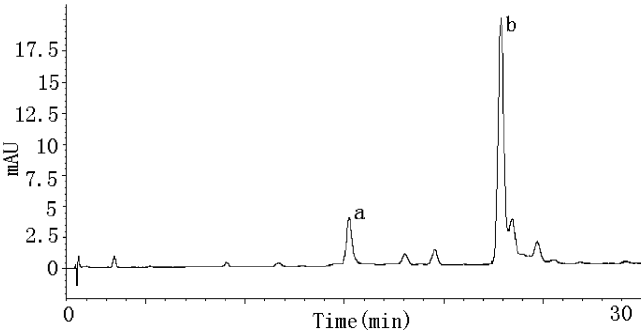

Fig. 1. Chromatogram of the hydrolyzate of gingko leaves (peak (a) was identified to be quercetin and (b) was identified as kaempferol).

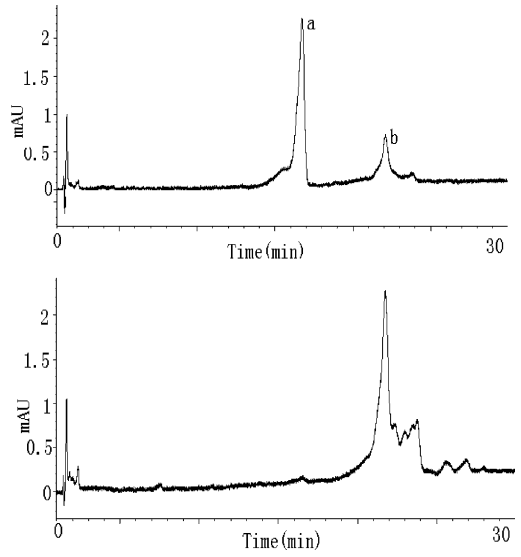

Fig. 2. Chromatograms of the eluent from the MIP cartridge (top) and that from the BP cartridge (bottom) (peaks (a) and (b) were corresponding to those identified in Fig. 1).

After the MIP cartridge process, the chromatograms of the eluents were simplified as a result of the removal of matrix components by washing with methanol, and the template quercetin as well as its analogue kaempferol was purified from the hydrolyzate of gingko leaves. The recovery yield for quercetin was satisfactory (89%). In contrast, after the BP process, except for kaempferol, whose content was the highest in the initial hydrolyzate, almost no quercetin was detected. In view of the capacity test, total amount of quercetin in the hydrolyzate could be retained on the MIP column by specific binding at the 1 ml loading amount, so most of this component was recovered after the MISPE process. This demonstrated that in the complex system of the crude herb extract did the MIP still exhibit the strongest specific affinity to its template. While for the analogue kaempferol, the retention mechanism on the MIP and BP cartridges might be different although it was finally trapped by both of these polymers. Kaempferol displayed stronger affinity to the quercetin-imprinted polymer than to the BP since its shape, size and functionalities almost could match the microcavity formed by quercetin. This SPE process based on a quercetin-imprinted polymer has shown that MIPs can provide efficient extraction of certain active components directly from herb matrix. The MIP showed excellent workability in the crude extract of herb and exhibited the highest affinity to the template. This polymer did retain a range of molecules structurally similar and dissimilar to the template molecule, however, such matrix compounds could be removed by careful washing with solvents; also close structural analogues and the template were purified simultaneously in this way.

Not only MIP could trap selectively the template molecule from the crude extract of natural products, but also it could be used for direct clean-up of the biological samples for the analysis of functional components in vivo originating from the extract of medicinal herbs [44]. As an example, an anti-quercetin AA-co-TRIM MIP selectively extract the template, the effective component in the plasma of rats fed the hydrolyzed extract of *Gingko biloba* L., through an effective SPE process. In that case, several washing solvents were tested before MISPE of the plasma sample, and the results showed that the less polar solvent acetonitrile was the most appropriate for the SPE wash step. The chromatographic traces of the rat plasma sample before and after the MIP pretreatment revealed obviously the extraction effect of the polymer (shown in Fig. 4).

At the early stage of the development of SPE technique, most applications deal with the template molecules themselves. As the most often used pre-concentration technique for trace analysis, MISPE provided more effective extractions with the high predetermined affinity to the templates than other usual commercial sorbents. Gradually, this technique was extended to the selective extraction of the analogues of the templates since the MIPs also specifically re-bind the closely related structures of the templates in preference to other unrelated compounds. Several groups have presented the use of MIPs made against a structural analogue of the target analyte, since the imprinting of a structural analogue instead of the target analyte could

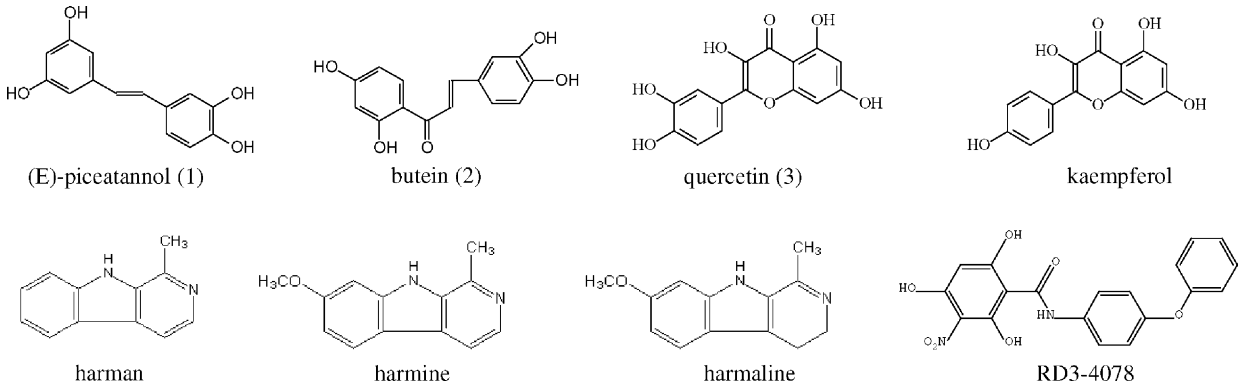

Fig. 3. Chemical structures of some compounds listed in the text (the characters 1–3 were corresponding to those in Table 3).

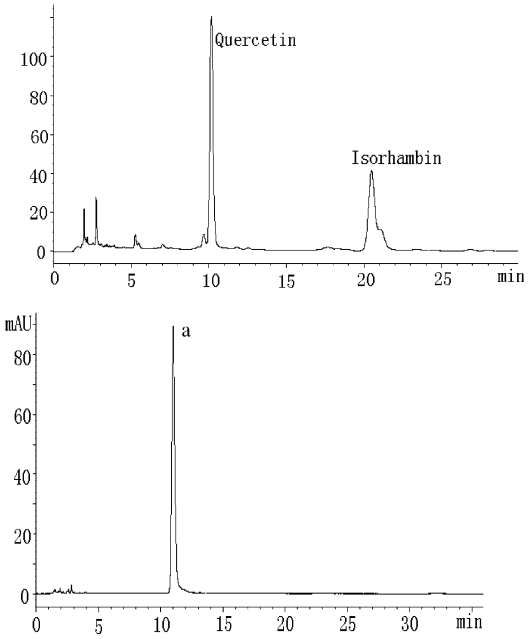

Fig. 4. Chromatogram of the plasma from the rats fed with 0.40 g of the hydrolyzed EGB (extract of *Gingko biloba* L.) 4 h later on an analytical column (Zobax Eclipse XDB-C8 4.6 mm × 150 mm i.d.) with the sample pretreated by (top) a C18 cartridge or (bottom) an anti-quercetin AA-co-TRIM cartridge. The mobile phase was acetonitrile-phosphoric acid solution (0.33%) (25:75, v/v). The flow rate was 1.0 ml/min. The wavelength was set at 365 nm. The column temperature was ambient. Peak (a) was corresponding to quercetin.

avoid problems encountered with template leakage, which sometimes prevents the efficient application of MISPE.

2.1.2. Analogues of the templates

Efficient extraction of some analogues of interests of the templates with the MISPE technique has attracted more and more attention in recent years [46–48]. Especially for those analytes difficult to obtain and thus difficult to synthesize their corresponding MIPs, the requirement of a substantial amount of pure template prior to the polymerization become a challenge for the application, while using the analogues easy to get to replace the template for synthesizing the MIPs provides a complementary method.

In our previous study of affinitive separation of different unknown anti-tumor components directly from the crude extract of a Chinese traditional Tibetan medicine, *Caragana Jubata*, the extraction was performed on a MIP prepared with a known inhibitor, quercetin, which exists widely in natural resources and is easy to get [49]. Using the quercetin-imprinted polymer as the sorbent material in an effective SPE procedure, two other analogues of the templates, (*E*)-piceatannol and butein, were selectively extracted from the herb, and the subsequent bioassay evaluation showed that these two analytes also possess potent anti-tumor inhibitory activities. It could be found for the detailed extraction of these two analytes from Table 2. Recovery of these two analytes in the elution step could be

Table 2
The SPE process of two analogues of the template as anti-EGFR inhibitors based on MIP

SPE step (loading[a] (total)) (ml)	Amount of the inhibitors (piceatannol and butein) (400 μg matrix components)			
	MISPE		BSPE (blank SPE)	
	Butein (μg)	Piceatannol (μg)	Butein (μg)	Piceatannol (μg)
Washing[a] 1–3	–[b]	–	–	23
4–7	–	0.3	16.7	1.8
8–10	–	0.2	1.6	0.9
Elution[a] 1	–	1.5	0.3	–
2	–	3.4	–	–
3	0.6	17.5	–	–
4	0.4	2.0	–	–
5	11		–	–
6	2.0		–	–
7	1.9		–	–
8	0.3		–	–
Elution (total)	16.2	24.4	18.6	25.7

[a] Loading and washing solvents: acetonitrile; elution solvent: methanol.
[b] Not possible to detect the analytes due to the matrix interferences.

calculated to be 76% for butein and 80% for piceatannol, which were satisfactory. The efficient extraction of the analogues possessing similar bio-characters (in this case bioactivities) studied in this work afforded a feasible approach for direct and selective separation of active components from herb, which would be very beneficial for the discovery of lead compounds and thus would help the development of drug candidates. The selective SPE procedure based on MIP provide a complementary technology for traditional isolation of herb, and it is expected that this novel application of MIP would be able to be extended to combinatory synthetic libraries since it had been applied successfully in herbs that possess more abundant complexity than synthetic libraries.

On another MIP prepared with a structural analogue, ditolyl phosphate, as the template, the analyst of interest, diphenyl phosphate, was selectively retained and concentrated in a solid-phase extraction [50]. In this case, two different MIPs, one prepared from methacrylic acid (MAA) and the other from 2-vinylpyridine (2-Vpy), were investigated for their ability to recognize diphenyl phosphate. The polymers were used in solid-phase extraction cartridges and evaluated by comparing their recovery and breakthrough parameters with those of the corresponding non-imprinted polymers (NIPs). The results showed that when applying the first extraction protocol, no difference in selectivity was observed between MAA-MIP and the corresponding MAA-NIP. On the other hand, when using the same extraction protocol, significantly higher selectivity was obtained for the 2-Vpy-MIP compared to 2-Vpy-NIP, whose total recoveries were nearly 100% and 30%, respectively. Moreover, another analogue, di(2-ethylhexyl) phosphate, was also evaluated in this study for its selectivity on the 2-Vpy-MIP cartridge. The similar SPE procedure for this analyte showed that the interaction

between di(2-ethylhexyl) phosphate and the polymer due to the imprinting effect was weaker, resulting in approximately 20–30% of the analyte being desorbed prior to the elution step, and the overall recovery was significantly lower than that for diphenyl phosphate. However, there was still a significant difference between the retention of di(2-ethylhexyl) phosphate on the MIP and that on the NIP. This work elucidated that the polymer made from 2-Vpy displayed the most selective recognition to the acidic analyte. Diphenyl phosphate was adsorbed on the basic MIP (2-Vpy-MIP) when methanol was used as mobile phase, and approximately 80% of the analyte was recovered when eluted from this polymer using a mixture of methanol and trifluoroacetic acid. The authors explained that the interaction between the basic MIP and the acidic analyte probably aroused mostly from an ionic interaction. Anyway, just as described in the literature, "the work has shown the potential of the MISPE technique for selective enrichment of ionic diphosphate esters in protic matrices".

2.2. Liquid chromatography

The use of MIPs as the stationary phase for analytical chromatographic and electrophoretic separations is by far the most extensively studied application of imprinted polymers and has been reviewed in some excellent literatures [51,52]. The objects analyzed in the liquid chromatography using MIPs media cover amino acids and peptides [53–56], nucleotide bases [57], drugs [58–60], sugars [61–64], steroids [65–68], etc. While mainly the bioactive components in natural resources separated and analyzed based on MIPs were summarized in this article, especially for those analytes of interest with potent inhibitory activities existed in Chinese traditional herbs.

On a polymer imprinted with an analogue harman as the template, two anti-tumor components, harmine and harmaline, were investigated for their affinity and selectivity chromatographically, for which three MIPs were prepared with different porogens bearing different hydrogen bonding capacities [69]. The structures of these three analytes could be seen in Fig. 3. The influence of the porogens and sample loads on the retention of the target compounds was also evaluated. Results showed that on the MIP1 (using toluene–acetonitrile, 1:1 (v/v) as the porogen) and MIP2 (using acetonitrile as the porogen) columns, the attempt of direct separation of target components from the crude extract of herb was workable although the shape of the chromatographic peak was tailing, which could be observed from Fig. 5. Among these two polymers, MIP1 possessed higher affinity and specificity for the targets than MIP2, and this resulted in partial separation between harmine and harmaline on MIP1 column while no detachment of these two targets on MIP2 column. In this work, the authors also suggested that the LC separation should be investigated with the sample pretreated with C18 Bond-Eluts since matrix interferences in herb extract disturbed the chromatographic performance

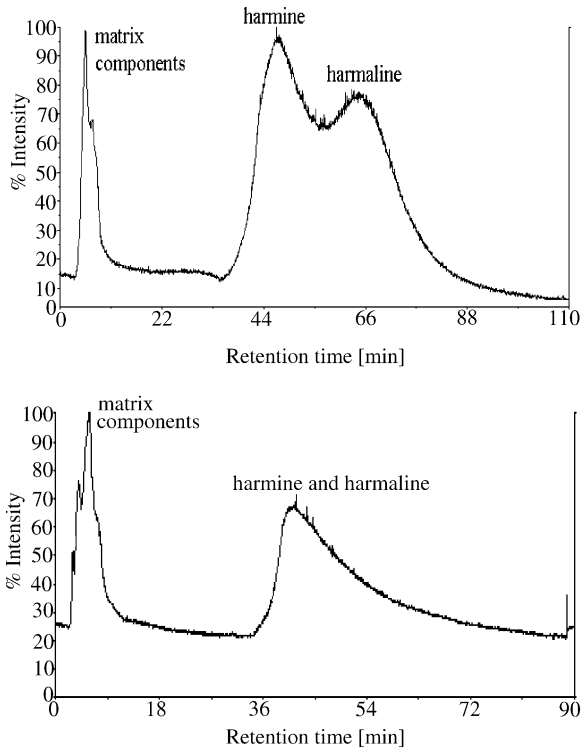

Fig. 5. Chromatogram of total ion current for the LC-MS separation on the column packed with MIP1 (top) and MIP2 (bottom).

of the polymer. They found that the chromatographic peaks of the target components on the MIP1 column could be explicitly seen after the treated sample was injected and the chromatographic shape for MIP2 also became better as the sample was treated previously. Actually, asymmetric shape of the chromatographic peaks on the MIP columns due to the imprinted site heterogeneity is always one of the disadvantages of imprinted polymers and limits the application of MIP in chromatographic systems. The preparation of uniform-sized particles for the polymers as the stationary phase will improve column efficiency remarkably. Owens et al. [6] explained that the 'good' recognition sites became saturated and the separation predominantly occurred on poorer sites which subsequently resulted in decreased retention and unfavorable chromatographic performance.

In another case, an anti-tumor (against the epidermal growth factor receptor which is widely studied as an anti-tumor target enzyme) small molecule inhibitor, (*E*)-piceatannol, was imprinted using 4-vinylpyridine and ethylene glycol dimethacrylate [70]. When the polymers were employed as chromatographic supports, different potent inhibitors existed in the crude extract of *Caragana Jubata* (a Chinese traditional Tibetan medicine) were selectively retained over other components with low inhibitory activities.

Also there is an efficient separation based on a MIP applied in *Phyllanthus urinaria* L., from which several novel inhibitors of Hepatitis C virus (HCV) NS3 protease were

screened out [71]. There were three MIPs prepared in this study using different functional monomers including acrylic acid, 4-vinylpyridine and acrylamide, while RD3-4078, a known active inhibitor against HCV NS3 protease, was adopted as the template. Resulted data indicated that the MIP imprinted with the template and 4-vinylpyridine as the functional monomer possessed the highest affinity for the targets. More important, five compounds with potent inhibitory activities against HCV NS3 were selectively separated from fifteen main components existed in the crude extract of *Phyllanthus urinaria* L. These analytes were screened out resulted from their stronger affinity on the MIP chromatographic column, and they were identified by MS detector to be ellagic acid, corilagin, geraniin, 1,3,6-tri-o-galloy-β-D-glucose and phyllanthusiin U.

One of the main objects studied in our group mainly focus on natural resources, especially Chinese traditional medicines. Effective separation based on MIPs represented in our work provides a powerful approach for analysis and discovery of certain pharmacophoric components directly from herb. As a known inhibitor or drug candidate or drug itself was adopted as the template to prepare for the corresponding MIP, the polymer is expected to possess the ability for selecting the potent inhibitory components with chemically related structures from matrix interferences of herb. From the viewpoint of structure-activity relationship, some inhibitors against one enzyme or receptor are often inclined to adopt a similar or even common binding mode to the binding pocket of the enzyme, and therefore they usually possesses similarity in their chemical structures to certain extent. The size, shape and functionality of the template determining the selectivity of the imprinted polymers also play important roles in deciding the bioactivities of the analytes. To the binding pocket of one enzyme, only those molecules belonging to some size (too large could not enter into the pocket and too small could not occupy the binding sites efficiently) could enter easily and retain stably and thus to be potential inhibitors. Moreover, the possible interactions between the enzyme and the inhibitors such as hydrogen bond, ionic interactions and hydrophobic effects are also utilized in the molecular imprinting. Taken these two important characteristics, one is steric memory (size and shape), and the other is chemical memory (spatial arrangement of the complementary functionality) of the MIP material into account, using MIP to selectively separate and analysis different small molecular components possessing certain pharmacophoric characters from herb, as it turned out in our work, has proven to be efficient.

2.3. Screening systems

Development of a feasible and efficient approach using MIPs as the stationary phase to mimic enzyme for screening different potent inhibitory components directly from Chinese traditional medicines is always one of the main projects undergoing in our group. Theoretically speaking, as the more active inhibitory compound was adopted as the template, the resulted imprinted polymer would possess more excellent ability for screening other different inhibitors, and the microcavities of the corresponding polymer would simulate the binding pocket of the enzyme more successfully considering that the template with higher bioactivity could conjugate to the biding pocket more tightly in terms of its shape, size and functional groups. Up to now, part of our work has validated this viewpoint.

First, a MIP was prepared with a known inhibitor against the epidermal growth factor receptor (EGFR), quercetin, as the template and applied to screen novel anti-EGFR inhibitory components [49]. Through an efficient solid-phase extraction procedure, two different anti-EGFR inhibitors were screened out from the crude extract of a Chinese traditional Tibetan medicine, *Caragana Jubata*. These two compounds were identified to be (*E*)-piceatannol and butein possessing anti-EGFR inhibitory activities with IC_{50}s of 4.9 and 10 μM, respectively; which were higher than the bioactivity of the template quercetin. And then the most potent inhibitor (*E*)-piceatannol was imprinted with 4-vinylpyridine and ethylene glycol dimethacrylate. Using this polymer to mimic the receptor for recognizing different anti-EGFR inhibitors existed in *Caragana Jubata*, the effect was more successful than that of the polymer imprinted with the template quercetin showing lower inhibitory activity (IC_{50} = 15 μM) [72]. The contrast between the effects of these two polymers for recognizing different anti-EGFR inhibitors existed in *Caragana Jubata* was shown in Fig. 6, in which the factor $-\ln(IC_{50})$ was adopted to indicate the bioactivities of the analytes: the bioactivity was higher as this factor was larger; IF was used to evaluate the effect of the polymer for retaining the analyte and it was defined by the equation $IF = k'(MIP)/k'(NIP)$. From the comparison it could be concluded that the polymer imprinted with quercetin could not recognize these three components according their inhibitory activities although it had extracted two other inhibitors through a SPE procedure; while the one prepared with piceatannol was able to achieve the recognition, on which the inhibitor with higher activity was retained longer.

Quercetin and piceatannol are belonging to two different kinds of chemical structures, but they were both of

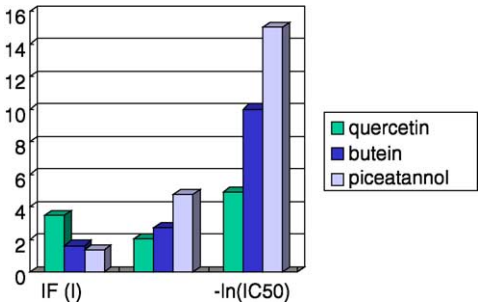

Fig. 6. Comparison of two MIPs in their recognition abilities for different anti-EGFR inhibitors.

inhibitors against the enzyme EGFR, and therefore the effects of these two resulted polymers for mimicking the enzyme to recognize different inhibitors were contrasted. Results showed that the polymer imprinted with the inhibitor possessing higher activity could fulfill the simulation more successfully, which validated the viewpoint described above. In order to improve this point, another control polymer was prepared with another template displaying rather low inhibitory activity against EGFR, calycosin, which was one of the non-retaining compounds on the piceatannol-imprinted polymer. And the corresponding results revealed that the retention was indeed improved for the template calycosin, but lost for the analytes bearing potent anti-EGFR bioactivities including piceatannol, butein and quercetin. Of course the attempt for this polymer to recognize different inhibitors was unsuccessful, since these inhibitors were all washed out in less than 20 min in an asymmetric peak on the column packed with calycosin-imprinted polymers [70].

It could be excepted that using one representative inhibitor possessing the highest activity as the template to synthesize the corresponding polymer, the efficiency may be improved further and thus could be applied to more complicated systems, which is another program undergoing.

3. Comparison of the screening of active inhibitors from herb based on MIPs and antibodies

Using polyclonal antibodies raised against an active inhibitor of one enzyme to mimic the enzyme for efficient recognition of other different active compounds from herb is another complementary technology to screen out certain pharmacophoric target components. For these small molecular analytes, MIPs and polyclonal antibodies could offer similar advantageous features for the direct recognition, which had been proven in our study [49,70–72].

Such an application of antibodies was performed in a system in which the polyclonal antibody (PcAb) was raised against piceatannol that was linked with bovine serum albumin (BSA) to be covalently bound to the chromatographic packing for mimicking the epidermal growth factor receptor (EGFR), different inhibitors including the half-antigen itself were recognized from the extract of this herb. In this study, frontal affinity chromatography (FAC) connected with mass spectrometry (MS) was adopted as the analytical method due to its inherent merits [72]. As the crude extract of *Caragana Jubata* was loaded on the antibody-based affinity column, six main components were analyzed using FAC-MS technology. According to the principle equation of FAC, the corresponding dissociation constants (K_d) for these six analytes were estimated and contrasted to the respective bioactivities (IC_{50}). Where an auxiliary coefficient factor, relative binding strength (S_r), was evaluated for each compound using the half-antigen (piceatannol) as the standard, which means the S_r value for piceatannol was set to be 1.00, and those for other analytes were calculated according to the ratio between the K_d value for piceatannol and that for the analyte. The binding affinity of the polyclonal antibody for the analyte was stronger as the factor K_d was smaller, while the bioactivity of the molecule was higher as the IC_{50} was smaller. Then the recognition based on the polyclonal antibody was expressed through the contrast between the S_r value for each analyte and their corresponding IC_{50} value, which was shown in Table 3. All of the chemical structures of compounds 1–6 were displayed in Figs. 3 and 7. From the results it could be concluded that the recognition based on the polyclonal antibody was efficient: the analyte with higher activity was retained on the affinity column longer.

Table 3
The recognition effect based on the polyclonal antibody expressed in the contrast

Compound	S_r (relative binding strength)	IC_{50} (against EGFR)
1	1.00	4.9 μM
2	0.21	10 μM
3	0.18	15 μM
4	0.04	1.1 mM
5	0.03	1.2 mM
6	0.005	59.0 mM

Obviously, these two materials (MIPs and antibodies) both possess inherent pre-determined specific binding affinities to the template (in MIP) or antigen (in PcAb) and those compounds chemically similar to the target molecule, and thus enabling them to simulate the enzyme for direct screening the analytes of interest from natural resources. Contrasted to MIP, the application of PcAb has its own merits and demerits. On the one hand, more amounts (often 1 mmol) of pure template molecule was demanded in molecular imprinting, while usually only trace amount (0.01–0.1 mmol) of half-antigen was needed for producing PcAb. So the biological technology (preparing PcAb) would be more favorable as the target molecule was expensive or difficult to be obtained, especially for some drug candidates or lead compounds existed with only trace contents in natural resources. Moreover, for those high molecular mass compounds, it is still difficult to prepare the corresponding MIPs. Accordingly, to date MIPs cannot be employed for analytes such as proteins and polynucleotides, wherefore antibody-based affinity chromatography provides a complementary method. On the other hand, MIP possesses better chemical and physical robustness than PcAb, and it is more inert towards acids, bases, metal ions and organic solvents as well as elevated temperatures and pressures than the latter. These factors provide more extensive applications for MIP. While the devel-

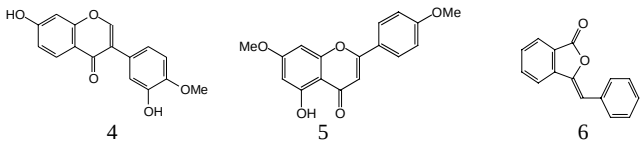

Fig. 7. Structures of compounds 4–6.

opment of antibodies against the compounds of interest may prove more difficult than the synthesis of MIP. However, as the complementary approaches to traditional isolation following with bioassay guidance, both of these two techniques displayed potential efficiency for high-throughput screening of analytes of interest. It was expected that the novel application of these two materials (MIP and PcAb) would be beneficial for efficient screening of lead compounds or drug candidates from both natural resources and synthetic combinatorial libraries.

4. Conclusion

There have been more and more literatures published each year indicating the growing interest in molecular imprinting technology. But the novel applications of MIPs to mimic enzyme or receptor for efficient recognition of active components from natural resources (especially in Chinese traditional medicines) have rarely been reported. As a consequence, mainly this aspect of the application of MIPs was summarized according to the studies of our group in this paper.

Traditional Chinese medicines (TCM) are rich in natural resources and have been used for thousands of years in China. The long period tryout of TCM on Chinese people has confirmed the excellent effects. The TCM system can be considered as natural combinatorial chemical libraries. Comparing with synthetic combinatorial libraries, TCM have more abundant diversities in chemical structures with extraordinary potent pharmacological activities and rather low toxicities. But the acting mechanisms of TCM are often difficult to be clearly clarified resulting from the lack of effectual techniques for isolation or separation of effective components. Conventional isolation or separation of lead compound from TCM following with the bioassay guidance such as the bioactivities evaluation by enzyme-linked immunoassay (ELISA) has always been taken an important part in drug development. However, it is often time-consuming and inefficient for this procedure resulting from the poor affinity and selectivity of conventional materials (e.g., silica-gel, polyamide, ion-exchange types and reversed-phase column). New types of sorbent materials, such as highly cross-linked polymers and chemically modified polymers, are therefore currently being developed to allow more effective extraction and separation.

As a promising technology with inherent high selectivity and affinity, molecular imprinting could thus afford an efficient complementary approach for traditional isolation based on normal materials. Using MIPs to mimic the enzyme or receptor for direct extraction and separation of active components from natural resources such as TCM, as it turned out, has proven to be feasible. Moreover, because of the inherent advantages of MIPs, e.g., physical robustness, resistance to elevated temperatures and pressures, and inertness towards acids, bases, metals ions and organic solvents, this material can be well employed in various kinds of samples, including some biological and pharmaceutical samples as urine, plasma, serum, or some environmental samples as water, apple extract, and also the crude extract of traditional Chinese medicines. The novel application of MIPs in TCM for effective recognition of active inhibitors in existence of plenty of interferences afforded us a new approach for the discovery of lead compounds or drug candidates, which will be very beneficial in drug development.

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