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Affinity screening by packed capillary high performance liquid chromatography using molecular imprinted sorbents II. Covalent imprinted polymers

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Abstract

This study concentrates on the production of covalent molecular imprint polymers (MIPs) as highly selective sorbents for nortriptyline (NOR), a representative tricyclic antidepressant (TCA). The functionalized template contains a polymerizable 4-vinylphenyl carbamate moiety used to bind the template molecule to the polymer matrix. Polymerization with a cross-linker followed by hydrolytic cleavage of the labile carbamate functionality leaves an MIP with selective binding sites capable of binding template through hydrogen bonding interactions. Demonstrated chromatographically through a ''selection index'', these MIPs showed high selectivity for the template molecule (NOR) among a library of structurally similar compounds. The recognition was found to correlate with structural similarity to the template compound. A direct comparison between covalent and non-covalent molecular imprinting strategies reveals a great deal of improvement in the peak shape of the retained compound resulting from covalent imprinting (evidenced by peak asymmetry factors A_s).

Keywords: Molecular imprinted sorbents; Molecular imprinting; Covalent imprinting; Peak shape; Nortriptyline

1. Introduction

Molecular imprint polymers (MIPs) are highly cross-linked polymers formed using, as a template compound, a molecule for which high selectivity is desired. The template compound interacts with monomers in solution, covalently or non-covalently, and a highly cross-linked polymer is formed around it. After polymerization is complete, the template compound is removed from the polymer matrix leaving selective recognition sites on the MIP that have demonstrable ''memory'' of the template. Molecular imprinting can therefore be utilized to produce synthetic mimics of biological recognition elements such as enzymes, receptors and antibodies [1–7]. The resulting MIPs can be used as surrogate receptors in the screening of a library of potential chemical activators [15,21]. While MIPs cannot completely substitute for biological recognition elements, they may provide an inexpensive, stable and simple means for preliminary library screening that can be used as a guide for further investigation.

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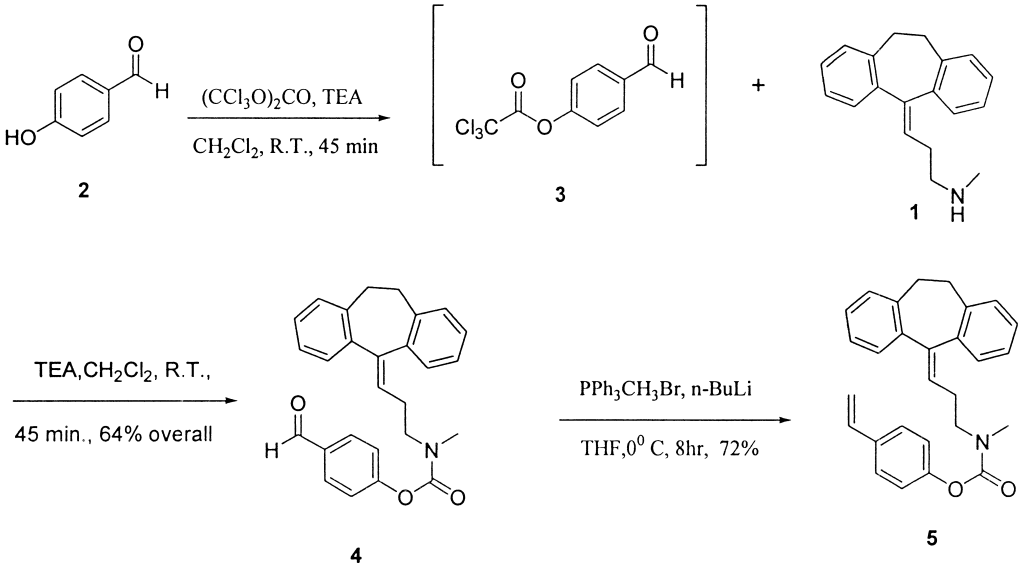
Furthermore, MIPs allow, to some degree, for harsh conditions such as high temperatures, high pressures, pH extremes and the use of organic solvents that may not be possible with biological recognition elements. Earlier studies have demonstrated the usefulness of MIPs in reaction catalysis [1–3], sensor design [4–6], immunoassays [7], protein separations [8] and chiral separations [9–13]. The utilization of MIPs as a combinatorial library screening tool is still in its initial stages but may be promising [14,15].

Two general approaches are usually employed in the synthesis of MIPs: covalent and non-covalent. In the latter approach, the template molecule is mixed with functionally complementary additives (usually methacrylic acid for basic template molecules or divinyl pyridine for acidic template molecules) along with a cross-linker. Polymerization is then effected, usually in bulk, forming a highly cross-linked polymer around the template molecule. The template molecule is fixed in the polymer matrix by combination of non-covalent interactions such as electrostatic, H-bonding or $\pi-\pi$ interactions. The template molecule is then removed from the polymer matrix leaving binding ''pockets'' that have size and functional memory of the template molecule. By virtue of its simplicity and generality, the non-covalent approach is by far the most widely used approach for molecular imprinting and the literature is rich with examples of non-covalent imprinting [7–17]. An example is the use of a benzodiazepine imprinted polymer for the chiral separation of benzodiazepines [18] in which recognition was found to be significantly dependent on analyte–MIP chiral match or mismatch.

In the covalent approach, on the other hand, the template molecule is chemically bound to a polymerizable monomer via a labile covalent bond. The functionalized template is copolymerized with a cross-linker in a suitable porogenic solvent. The template molecule is fixed in spatial arrangement within the polymer matrix by means of this chemical bond. The labile bond is then cleaved to remove the template molecule, leaving binding pockets that are claimed to be more uniform in placement of a single complementary functional group than those obtained by the non-covalent approach. The covalent approach is more difficult to apply since it requires multiple heteroatom functionality to be available in the template molecule; hence, examples that utilize this approach are relatively rare in the literature. One of the earliest examples utilizing this approach is the work of Wulff et al. in which phenyl-α-D-mannopyranoside was used as a template through functionalization of the sugar hydroxyl groups with 4-vinylphenyl boronic acid followed by copolymerization [19]. The boronic acid undergoes a rapid and reversible reaction with sugar diols facilitating the recognition of the template molecule. Another important example of covalent molecular imprinting approach is the work of Whitecombe et al. [20] in which cholesterol was covalently incorporated into the polymer matrix via a 4-vinylphenyl carbonate ester functionality. Hydrolytic cleavage of this functionality with the loss of CO_2 leaves a phenolic residue that is capable of interacting with the template molecule in the recognition process. In this work, H-bonding was demonstrated to play a key role in the recognition process in this system.

We report herein the application of the covalent molecular imprinting approach to obtain a highly selective sorbent for nortriptyline (NOR) as a model template for the tricyclic antidepressant (TCA) family. This effort represents a continuation of our previous work in which NOR imprinted MIPs were prepared via the non-covalent approach [21]. NOR has a pendant secondary amine group that is functionalized to introduce 4-vinylphenyl carbamate functionality as outlined in Scheme 1. This functionalized template molecule is then incorporated into a highly cross-linked polymer using trimethylolpropane trimethacrylate (TRIM) as the building block. The carbamate functionality is then hydrolytically cleaved to remove the template from the polymer matrix leaving a phenolic residue with the loss of CO_2 (Scheme 2). This is one of the few examples in which a template with a minimal degree of heteroatom functionality has been covalently imprinted.

One of the drawbacks of MIPs is their low chromatographic efficiency arising from peak tailing, which is often observed for the retained components. This peak broadening is a prime reason for the limited usefulness of MIPs in analytical applications (for a review, see Ref. [22]). Sellergren and Shea reported on some improvement in chromatographic



Scheme 1. Functionalization of the template molecule (NOR, 1) used in this study.

performance for L-phenylalanine anilide imprinted polymers after heat treatment of these polymers [12]. They also found that the main cause for peak asymmetry in this system is the non-linear adsorption isotherm [23]. A partial solution to the peak tailing problem encountered with MIPs may be found in the covalent molecular imprinting strategy [24], which is the theme of this paper.

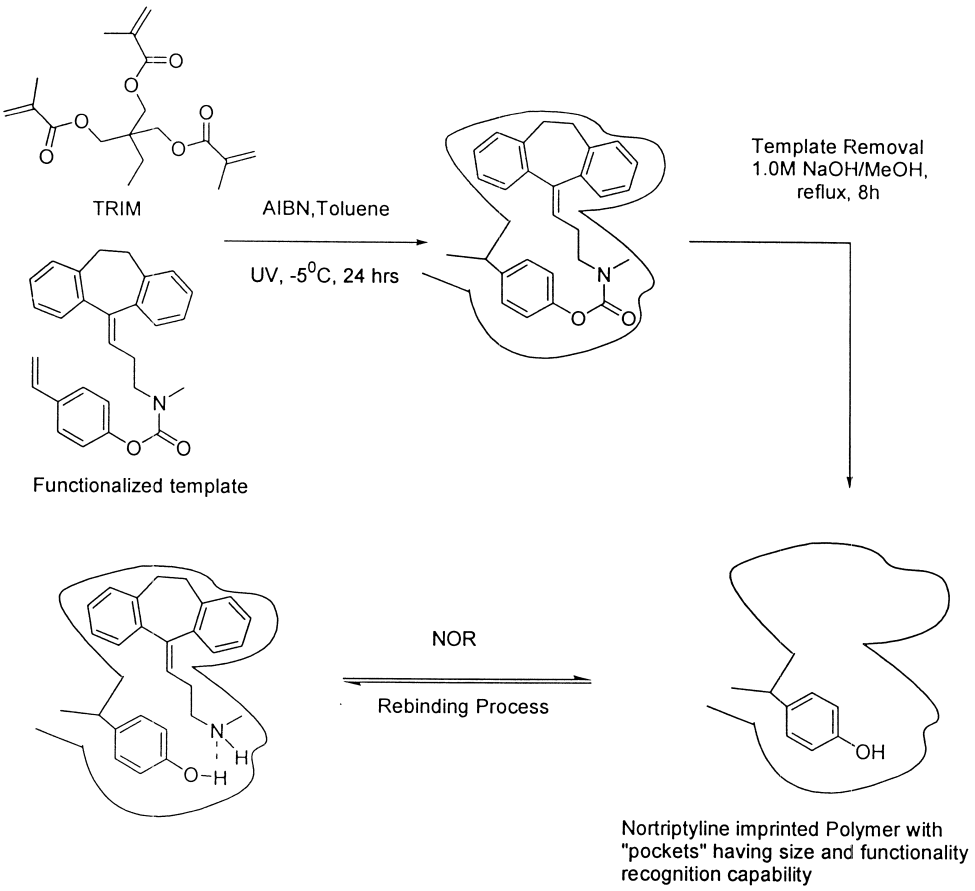
2. Experimental section

2.1. Materials and methods

HPLC grade dichloromethane (Fisher Scientific, Pittsburgh, PA, USA) and reagent grade tetrahydrofuran (Mallinckrodt Baker, Paris, KY, USA) were distilled from Na/benzophenone before use. All other solvents were HPLC grade and used as received. Glassware was thoroughly cleaned (alcoholic KOH), rinsed with water, acetone and then oven dried overnight before use. All reactions were performed under a nitrogen atmosphere. ^{1}H and ^{13}C NMR experiments were performed on an AVANCE-300 instrument (Bruker Analytik, Ettlingen, Germany) and IR experiments were performed on an Nicolet 5DXB (Nicolet Analytical, Madison, WI, USA). NOR and other TCAs were purchased from Sigma (St. Louis, MO, USA). Triphosgene, triethylamine, 4-hydroxybenzaldehyde, methyltriphenylphosphonium bromide, *n*-butyl lithium (1.0 *M* solution in hexane), trimethylolpropanetrimethacrylate (TRIM), 2,2′-azobisisobutyronitrile (AIBN), methacrylic acid (MAA) and styrene were all purchased from Aldrich (Milwaukee, WI, USA) and used as received.

2.2. Synthesis of aldehyde **4**

To a solution of triphosgene (0.153 g, 0.546 mmol) in CH_2Cl_2 (3.0 ml) stirred at room temperature was added, over a period of 30 min, a mixture of 4-hydroxybenzaldehyde (0.20 g, 1.64 mmol) and triethylamine (0.45 ml, 3.26 mmol) in CH_2Cl_2 (5.3 ml) [25]. The resultant mixture was stirred at room temperature for 15 min and then a solution of nortriptyline hydrochloride (0.492 g, 1.64 mmol) and triethyl amine (TEA) (0.45 ml, 3.26 mmol) in CH_2Cl_2 (5.3 ml) was added in one portion. The mixture was stirred at room temperature for 2 h and then quenched with water (10 ml). The two layers were separated and the aqueous layer was extracted with diethyl ether (3×25 ml). The combined organic layer was washed with brine, dried over anhydrous



Scheme 2. Covalent imprinting strategy: polymerization, template removal and rebinding processes.

$MgSO_4$, filtered and concentrated in vacuum to give a reddish brown residue. Purification by column chromatography (silica gel, elution with 3 ethylacetate:1 ether:2 hexane) gave the title product (0.431 g, 1.05 mmol, 64% yield over two steps) as a yellow oil. The product was identified by FTIR, ^{1}H NMR, ^{13}C NMR and MS as indicated in Appendix A.

*2.3. Synthesis of alkene **5***

To an ice-bath cooled suspension of methyltriphenylphosphonium bromide (0.347 g, 0.972 mmol) in THF (20 ml) was added *n*-BuLi (1.0 *M* solution in hexanes, 0.97 ml, 0.97 mmol) slowly with vigorous stirring. The mixture was stirred for 45 min to give a clear yellow solution to which was added a solution of aldehyde **4** (0.20 g, 0.486 mmol) in THF (12 ml). The resultant mixture was stirred at 0°C for 10 h. The reaction was quenched by addition of a saturated aqueous solution of ammonium chloride (25 ml). The two layers were separated and the aqueous layer was extracted with ether (3×25 ml). The combined organic layer was washed with brine followed by water, dried ($MgSO_4$), filtered and concentrated in vacuum. The residue was purified by column chromatography (silica gel, elution with 33% ethylacetate in hexane) to give the title product (0.143 g, 0.35 mmol, 72% yield) as a thick oil. The product was identified by FTIR, ^{1}H NMR, ^{13}C NMR and MS as indicated in Appendix A.

2.4. Polymer synthesis

The functionalized template **5** (75 mg, 0.183 mmol, 5 mol.% of the cross-linker) or the template molecule (NOR in the case of non-covalent imprinting, 50 mg, 0.189 mmol, 5 mol.% of the monomer) and TRIM (1.18 ml, 3.696 mmol) were dissolved in toluene (1.0 ml) in a 5-ml scintillation vial. AIBN (15.4 mg, 0.094 mmol) was added to the mixture. The mixture was degassed (bubbling nitrogen while immersed in an ice-bath) for 5 min and then placed in a freezer (−5°C) for UV irradiation (λ=350 nm) for 24 h. The resultant polymer was a colorless, brittle solid, which was air-dried, and then ground (pestle and mortar). The methanol suspended particles were successively filtered through a 30-μm mesh nylon filter. The filtrate was re-filtered through a 20-μm mesh nylon filter. The 20–30-μm nominal size particles were dried and kept for later use.

2.5. Polymer hydrolysis and template recovery

In the case of the covalently imprinted polymer, the ground polymer particles were suspended in 1.0 *M* NaOH in methanol and heated to reflux. After 8 h, the particles were filtered, and then washed sequentially with methanol, water and ether. In an attempt to recover the template molecule, the filtrate was extracted with ether (3×25 ml). The organic layer was washed with water, dried (anhydrous $MgSO_4$), filtered and concentrated to give an oil residue which was examined by ^{1}H NMR spectroscopy and determined to be a complex mixture, which indicates decomposition of NOR making it difficult to quantify the NOR recovery yield. For the non-covalent polymers, the packed capillary columns were flushed with 10% acetic acid in methanol to remove the template molecule.

2.6. Packing of the capillary columns

The finished polymer particles were packed in fused-silica capillary columns for chromatographic evaluation following a version of slurry packing technique developed in our laboratory [21]. In this technique, capillary columns (250 μm I.D., Polymicro Technologies, Phoenix, AZ, USA) equipped at the outlet with frits made of porous silica particles were slurry packed. The slurry was prepared by mixing 15 mg of the sieved MIP particles and 1 ml of a 50% acetonitrile in water. The slurry was placed in a 1-ml packing reservoir equipped with a small magnetic bar and the system was placed on a magnetic stirrer. With continuous stirring, the slurry was forced into the capillary by applying increasing pressure created by a syringe pump (model 100DX with series D pump controller, ISCO, Lincoln, NE, USA). The packing progress was observed under a microscope to ensure the uniformity of the chromatographic bed. Packing was continued until the bed length was ~25 cm; at this point the syringe pump was stopped and the pressure was allowed to bleed slowly to zero.

2.7. Microscale HPLC measurements

Packed capillary columns were assembled in a micro HPLC set-up consisting of the ISCO syringe pump, a Valco microinjection valve (Valco Instruments, Houston, TX, USA) with a 60-nl loop volume and a Unicam 4225 variable wavelength UV–Vis detector (ThermoSeparation Products, San Jose, CA, USA). Data were recorded and analyzed on a PowerChrom system 2.0 (ADInstruments, Milford, MA, USA) with a Power Macintosh 6100/66 (Apple Computers, Cupertino, CA, USA).

3. Results and discussion

3.1. Template functionalization and polymer synthesis

Nortriptyline (**1,** Scheme 1) was chosen in this study as a model for the widely available tricyclic antidepressants (TCAs). The pendant secondary amine of NOR was derivatized to form a labile carbamate functionality as outlined in Scheme 1. To this end, 4-hydroxybenzaldehyde (**2**) was reacted with triphosgene to give intermediate (**3**) which was immediately reacted with NOR to obtain aldehyde (**4**) in a 64% overall yield according to a literature procedure [25]. Wittig olefination of aldehyde (**4**) with methyl triphenylphosphonium bromide gave rise to functionalized template (**5**) in a 72% yield [26]. Low temperature photochemical initiation of

the polymerization of the functionalized NOR (**5**) and the cross-linker (TRIM) was conducted with toluene as a porogenic solvent (Scheme 2). The template was then removed from the resulting polymer (P3, Table 1) by hydrolyzing the carbamate functionality with the loss of CO_2 under standard conditions (8-h reflux with 1.0 *M* NaOH in methanol). For the purpose of comparison, non-covalent imprinted polymers were prepared using, in addition to NOR, either MAA or 4-vinylphenol as the functional monomer. The template molecule was extracted from the non-covalent MIPs (P4, P5 and P6) by extraction with 10% acetic acid in methanol.

A series of highly cross-linked MIPs were synthesized for chromatographic evaluation (Table 1). Polymer P1, which contains TRIM and 4-vinylphenol, serves as the blank for the covalent imprinted polymers since it exhibits phenolic residues in the polymer matrix. In the covalent polymer P3, these residues are left behind after the hydrolytic removal of the template molecule. Polymer P2 contains, in addition to TRIM, methacrylic acid (MAA) and styrene. This polymer contains no template and serves as an appropriate blank polymer for the non-covalent imprinted polymers P4 and P5. P4 is a control polymer in which no MAA or styrene were added in an effort to test whether the functional complementarity between the analyte and the MIP rebinding sites is essential for the recognition process. The addition of MAA to P2 and P5 provides an insight of the role of these additives on the rebinding interactions since they enhance interaction (H-bonding or ionic) with the polymer matrix. P6 is a non-covalent MIP in which 4-vinyl phenol was used in place of MAA. This polymer will have, after the removal of the template, a phenolic residue similar to that in the covalent polymer (P3). P7 is prepared in the same way as P6, but in the absence of the template compound.

This experimental design provided a basis for a thorough study comparing the chromatographic performance of covalent and non-covalent MIPs, particularly with regard to peak shape.

3.2. Simulated chemical library

The finished polymers were packed in capillary columns for chromatographic evaluation (Experimental section). The method used for evaluating the performance of these MIPs is microscale HPLC, selected by virtue of its minimal consumption of MIP packing material, solvent and sample. This provides important advantages, aside from economic and environmental advantages, for applications in which only small quantities of analyte may be available [27]. In addition, microscale HPLC is amenable to direct hyphenation with MS and NMR detection making it a powerful tool for separation and on-line structural identification. A simulated chemical library (structures and acronyms for the library compounds are shown in Fig. 1) was assembled from the template molecule, a number of structurally related TCAs and other control compounds (IDB, which has a tricyclic moiety similar to the TCAs) and structurally unrelated control (CAF). BUP was included because it is pharmacologically similar to the TCAs though its structure is different.

Table 1
Compositions of polymers synthesized in this study

Polymer	Template (mol.%)	Cross-linker (mol.%)	Functional additive (mol.%)
P1	None	TRIM (96)	4-Vinylphenol (4)
P2	None	TRIM (64)	MAA(24)+styrene (12)
P3	Alkene **5** (4)	TRIM (96)	None
P4	NOR (2)	TRIM (98)	None
P5	NOR (2)	TRIM (62)	MAA (24)+styrene (12)
P6	NOR (4)	TRIM (60)	4-Vinylphenol (12)+styrene (12)+MMA (12)
P7	None	TRIM (63)	4-Vinylphenol (12)+styrene (12)+MMA (13)

Polymerization was initiated photochemically (at −5°C) with toluene used as the porogen and AIBN (1 mol.%) as the initiator. Polymerization yields around 90% were achieved in all cases.

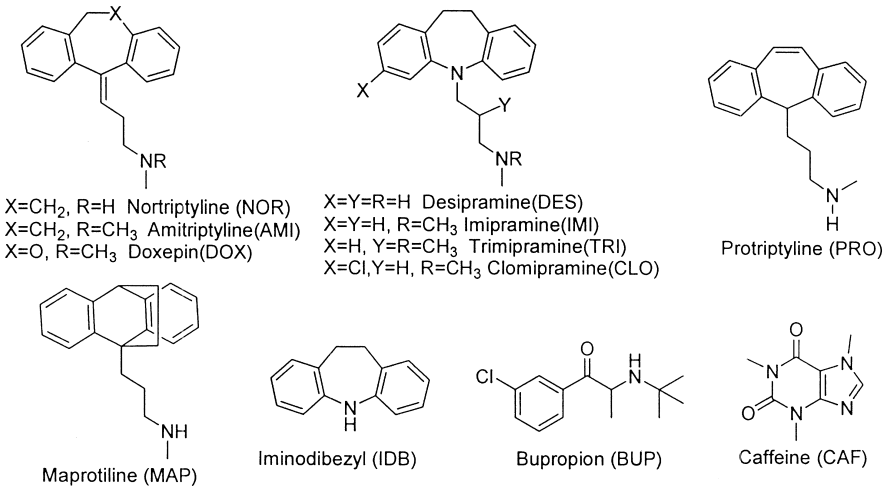


Fig. 1. Structures of library compounds used in this study.

3.3. Chromatographic evaluation

Chromatographic retention factor values (k) are summarized in Table 2 for all the simulated combinatorial library compounds. An examination of the data in Table 2 reveals that the template molecule has, as might be expected, the highest retention factor value on imprinted polymers P3, P5 and P6. However, P4, in which no MAA was incorporated, is an exception in which no retention was observed for any of the library compounds, including the template. This observation supports the hypothesis that the acidic functional residue incorporated by the addition of MAA to P5 is essential to recognition; no recognition is observed without that functionality. For polymers P3, P5 and P6, the secondary amine TCAs (PRO, DES and MAP) exhibit lower values of k than NOR (also a secondary amine) but higher than those of tertiary amine TCAs (AMI, IMI, CLO and TRI). Structurally different test compounds (IDB and CAF) have the smallest k values. Fig. 2 shows that NOR (the template) is retained selectively relative to AMI, IMI, TRI and CLO for P3, P5 and P6. No separation is achieved on the corresponding blanks (P1, P2, and P7). It should be mentioned that due to the relatively low plate numbers on these MIP columns (typical values were 4200 plates/m for acetone and 1700 plates/m for NOR), no separation of NOR from the other secondary amine TCAs (PRO, DES, and MAP) could be obtained.

The fact that secondary amines have higher k values than, and are well separated from, their tertiary counterparts suggests that the pendant secondary amine functionality is critical for the recognition process. This behavior was observed in our

Table 2
Retention factor (k) values for library compounds studied on different MIPs

Compound	k (P3)	k (P4)	k (P5)	k (P6)
NOR	2.83	0.16	2.68	4.55
DES	2.59	0.20	2.48	3.53
PRO	2.35	0.18	2.24	3.58
MAP	2.49	0.20	2.44	3.96
DOX	1.43	0.14	1.13	0.75
TRI	1.33	0.15	1.03	0.53
AMI	1.57	0.14	1.11	0.85
IMI	1.52	0.14	1.12	0.88
CLO	1.61	0.16	1.09	0.97
BUP	1.42	0.20	0.88	0.58
IDB	0.17	0.27	0.37	0.53
CAF	0.19	0.13	0.68	0.40

Mobile phase 0.01% TEA and 0.0065% TFA (v/v) in ACN. Typical column dimensions: 15–25 cm long, 250 μm I.D. The retention factor is defined as $k=(t_r-t_0/t_0$, where t_r is the retention time of a given analyte and t_0 is the retention time of the void peak (acetone was used as an unretained marker). Data represent the mean of three values. Relative standard deviation was less than 5% in all cases.

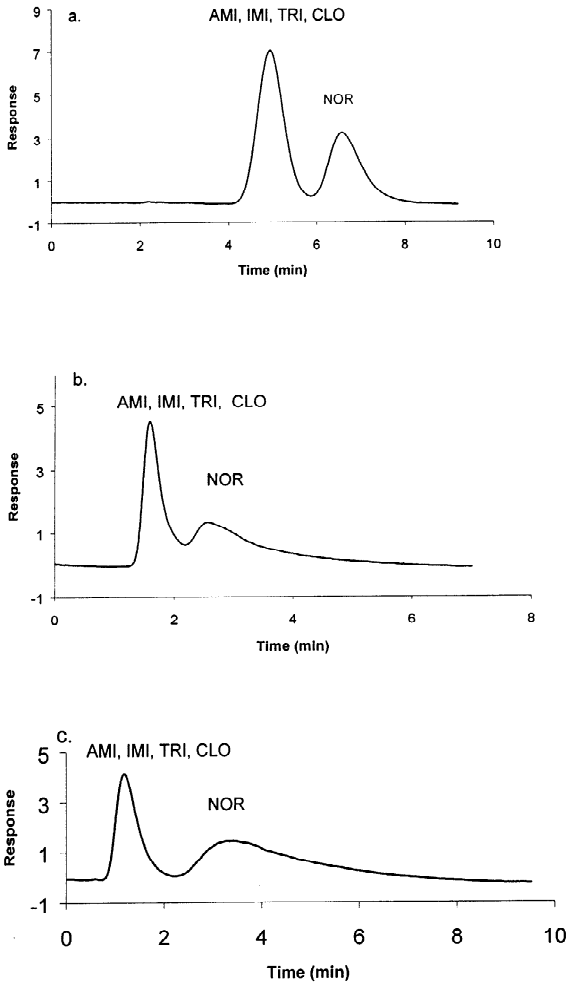


Fig. 2. Chromatograms showing the performance of different studied MIPs. (a) Covalent polymer (P3), (b) non-covalent with MAA (P5), and (c) non-covalent with 4-vinylphenol (P6) under identical conditions (Table 2).

previous work with non-covalent NOR imprinted polymers [21]. The additional methyl group on the amine functionality in the tertiary amines seems to increase steric hindrance around nitrogen impeding its capability of interacting with the functional complement in the binding sites. This is evident through a comparison of the retention behavior of NOR and its tertiary amine analog AMI (e.g. on P3, $k_{NOR}=2.83$ while $k_{AM\ I}=1.57$). The observation that BUP and IDB have smaller k values although they are secondary amines suggests that the size match between the analyte and the binding sites may also play a role in the recognition process. These findings support the assertion that in order for recognition to take place on an MIP, the functionalities on the analyte and those of the binding site should be complementary to one another. The recognition observed on P5 in contrast to its absence on P4 (which is identical to P5 except that no MAA is added) suggests that the presence of this moiety is crucial for the recognition process. It is also notable that the secondary amine TCAs are more retained than tertiary amines.

3.4. Comparison of covalent and non-covalent imprinting strategies

As can be seen from the data in Table 2, for non-covalently formed MIPs (P4 and P5), recognition is observed only when functional complementarity is a characteristic of the polymer matrix (P6), while no functional additives are necessary for the covalently formed MIP (P3). Interestingly, with the approach presented here, no additives are necessary in polymer synthesis since a phenolic residue is left after the removal of the template molecule. It is this functionality that apparently plays a vital role in the analyte binding process.

As was mentioned previously, MIPs suffer from poor efficiency of separation as a result of the excessive peak tailing observed for the retained component using these phases. This peak tailing makes the practical applications of MIPs in analytical purposes difficult especially quantitatively. There have been several studies performed on MIPs in an effort to gain a better understanding of the factors governing chromatographic performance of these MIPs [12,23,28]. It should be mentioned that with our approach of covalent imprinting, there is a substantial improvement in the peak shape for the template compound relative to a non-covalent MIP prepared for the same template (Fig. 2). This peak shape improvement is evident from peak asymmetry factor data. Asymmetry factors A_s, determined at 10% of the peak height as described in the literature [29], were calculated for the template on the covalent and non-covalent MIPs. A_s values for NOR were

determined to be 1.72, 5.43 and 4.85 on P3, P5 and P6, respectively. From these values, it can be seen that peak asymmetry factor is much smaller (the peak is more symmetrical) for the covalent polymer (P3) as compared with the non-covalent polymers (P5 and P6). A closer look at the compositions of P3 and P6 and their corresponding blanks reveals that for both polymers, the same functional moiety is responsible for recognition. The asymmetry data therefore suggest that the improvement in peak shape is mainly due to the difference in imprinting strategy — covalent versus non-covalent. This improvement in the peak shape of the template compound is very important since it could help in the quantitative analytical applications of covalently formed MIPs. The improved peak shape observed for the covalent MIP may indicate better specificity of imprinting since the prepolymerization complex is held together by means of a covalent bond as compared to that in the non-covalent approach, which depends only on weaker non-covalent interactions. This conclusion is in good agreement with that made by Shimizu et al. [24] in which the covalent imprinting strategy was concluded to be more appropriate for chromatographic applications since chromatography requires a narrow distribution of imprinted sites. Further support for this conclusion may come from a study by Wulff et al. [30] in which a covalent MIP was evaluated based on frequency distribution of binding sites as a function of their specificity. The covalent imprinted polymer showed a relatively narrow frequency distribution. An investigation of the binding site distribution of the polymers used in this study is currently underway.

Although the selectivity (α) describes the separation of zone centers, it takes no account of non-specific interactions or blank corrections. A parameter that accounts for non-specific interactions is therefore needed to more accurately evaluate molecular recognition on these sorbents. The selection index (SI) introduced earlier by our group is defined as [21]:

$$SI = \frac{\left[\left(k_a^{MIP}/k_a^{Blank}\right) - 1\right]}{\left[\left(k_t^{MIP}/k_t^{Blank}\right) - 1\right]}$$

where k_a^{MIP}, k_a^{Blank} are the retention factors for analyte on the MIP and blank, respectively and k_t^{MIP}, k_t^{Blank} are those for template molecule on MIP and blank, respectively. The selection index thus provides a good measure of the extent of ''recognition'' of a test compound relative to the template compound. The template molecule has, by definition, an SI of 1. For a test probe having identical retention factors on an MIP and blank sorbent, $SI = 0$. The selection index values for P3, P5 and P6 are summarized in Table 3. The selection index then provides a reasonable measure of the quality of fit between the analyte and the MIP binding sites.

The correlation between the selection index values for the various test compounds and their structural similarity to the template molecule should be pointed out. For all of the imprinted polymers, NOR exhibits the highest selection index value. On P3, other secondary amine TCAs (DES, PRO and MAP) exhibit lower selection index values than NOR but higher values for the tertiary amines (DOX, TRI, AMI, IMI and CLO). Structurally unrelated compounds (CAF and IDB) exhibit minimal selection index values. The same general trend is observed for other imprinted polymers except P5, in which a less obvious trend between tertiary and secondary amines can be observed. It should be noted that retention factor data for the test probes on ''blanks'' P1, P2 and P7 were used in the calculation of the SI data for the corresponding test probes on P3, P4 and P6, respectively. The extent of selective interaction with the MIP, reflected by the selection index data, is seen

Table 3
Selection index values for library compounds on MIPs P3, P5 and P6

Compound	P3	P5	P6
NOR	1.00	1.00	1.00
DES	0.83	0.71	0.68
PRO	0.75	0.45	0.63
MAP	0.87	0.62	0.74
DOX	0.51	0.56	0.20
TRI	0.52	0.70	0.16
AMI	0.57	0.50	0.22
IMI	0.57	0.49	0.24
CLO	0.63	0.40	0.25
BUP	0.53	0.15	0.10
IDB	0.01	0.09	0.03
CAF	0.02	0.27	0.05

to correlate with structural similarity of the library compound to the template.

4. Conclusions

This study describes the synthesis of a nortriptyline covalent molecular imprint polymer, its characterization, and chromatographic evaluation. The template molecule was covalently attached to 4-vinylphenyl carbamate functionality and co-polymerized with TRIM as a building block (cross-linker). The template was then removed from the polymer matrix by hydrolytic cleavage of the labile carbamate functionality with the loss of CO_2. In all cases, the synthesized MIP was found to be highly selective for the template molecule used. The degree of retention was found to correlate with structural similarity of test probes to the template compound. The retention data was interpreted by a ''selection index''. Selection index values were found to be higher for secondary amine TCAs as compared with those of tertiary amine TCAs when tested on the NOR imprinted polymer, indicating the importance of the pendant amine moiety in the recognition process. Structurally unrelated test compounds yielded minimal selection index values. Incorporation of methacrylic acid in the polymer matrix was necessary for the non-covalent imprinting approach. In the covalent approach however, the phenolic residue left in the polymer matrix after template removal is sufficient to yield the selectivity towards the template molecule. It was also found that covalent MIPs resulted in much improved peak shape for the template compound as compared with non-covalent MIPs as quantified using peak asymmetry factor data.

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Appendix A

*Spectroscopic data for aldehyde **4***

FTIR (thin film) 3022, 2956, 1725, 1602, 1192 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz) δ(ppm) 9.98 (s, 1 H), 6.8–7.7 (m, 12 H), 5.9 (t, $J=4.2$ Hz, 1H), 3.25–3.60 (brm, 4 H), 3.05 (t, 2 H), 2.95 (t, 2 H), 2.50 (s, 3 H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ(ppm) 191.4, 154.0, 139.9, 139.6, 137.4, 133.7, 132.5, 131.4, 130.5, 130.4, 128.9, 128.8, 128.6, 128.4, 128.0, 127.7, 127.6, 126.5, 122.7, 116.6, 46.3, 34.1, 32.4, 28.1, 25.1. Molar mass calculated for $C_{27}H_{25}NO_3$ requires 411.1834, found 411.1838.

*Spectroscopic data for alkene **5***

FTIR (thin film) 3031, 2966, 1609, 1201 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz) δ(ppm) 6.8–7.7 (m, 12 H), 6.7 (dd, $J=11.0$, 6.5 Hz, 1 H), 5.92 (t, $J=5.6$ Hz, 1 H), 5.60 (dd, $J=11.0$, 6.5 Hz, 1 H), 3.4 (brm, 4 H), 2.85 (s, 2 H), 2.80 (m, 2 H), 2.50 (s, 3 H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ(ppm) 155.0, 136.5, 136.4, 135.1, 134.3, 134.0, 132.7, 132.5, 130.50, 130.40, 124.0, 128.9, 128.5, 128.0, 127.6, 127.4, 127.3, 126.5, 126.2, 122.2, 113.9, 49.4, 35.2, 34.1, 32.4, 28.8. Molar mass calculated for $C_{28}H_{27}NO_2$ requires 409.2042, found 409.2035.

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