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Affinity screening by packed capillary high-performance liquid chromatography using molecular imprinted sorbents I. Demonstration of feasibility

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Abstract

Molecular imprint polymers (MIPs) are synthetic polymers capable of selectively binding a template molecule. In this work, the potential utility of MIP-based chromatographic sorbents for affinity screening of structurally similar compounds was investigated as alternatives to in vitro bioassays and biological targets bound to chromatographic supports. A group of structurally similar tricyclic antidepressant drugs and related compounds were used to simulate a combinatorial library. One of the antidepressants, nortriptyline (NOR), was selected as the template species. Using capillary HPLC columns packed with NOR-imprinted MIP particles, the simulated library was screened and the degree of selective interaction of each compound was determined. This correlated with each compound's affinity for the NOR binding site in the polymer. The results of the study revealed that library species which possess the major structural features of the template, specifically the ring structure and pendant secondary amine, were best ''recognized'' by the MIP, while the most structurally dissimilar compounds exhibited the least selective interaction. An investigation of the retention mechanism on these MIPs provided evidence that hydrogen bonding between the pendant amine group on the antidepressants and a methacrylic acid moiety on the polymer surface was critical in the molecular recognition process.

Keywords: Molecular imprinted sorbents; Feasibility

1. Introduction

Molecular imprint polymers (MIPs) are synthetic polymers that can be made to exhibit spatial ''memory'' of a template molecule that is incorporated prior to polymerization [1–4]. One of the most promising applications of MIPs is as synthetic mimics of biological receptors and antibodies. In this approach, a template molecule is chosen that is a potent activating ligand of the receptor to be mimicked. MIP synthesis around this ligand yields a material with complementary cavities that can subsequently ''recognize'' the template species, and thereby function as a surrogate biological receptor. Immunoassays, in which synthetic antibodies have been prepared from MIPs, have shown excellent correlation with their biological counterparts [5]. In a similar vein, MIPs have been successfully utilized as mimics of biological recognition elements in biosensors [6].

Combinatorial chemistry techniques, from which a library of diverse compounds can be produced from an initial skeleton structure, have dramatically

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increased in popularity over the past ten years and are becoming powerful tools in the search for novel drug compounds [7]. Techniques used to identify lead compounds from the multitude of reaction products generally involve screening for activity against some biological recognition element (e.g. enzyme, receptor, or antibody), with which the proposed new drug will interact. Included among these are enzymatic and cell-based assays, enzyme linked immunosorbent assay (ELISA), radioimmunoassay, ultrafiltration, affinity chromatography and scintillation proximity assay (SPA) [8,9].

An important subset of the receptor binding techniques is based on affinity chromatography and electrophoresis. Screening methods derived from the former, known as affinity selection chromatography [9], make use of a receptor immobilized on a solid support as the chromatographic stationary phase. In affinity selection chromatography, elution profiles are monitored with the retention time of a given analyte correlating to its affinity for the target receptor. An advantage of this technique is that the affinity column can be coupled to a mass spectrometer, which can provide on-line structural characterization of the analytes in addition to receptor binding data (e.g. approximate K_d values). Furthermore, multidimensional information can be gleaned by means of a second column [10] (e.g. reversed-phase) in series with the affinity column. The need to immobilize the receptor, however, is a limitation of this approach. In addition to the often-difficult chemistry required, one must ensure that the receptor is immobilized in such a way that its binding domain for the target ligand is left intact. Similarly, during chromatographic analysis, careful consideration must be given to the eluent composition to ensure that no conformational change, deleterious to molecular recognition, is induced (e.g. by pH or organic solvent composition). Ideally, the eluent should very closely approximate physiological conditions, but to fully optimize the separation often requires deviation from these ''ideal'' conditions.

Recently, the use of MIPs as chromatographic stationary phases for library screening has garnered attention [11,12]. This approach is essentially affinity selection chromatography employing MIPs as receptor mimics. A ligand having a known, high binding affinity for the target receptor is chosen as the template molecule. The resultant MIP, having binding sites complementary to the high affinity ligand, then serves as a surrogate receptor against which a library of potential activators can be screened. As in affinity selection chromatography, the elution profiles of the analytes are used as the basis to determine affinity for the MIP binding site.

This MIP-based approach, in principle, has several important advantages over methods that employ biological receptors. Among these are the favorable physico-chemical properties of MIPs, specifically the ability to tolerate organic solvents, pH extremes, high pressures, and elevated temperatures. The low cost and relative ease of preparation are further advantages of these polymers. In addition, the need for receptor immobilization (and its associated difficulties) is obviated. It is when the target biological receptor is unusually expensive, difficult or impossible to obtain that an MIP-based screening approach would be most useful. In the search for novel drug entities, the compounds generated by combinatorial synthetic techniques typically have the following features [7]: relatively low molecular weights, at least one aromatic ring, one ionizable group, and moderate polarity. These types of molecules are amenable to imprinting with existing technologies; and to chromatographic analysis.

The work presented here is a preliminary study undertaken to evaluate the use of MIPs for library screening using packed capillary HPLC. The advantages of column miniaturization in HPLC have been well documented [13] A number of these are important from the standpoint of combinatorial library screening. The enhanced mass sensitivity achieved with miniaturized columns is of particular importance when only small quantities of analyte are available for screening. Reduced solvent consumption, a boon for economic as well as environmental reasons, is desirable when large numbers of samples are to be screened. In addition, the ease with which capillary HPLC can be coupled to a mass spectrometer (in particular with electrospray ionization) allows invaluable structural information and affinity data to be obtained concurrently. The emergence of capillary LC-NMR [14] has the potential to provide even more thorough on-line structural characterization of library compounds.

In this study, a group of structurally similar

tricyclic antidepressant drugs (TCAs) and related compounds were used to simulate a combinatorial library. One of these, nortriptyline (NOR) was selected as the template molecule. The library was screened in packed capillary HPLC mode and the affinity of each library compound for the MIP, a measure of each compound's ''goodness of fit'' in the NOR binding site, was determined. No effort was made in this initial study to select a template molecule on the basis of its enhanced affinity for a target receptor or pharmacological activity with respect to the other test probes in the library. The specific goals of this work were: (1) to quantitate the degree of selective interaction of each library compound with the MIP; and (2) based on the data generated in (1), to assess the structural features found to correlate with enhanced molecular recognition and to propose a mechanism(s) of molecular recognition on these MIPs.

2. Experimental

2.1. Materials

Functional monomers, methacrylic acid (MAA) and styrene (STYR), cross linking monomer ethylene glycol dimethacrylate (EDMA), and free radical initiator, 2,2′-azobisosobutyronitrile (AIBN) were purchased from Aldrich Chemical Co. (Milwaukee, WI, USA) and used as received. HPLC-grade toluene, purchased from Fisher Scientific (Pittsburgh, PA, USA), was dried over 4-Å molecular sieves prior to use. The antidepressant drugs and other compounds employed in the study were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

2.2. Preparation of molecular imprint polymers

Nortriptyline (NOR), obtained in its hydrochloride salt form, was converted to the free base by extraction into toluene using 1 *M* NaOH. An appropriate amount of free base NOR (approximately 0.35 mmol) was added to a solution of EDMA (6.25 mmol), MAA (1.25 mmol) and STYR (1.25 mmol) in toluene. The volume ratio of toluene to total monomers was maintained at 2:1. The solution was prepared in a 20-ml glass scintillation vial, which was immersed in an ice bath during the addition of the reagents, and stirred constantly using a magnetic stirrer to ensure homogeneity. The total volume of the mixture of monomers and toluene was approximately 5 ml. Following the dissolution of 0.015 g AIBN, the mixture was purged with a stream of N_2 for 5 min, after which the vial was capped and transferred to a freezer ($^{-}10$°C) where it was illuminated with a UV lamp and allowed to polymerize for 24 h. Following polymerization, the resultant solid block of polymer was recovered by breaking the scintillation vial. The block of material was ground into fine particles using a mortar and pestle. It should be noted that the entire block of bulk polymer appeared uniform in its physical characteristics, e.g. optical properties (white, opaque), texture, and hardness. The particles were then wet-sieved with methanol through a nylon mesh filter having a nominal mesh opening of 30 μm (Spectrum Inc., Laguna Hills, CA, USA). Fines were removed by multiple sedimentation steps in acetone. The particles were then dried at 50°C for 12 h prior to use. ''Blank'' polymer was prepared for use as a control by the same procedure in the absence of template. Although the bulk polymerization methodology employed here yields rather large and irregularly shaped particles, which are undesirable from the viewpoint of chromatographic efficiency, the synthesis is less complex and easier to optimize relative to other strategies designed to provide smaller and more uniform particles.

2.3. Preparation of packed capillary columns

Fused silica capillary tubing (I.D. 250 μm, O.D. 360 μm) was purchased from Polymicro Technologies (Phoenix, AZ, USA) and cut to lengths of approximately 40 cm prior to packing. A retaining frit was prepared at the column outlet by tapping the capillary tip into a paste consisting of 7-μm Nucleosil Si Particles (Meta Chem Technologies, Torrance, CA, USA) and potassium silicate (Kasil #1, PQ Corp., Valley Forge, PA, USA) followed by curing at 80°C for approximately 24 h. A slurry was prepared by adding 10 mg finished MIP particles to 1 ml acetonitrile. The slurry was pumped into a fritted capillary using an Isco 100 DX syringe pump (Isco Inc, Lincoln, NE, USA) in constant pressure mode

with which a pressure of 1500–2000 p.s.i. was maintained. After packing the desired length of capillary, the pump was stopped and the pressure allowed to slowly bleed to zero. The packed capillary was subsequently coupled to a section of bare fused-silica tubing of the same inner diameter via a Teflon sleeve. In order to minimize dead volume at the coupling, the following measures were taken. Firstly, the ends of the capillaries were cut squarely using a specially designed cutter with a rotating diamond blade (Hewlett-Packard, Waldbronn, Germany). While viewed under a microscope, the capillaries were carefully butted together inside Teflon tubing. This provided a liquid tight joint with a minimum of dead volume. For subsequent on-column detection, a detection window was made in this open section of capillary by removing a small (1 mm) section of the polyimide coating. Prior to chromatographic evaluation, the packed capillary was flushed with a solution of acetic acid in methanol (10:90 v/v) to extract any residual template from the polymer. No effort was made to ascertain the recovery of template molecule from the polymer. To serve as a control, an additional capillary column was packed with ''blank'' polymer and conditioned using an identical procedure.

2.4. Instrumentation

All reagents employed in the capillary HPLC experiments were HPLC-grade and were used as received from Fisher Scientific (Pittsburgh, PA, USA) or Mallinkcrodt (St. Louis, MO, USA). The HPLC system consisted of an Hitachi L-6000 HPLC pump (Hitachi Ltd., Tokyo, Japan) or an Isco 100 DX syringe pump (Isco Inc., Lincoln, NE, USA), a Unicam 4225 UV detector (Thermo Separation Products, San Jose, CA, USA), and a Valco microinjection valve (Valco Instrument Co., Houston, TX, USA) with a 60 nl (fixed) loop volume. Data were collected using an Apple Power Macintosh 6100/66 (Apple Computer, Cupertino, CA, USA) equipped with a PowerChrom System 2.0 (AD Instruments, Milford, MA, USA) chromatography data system. Unless otherwise specified, in all HPLC experiments, the mobile phase was delivered in constant-pressure mode with a detection wavelength of 220 nm. The void time (t_o) was determined by the elution time of a small negative peak observed upon injection of the sample solvent (acetonitrile).

3. Results and discussion

3.1. Library screening

A simulated combinatorial library was assembled from a group of tricyclic antidepressant drugs and related compounds, which are shown in Fig. 1. Caffeine was included in the library as a structurally dissimilar and unrelated test probe. MIPs were prepared as described previously using NOR as the imprint molecule. Following careful optimization of the chromatographic conditions, the library was screened by capillary HPLC using packed MIP capillary columns prepared as described previously. The retention factor (k) was then calculated for each library compound. As expected, the template species, NOR, was the most strongly retained of the library compounds. To determine the extent of non-specific retention on the MIP, the library was run under identical chromatographic conditions on a capillary packed with blank polymer (herein referred to as ''blank'' capillary). The k values of the library compounds on the MIP and blank columns are shown in Fig. 2.

For a given compound, the ratio of its retention factors on the MIP and ''blank'' columns ($k^{\mathrm{MIP}}/k^{\mathrm{blank}}$) is of interest, as it provides a quantitative measure of the compound's selective affinity for the MIP. This ratio, for which the term *selection factor* will be introduced, would be expected to be highest for the template molecule, in this case NOR. The selection factors for the other compounds in the library are useful in comparing their relative affinities for the MIP. A more useful parameter with which to obtain a relative measure of affinity of a compound for the MIP recognition cavity is the *selection index*, which we introduce as follows:

$$\text{selection index(compound } n) = \frac{\left(\frac{k_n^{(\mathrm{MIP})}}{k_n^{(\mathrm{Blank})}}\right) - 1}{\left(\frac{k_{\mathrm{template}}^{(\mathrm{MIP})}}{k_{\mathrm{template}}^{(\mathrm{Blank})}}\right) - 1} \quad (1)$$

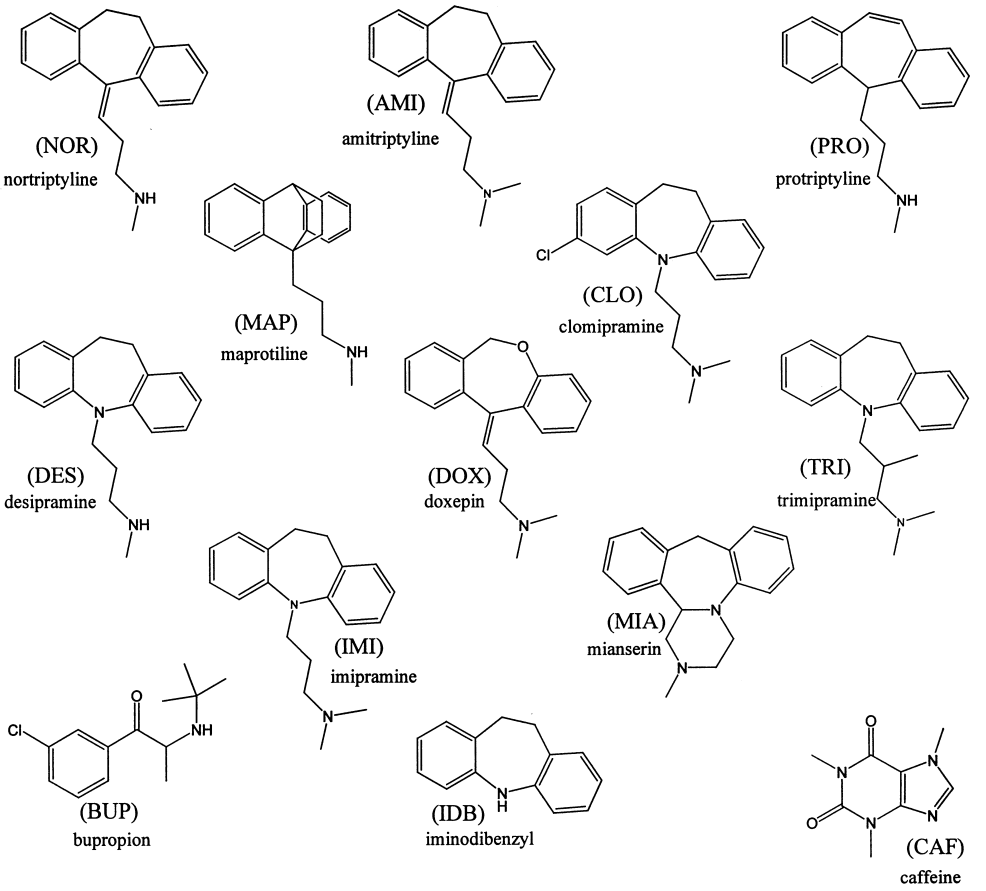

Fig. 1. Structures of the compounds in the simulated combinatorial library.

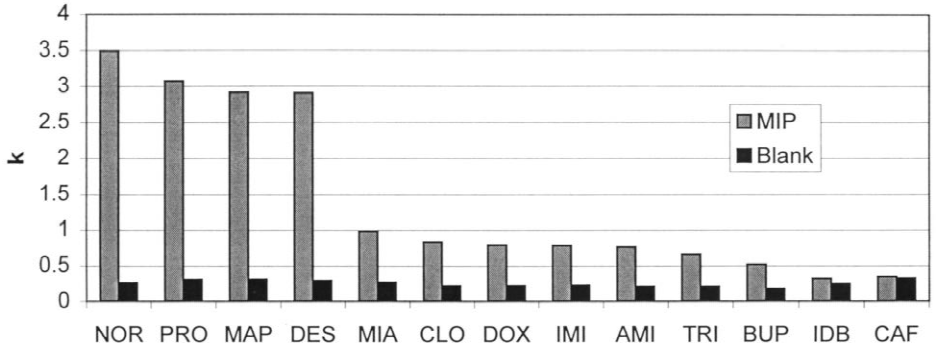

Fig. 2. Comparison of k values for library compounds on NOR-imprinted MIP and blank polymer capillaries. Eluent: 0.02% v/v TFA, 0.015% v/v TEA in CH_3CN.

The selection index is obtained by normalizing the selection factor for compound n to the selection factor calculated for the template molecule. It is useful to note several cogent features of this equation. By definition, the selection index for the template is 1, thus as the ratio $k^{(MIP)}/k^{(Blank)}$ (the selection factor) for library compound n approaches the template selection factor ($k^{(MIP)}_{template}/k^{(Blank)}_{template}$) the selection index for that compound approaches 1. If library compound n exhibits very little selective retention on the MIP (i.e. when the selection factor $\rightarrow 1$), the selection index approaches zero. This equation is similar in form to the retention index used by Ramström et al. [12], differing in the limit $k_n^{(MIP)}/k_n^{(Blank)} \rightarrow 1$. The selection index, then, provides a measure of the relative ''goodness of fit'' of the various library compounds into the NOR binding pocket in the MIP.

Using Eq. (1), selection indices for the library were calculated and are shown in Fig. 3. Upon inspection of these data, some trends are observed. The secondary amine antidepressants, PRO, DES and MAP exhibit greater affinity for the MIP than do the tertiary amines; selection indices for the former are approximately a factor of three greater than the latter. This behavior is not surprising in view of the fact that NOR (the template) is a secondary amine. AMI, the tertiary amine analog of NOR, exhibits relatively weak affinity for the MIP (selection index = 0.216). One possible explanation of the more favorable interaction of the secondary amine TCAs with the MIP is steric hindrance at the pendant amine. It is plausible that the additional methyl group on the tertiary amine TCAs impedes hydrogen bond formation with a methacrylic acid moiety on the MIP. The importance of hydrogen bonding in molecular recognition is supported by the results of studies of the effect of eluent composition on retention.

The compounds with the least affinity for the MIP are BUP, IDB, and CAF. BUP is an antidepressant that is pharmacologically similar to the TCAs [15], but lacks the tricyclic structure. Interestingly, although BUP contains a secondary amine (Fig. 1), it exhibits rather weak affinity for the NOR binding pocket, having the lowest selection index of the antidepressants tested. This behavior implies that the secondary amine functionality and tricyclic structure are necessary to maximize interaction (''fit'') with the binding cavities in the MIP. IDB is a structural precursor to the dibenzazepine TCAs (IMI, DES, CLO, and TRI), which lacks the pendant group. This compound exhibited minimal recognition, having a selection index of only 0.025. Thus, the ring structure alone is insufficient for a favorable interaction with the binding cavity. As expected CAF, a test probe included in the library to evaluate the MIP's ability to discriminate against a structurally dissimilar (and pharmacologically unrelated) compound, showed virtually no selective interaction (selection index = 0.006). These observations are generally in agreement with the anticipated result: the most

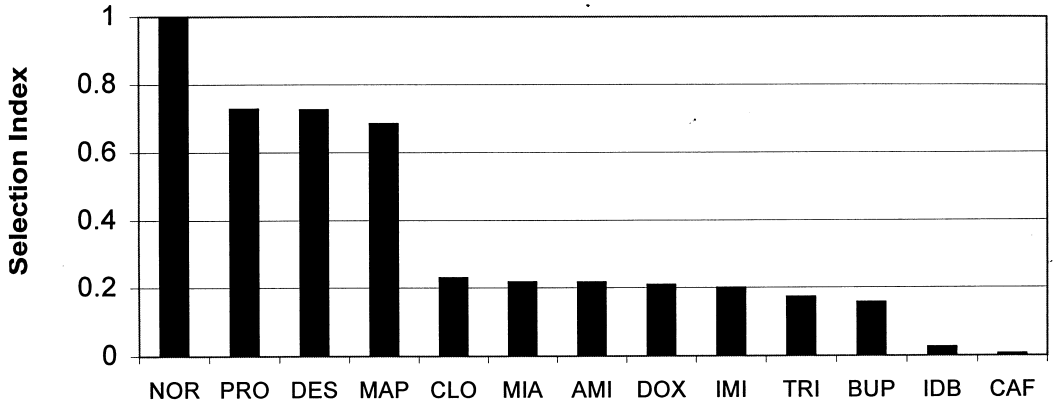

Fig. 3. Selection indices for simulated combinatorial library.

structurally dissimilar compounds in the library (with respect to the template) were the least ''recognized'' by the MIP. Clearly the tricyclic ring structure and pendant amine together are important structural elements in the recognition process.

3.2. Chromatography

The MIP sorbent was employed to separate NOR from a series of tertiary amine structural analogs. As illustrated in Fig. 4a, resolution of NOR from the structural analogs is achieved in about 5 min. Although a measure of selectivity was obtained between NOR and the various secondary amine antidepressants on this sorbent (refer to the *k* values in Fig. 3), no separations could be obtained due to low efficiencies and poor peak shapes. Similarly, in the chromatogram shown in Fig. 4a, the tertiary amine TCAs (AMI, IMI, CLO, DOX, and TRI) coelute despite slight differences in their *k* values. Also apparent in this chromatogram is the severe tailing of the template molecule. Such behavior is well documented with MIP sorbents and has been attributed to slow adsorption/desorption kinetics and nonlinear binding isotherms [16,17]. It should be noted that the MIP particles employed here were obtained by grinding and sieving bulk polymer, which yielded irregular particles of the order of 15–25 μm in diameter. Chromatographic theory predicts low efficiencies for such particles and undoubtedly this is a factor in observed peak shapes. Efforts have been made to synthesize more chromatographically amenable MIP particles (i.e. uniformly sized, small and spherical), most notably by means of dispersion [18] and ''seed'' polymerization [19]. Although successful in the end goal, there is unfortunately a precipitous increase in complexity with these approaches relative to bulk polymerization. Because optimization of selectivity was the primary focus in this initial phase, bulk polymerization was employed. However, work is currently underway in our laboratory to develop MIPs with more favorable chromatographic properties.

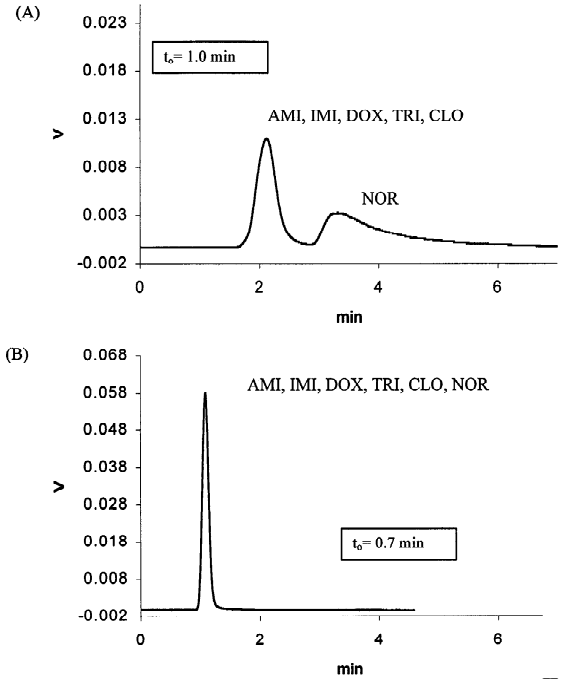

Fig. 4. Analysis of TCA test mixture. Run conditions: Pressure 50 bar; Eluent: 0.02% TFA v/v, 0.0075% TEA v/v in CH_3CN. Sample:0.5 mg/ml (each) AMI, IMI, DOX, TRI, CLO; 1.5 mg/ml NOR in CH_3CN. (A) MIP Capillary: I.D: 250 μm; L_{bed}: 28 cm. (B) Blank Capillary: I.D: 250 μm; L_{bed}: 29 cm.

Fig. 4b shows the same test mixture run on the blank column. In addition to the absence of molecular recognition, it is interesting to note the overall improvement in the shape of the coeluted peak in this blank run, which suggests very little interaction between the analytes and sorbent. The selection index values (Fig. 3) for the tertiary amine test probes range from about 0.18 to 0.25 (i.e. there is some ''recognition'' on the MIP). It can be seen from a comparison of Fig. 4a and b that AMI, IMI, CLO, DOX, and TRI are preferentially retained on the MIP sorbent, although to a much lesser extent than the template species.

Shorter columns and slightly faster linear velocities were employed to effect faster separations of NOR from the five tertiary amine TCAs, shown in Fig. 5a and b. Under these conditions quite rapid separations were achieved; the run in Fig. 5b was completed in less than 60 s. It must be emphasized that all of the separations presented here were performed isocratically and at ambient temperature (approximately 25°C). Gradient elution and elevated temperature are often employed in MIP chromatography to improve peak shape [20], thereby enhancing

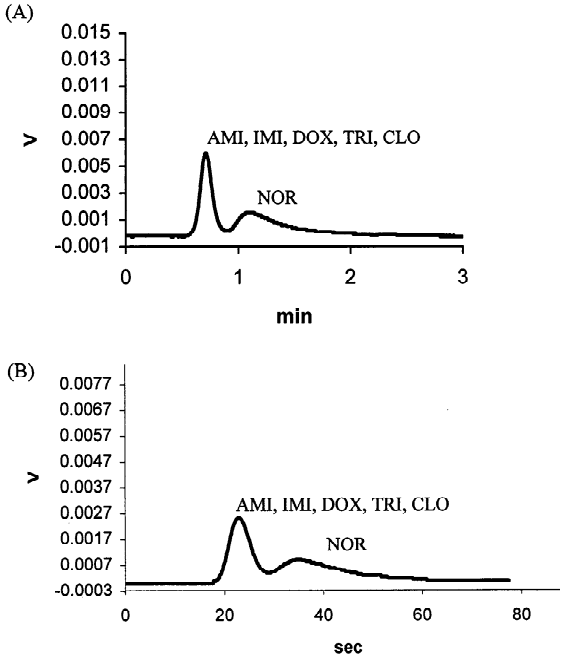

Fig. 5. Rapid MIP separations of NOR from a series of structural analogs. Eluent: 0.02% TFA v/v, 0.0075% TEA v/v in CH_3CN. (A) Capillary I.D.: 250 μm; Pressure: 50 bar; L_{bed}: 14.5 cm; $\mu = 10$ mm/s. (B) Capillary I.D.: 250 μm; Pressure: 50 bar; L_{bed}: 11.5 cm; $\mu = 13$ mm/s.

resolution and reducing analysis time. Under the conditions employed here, however, rapid separations were achieved without the need for these tools, thus simplifying the analysis.

3.3. Investigation of separation mechanism

In an effort to elucidate the mechanism of molecular recognition on the MIP, a series of experiments was conducted in which the eluent composition was varied. The mixture of NOR and the five tertiary amine TCAs was used as a test sample and the effect on the retention behavior of the TCAs (i.e. retention factors) was observed. The optimum eluent for the separation consisted of 0.02% trifluoroacetic acid (TFA) v/v, 0.0075% triethylamine (TEA) v/v in CH_3CN, and a representative chromatogram is shown in Fig. 4a. This eluent was used as a reference point from which to begin the eluent composition study. The test mixture was run on the MIP and blank columns under the following conditions: (1) optimized eluent (control), (2) vary TFA concentration, holding TEA constant, (3) vary TEA concentration, holding TFA constant, (4) omit TFA and TEA, and (5) incorporate an aqueous component. Finally, the test mixture was analyzed on an MIP prepared without methacrylic acid.

The TFA concentration in the eluent was varied over the range of 0 to 0.1% v/v. The results for the MIP and blank columns are shown in Fig. 6. Surprisingly, it was found that when the TFA was omitted, all six TCAs were strongly retained on the MIP *and* blank columns. So strong was the retention that no peak elution was observed and hence k values could be estimated only.[1] As such, the extent of selective retention on the MIP could not be determined. As the TFA concentration is increased there is an overall decrease in k on both columns. On the MIP, selective retention of NOR is readily apparent and decreases with increasing TFA content. At 0.1% TFA, selectivity is decreased to the point where NOR retention on the MIP and blank are comparable. Extrapolation of the MIP data to 0% TFA would predict a relatively large measure of molecular recognition superimposed on a strong nonspecific component of retention. TFA evidently plays a role not only in disrupting molecular recognition, but in attenuating nonspecific sorption on these polymers.

Next, the percentage of TEA was varied from 0 to 0.075% v/v while the TFA concentration was fixed at 0.02%. As shown in Fig. 7, in the absence of TEA, the tricyclic antidepressants were minimally retained ($k < 1$) on both columns. Increasing the concentration up to 0.015% results in a moderate increase for each compound on the blank polymer. Although the same general trend of increased k with % TEA is observed on both columns, selective retention of NOR on the MIP sorbent becomes apparent upon addition of TEA, and increases with the amount added. In contrast, no noticeable difference in retention exists between the template and the other TCAs on the blank polymer. At 0.075% TEA, extremely strong retention (not measurable) is once again observed on both columns.

[1] Due to low plate numbers and the sensitivity of the available detector, k values greater than approximately 15 could not accurately be determined on these columns.

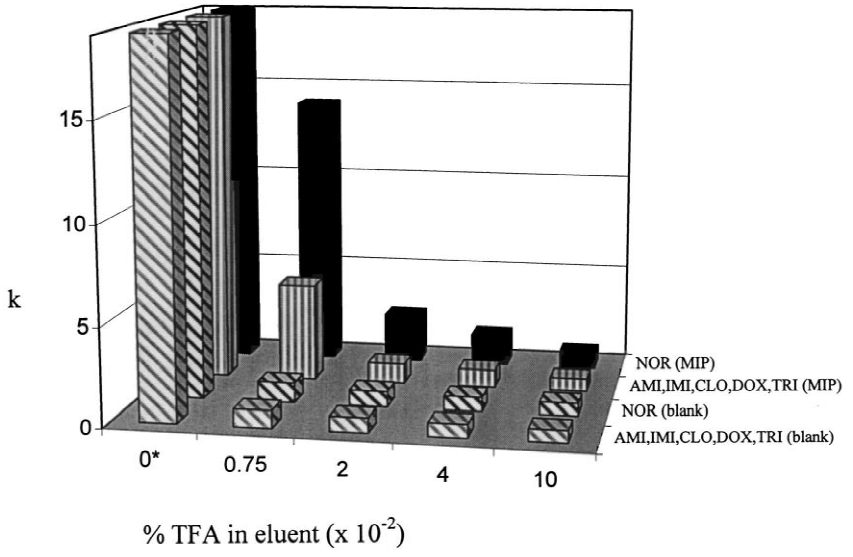

Fig. 6. Effect of TFA concentration on retention. TEA concentration held constant at 0.0075% v/v. *k>15; exact value not determined.

These data indicate the presence of specific and nonspecific components of retention acting in concert on this MIP. Upon inspection of Figs. 6 and 7, it is seen that near the optimum TFA and TEA concentrations, the nonspecific component is effectively disrupted while the specific component is sufficiently maintained such that resolution of NOR from the tertiary amine analogs is possible.

When the acid and base modifiers were omitted from the eluent (neat CH_3CN), runs of the test mixture were characterized by strong retention of the TCAs on both the MIP and blank columns. In addition, the peaks were unusually broad and poorly shaped. For this reason molecular recognition of NOR on the MIP, though observed, was not quantitated.

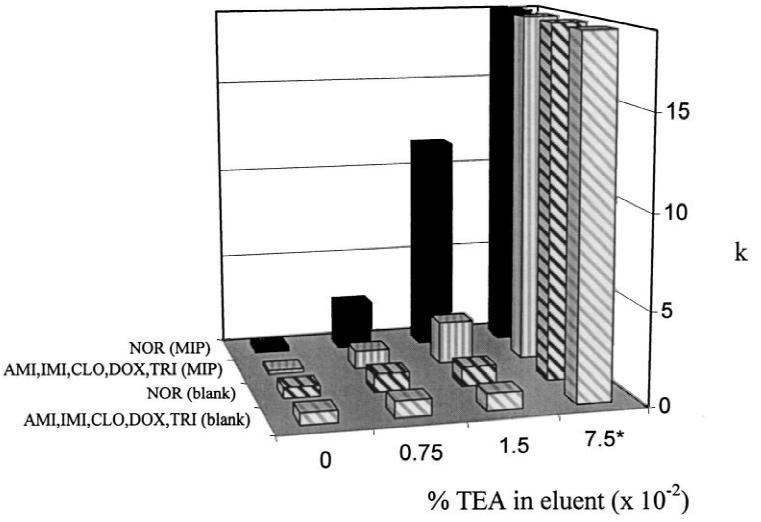

Fig. 7. Effect of TEA concentration on retention. TFA concentration held constant at 0.02% v/v. *k>15; exact value not determined.

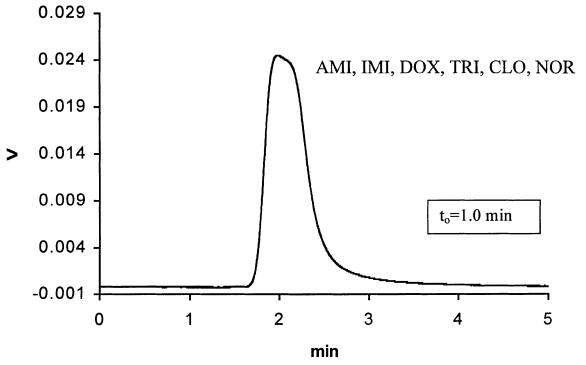

Fig. 8. Effect of an aqueous modifier on molecular recognition (MIP capillary). Eluent: CH_3CN: 10 m*M* sodium acetate pH 3.0 (95:5) containing 0.02% TFA, 0.0075% TEA v/v (final volume). Sample: 0.5 mg/ml (each) AMI, IMI, DOX, TRI, CLO; 1.5 mg/ml NOR in CH_3CN; Pressure: 50 bar.

Incorporation of an aqueous component was next studied. The optimized ''control'' eluent was modified by the addition of a 10 m*M* sodium acetate buffer to yield the following: (95:5) v/v CH_3CN: 10 m*M* sodium acetate pH 3.0 containing 0.02% TFA, 0.0075% TEA v/v (final volume). From the resultant run of the test mixture, shown in Fig. 8, it is evident that the aqueous component disrupts molecular recognition. Retention of the TCA on the blank column was essentially unchanged relative to the control eluent, where only weak retention was observed.

Results obtained for the test mixture on an MIP prepared without methacrylic acid are presented in Table 1. These data show that very little retention and no molecular recognition were obtained with the control eluent. From these data it could be argued that an ionic interaction between a protonated amine on an analyte and dissociated methacrylic acid moiety on the MIP is important in the recognition process. This is unlikely, however, in view of the fact that selective retention of NOR increases upon addition of TEA to the eluent. If ionic interactions were dominant, one would expect that TEA would compete for the ionized sites on the polymer, resulting in an overall decrease in selectivity. Indeed, addition of TEA to a (nonaqueous) CH_3CN eluent has been shown to decrease retention and selectivity in separations of diaminonapthalene isomers on methacrylic acid-based MIPs [19]. Such behavior is consistent with an ion-exchange model [21], developed to describe retention on MIPs in the presence of aqueous/organic eluents. This behavior was not observed in the present study. Instead, the addition of TEA through the range studied resulted in an increase in selective retention (Fig. 7). Furthermore, in the presence of ionic interactions between the pendant amine and the polymer, a correlation might be expected between selection index values and analyte basicities. Such a correlation, however, was not observed. Consider the following six TCA test probes ranked in order of decreasing pK_a [22]: PRO > MAP > DES > NOR > IMI > CLO > DOX. Selection index values, however, rank as follows: NOR>PRO>DES>MAP>CLO>DOX>IMI. No apparent correlation is found. It must be noted, however, that these pK_a values were determined in aqueous solution and the actual values may be expected to vary considerably in the nonaqueous conditions employed here. Nevertheless, these data are useful in providing a measure of relative base strengths.

Table 1
Retention data for NOR and selected TCAs on an MIP sorbent prepared without methacrylic acid[a]

TCA	*k*
NOR	0.19
AMI	0.18
CLO	0.19
DOX	0.18
IMI	0.18
TRI	0.18

[a] Eluent: 0.02% TFA v/v, 0.0075% TEA in CH_3CN.

These results suggest that a critical element of molecular recognition on this MIP is hydrogen bonding between the pendant amine of the TCAs and a methacrylic acid moiety on the polymer. The interaction is apparently more favorable at the less sterically hindered secondary amine group. This hypothesis is supported by the disruption of molecular recognition observed upon addition of a potent hydrogen bonding agent (e.g. water, TFA) to the eluent. The nonspecific component of retention on these polymers was disrupted by the addition of TFA. It is likely, then, that hydrogen bonding also plays a role in this nonspecific sorption. With MIP sorbents, it is generally desirable to eliminate or at least minimize any nonspecific retention. In this

work, attenuation of nonspecific retention was accomplished by judicious use of TFA and TEA as mobile phase modifiers. The precise role of these modifiers is complex and probably involves multiple effects that include influence on the charge states of the polymer acid groups and analytes as well as providing hydrogen bonding competition. In this study, the net effect of the modifiers is to disrupt of nonspecific sorption while maintaining to an acceptable degree the interaction(s) responsible for molecular recognition. Eluents of similar composition (i.e. acid and base modifiers in an anhydrous polar organic solvent such as CH_3CN) have been employed in chiral HPLC with cyclodextrin stationary phases [23], where they are thought to affect the interaction of polar functional groups such as amines with the hydroxyl groups on the cyclodextrin. Additional mechanistic studies on MIPs are needed to more fully explore the role of these modifiers under these conditions.

4. Conclusion

The goal of this study was to prepare an MIP using one of a group of structural analogs as a template and subsequently to screen this group of analogs against the MIP. The primary purpose of the screening analysis was to determine if certain compounds in the ''library'' would interact more favorably with the MIP relative to others and if so, to evaluate any observed differences in terms of the structures of the test probes. The selection index data provided a quantitative measure of the ''goodness of fit'' of each library compound into the NOR binding pocket and revealed that some of the test probes did in fact exhibit better ''fit'' than others. The results of the screening analysis also demonstrated the ability of the MIP to discriminate against structurally dissimilar compounds.

The ''best fit'' compounds in the library were those that possessed both the tricyclic structure and a pendant secondary amine group-structural features shared by the template molecule. Tricyclic compounds with a tertiary amine exhibited an intermediate degree of interaction with the MIP while the most structurally dissimilar species exhibited the least. The experimental data suggest that hydrogen bond formation between the test compounds and the polymer is important in molecular recognition. In general, multiple types of intermolecular forces (e.g. hydrogen bonding, $\pi-\pi$ interactions, etc.) acting in concert can serve to maximize molecular recognition in noncovalent MIPs.

Comparison of MIP screening data with that obtained by bioassay or affinity chromatography, while beyond the scope of this paper, will be the subject of future research. In principle, the MIP-based technique could be useful as a preliminary step in screening libraries generated by combinatorial techniques. This approach has the advantages that MIPs are inexpensive and relatively simple to produce, especially important when the target receptor(s) are difficult or impossible to obtain, and are stable in a broad range of chemical environments. Utilizing MIPs for an initial screening step could serve to identify those compounds in the library worthy of immediate study by bioassay techniques, thereby enabling a limited supply of biological material to be conserved and used to screen a more highly focused group of compounds.

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