MOLECULAR IMPRINTING
Research Article • DOI: 10.2478/molim-2012-0004 • MOLIM • 2013 • 27–34

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Molecularly Imprinted Polymer Receptors for Nicotine Recognition in Biological systems

Abstract
Molecular imprinting of nicotine and related carcinogenic chemicals in itaconic acid – ethylene glycol dimethacrylate copolymer is described. Molecularly imprinted polymers (MIPs) are made to contain binding sites capable of recognizing nicotine; thus the fingerprint of the nicotine created in the polymer allows it to serve as an ideal molecular recognition element. We demonstrate that the imprinted polymers show high selective binding affinity in biological buffers. This is a previously un-described initiative for molecular imprinting, since the binding occurs under conditions relevant to biological systems. Due to effect of molecular imprinting nanocavities with size 24±5 nm were formed and these nicotine receptor sites were distributed homogeneously in the nicotine imprinted polymer. The nicotine receptors showed highly selective to nicotine with Kd values as low as 10^{-5}M, and the levels of selectivity similar to those of natural molecules - acetylcholine esterase (AChE). The recognition properties of the polymer receptor were analyzed using ultra-violet spectroscopy, computer simulations and adsorption assay. Importantly, the receptors were effective in wide pH range (6.8-8.2) while the natural nicotine receptors showed high binding only at pH 7.6. The high specificity and stability of artificial receptors rendered them promising alternatives to enzymes, antibodies, and other natural receptors useful in biomedical assays, sensors and drug development.

Keywords
Molecular imprinting • Molecular modeling • Nicotine receptors • Polymer receptors • Selective binding

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Received 25 July 2012
Accepted 16 December 2012

1. Introduction

The design and synthesis of artificial receptor systems capable of binding a target molecule with similar affinity and specificity to antibodies has been a long-term goal of biomedical sciences and biotechnology. The mimicking of antibodies function primarily requires selective binding of antigen to a natural or synthetic antibody. The binding utilizes the exquisite recognition properties of an antibody for the antigen, in which the antigen fits precisely into the antibody's binding site, whereas even structurally related compounds are excluded from the site. However, antibodies exhibit characteristics that limit their applications. For examples, bio-macromolecules such as proteins are large (M_r≈150 K) complex molecules that need to be stored carefully [1]. As antibodies are produced by living cells it is sometimes difficult to control their quality. Furthermore, it is difficult to raise antibodies against some bio-macromolecules. The preparation of artificial receptors for bioactive molecules such as carcinogens, endocrine disruptors, growth inhibitors and enzymes is an important area of research. In particular it is a need of the day to develop artificial receptors capable of facilitating the screening of peptide mixtures or assist in the evaluation of peptidomimetics that can be used to either enhance or inhibit receptor responses [2].

It is known that when monomers are polymerized in the presence of the molecular, collective weak interactions between the monomers and the target formed during polymerization produce populations of complementary binding sites in the resulting polymer. This molecular imprinting approach has been extended from bulk materials to nanoparticles and dendrimers [3]. Molecular imprinting involves formation of a recognition site or binding pocket at the surface or inside a polymer matrix (e.g., thin film or particles) by incorporating functional monomers with side groups that can interact with the target molecule. After polymerization the template molecule is removed from the polymeric matrix, leaving recognition sites that are complementary in shape, size, and spatial distribution of functional groups to the target [4]. Molecularly imprinted polymers (MIPs) are certainly very different from the antibodies; they are large, rigid and insoluble, whereas antibodies are

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relatively small, flexible, and soluble. Nevertheless the polymers were developed successfully that exhibit selective recognition of imprinted proteins (albumin, immunoglobulin G, lysozyme, RNAase, and streptavidine) through H-bonding with the target [5]. Matsui and Takeuchi (1997) prepared nicotine imprinted polymer using 2-(trifluoromethyl) acrylic acid (TFMAA) as a functional monomer which showed high binding capacity and selectivity factors (28.4 and 2.4, respectively) in organic solutions [6]. Nicotine selective MIP was used as solid phase extraction (SPE) material to improve the sensitivity of liquid chromatographic determination of nicotine in biomedical analysis [7]. A detection limit 1.8μg mL^{-1} and a linear dynamic range up to 1000 μg mL^{-1} were obtained using this nicotine SPE material. Sevenson et al., (2004) conducted ^{1}H nuclear magnetic resonance (H_1 NMR) studies on molecular imprinting of (-)-nicotine and determined the dissociation constants for complexation of templates by functional monomer analogues and acetic acid [8]. However the above cited references were successful examples of nicotine imprinting for the non-aqueous systems. It is quite important to study nicotine/MIP models systems in aqueous conditions, where the hydrophilic surface of the MIPs leads to substantial non-specific binding of the template nicotine, as well as non-specific binding of non-related and non-polar structures. The binding affinity of imprints in aqueous buffers is very relevant to biological systems application.

In this study, a mimic of nicotine receptor has been prepared using bio-compatible polymer precursors for nicotine selective binding in biological buffers. The imprint designed has shown excellent potential comparable with natural receptor - acetylcholine esterase (AChE) for recognition of nicotine in biological buffers. Further, the binding mechanism between nicotine and the artificial receptor has been studied using ultra-violet spectroscopy and computer aided molecular simulations. It was found that the strong nicotine-imprint interactions require the use of apolar organic solvents during imprinting process, which is a disadvantage for biological systems application. Hence, this issue was addressed and some basic procedures for the optimization of nicotine binding to artificial receptors in biological buffer were developed. A comparison is made among the binding properties of nicotine imprints and AChE, and structural and functional analogues of nicotine. The results reported in this study provide basis for development of nicotine imprints useful in clinical analysis nicotine in blood and serum and design of nicotine receptors for nicotine addicted patients treatment.

2. Experimental Procedure

2.1. Chemicals and Reagents

The template nicotine and functional monomer, itaconic acid and cross-linking monomer ethylene glycol dimethacrylate (EGDMA) were obtained from Acros Organics (Geel, Belgium). Methacrylic acid (MAA), Trimethylolpropane trimethacrylate (TRIM), 1,10-azobisisobutyronitrile (AIBN), solvents of HPLC grade (acetonitrile, methanol and acetic acid), and the buffer salts, sodium citrate, sodium carbonate and sodium phosphate were procured from Merck (Darmstadt, Germany). AIBN was re-crystallized from methanol before use. Bi-functional monomer *N, O* – bis methacrylol ethanolamine (NOBE) was synthesized using a previously reported procedure [9]. Acetylcholine esterase (AChE) was purchased from Hycult Biotech (PB Unden, The Netherlands). The structural analogues myosmine and cotinine were purchased from Chemsky International Ltd. (Shanghai, China) and the functional analogues – epinephrine and physostignine were procured from Aldrich (St. Louis MO, USA). The molecular structures of chemicals used in the study are given as support information (SI Figure 1). Freshly prepared Millipore de-ionized water (Milli-Q3 RO/MilliQ2 model Millipore, Singapore) was used in the preparation of biological buffers and all binding and cross-selectivity experiments.

2.2. Preparation of MIPs

The imprinted polymer receptors of nicotine were prepared with three different compositions. The first receptor (MIP-1) was synthesized using 1.62 mg (1.0μmol) of nicotine as a template, 5.3 mg (4.0 μmol) of functional monomer itaconic acid (IA), 0.39 g of cross-linking monomer EGDMA (20 μmol), 0.5 g of acetonitrile (ACN), and 5 mg of 1,1'-azobis(isobutyronitrile). The composition of second polymer receptor (MIP-2) included functional monomer methacrylic acid (MAA) 8.6 mg (1.0μmol) and cross-linking monomer TRIMA 1.34 g (20 μmol). The third polymer receptor (MIP-3) was prepared with functional monomer (IA), 5.3 mg (4 μmol) and cross-linking monomer NOBE , 1.97 g (20 μmol). The solvent and initiator use during polymerization was the same for all polymers (see composition of three polymers in Table 1). Initially, the polymer mixtures were saturated with nitrogen and then glass tubes were sealed before polymerization was initiated by UV irradiation (352 nm) at 4°C in a photo-reactor.

The polymerization was continued for 16 h. After synthesis, all polymers were ground and sieved to obtain the fractions with size 30-100 μm separately. The template removal from the

Table 1. Composition of polymer receptors

Polymer receptor	Functional monomer	Cross-linking monomer	Initiator	Solvent
MIP-1	IA (1.6 mmol)	EGDMA (32 mmol)	AIBN (50 mg)	DCM (5 mL)
MIP-2	MAA (1.6 mmol)	TRIM (32 mmol)	AIBN (50 mg)	DCM (5 mL)
MIP-3	IA (1.6 mmol)	NOBE (32 mmol)	AIBN (50 mg)	DCM (5 mL)

IA, itaconic acid; MAA, methacrylic acid; EGDMA, ethylene glycol dimethacrylate; TRIM, Trimethylpropane trimethcarylate; NOBE, N-O bismethacryloyl ethanolamine; AIBN, 2,2-azobis isobutyronitrile; DCM, dichloromethane

polymer matrix is very important step in molecular imprinting and to ensure that no template remained in the polymer matrix, the polymer was thoroughly washed in Soxhlet using methanol followed by regular changes of acidic methanol, acidic water, water and methanol, and then dried in oven at 20°C. Washing continued for 2 days until the concentration of the template in the eluent measured using HPLC-MS became below quantification level (2 ng mL^{-1}).

2.3. Surface properties of synthetic receptors

The surface morphology of the imprinted polymer receptors was characterized using JOEL JSM-5200 scanning electron microscope, operated at a voltage of 15 kV, a current of 10^{-12} to 10^{-9} A and 10 000 x magnification. A high resolution transmission electron microscope (HRTEM, JOEL 3010) with a ultra-high resolution pole piece was used to detect tailor-made nanopores. The polymer samples were gold sputtered at 15 kV for 200 s using JOEL, JFC-1000E ion sputter fine coat under vacuum (~10^{-3} Torr). The porosity and surface area of the polymers was determined by nitrogen adsorption/desorption analysis using nitrogen surface area analyzer (JOEL JSM 6400).

2.4. HPLC-MS analysis of nicotine and its analogues

To quantify the binding of nicotine and its analogues by polymer receptor, HPLC-MS was used. HPLC separation was conducted using Waters 2975 HPLC system equipped with µBondapak C_{18} column (10µm, 3.9 x 300 mm, Waters). The column was washed with methanol during initial conditioning of the column. Mobile phase: (A) water and (B) methanol in a binary system with 0.1% formic acid as an additive. The elution gradient: linear gradient from 50% methanol/0.1% formic acid to 100% B from 0 to 10 min then for 5 min solution B (100% methanol/0.1% formic acid), and return to 50% methanol at 20 min (total time 10 min). The flow rate was 0.2µl min^{-1}, the injection volume was 10 µL, and the column temperature was +20°C. The 80.1 m/z fragment is common to both tested isomers of nicotine, and it was detected in positive ion mode using a mass spectrometer (Micromass Quantro and ESI interface). The MS parameters set during analysis were the following; desolvation gas 850 L h^{-1}, cone gas 50 L h^{-1}, desolvation temperature +350°C, collision energy 20 V and photomultiplier 650 V. The nicotine content was identified and quantified by comparing peak areas in the chromatogram with a calibration curve nicotine standards. Peak areas of standards were plotted against concentration using linear regression. The quantification of nicotine and other analogues was conducted using GraphiCard software. The co-relation coefficient r^2 was found to be 0.991 which indicates the precision of chromatograph analysis.

2.5. Binding studies

Polymer (10 mg) was weighed into a 5 mL capacity scintillation vials, and 2 mL of nicotine solution (1 µgmL^{-1}, in 20 mM phosphate buffer, pH 7.8) was added. The vials were closed and shaken for 3 h at 20°C to be certain of attaining equilibrium. Polymer suspensions were filtered through syringe filters with pore diameter 0.2µm glass fiber filters attached to the barrel of a disposable 5 mL syringe into sample vials which were sealed with septum caps. The concentration of free nicotine was determined by HPLC-MS, and the amount of bound nicotine was calculated by subtraction. The binding of nicotine at 20°C in 20 mM in phosphate buffer to polymers and AChE was determined at 0.1,5,10 and 20 µg mL^{-1} concentration of nicotine in duplicate experiments as described above. The binding selectivity of polymers and natural receptor (AChE) was determined by following above procedure with mixture of nicotine, cotinine, myosmine, epinephrine, and physostigmine in 1µg mL^{-1} concentrations in aqueous buffer in place of nicotine. The dependence of binding on pH was determined as described above expect that the pH's of stock solutions of nicotine in 20 µM phosphate buffer solution were adjusted by the addition of NaOH/HCl solution before pipetting onto the polymer. Individual HPLC calibration curves were determined for each pH. All experiments were conducted in duplicate. The binding performance of polymer receptors and natural receptor (AChE) was compared using the experimental data.

2.6. Computer simulations and UV spectroscopy for nicotine-itaconic acid complex

The typical procedure for the UV titration experiments of nicotine with functional monomers (IA, MAA and NOBE) was the following: stock solutions of the template nicotine (10^{-5} M) and IA (10^{-2} M) were prepared in ACN. A 5 mL aliquot of the stock solution of the template was transferred to six glass tubes, and different aliquot of the stock solution of IA and ACN were added to have molar ratios of IA to the template from 0 to 2 in a total volume of 5 mL. Total concentration of nicotine and IA was kept constant and molar fraction of IA was varied at the total volume of 5 mL. UV spectra were recorded and the shift of the absorbance intensity of the template was followed. The change in UV absorbance shift was used to calculate the complex concentration. The complex concentration was plotted against the molar fractions of MAA. The stoichiometry of the complex between the nicotine and the functional monomer (IA) was determined by the Job's plot. The similar procedure was followed to understand MAA -nicotine and NOBE- nicotine complex formation in polymerization reaction medium (acetonitrile).

Complimentary to UV spectral studies, computer simulations were performed to determine the nature of interactions between functional monomers and nicotine in pre-polymerization systems. For this purpose, Dell ™ Precision T7500 Workstation was used to run the software Gaussian 4.21 (Gaussian, Inc. Willingford CT, USA). Initially, 2-D chemical structures of the functional monomers (IA, MAA and NOBE), template nicotine, and cross-linking monomers (EGDMA and TRIM) were prepared, then using molecular builder option 2 D structures were converted to 3-D structures. The geometric optimization was carried out on the semi-empirical (SE) quantum mechanical approach (PM3 method) to obtain minimum energy structures. The molecular structures were optimized using the Polak – Ribiere algorithm until the root mean square gradient was 0.01; which

is the acceptable in molecules optimization. Then, the binding energy of monomer-template complexes were computed on Haltree-Fock (HF) method with 6-31G basis set. The minimum binding energies between the optimized conformations of 1:1 ratio of template–monomer complexes was computed. In the similar lines, optimum F/M ratio was also determined. Using conformation optimization the most stable template–monomer complexes combinatorially were screened in solvent (acetonitrile) based on interaction energy scores.

3. Results and Discussion

One of the objectives of this study is to prepare a series of polymers to be imprinted with nicotine compatible with biological systems. The assembly of functional groups around the template in the solution, that is, formation of prepolymer complex, is the first step in molecular imprinting. In previous research papers, the nicotine imprinted polymers were tested for selective binding of nicotine in organic solutions. Nicotine has two amine functional groups, which are capable of multiple interactions that must be accounted for when modeling the system, complicating the overall understanding of the model. Initially, we used computer simulations and UV spectroscopy in studying a system with functional monomer itaconic acid, methacrylic acid and NOBE. A series of simulations were performed to obtain interaction energy score of functional monomers and nicotine based on *ab initio* calculations. The simulations demonstrated that the pyridine and pyrrolidine structures of nicotine are the most accessible to IA. Interestingly, binding of IA to the nitrogen atom in the pyridine and pyrrolidine of nicotine was clearly the most dominant interaction observed in the ensemble of IA-nicotine complexes formed (Figure 1). The strengths of these complexes were dependent on the relative orientation of IA and nicotine. Similar interactions were observed with MAA-nicotine and NOBE-nicotine complexes.

The binding energies of nicotine with functional monomers follow the decreasing order: NOBE (-8.746 a.u) > IA (-7.384 a.u) > MAA (-6.481 a.u). NOBE also showed stronger bonding with nicotine compared with IA and MAA nicotine interactions. The existence of N-H group of NOBE orients functional groups in a

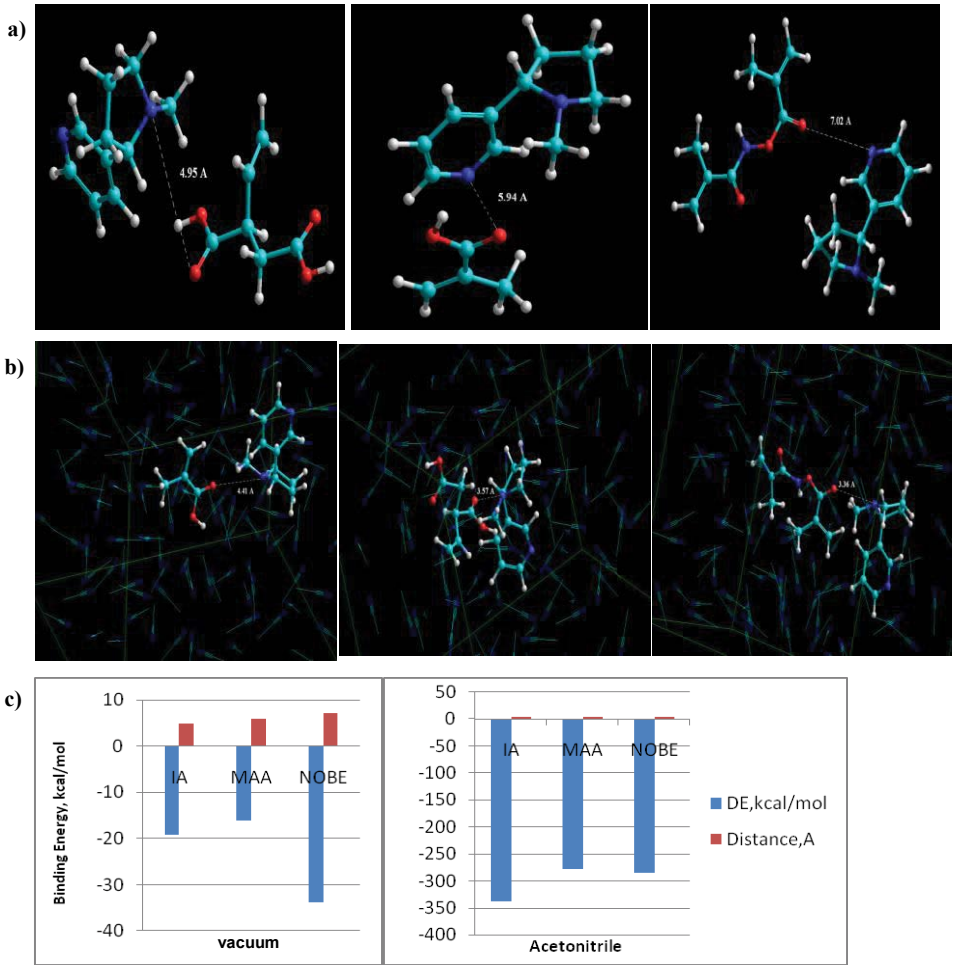

Figure 1. A view of simulated binding interactions between nicotine and functional monomers (IA, MAA and NOBE) in (a) vacuum and (b) polymerization reaction medium acetonitrile (c) Relation between nicotine and functional monomers interaction energy and binding distance in vacuum and acetonitrile.

binding cavity with better fit than IA and MAA monomers. These theoretical investigations provide information about nature of interactions between nicotine and functional monomers in acetonitrile solvent and also ranks suitable functional monomers based on energy scores computed for functional monomers for the template nicotine.

Experimentally, the presence and stoichiometry of pre-polymer complexes were determined by Job's plots of functional monomers (IA, MAA and NOBE) with nicotine (Figure 2). Solutions of template and functional monomer were formulated in such a way that the combined concentration of IA and template nicotine (C_0) were kept at constant molarity but the relative molar fractions of IA and the template were varied in a compensatory manner to establish the Job's plot. The complex concentration was determined by measuring change in UV absorbance intensity. Different values of *C0* were used to plot the complex concentration versus the molar fractions of IA at concentrations above the necessary threshold value. The Job plot for nicotine (Figure 2a) shows that a maximal complex was formed at a 0.8 molar fraction of IA for all values of *C0.* This result provides evidence for the formation of 1:4 complex between the functional monomer IA and the template nicotine in acetonitrile, while the template nicotine formed 1:1 complex with the functional monomers MAA and NOBE (Figure 2a). Furthermore, the association between IA and nicotine was determined using variations in UV spectral intensity at 264 nm (Figure 2b) and it was concluded that these results complimentary to the theoretical studies.

The imprinted polymer receptors for nicotine were prepared by considering above F/M molar ratio in acetonitrile in the presence of cross-linker (EGDMA) and small amount of initiator (AIBN). The nicotine was removed from cavities that were molecular size dependent. Nitrogen sorption measurements, performed with synthetic receptor (BET surface area 413 $m^2\ g^{-1}$; cumulative pore volume 0.604 cm^3g^{-1}) displayed a type IV isotherm with large adsorption capacities of 0.42-0.3 $cm^3\ g^{-1}$. About 90% of the total surface area of the synthetic receptor (390 $m^2\ g^{-1}$) showed presence of cavities with diameter 19-29 nm. The control polymer receptors on the other hand, showed lower surface area and lower pore volume (BET, 98.5 m^2g^{-1}; total pore volume, 0.023 $cm^3\ g^{-1}$). The surface properties of nicotine imprinted polymers are given in Table 2. Scanning micrograph of synthetic receptors showed existence of nanopores in the polymer matrix (Figure 3a-e). The narrow pore size distribution and lower average pore diameter derived from the nitrogen adsorption isotherm plots indicate that a homogeneous continuum of cavities exists in the polymer (Figure 3b). It would be reasonable to assume that the size of the cavities formed in the polymer had an important role in selective binding of nicotine.

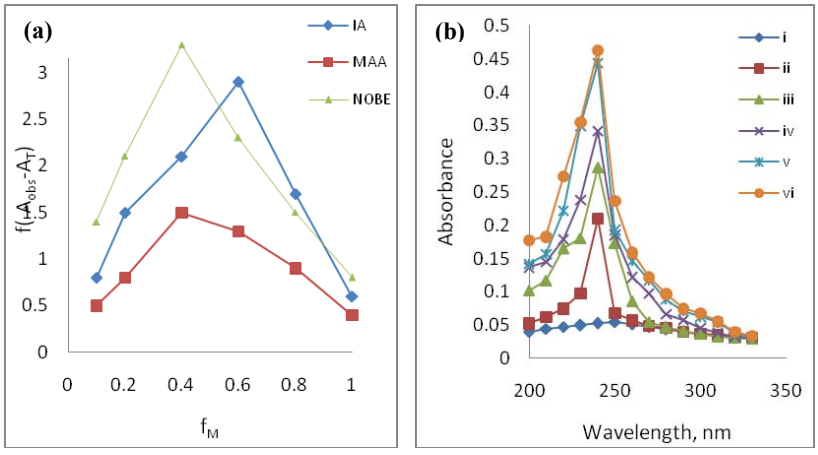

Figure 2. (a) Job's plots for the association between functional monomers (IA, MAA and NOBE) and nicotine; where fM is the molar fraction of functional monomer and f(Aobs-AT) is the change UV spectral intensity due to the complex concentration between functional monomer and nicotine at different concentrations (b) UV spectra of titrations between functional monomer IA (10-2M) and different equivalents of nicotine (10-5M) i.e., (i) 5 eq (ii) 3 eq (iii) 2 eq (iv) 1 eq (v) 0.5 eq and (vi) 0 eq.

Table 2. Surface properties of polymer receptors

Polymer receptor	BET Surface area, m^2g^{-1}	Cumulative pore volume, cm^3g^{-1}	Average pore diameter, Å
MIP-1	413	0.604	27
MIP-2	361	0.487	39
MIP-3	396	0.528	31
AChE	278	0.325	40

The values reported in the table are mean of three instrumental measurements.

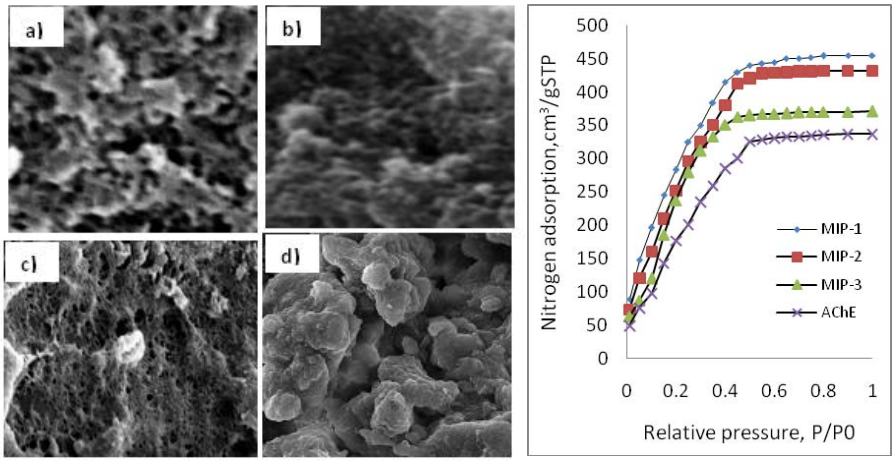

Figure 3. *Scanning electron micrographs of MIP-1 (a), MIP-2 (b) MIP-3 (c), AChE (d) and (e) nitrogen adsorption isotherms of polymer and natural receptors.*

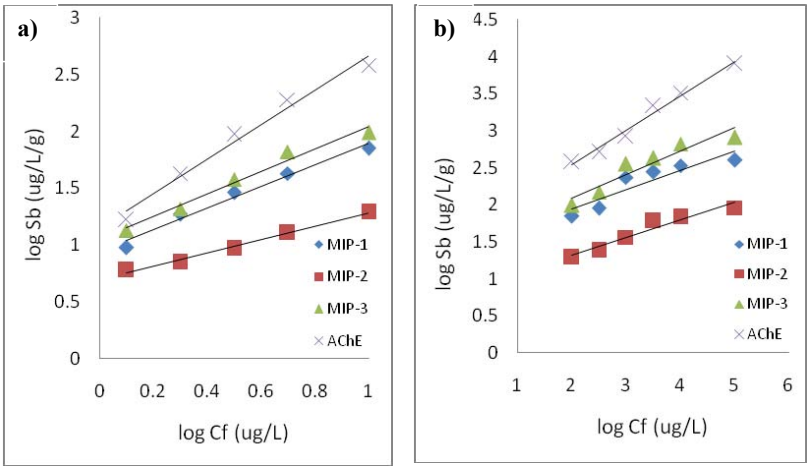

Figure 4. *Freundlich isotherms for (a) Region – I nicotine with 0.1, 0.3, 0.5, 0.8, and 1 ug/L and (b) Region – II with nicotine concentration between 2 and 5 ug/L.*

Three types of polymers were prepared using three different functional monomers (the composition of each polymer is listed in Table 1), and then the template nicotine was extracted from polymer with methanol. Polymer was ground and fraction with size 50-70 µm collected.

The molecular recognition properties of these imprinted polymers were evaluated by batch rebinding studies.

For the batch rebinding experiments, a constant volume of a solution with various concentrations of nicotine was added to a fixed amount of the polymer and shaken for 3 h allowing equilibrium to be reached. The amount of free nicotine (Cf, µg L^{-1}) was determined by HPLC-MS of the supernatant, and the amount of bound nicotine (Cb, µg L^{-1}) was determined by subtracting the amount of free nicotine from the total amount of nicotine (i.e., *Cb=CT - Cf)* added to the polymer solution. A plot of *Cb* versus *Cf* is often used to graph the binding isotherms for imprinted polymers. Because the polymers are solid, *Cb* is converted to *Sb* which has dimensions in micrograms of substrate per gram of polymer (µg/g). Therefore, the binding isotherms for the polymers are plotted as *Sb* versus *Cf.* As previously mentioned, it has been shown that MIP isotherms fit well to the Freundlich equation [10]. The Freundlich equation is an exponential model that is best represented in a log *Sb* versus log *Cf* format, which is used to ploh the isotherms presented in Figure 4. For each of the three polymers, nine isotherms are obtained for template nicotine and other non-templated nicotine structural analogues (myosmine and cotinine) each of the MIP formulation. In the similar way isotherms of non-imprinted polymer were also evaluated. The isotherms for the polymers imprinted with nicotine are shown in Figure 4; isotherms for the MIPs to the other templates can be found in the Supporting Information (SI Fig.2). All nine isotherms are divided into two different portions, the region I for

those with 0.1 to 1 μg L^{-1} and the region II for those with 2 to 10 μg L^{-1} of nicotine. Region I illustrates the upward movement (indicating increasing binding ability) of the isotherms from 0.1 to 1 μg L^{-1} template (Figure 4a), whereas region II illustrates a downward movement (indicating decreasing binding ability) from 2 to 10 μg L^{-1} template. In addition to trends in binding ability, Figure 4 also illustrates good linearity, supporting the Freundlich model of binding isotherm. The similar binding analysis was carried out using AChE as a receptor (Figure 4b). The specific binding displayed by the MIP-3 can be attributed to the favourable and stronger interactions existing between the NOBE monomer and the nicotine template. The MIP-3 have better binding selectivity for nicotine as the NOBE contain free carboxyl groups responsible for formation of hydrogen bonding with the amide moiety of NOBE.

The selectivity of the binding sites was further studied by determining uptake of structural analogues (myosmine and cotinine) by the MIPs and AChE. Additionally, epinehprine and physostigmine were also used for evaluating MIPs selective binding (Table 3). These two endocrine disruptors are found in pharmaceutical wastes along with nicotine during environmental monitoring of drugs and pharmaceutical industrial effluents. While epinephrine and physostigmine showed quite low binding, the nicotine pyridine and pyrrolidine moieties fit precisely into well defined receptor site. The polymer receptor (NOBE) was exceedingly specific even compared with AChE. This could be explained by the fact that in evolutionary terms the AChE was not required to discriminate between these compounds, while the synthetic receptors are tailored to recognize a specific template. Since physostigmine and epinephrine showed very low binding, it can be postulated that pyridine and pyrrolidine moieties of nicotine itself contribute to specific recognition. The two other MIPs prepared using functional monomers – IA (MIP-1) and MAA (MIP-2) - displayed almost identical binding profiles with 20 to 30% lower binding capacity compared with MIP-3. Thus it is possible to conclude that the specific binding of the nicotine takes place mainly through hydrogen bond interaction between the NOBE units and pyridine and pyrrolidine nitrogen atom of the nicotine. From these selective binding experiments it could be concluded that the MIPs show reasonably good binding affinity and specificity in biological buffers. It is an important finding, since the binding can now be performed under conditions compatible with biological systems. The non-covalent interactions, mainly hydrogen and electrostatic bonds that are employed for imprinting are highly dependent on the medium used for polymerization.

To create efficient binding sites in MIPs, the polymerization reaction was performed in apolar solvent-ACN. The performance of MIPs for recognizing nicotine in aqueous solutions has been as good as performance of previously designed MIPs examined in organic solvents [11]. However, the nicotine binding by polymer receptors in water is 30-40% lower compared with organic solvents (the reverse trend was noticed in the case of natural receptor-AChE) (Figure 5a). This could be explained as the water molecules strongly interfere with polar interactions, including hydrogen bonding, whereas hydrophobic effects are very strong in water.

Table 3. Selective binding of nicotine and its analogues to the nicotine imprinted polymers and AChE.

S.No	Nicotine and its analogues	MIP-1	MIP-2	MIP-3	AChE
1	Nicotine	100	100	100	100
2	Myosine	28	33	15	22
3	Cotinine	24	35	12	15
4	Epinephrine	17	21	6	11
5	Physostigmine	16	18	5	12

Selective binding expressed as the molar ratio (in percent) of nicotine and its analogues in biological buffer

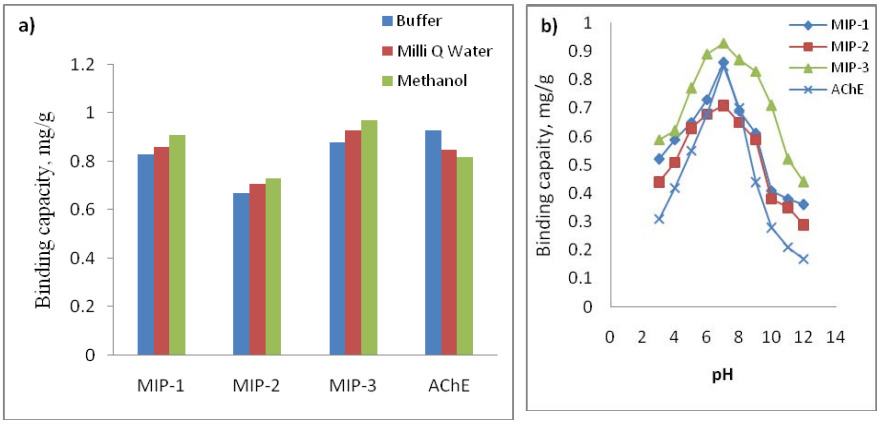

Figure 5. (a) Effect of medium and (b) pH on nicotine binding capacity of polymer and natural receptors.

The pH profile for nicotine binding to MIPs revealed that the MIP's selectivity is a function of pH (Figure 5b). It has been shown previously for MIPs exhibiting an underlying ion-exchange mechanism that the optimum binding affinity and selectivity occur approximately at a pH that maximizes the reciprocal charges on the polymer and nicotine. The optimum selectivity is observed at pH values < 6.8 for all polymers and is lost at higher pH values. As pH values are lowered, there will be an increase in the amount of protonated pyrrolidine and pyridinium groups of nicotine which favours selective recognition by polymer receptors. The optimum results are seen in the pH range where the positively charged pyrrolidine and pyridinium group is fully protonated (<pH 7.0). While the natural receptor AChE showed an excellent selective biding at pH 7.0 and other pH values there is a drastic reduction (40 to 60%) in nicotinebinding. The pH experiments conclude that the MIPs work in wide pH range 6.5 to 8.9 while natural receptor are highly sensitive to pH of the binding medium i.e., biological buffers.

4. Conclusions

The results reported in this study demonstrate the ability to use chemically prepared synthetic receptor with pre-selected specificity as receptor-binding-site mimics. It is important to note however that the natural and synthetic receptors are different species which were not designed (by evolution or by rational selection) to perform the same functions. Thus it would not be wise to use MIPs as direct alternative to natural receptors in drug screening. However, MIPs would constitute perfect recognition elements for assays and sensors for particular niche applications. Besides, MIPs can be very useful objects for the fundamental studies of molecular recognition. Furthermore, the high binding affinity and selectivity obtained in biological buffers could be way forward to prepare MIP based artificial receptors for isolation and separation of water-soluble biologically related compounds and particularly development treatment for nicotine addicted patients.

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