THE INFLUENCE OF ALIPHATIC SIDE CHAIN OF ANACARDIC ACID ON MOLECULAR RECOGNITION PROPERTIES OF IMPRINTED POLYMERS

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ABSTRACT

The objective of this work was to determine the influence of the aliphatic side chain of anacardic acid on molecular recognition properties of imprinted polymers made from anacardanyl methacrylate (AnMcr). Salicylic methacrylate (SaMcr), a structural analog of AnMcr, was synthesized and used as a functional monomer to prepare imprinted polymers for comparison with AnMcr-based polymers. Using divinylbenzene (DVB) as a cross linker and racemic propranolol as a model template, irregular monolithic particles of poly(SaMcr-co-DVB)m were synthesized in toluene, and spherical beads of poly(SaMcr-co-DVB)b and poly(AnMcr-co-DVB)b were synthesized in acetonitrile by precipitation polymerization. Although imprinted irregular monolithic particles, poly(SaMcr-co-DVB)m, tested in toluene containing 0.5% acetic acid, displayed relatively low specific propranolol binding, they showed high molecular selectivity. For the spherical beads tested in acetonitrile, both imprinted poly(AnMcr-co-DVB)b and poly(SaMcr-co-DVB)b showed obvious specific propranolol binding despite the use of polar organic solvent during imprinting. Imprinted poly(AnMcr-co-DVB)b showed higher molecular selectivity than imprinted poly(SaMcr-co-DVB)b. Interestingly, the presence of the aliphatic side chain in AnMcr resulted in more uniform imprinted beads as compared to particle agglomerates obtained from SaMcr in the presence of propranolol template. Therefore, the aliphatic side chain of anacardic acid improves both molecular recognition of imprinted polymers as well as the formation of uniform imprinted spherical beads.

KEYWORDS: Molecular imprinting, anacardanyl methacrylate, salicylic methacrylate, propranolol, precipitation polymerization

INTRODUCTION

In non-covalent molecular imprinting, template-functional monomer interactions such as hydrogen bonding, ionic pairing, van der Waals forces and hydrophobic effects are crucial molecular events that at least one of them must be involved to generate intended molecularly imprinted polymers (MIPs) with high affinity and specificity. These molecular interactions are equally essential for MIP applications in different analytical fields (Zhang and Mosbach 2006, Mosbach and Haupt 1998, Caro *et al*. 2004, Parmpi and Kofinas 2004, Matsui *et al.* 1997). Practical conditions that tend to influence the stability of the complex formed between the template and the functional monomer, and between the template and the obtained imprinted sites influence the molecular recognition efficiency of a particular MIP. Polarity of the solvent in which the binding process is being performed and the functional groups available from the imprinted polymers (such as –COOH, –OH and $–NH_2$) are among the most critical factors (Piletsky *et al*. 1998,

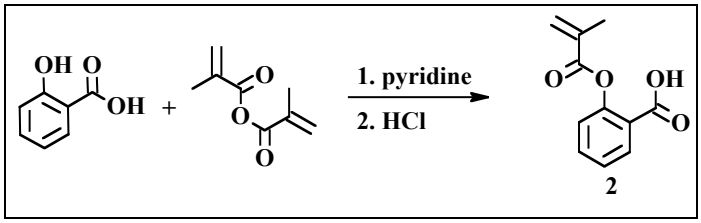
1999). Thus, optimization of parameters during preparation and application of MIPs has been of great research concern (Zhu *et al*. 2007, Wei *et al*. 2007).

In a recent effort to exploit natural byproduct to produce valuable affinity adsorbents, anacardic acid derived from cashew nut shells was used to prepare molecularly imprinted polymers (Philip *et al*. 2007). The study entailed blocking the phenol group by acrylation or methacrylation to suppress the formation of intramolecular hydrogen bonding within anacardic acid, so that the carboxyl group can be freed to form stable interaction with the propranolol template (Figure 1). Methacrylation of anacardic acid gave AnMcr (**1**), which was proved to be a good functional monomer for non-covalent imprinting in organic solvent (Philip *et al*. 2007). Despite the successful imprinting, the effect of the C_{15} alk(en)yl side chain of the functional monomer, AnMcr, remained unclear. Given the established chemical structure of anacardic acid and its close similarity to salicylic acid (Gedam and Sampathkumaran 1986), it was possible to design an investigation on the influence of the aliphatic side chain of anacardic acid on molecular recognition achievable with different imprinted polymers. This was done by comparing binding properties of different MIPs prepared using AnMcr and SaMcr (**2**) as functional monomers under otherwise identical conditions. On a second aspect we were also interested to see how the side chain of AnMcr can affect polymer morphology, more specifically for MIP beads prepared in acetonitrile using precipitation polymerization method.

MATERIALS AND METHODS

Chemicals. Acetone (99.0%, extra pure), n-hexane (over 85%), methanol (99.9%), chloroform (99.8%), toluene (99.8%) and acetonitrile (99.9%) were HPLC grade. These solvents were purchased from Fisher Scientific AB (Västra Frölunda, Sweden) and used without further purification. Diethyl ether (99.5%), hydrochloric acid (37-38%), anhydrous sodium sulphate (99.0%), pyridine (99.0%), potassium bromide (99.0%), aluminium oxide (90 active) and azobisisobutyronitrile (AIBN, 98%) were purchased from Merck (Darmstadt, Germany). Apart from azobisisobutyronitrile, which was re-crystallized from methanol before use, others were used as supplied. Methylacrylic anhydride (~94%) and acetic acid (99.8%) were used as obtained from Fluka (Buchs, Switzerland). Salicylic acid (99+%), chloroform-*d* (99.8 atom %D) and divinylbenzene (DVB, 80%, isomeric mixture) were purchased from Sigma-Aldrich (Chemie GmbH, Germany). DVB was passed through a column of aluminium oxide to remove the polymerization inhibitor. (*R*,*S*)-Propranolol hydrochloride (99%), (*S*)-propranolol hydrochloride (99%) and (*R*)-propranolol hydrochloride (99%) supplied by Fluka (Dorset, UK) were converted into free base form before use. ^{3}H-(*S*)-propranolol (specific activity 555 GBq mmol^{-1}, 66.7 mM solution in ethanol) was purchased from NEN Life Science Products Inc. (Boston, MA, USA), and scintillation liquid (Ecoscint A) was from National Diagnostics (Atlanta, GA, USA).

Synthesis of AnMcr and SaMcr. Synthesis and characterization of AnMcr (**1**) from anacardic acid was performed as reported elsewhere (Philip *et al*, 2007). SaMcr (**2**) was synthesized using a protocol described by Lübke *et al*. (1998), with minor modification (Scheme 1).



Scheme 1: Synthesis of SaMcr.

Salicylic acid (2.49 g, 18 mmol) was measured directly in to a reaction flask followed by addition of pyridine (9 mL). The mixture was cooled by dipping the reaction flask in ice water. Into a Falcon tube, pyridine (12 mL) and methylacrylic anhydride (5.6 mL, 36 mmol) were mixed, cooled and dropwise added to the reaction flask kept in ice water. The reaction was then left to proceed at room temperature. After 12 hrs, the mixture was cooled and added into excess of stirred ice-cold 3 M HCl. The product was extracted with diethyl ether. The organic phase was dried over anhydrous sodium sulphate, filtered, and concentrated under reduced pressure at 40 ºC by means of a rotary evaporator. The oily substance obtained was recrystallized for 5 times from n-hexane to give white, needle-like crystals (2.16 g, 58%). FT-IR (KBr): 3000-2530 cm^{-1} (alkyl C-H, mixed with carboxylic O-H), 1739-1691 cm^{-1} (carboxylic C=O), 1634-1596 cm^{-1} ($–CH_3$ and vinyl C=C). ^1H NMR (400 MHz, $CDCl_3$, d (ppm)): 8.10 (d, 1H, arom.), 7.61 (t, 1H, arom.), 7.33 (t, 1H, arom.), 7.15 (d, 1H, arom.) 6.31 (s, 1H, vinyl), 5.77 (s, 1H, vinyl), 2.05 (s, 3H, $-CH_3$).

Synthesis of molecularly imprinted polymers. Two types of imprinted polymers were prepared using SaMcr and AnMcr as functional monomers: (1) irregular monolithic particles obtained by pulverization of bulk polymers synthesized in toluene, and (2) polymer microspheres obtained by precipitation polymerization in acetonitrile. Synthesis of irregular poly(SaMcr-co-DVB)m particles was as follows: In two different screw capped borosilicate test tubes, SaMcr (0.31 g, 1.5 mmol) was dissolved in 1.5 mL of toluene. To one of the tubes racemic propranolol (**3**) (0.0650 g, 0.25 mmol) was added for preparing MIP (the other tube was used to prepare the non-imprinted polymer, NIP, which is the control polymer). The tubes were vortexed and sonicated to dissolve all the solid materials. Into each test tube, DVB (1.24 mL, 7 mmol) and 1 mL of initiator solution (containing 0.03 g AIBN dissolved in toluene) were added. The solutions were purged with nitrogen gas for 10 min, sealed and then transferred to a water bath with temperature maintained at 60 ºC, and kept for 24 hrs. The resulting bulk polymers were removed from the tubes and mechanically ground for 1 min using a planetary micro mill (Pulverisette 7, Laval Lab, Inc). Polymer particles were suspended in 5% methanol and passed through a 25 mm test sieve (Retsch, Haan, Germany). Large particles were repeatedly ground until almost all the polymer particles passed through the sieve. After settling for 12 hrs and decanting, polymer particles were collected by centrifugation. The template was removed by batch-mode solvent extraction with methanol containing 10% acetic acid. The supernatant after each washing step was analyzed by UV-Vis (BECKMAN COULTER, DU 800 Spectrophotometer) to detect the presence of propranolol. When propranolol peak (λ_{max} =

290 nm) was absent, the particles were soaked in acetone, centrifugated, decanted and dried *in vacuo*.

Polymer microspheres were synthesized using a precipitation polymerization method adopted from the literature (Yoshimatsu *et al*. 2007). Functional monomers SaMcr (0.5 g, 2.4 mmol) and AnMcr (1.0 g, 2.4 mmol)) were measured directly into different screw capped borosilicate test tubes, followed by addition of acetonitrile (80 mL). For the synthesis of the MIP microspheres, racemic propranolol (0.28 g, 1.12mmol) was added in the respective borosilicate test tubes. DVB (1.2 mL, 8.6 mmol) and AIBN (0.0824 g, 0.5 mmol) were then added in each test tube. The tubes containing the reaction mixtures were then sonicated for 2 min, purged with nitrogen gas for 10 min, sealed and transferred to an oil bath with temperature maintained at 60 ºC and kept for 24 hrs. Polymer beads were collected by centrifugation and extracted in batch mode to remove the template, following the same procedure as for the monolithic irregular particles. In all cases the control polymers (NIPs) were treated in the same way as for the imprinted polymers.

Saturation radioligand binding analysis. These experiments were performed in solvents similar to those used for polymer synthesis to avoid the possibility of destroying the imprinted sites by polymer swelling or shrinking. All measurements were performed in triplicate. For experiments that involved irregular polymer particles, the incubating solvent was toluene containing 0.5% acetic acid. From a stock polymer suspension, a series of dilution was performed to obtain polymer suspensions with concentration ranged from 0.0625 to 2 mg/mL. Polymer suspensions (1 mL at each polymer concentration) were transferred into Eppendorf tubes. A 20 mL toluene containing 1.2 pmol radiolabeled (*S*)-propranolol was added to each Eppendorf tube. In reference tubes, 1 mL of incubating solvent was mixed with 1.2 pmol radiolabeled (*S*)-propranolol dissolved in 20 mL toluene. The samples were incubated at room temperature for 12 hrs under gentle mixing using a rocking table. After the incubation, samples were centrifugated at 13500 rpm for 10 min. A 700 mL supernatant was withdrawn and thoroughly mixed with 10 mL of scintillation liquid. The radioactivity of the solution was measured by liquid scintillation counting using a 1219 Rackbeta Liquid Scintillation Counter (LKB WALLAC, Sollentuna Sweden). The quantity of the radiolabeled (*S*)-propranolol bound to the polymer particles was calculated as the difference of radioactivity between the supernatants withdrawn from the reference tubes and the tubes containing polymer particles. For polymer microspheres prepared in acetonitrile, the incubating solvent was pure acetonitrile and the concentrations of the polymer microspheres were varied from 0.03125 to 4 mg/mL. The remaining procedures were identical to those used for the monolithic irregular particles.

Competition radioligand binding analysis. As for the saturation experiments, each polymer was tested in the same solvent as the one used for polymer synthesis. For monolithic irregular polymer particles, competition experiments were performed using toluene containing 0.5% acetic acid as incubating solvent, whereas for imprinted spherical beads, the incubating solvent was pure acetonitrile. With ^{3}H-(*S*)-propranolol as a tracer, two kinds of competitors were used: (1) chiral competitors: (*S*)-propranolol and (*R*)-propranolol, and (2) structurally related competitors: (*R*,*S*)-metoprolol (**4**) and (*R*)-timolol (**5**). The procedure used for sample preparation was similar to that used for the saturation experiments, except that the concentrations of the polymer (0.5 mg/mL) and radiolabeled (*S*)-propranolol (1.2 pmol/mL) were fixed, and an increasing

amount of competitors (from 3.85×10^{-4} to 3.85×10^{4} nmol/mL) was added in the incubating solvent.

RESULTS AND DISCUSSION

Synthesis of SaMcr - a structural analog of AnMcr. In order to study the influence of the side chain of AnMcr on molecular imprinting effect, we considered SaMcr to be a suitable analog to AnMcr because the only difference is lack of the aliphatic side chain in SaMcr (Figure 1). In our initial attempt to synthesize salicylic acid-based monomer, we found acrylation of salicylic acid to give an unstable product that quickly polymerized during work up. For this reason, in this study we focused only on methacrylated salicylic acid. The FT-IR spectrum of SaMcr showed strong bands extending from 3000 to 2530 cm^{-1} for the alkyl C-H, which are mixed with carboxylic O-H signals. The strong and sharp bands of carboxylic C=O in salicylic acid (1663 and 1610 cm^{-1}) were shifted to 1739 and 1691 cm^{-1}, respectively, after the phenolic –OH was methacrylated. The blue-shift of these signals in SaMcr can be explained by the weakened intra-molecular hydrogen bonding (Taka**c** and Topi**c** 2004). Compared to the spectrum of salicylic acid, new bands at around 1634 and 1596 cm^{-1} appeared, indicating the presence of $–CH_3$ and vinyl C=C in SaMcr. The FT-IR data are in agreement with results obtained previously by Licea-Claveríe *et al.* (2003). In the ^1H NMR spectrum of SaMcr, the original –OH peak (δ = 11.04 ppm) in salicylic acid disappeared after the methacrylation. Instead three new peaks appeared at δ = 6.31 and δ = 5.77 ppm (for the vinyl $=CH_2$), and at δ = 2.05 ppm corresponding to three methyl protons. Collectively, these spectroscopic analyses confirmed the successful chemical conversion of the phenolic O-H of salicylic acid into a methacrylic ester bond.

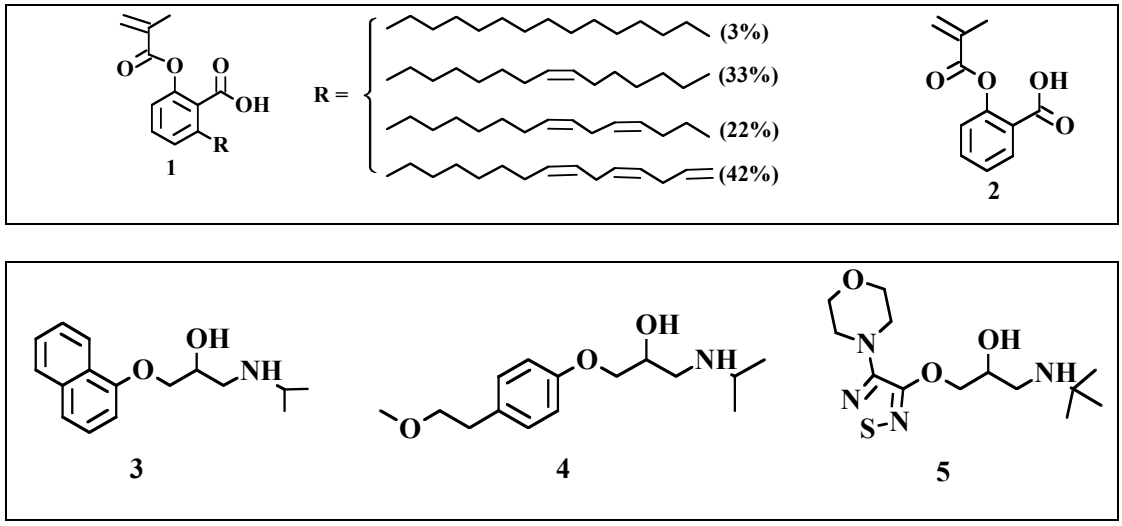


Figure 1: Chemical structures of functional monomers and test compounds: (**1**) AnMcr, (**2**) SaMcr, (**3**) propranolol, (**4**) metoprolol, (**5**) timolol

FT-IR analyses of poly(SaMcr/AnMcr-co-DVB)m. The efficiency of functional monomer incorporation in crosslinked co-polymers can be conveniently estimated by FT-IR analysis. Figure 2 shows representative FT-IR spectra for molecularly

imprinted polymers and the corresponding control polymers prepared using SaMcr and AnMcr as the functional monomers. The spectra were taken from the irregular particles synthesized in toluene. As can be observed in this figure, in each case the NIP and MIP spectra were very similar, suggesting that the imprinted polymer and the control polymer have almost identical chemical composition. Importantly, all four polymers had the characteristic peaks for carboxylic –OH at around 3200 – 2800 cm^{-1}, as well as broad and strong carboxylic C=O bands at around 1800 – 1650 cm^{-1}, signifying the presence of carboxylic acid group in all the four polymers. Previous studies have demonstrated that the carboxylic acid moieties in imprinted polymers prepared using –COOH containing functional monomers are vital for the ability of the imprinted polymers to offer hydrogen bond or ionic interaction with propranolol template (Zhang and Mosbach 2006, Mosbach and Haupt 1998, Andersson 1996, Kempe 1996, Kim and Spivak 2003).

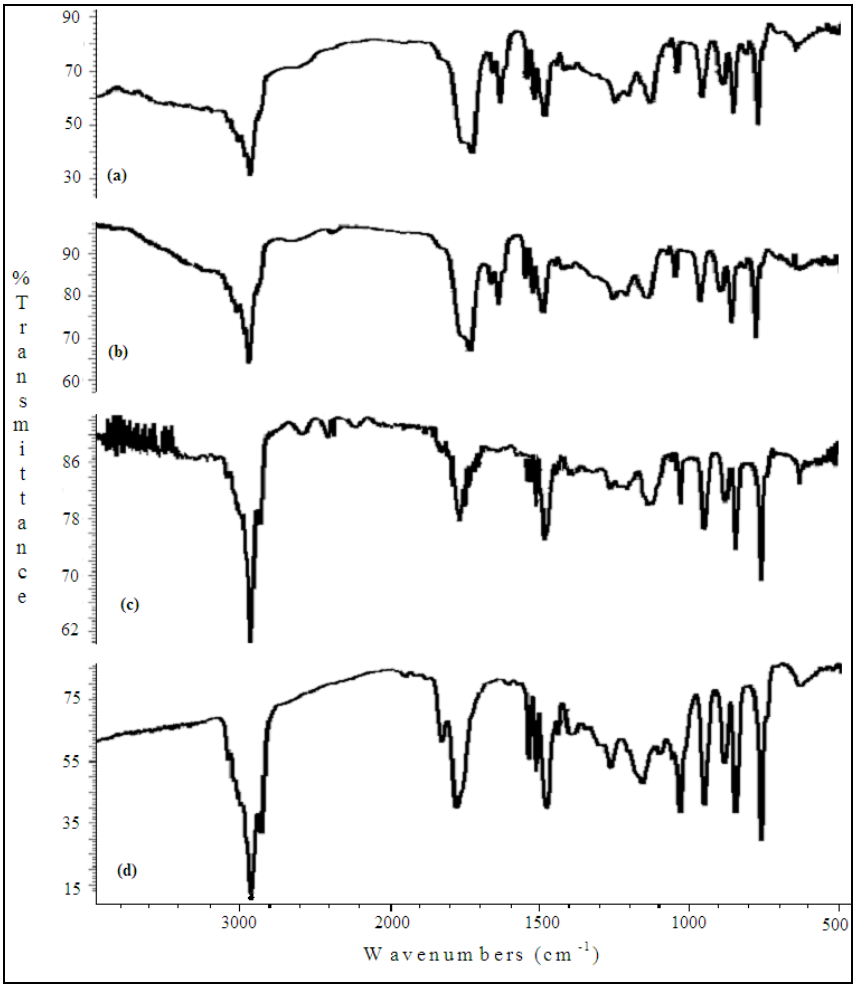


Figure 2: FT-IR spectra for poly(SaMcr-co-DVB)m NIP (a), poly(SaMcr-co-DVB)m MIP (b), poly(AnMcr-co-DVB)m NIP (c) and poly(AnMcr-co-DVB)m MIP (d).

Scanning electron micrographs of poly(SaMcr-co-DVB)b and poly(AnMcr-co-DVB)b microspheres. Scanning electron micrographs of imprinted and non-imprinted poly(SaMcr-co-DVB)b and poly(AnMcr-co-DVB)b microspheres are shown in Figure 3. While the non-imprinted poly(SaMcr-co-DVB)b were obtained as regular beads with diameter of around 0.5-2 µm (Figure 3a), the imprinted poly(SaMcr-co-DVB)b formed severe particle aggregates (Figure 3b). For the poly(AnMcr-co-DVB)b system, both the non-imprinted and the imprinted polymers gave spherical beads with diameter of roughly 0.5-2 µm and 1-3 µm, respectively (Figure 3c-d). Most probably the side chain of AnMcr presented a better chemical environment for the formation of spherical beads, making the precipitation polymerization system less sensitive to the influence of the propranolol template.

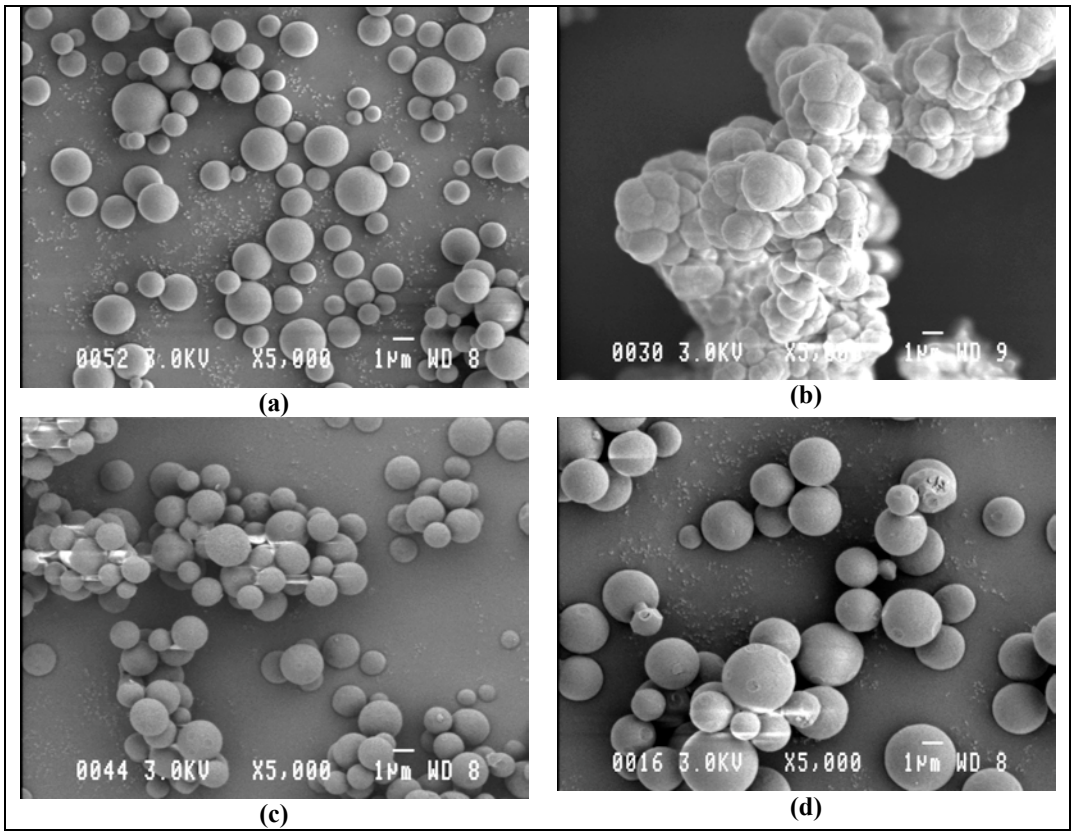


Figure 3: Scanning electron micrographs of poly(SaMcr-co-DVB)m NIP (a), poly(SaMcr-co-DVB)m MIP (b), and poly(AnMcr-co-DVB)m NIP (c) and poly(AnMcr-co-DVB)m MIP (d).

Saturation radioligand binding analysis of irregular particles in toluene containing 0.5% acetic acid. Figure 4 shows the binding of (*S*)-[^{3}H]-propranolol as a function of concentrations of poly(SaMcr-co-DVB)m, for both the imprinted (MIP) and

the non-imprinted (NIP) polymers tested in toluene containing 0.5% acetic acid. As can be seen, the imprinted poly(SaMcr-co-DVB)m showed an apparent propranolol affinity. With polymer concentration of 2 mg/mL, imprinted poly(SaMcr-co-DVB)m registered about 45% propranolol uptake. More apparent information was exposed when taking in to account the background (nonspecific) binding of the corresponding control polymers (NIPs). At a concentration of 2 mg/mL, poly(SaMcr-co-DVB)m NIP showed high background binding (15%). Therefore, specific binding, defined as the difference in template binding between imprinted (MIP) and non-imprinted (control, NIP) polymer, for poly(SaMcr-co-DVB)m (30%) is lower than that shown by poly(AnMcr-co-DVB)m (40%) (Philip *et al* 2007). Higher specific binding of poly(AnMcr-co-DVB)m can be associated with the structural difference of the two functional monomers (structure **1** and **2**). The AnMcr molecule has C_{15} alk(en)yl side chain which is positioned *ortho* to the carboxyl group. On average, this alkenyl side chain consists of more than two C=C bonds that can co-polymerize with DVB (Philip *et al.* 2007), thereby enhancing the rigid positioning of -COOH groups to form better defined binding sites.

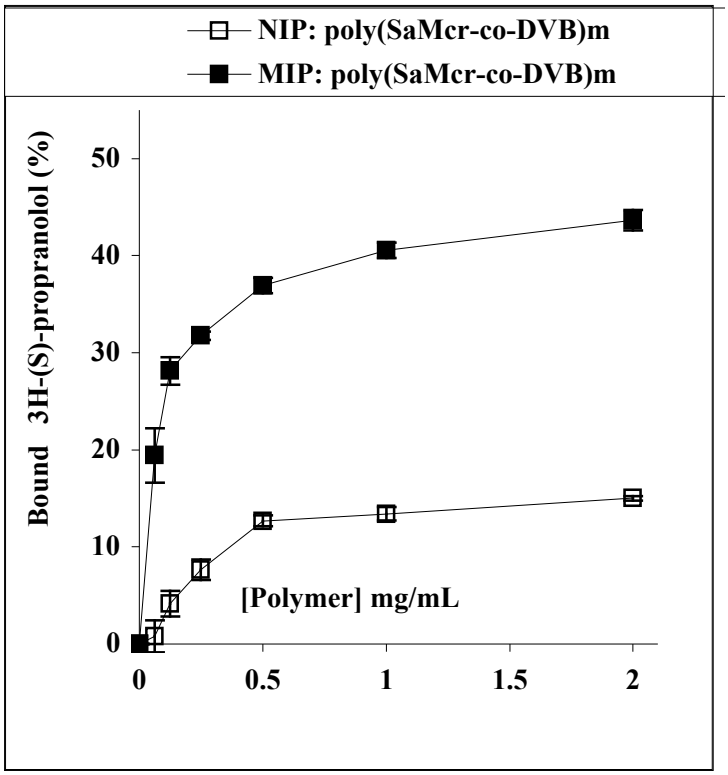


Figure 4: Binding of (*S*)-[^{3}H] propranolol as a function of concentration of imprinted and non-imprinted poly(SaMcr-co-DVB)m particles in toluene containing 0.5% acetic acid.

Competition radioligand binding analysis of irregular particles in toluene containing 0.5% acetic acid. Figure 5 shows the displacement curves obtained when increasing concentration of competitor compounds were added to compete for limited recognition sites in the imprinted particles. Chiral selectivity of these MIPs can be studied by comparing displacement curves for (*S*)- and (*R*)-propranolol. It is clear from Figure 5 that (*S*)-propranolol is always more potent to displace ^{3}H-(*S*)-propranolol than (*R*)-propranolol. This indicate that the formulation of both poly(SaMcr-co-DVB)m successfully resulted in polymers with chiral-imprinted sites. Imprinted polymers prepared in the presence of racemate template are assumed to contain nearly 50% of (*S*)-enantiomer-imprinted and 50% of (*R*)-enantiomer-imprinted recognition sites. It follows that (*S*)-propranolol can reversibly displace ^{3}H-(*S*)-propranolol bound to both (*R*)- and (*S*)-enantiomer-imprinted sites. On the contrary, (*R*)-propranolol can only displace ^{3}H-(*S*)-propranolol bound to (*R*)-enantiomer-imprinted sites, but not ^{3}H-(*S*)-propranolol bound to (*S*)-enantiomer-imprinted sites. This means, the correct positioning of ^{3}H-(*S*)-propranolol to the (*S*)-enantiomer-imprinted sites hamper the chances of (*R*)-propranolol to displace it.

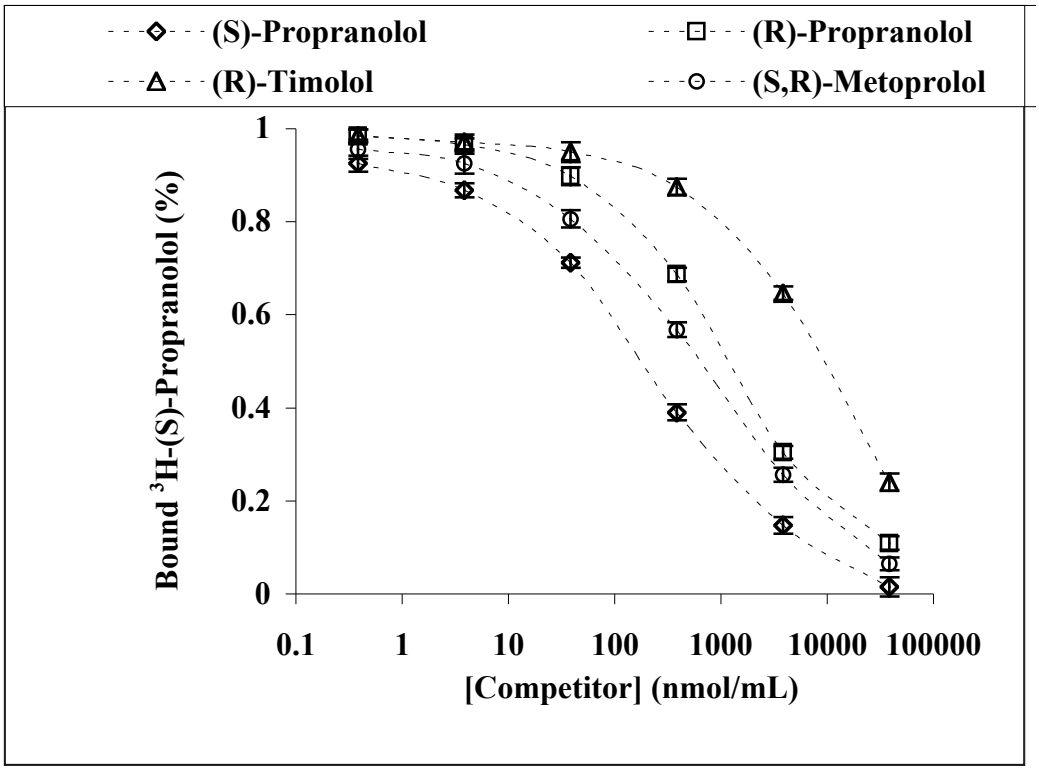


Figure 5: Displacement curves of (*S*)-propranolol, (*R*)-propranolol, (*R,S*)-metoprolol and (*R*)-timolol obtained with 0.5 mg/mL of imprinted poly(SaMcr-co-DVB)m. The concentration of ^{3}H-(*S*)-propranolol tracer was fixed at 1.2 nM in toluene containing 0.5% acetic acid.

The imprinted poly(SaMcr-co-DVB)m displayed similar cross-recognition profiles for the structural analogues of propranolol as for poly(AnMcr-co-DVB)m particles stated elsewhere (Philip *et al.* 2007). (*R*)-timolol showed lower potency than (*R*)-propranolol to displace ^{3}H-(*S*)-propranolol, although both were less potent compared to the remaining competitors (Figure 5). These displacement results are similar to those displayed by imprinted poly(AnMcr-co-DVB)m particles (Philip *et al.* 2007). The displacement curves of (*R,S*)-metoprolol appeared between (*S*)- and (*R*)-propranolol, indicating that the presence of (*S*)-metoprolol in the racemate enhanced the capability of (*R,S*)-metoprolol to compete with ^{3}H-(*S*)-propranolol for the chiral recognition sites.

Saturation radioligand binding analysis of spherical beads in pure acetonitrile. In order to get a further insight into the effect of the non-polar side chain of AnMcr in imprinted polymers, microspheres prepared by precipitation polymerization were tested for their performance in propranolol binding in acetonitrile. The results presented in Figure 6 indicate that imprinted poly(SaMcr-co-DVB)b aggregates provided slightly higher propranolol uptake than poly(AnMcr-co-DVB)b microspheres, which was accompanied by a slightly higher background binding. These results are somehow different from those obtained with poly(SaMcr-co-DVB)m and previously reported poly(AnMcr-co-DVB)m particles synthesized in toluene (Philip *et al.* 2007), for which the binding experiments were carried out in toluene containing 0.5% acetic acid. Presumably, the more polar acetonitrile makes propranolol imprinting and rebinding less effective than the non-polar solvent, toluene. It is also obvious from Figure 6 that, even at low polymer concentrations, non-specific propranolol binding in acetonitrile is high. This high non-specific propranolol binding shown by the non-imprinted poly(SaMcr-co-DVB)b and poly(AnMcr-co-DVB)b may be accounted for by the aromatic moieties of the functional and crosslinking monomers that strongly interact with propranolol through its naphthalene part.

Competition radioligand binding analysis of spherical beads in pure acetonitrile. It is noted form Figure 7 that in both cases, (*S*)-propranolol is more effective to displace ^{3}H-(*S*)-propranolol than (*R*)-propranolol, signifying a successfully creation of chiral-imprinted sites in both poly(SaMcr-co-DVB)b and poly(AnMcr-co-DVB)b. In general, (*R*)-timolol was the least potent competitor followed by (*R,S*)-metoprolol. Apparently, this may be attributed to the structural and chiral differences of (*R*)-timolol compared to the radioligand ^{3}H-(*S*)-propranolol (Figure 1). Racemic metoprolol, which contains both (*S*)- and (*R*)-enantiomers would be expected to be more potent to displace ^{3}H-(*S*)-propranolol than (*R*)-propranolol. However from the obtained results it is clear that racemic metoprolol is less potent than (*R*)-propranolol (Figure 7). This is probably because its chemical structure did not give a perfect fit to the imprinted sites, especially for the aromatic moiety of the molecule. This is much more obvious with poly(AnMcr-co-DVB)b which seems to be more structural discriminative than poly(SaMcr-co-DVB)b. By comparing the displacement curves of poly(AnMcr-co-DVB)b and poly(SaMcr-co-DVB)b (Figure 7), it is probable that the presence of about two C=C bonds on the side chain of AnMcr, that co-polymerize with DVB (Philip *et al.* 2007), provide additional cross-linking, thereby improving the positioning of -COOH groups inside the binding sites to give high fidelity recognition cavities.

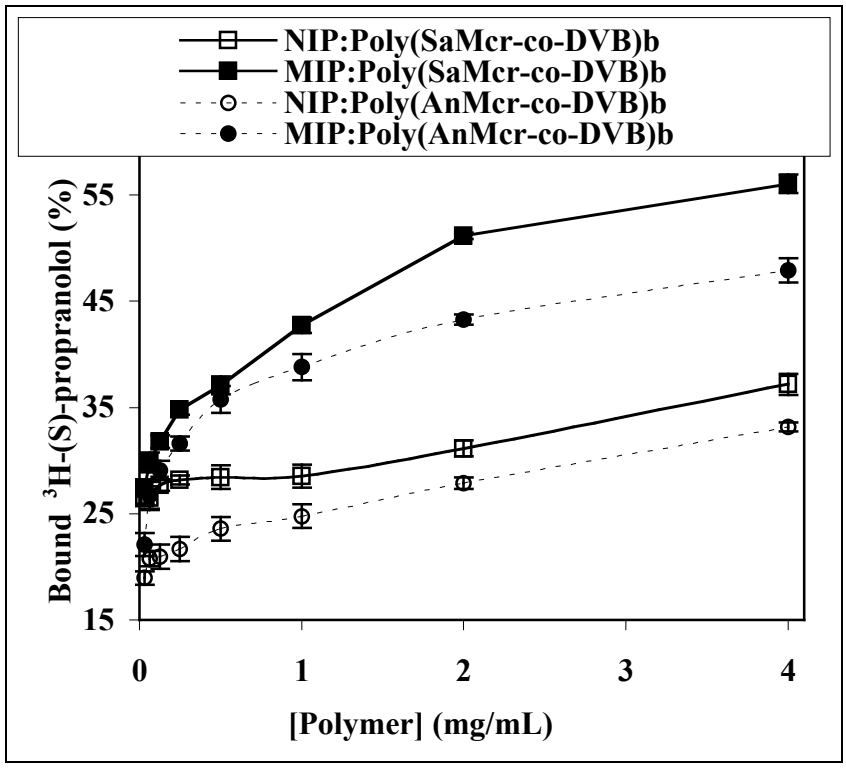


Figure 6: Binding of (*S*)-[^{3}H] propranolol as a function of concentration of imprinted and non-imprinted poly(SaMcr-co-DVB)b and poly(AnMcr-co-DVB)b in acetonitrile

By putting together the results in Figures 5 and 7**,** additional valuable information can be revealed for these MIPs (Table 1). Although the spherical beads synthesized in acetonitrile displayed lower apparent binding affinity than the monolithic particles synthesized in toluene, the competition radioligand binding analysis showed a better chiral and structural selectivity of the spherical beads. The observed phenomenon can be explained in terms of the nature of the solvents used during polymerization and the chemical structures of the monomers and the template: when toluene is used as a solvent, it is likely that the π-π interaction between DVB and the naphthalene ring of propranolol is weakened due to the large excess of toluene. In this case the imprinting effect is generated mainly via the interaction of AnMcr or SaMcr with the aminopropanediol moiety of propranolol. Because the naphthalene moiety of the template did not participate in the interaction with the functional monomer, it had little effect on the formation of the imprinted sites. As a result, the imprinted sites in monolithic MIPs had high cross-reactivity towards (*R,S*)-metoprolol, because (*S*)-metoprolol has the same chiral configuration as (*S*)-[^{3}H]-propranolol tracer (Table 1). For the spherical beads prepared in acetonitrile, it is possible that the π-π interactions and van de Waals forces between the aromatic monomers and propranolol are enhanced during polymerization. When this effect is combined with functional monomer interactions via the aminopropanediol part of the template, the resulting crosslinked

polymer exhibits improved chiral and structural discriminations. The improved selectivity of the imprinted spherical beads is clearly indicated by the lower cross-reactivity of metoprolol and timolol (Table 1).

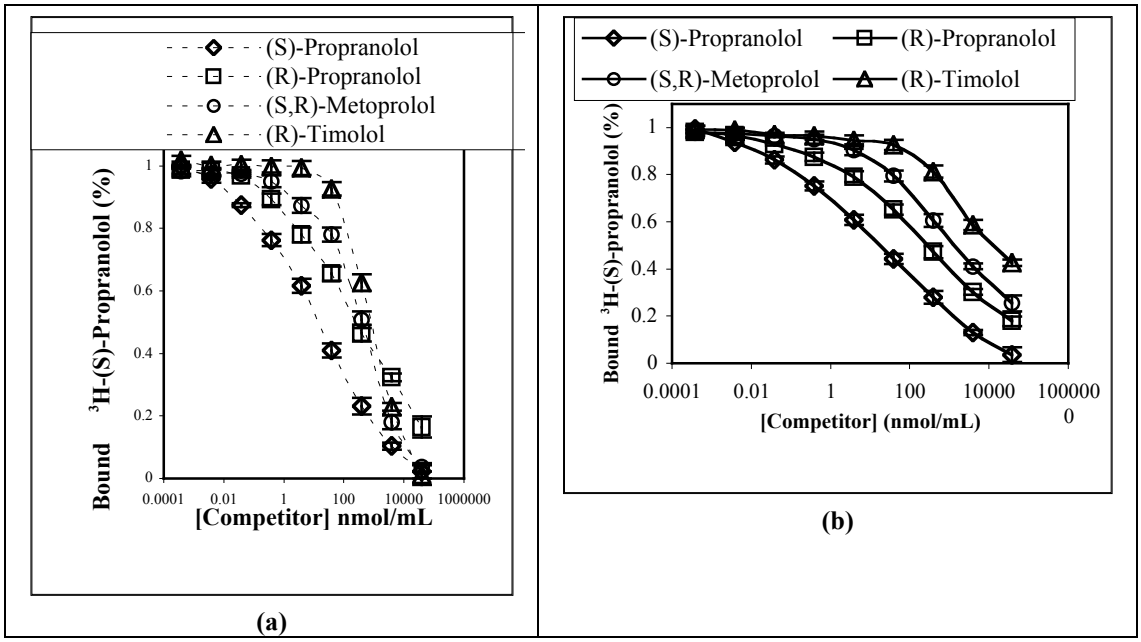


Figure 7: Displacement curves of (*S*)-propranolol, (*R*)-propranolol, (*R,S*)-metoprolol and (*R*)-timolol obtained with 0.5 mg/mL of imprinted poly(SaMcr-co-DVB)b (a), and poly(AnMcr-co-DVB)b (b). The concentration of ^{3}H-(*S*)-propranolol tracer was fixed at 1.2 nM in pure acetonitrile.

Table 1: Results of competitive radioligand binding analysis

Imprinted polymer	***IC*$_{50}$ (nM)[a] [Cross-reactivity (%)][b]**			
	(*S*)-Propranolol	(*R*)-Propranolol	(*R,S*)-Metoprolol	(*R*)-Timolol
poly(SaMcr-co-DVB)m	177 [100]	1144 [16]	632 [28]	8760 [2]
poly(SaMcr-co-DVB)b	13.8 [100]	254 [5.4]	406 [3.4]	805 [2]
poly(AnMcr-co-DVB)b	17.5 [100]	266 [7]	1356 [1.3]	13928 [0.2]

[a] IC_{50} is defined as the concentration of analyte at which the labeled ^{3}H-(*S*)-propranolol bound to MIP is reduced by 50%.

CONCLUSION

The successful synthesis of SaMcr function monomer, which is structurally similar to AnMcr, made it feasible to study the influence of the alk(en)yl side chain of anacardic acid on molecular recognition. After preparation of imprinted polymers using SaMcr and AnMcr functional monomers, it was verified that both poly(SaMcr-co-DVB) and poly(AnMcr-co-DVB) contained –COOH groups. It was also established that NIP and MIP of either of

these polymers had identical chemical composition. Based on these fundamental results the following can be derived from the experimental outcomes we obtained: (i) from the observed high propranolol recognition properties displayed by poly(AnMcr-co-DVB)m over poly(SaMcr-co-DVB)m in toluene containing 0.5% acetic acid, it can be concluded that the side chain of anacardic acid improve the capacity of AnMcr as a functional monomer for propranolol non-covalent imprinting in apolar solvents, (ii) the competition radioligand binding results obtained in acetonitrile suggest that the side chain of anacardic acid enhance the fidelity of the recognition sites of imprinted polymers, (iii) the results pointed out by scanning electron micrographs recommend AnMcr over SaMcr for preparation of MIP beads using precipitation polymerization, particularly for small molecular templates. Moreover, the achievement regarding the preparation of spherical co-polymer beads using monomers derived from anacardic acid is a step forward towards exploiting renewable sources from cashew nut shells. We therefore further predict a wide range of possibilities of using anacardic acid monomer and its chemically modified forms to develop novel –COOH containing spherical polymer beads for diverse practical applications.

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