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Preparation of Molecularly Imprinted Polymers Using Anacardic Acid Monomers Derived from Cashew Nut Shell Liquid

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The objective of this work was to use monomers from cashew (*Anacardium occidentale* L.) nut shells to develop molecularly imprinted polymers. Cashew nut shell liquid (CNSL) is a cheap and renewable agro byproduct consisting of versatile monomers. Solvent-extracted CNSL contains over 80% anacardic acid (AnAc) with more than 90% degree of unsaturation in its C_{15} side chain. From AnAc monomer, anacardanyl acrylate (AnAcr) and anacardanyl methacrylate (AnMcr) monomers were synthesized and their chemical structures were characterized by Fourier transform IR and NMR. Different imprinted bulk polymers based on AnAc, AnAcr, and AnMcr functional monomers have been prepared. In the present study, each functional monomer was separately copolymerized in toluene with ethylene glycol dimethacrylate and divinylbenzene as cross-linkers, using racemic propranolol as a model template. While the AnAc based polymer revealed a meager rebinding ability, the imprinted polymers made from AnAcr and AnMcr displayed highly specific propranolol binding. At a polymer concentration of 2 mg/mL, AnAcr and AnMcr based imprinted polymers were able to bind over 50% of trace propranolol (initial concentration 1.2 nM). Under the same condition propranolol uptake by the two nonimprinted control polymers was less than 20%. Chiral recognition properties of these polymers were further confirmed using tritium-labeled (*S*)-propranolol as a tracer in displacement experiments, suggesting that the apparent affinity of the imprinted chiral sites for the correct enantiomer is at least 10 times that of the mismatched (*R*)-propranolol. Moreover, cross reactivity studies of these polymers showed that the (*S*)-imprinted sites have higher cross-reactivity toward (*R,S*)-metoprolol than (*R*)-propranolol and (*R*)-timolol.

KEYWORDS: Molecular imprinting; anacardic acid; anacardanyl acrylate; anacardanyl methacrylate; propranolol; metoprolol; timolol

INTRODUCTION

Cashew nut shell liquid, a byproduct in cashew-processing factories, is among the sources of renewable alkenyl phenols, whose structural properties permit their broad applications including the synthesis of highly cross-linked polymers (*1, 2*) and bioactive compounds (*3*). It is well-known that anacardic acid (AnAc) is the main component of natural cashew nut shell liquid (CNSL) constituting more than 80% of the total solvent-extracted CNSL. From a number of investigations described in the literature, the composition of AnAc has been confirmed to be a mixture of four monophenol compounds (**1a–d**) differing from each other only in the unsaturation level of their aliphatic side chain ($C_{15}H_{31-n}$, where $n = 0, 2, 4, 6$). The composition of anacardic acid, given as weight percentage, is shown in **Figure 1**. The overall alkenyl-containing components (**1b–d**) in AnAc are over 90% by weight (*4*).

In recent years, interest and requirements to synthesize cross-linked polymers based on vinyl monomers have increased tremendously, particularly in the area of molecular imprinting (*5, 6*). Conventionally, molecularly imprinted polymers (MIPs) are prepared in organic porogens by free radical copolymerization of functional and cross-linking monomers in the presence of a target compound. The target compound is used as a template to create binding cavities within the synthesized cross-linked polymer matrix. Templated cavities within the polymer particles, obtained either by pulverization of bulk polymers or by precipitation polymerization, readily bind the target compound as well as the analogue of the template, making MIPs a useful tool in separation and bioanalytical sciences (*7–11*). Classic functional monomers used in this technology are mainly alkenyl

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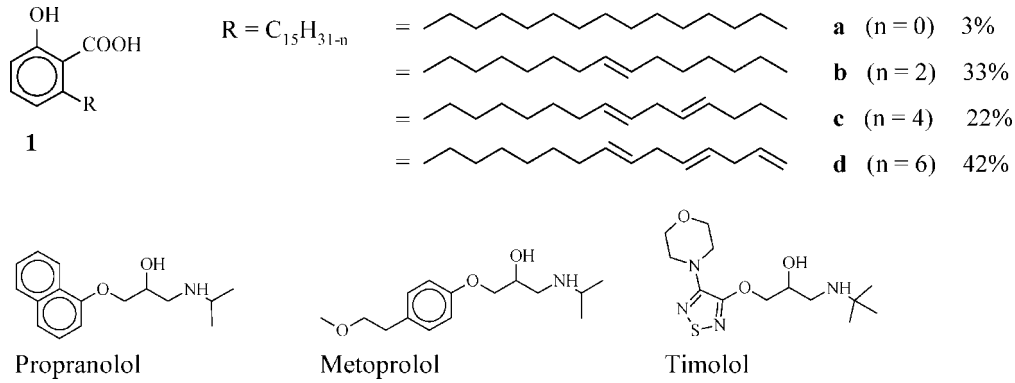


Figure 1. Chemical composition of AnAc (**1**) and structures of the test compounds used in this study.

carboxylic acid analogues such as acrylic acid, methacrylic acid, and vinylbenzoic acid (*12*). To achieve cross-linking, which is essential for molecular rebinding specificity, cross-linkers must be incorporated during MIP synthesis. Frequently used cross-linkers in numerous systems are divinylbenzene (DVB), ethylene glycol dimethacrylate (EGDMA), and trimethylolpropane trimethacrylate (*13, 14*). Nonetheless, a number of other functional and cross-linking monomers are still being developed and investigated so as to suit different predetermined goals of the synthesized MIPs (*15–21*).

The presence of double bonds in the side chain of anacardic acid in the mono-, di-, and triunsaturated components, and the aromatic moiety containing both phenolic and carboxylic acid groups are entirely persuasive chemical properties entailing considerable excitement to search for a strategy to use it to synthesize MIPs. The present study reports the propranolol recognition properties of MIPs prepared by copolymerizing EGDMA and DVB with both AnAc and the chemically modified AnAc functional monomers. Propranolol was used as a model template because its different chiral structures provide easy assessment of stereoselectivity of imprinted sites. For practical uses, MIPs with high specificity for propranolol can be used as affinity adsorbents (in solid phase extraction protocols) to enable trace analysis of propranolol and similar β-blocker drugs. This is the initial strive toward exploiting CNSL monomers in molecular imprinting technology, where for the first time the use of sustainable resources for preparation of highly specific molecular recognition materials (MIPs) is reported.

MATERIALS AND METHODS

Chemicals. Cyclohexane (90.0%) and ethyl acetate (99.8%) were analytical grade, acetone (99.0%, extra pure), methanol (99.9%), chloroform (99.8%), toluene (99.8%), and acetonitrile (99.9%) were HPLC grade. All the solvents were purchased form Fisher Scientific AB (Västra Frölunda, Sweden) and used without further purification. Diethyl ether (99.5%) and hydrochloric acid (37–38%), anhydrous sodium sulfate (99.0%), pyridine (99.0%), potassium bromide (99.0%), and azobisisobutyronitrile (AIBN, 98%) were purchased form Merck (Darmstadt, Germany) and used as supplied, except for azobisisobutyronitrile that was recrystallized from methanol before use. Methylacrylic anhydride (~94%), calcium hydroxide (96.0%), and acetic acid (99.8%) were used as obtained from Fluka (Buchs, Switzerland). Divinylbenzene (DVB, 80%, isomeric mixture), ethylene glycol dimethacrylate (EGDMA, 98.0%), and formic acid (95–97%) were purchased from Sigma-Aldrich (Chemie GmbH, Germany). DVB was freed from polymerization inhibitor by passing it through a column of aluminum oxide. Triethylamine (99%) and trifluoroacetic acid (98.0%) were purchased from Sigma-Aldrich and used as supplied. Acryloyl chloride (96.0%) purchased from Merck was used as supplied. (*R*,*S*)-Propranolol hydrochloride (99%), (*S*)-propranolol hydrochloride (99%), and (*R*)-propranolol hydrochloride (99%) supplied by Fluka (Dorset, U.K.) were converted into free base form before use. (*S*)-[4-^{3}H]-Propranolol (specific activity 555 GBq mmol^{-1}, 66.7μM solution in ethanol) was purchased from NEN Life Science Products Inc. (Boston, MA). Scintillation liquid, Ecoscint A, was from National Diagnostics (Atlanta, GA).

UV–vis and FT-IR Spectroscopy. UV–vis analysis of the materials was performed using a Beckman Coulter DU 800 spectrophotometer. FT-IR analysis was performed on a Nicolet Impact 410 spectrophotometer equipped with QuickIR software (Thermo Fisher Scientific).

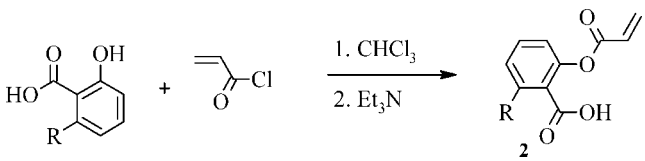
HPLC–DAD and HPLC–MS Analysis. HPLC–DAD analysis was carried out on a Chromolith Performance RP-18e column (Merck, Darmstadt, Germany) connected to a LaChrome system consisting of a L-7100 pump, a L-7200 autosampler, a L-7455 diode array detector, and software package D-7000 System Manager (Merck KgaA, Darmstadt, Germany): solvent A, H_2O/TFA = 100/0.1; solvent B, MeCN/H_2O/HOAc = 80/20/1. Separation of AnAc was achieved with isocratic elution using solvent A/B = 10/90 at a flow rate of 0.5 mL/min.

HPLC–MS analysis was carried out on a SunFire C_{18} column (3.5 μm, 2.1 × 50 mm) mounted on a Waters 2695 separation module: solvent A, 0.1% formic acid in water; solvent B, MeCN. AnAc was separated with isocratic elution using solvent A/B = 20/80 at a flow rate of 0.3 mL/min and detected with an electrospray Waters Quattro micro API mass spectrometer in positive mode, using MS scan (ES+, *m*/*z* 340.0 to 352.0).

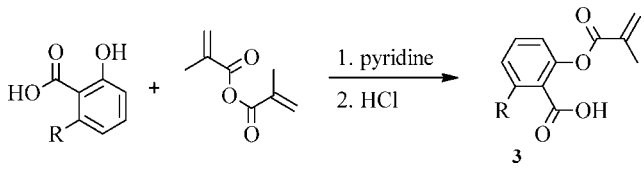
Extraction of CNSL and Isolation of Anacardic Acid. Fresh and dry cashew nuts were obtained from Pwani and Mtwara regions of Tanzania. Pretreatment processes including scrub washing and sun drying were performed to reduce field contaminants. Deshelling and size reduction of the shells were carried out to ascertain efficient extraction. While kept in the dark, the shell pieces (1000 g) were soaked in cyclohexane (2500 mL) for 1 day; thereafter shells were sieved out, and the solution was filtered. The clear solution was concentrated under reduced pressure using a rotary evaporator at 40 °C, which gave a brown oily product (170 g).

Isolation of AnAc was performed as per Paramashivappa et al. (*22*), with a few operational modifications. The process involved dissolving crude CNSL (100 g) in 5% aqueous methanol (400 mL) followed by reacting with $Ca(OH)_2$ (150 g) slurred in 5% aqueous methanol (300 mL) at 40 °C for 3 h. The calcium anacardate cake obtained was vacuum-filtered and washed thoroughly with 5% aqueous methanol. The wet crushed cake was slowly transferred into a beaker containing a stirred mixture of excess 6 M HCl (500 mL) and ethyl acetate (300 mL) and left for 1 h. The organic phase was washed five times with equal volume of distilled water, dried over anhydrous Na_2SO_4, filtered, and concentrated using a rotary evaporator at 40 °C, which gave 76 g of AnAc. IR (KBr, cm^{-1}): 3424 (Ar–OH), 3400–2400 and 1645 (–COOH), 3009 (Ar-H and vinyl-H), 2924 and 2849 (aliphatic C–H), 1607 (aliphatic C═C), 1446 (aromatic C═C), and 1304 (Ar-OH and -COOH). ^{1}H NMR ($CDCl_3$, δ (ppm)): 0.8896 (m, 3H, –CH_3), 1.3275, 1.601, 2.784, 2.981, and 5.018, (m, 28H, –CH_2-), 5.018 (m, mixed, 2H, –CH═CH–, Ar-OH/–COOH), 5.018, 5.369, 5.829 (m, 5H, –CH═CH–), 6.771, 6.870 (d, 2H, Ar-H), 7.374 (t, 1H, Ar-H), 11.043 (s, mixed, 1H, Ar-OH/–COOH).

Scheme 1. Synthesis of AnAcr



Scheme 2. Synthesis of AnMcr



Synthesis of Anacardanyl Acrylate (AnAcr). AnAc (1.056 g, 3 mmol) was transferred into a reaction flask followed by 2 mL of chloroform. Triethylamine (0.42 mL, 3.6 mmol) and acryloyl chloride (0.40 mL, 3.6 mmol) were transferred into two different test tubes followed by addition of 2 mL of chloroform into each tube. Prior to combining the reactants, contents in the reaction flask and tubes were cooled to 0 °C. After cooling, the triethylamine–chloroform solution was dropwise added to the stirred content in the reaction flask, followed by the acryloyl chloride–chloroform solution. The reaction flask was stirred at 0 °C for 10 min, after which the reaction was allowed to proceed at room temperature (≤25 °C) for 12 h while gently stirred. The impurities were removed by repeatedly washing the resulting mixture with equal volumes of water in a separating funnel. The organic phase was dried over anhydrous sodium sulfate and concentrated under reduced pressure by means of a rotary evaporator and then solvent freed in vacuo. AnAcr was obtained as yellowish-brown oil (0.9456 g, 65%). The structure of the resulting compound was identified by parallel comparison of its IR and ^{1}H-NMR spectra with those of AnAc. **Scheme 1** shows the synthesis of AnAcr (**2**) from AnAc.

Synthesis of Anacardanyl Methacrylate (AnMcr). The protocol reported by Lübke et al. on synthesis of 2-(methacryloyloxy)benzoic acid from salicylic acid was adopted (*17*). In a reaction flask, AnAc (1.060 g, 3 mmol) was dissolved in 6 mL of pyridine. The solution obtained was cooled to 0 °C. In a separate flask, 3 mL of pyridine and 0.5 mL (3.6 mmol) of methylacrylic anhydride were thoroughly mixed and cooled to 0 °C, then dropwise added to the reaction flask. After 10 min of cooling, the reaction mixture was left to proceed at room temperature (≤25 °C) for 12 h. The resulting mixture was cooled to 0 °C and then added dropwise into stirred ∼1 M HCl (40 mL) containing ice blocks. The organic phase was extracted using diethyl ether (40 mL), repeatedly washed with equal volumes of water, dried over anhydrous sodium sulfate, and concentrated under reduced pressure by means of a rotary evaporator. The product was dried in vacuo to completely evaporate the solvent. AnMcr was obtained as yellow-brown oil (0.8620 g, 55%). The structure of the resulting compound was identified by parallel comparison of its IR and ^{1}H-NMR spectra to those of AnAc. **Scheme 2** shows the synthesis of AnMcr (**3**) from AnAc.

Polymer Synthesis. The appropriate weight of the functional monomer (AnAc (**1**), AnAcr (**2**), or AnMcr (**3**)), corresponding to 3 mmol, was measured directly into a screw-capped borosilicate glass tube, followed by adding 5 mL of toluene. The template compound, (*R*,*S*)-propranolol (0.5 mmol), was then added. The content was well mixed, and then 15 mmol of the cross-linking monomer (DVB or EGDMA) and 0.25 mmol of the initiator (AIBN) were added. The solution obtained was purged gently with nitrogen for 10 min and sealed. Polymerization reaction was carried out by inserting the tube in a water bath with temperature maintained at 60 °C for 24 h. The resulting bulk polymer was mechanically pulverized and wet sifted through a 25 μm sieve by means of ∼5% methanol solution. The grinding and sieving procedures were repeated until nearly all the polymer passed through the sieve. After settling and decanting, polymer particles were collected by centrifugation. Initially, the template was removed by batch-mode solvent extraction with toluene, acetonitrile, and methanol containing 10% acetic acid. Later, Soxhlet extraction with toluene was employed to remove most of the template from the polymer particles, followed by batch-mode washing in methanol containing 10% acetic acid. The supernatant from each washing step was analyzed spectrophotometricaly to monitor the template removed. When the template peak (at 290 nm) was absent, the particles were soaked in acetone, separated, and dried in vacuo. The corresponding nonimprinted control polymers were synthesized and treated under the same conditions except that the template (*R*,*S*)-propranolol was omitted.

Radioligand Binding Analysis. *Saturation Experiments.* These experiments were performed in triplicate. Polymer suspensions with concentration range between 0.0625 and 2 mg/mL were made by successive dilution using incubating solvent that was toluene containing 0.5% acetic acid. Suspensions were transferred into Eppendorf tubes, 1 mL for each polymer concentration. A 20 μL portion of toluene containing 1.2 pmol of radiolabeled (*S*)-propranolol was added to each Eppendorf tube containing polymer suspension, as well as reference tubes that contained 1 mL of incubating solvent without polymer. The mixture was incubated at room temperature overnight. Gentle mixing was achieved by means of a rocking table. Each of the incubated contents in Eppendorf tubes were centrifugated at 13500 rpm for 10 min. A 700 μL supernatant was withdrawn and mixed with 10 mL of scintillation cocktail. 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 radioactivity difference between incubated solution without polymer and the supernatant taken from the polymer suspension.

Competition Binding Experiments. These experiments were carried out using polymers based on AnAcr and AnMcr functional monomers with DVB cross-linker. Competition binding experiments were performed by using (*S*)-propranolol and (*R*)-propranolol as chiral competitors. In addition, the structurally related (*R*,*S*)-metoprolol and (*R*)-timolol were also used as competitors. Polymer and radiolabeled (*S*)-propranolol concentrations were fixed at 0.5 mg/mL and 1.2 pmol/mL, respectively. The competitor concentrations were varied from 3.85×10^{-4} nmol/mL to 3.85×10^{4} nmol/mL. The incubating solvent and the remaining procedure were the same as used in the saturation experiments.

RESULTS AND DISCUSSION

Characterization of Anacardic Acid. UV–vis analysis of the material was performed with $CHCl_3$ as a solvent. The UV–vis spectrum obtained had peaks (λ_{max}) at 416, 317, and 245 nm. In the HPLC analysis, three major peaks appeared at retention time of 5.92, 7.41, and 10.27 min, with a ratio of the integrated peak area of 5:2:3, respectively. From LC/MS analysis three major peaks with ions of m/e = 343.375, 345.397, and 347.414 with intensity ratio of 5:2:3, respectively, were observed. On the basis of these results, the average number of unsaturated C═C bonds in the purified AnAc can be estimated to be

$$(5\times3+2\times2+3\times1)/(5+2+3)=2.2$$

which was in agreement with that reported by Paramashivappa et al. (*22*).

Preparation of Imprinted Polymers Using AnAc as Functional Monomer. Initially the imprinting experiment was performed using AnAc as a functional monomer. These polymer particles were washed in a batch-mode solvent extraction to remove the propranolol template. The process involved a series of washing: four times with toluene, once with acetonitrile, three times with methanol containing 10% acetic acid, and finally four times with toluene. It was observed spectrophotometricaly that toluene was more efficient to remove the template than other solvents, therefore in later experiments polymers were first treated by Soxhlet extraction in toluene, followed by a series of batch mode washing as described in Materials and Methods.

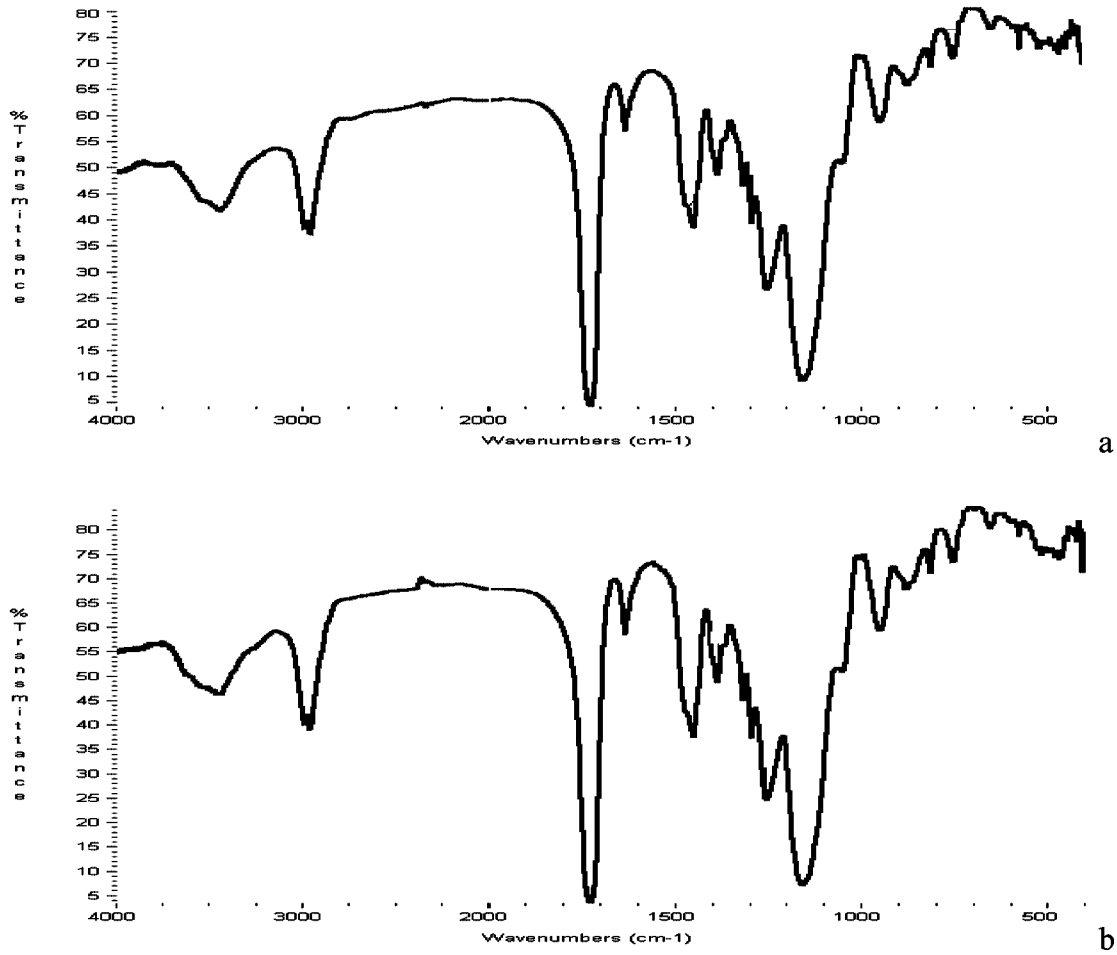


Figure 2. FT-IR spectra of molecularly imprinted (**a**) and nonimprinted poly(AnAc-*co*-EGDMA) (**b**).

Although the unsaturated C═C bonds in anacardic acid may have polymerization activity somehow different from that of the vinyl group in the cross-linker monomer (EGDMA), the brownish color of the polymers obtained after intensive purification suggests that anacardic acid has been successfully copolymerized into the cross-linked polymers. In a recent study, we have utilized a similar isolated vinyl group to copolymerize a surfactant monomer with EGDMA cross-linker in a miniemulsion polymerization system, which resulted in efficient incorporation of the surfactant monomer on the surface of molecularly imprinted nanoparticles (*21*).

To further confirm that AnAc has been copolymerized in the present system, we carried out FT-IR analyses for both the imprinted and the nonimprinted poly(AnAc-*co*-EGDMA) (**Figure 2**). As seen, both polymers have the characteristic peaks for the Ar-OH of AnAc at around 3400 cm^{-1}, and the two spectra are almost identical, suggesting the identical chemical composition of the two polymers.

Equilibrium binding tests were carried out in toluene containing 0.5% acetic acid, which provided a solvent condition similar to that used for polymer synthesis. A small amount of acetic acid was added to suppress nonspecific adsorption. The percentage of bound radioligand (*S*)-propranolol was plotted as a function of the amount of polymer used in each experiment (**Figure 3**). Both the imprinted and the nonimprinted polymers showed low propranolol affinity, and there was no clear distinction between template binding to the imprinted and to the nonimprinted polymers. The equilibrium binding result indicated that imprinting using anacardic acid did not generate high fidelity binding sites for propranolol, and the propranolol

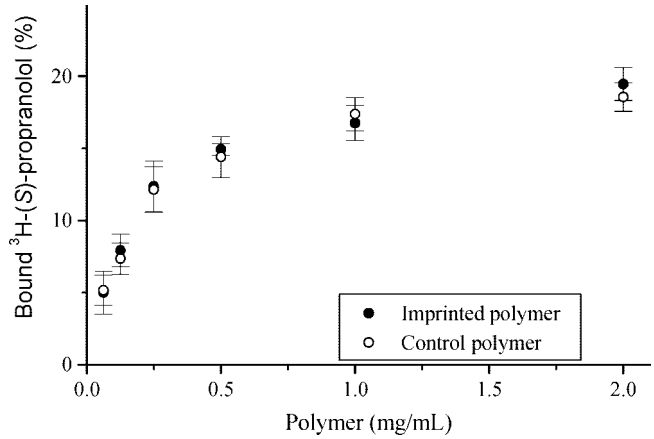


Figure 3. Uptake of (*S*)-[^{3}H]-propranolol by increased amount of poly(AnAc-*co*-EGDMA).

uptake by the two polymers was simply due to nonspecific adsorption. There are two possible reasons that may explain the present low imprinting efficiency: (1) the location of the phenolic and the carboxylic groups in AnAc makes it possible to form intramolecular hydrogen bonds, thus reducing the interaction between AnAc and the template and consequently the poor molecular recognition property; (2) the vinyl groups present in the side chain of AnAc are located at the closest after the C_8 position from the benzene ring, meaning that the functional groups responsible for template binding are attached to the polymer backbone via a long and flexible aliphatic chain resulting in poorly defined template recognition sites.

In order to prevent the intramolecular hydrogen bond in AnAc and to increase the imprinted site fidelity by extra cross-linking, we selected to block the phenolic group of AnAc by acrylation and methacrylation reactions (**Schemes 1** and **2**). The imprinted polymers were later separately prepared using these new functional monomers both with EGDMA and with DVB cross-linkers.

The FT-IR spectrum of the acrylate product (**2**) was analogous to that of AnAc (**1**) with the following significant exceptions: Lack of absorption band at around 3400 cm^{-1} indicates the absence of phenolic OH. The increased intensity of absorption bands at around 1000–1300 cm^{-1} suggests the presence of the ester C–O bond. Furthermore, the strong peaks at around 1740–1800 cm^{-1} are related to the C═O bond. In the ^{1}H-NMR spectrum of AnAcr, the phenolic OH peak at 11.04 ppm disappeared, and two new peaks at 6.28 and 6.56 ppm were observed, corresponding to the acryloyl protons.

Likewise, the FT-IR spectrum of the compound obtained from **Scheme 2** had no absorption band at around 3400 cm^{-1}, indicating the absence of the phenolic OH. The observed strong absorption bands at around 1000–1300 cm^{-1} implied the presence of the ester C–O bond. Also, the strong peak at 1740 cm^{-1} is associated with the C═O bond. In the ^{1}H-NMR spectrum of AnMcr (**3**), the phenolic OH peak at 11.04 ppm disappeared, and new peaks at $\delta = 6.31$ and 5.77 ppm appeared, with peak integration equivalent to two vinyl protons. In addition, an increased integration value for the peak at $\delta = 2.01$ ppm for the three methyl protons was observed. These spectroscopic analyses confirmed that the chemical modification of anacardic acid has successfully converted the phenolic OH into a (meth)acrylic ester bond.

The two new functional monomers (AnAcr (**2**) and AnMcr (**3**)) were used to replace AnAc to prepare propranolol imprinted polymers. **Figure 4** shows the uptake of (*S*)-[^{3}H]-propranolol as a function of polymer concentration in the range of 0–2 mg/mL for poly(AnAcr-*co*-EGDMA) and poly(AnMcr-*co*-EGDMA). The propranolol uptake with 2 mg of imprinted poly(AnAcr-*co*-EGDMA) and poly(AnMcr-*co*-EGDMA) reached over 50%, indicating that the phenol-blocked functional monomers indeed enhanced propranolol affinity for the new polymers, due to improved hydrogen bond interactions between the new functional monomers and the propranolol template. Despite improved propranolol uptake, the specific binding, as defined as the difference between the imprinted (MIP) and the nonimprinted polymer (control, NIP) was not very high (**Figure 4**). The apparently high nonspecific binding may be attributed to the cross-linking monomer (EGDMA) used, which after polymerization can also participate in hydrogen bond interaction with propranolol during the equilibrium binding experiment. One way to further suppress the nonspecific binding is to utilize an inert cross-linking monomer, such as divinylbenzene (DVB), that does not contain any hydrogen bond donor or acceptor.

With DVB as cross-linker, the imprinted polymers prepared from the chemically modified anacardic acid functional monomers showed much higher specific binding of propranolol. At a polymer concentration of 2 mg/mL, propranolol uptake with the imprinted poly(AnAcr-*co*-DVB) and poly(AnMcr-*co*-DVB) was 3- and 10-fold of the corresponding nonimprinted polymers, respectively (**Figure 5**). As expected, the use of DVB cross-linker effectively reduced nonspecific binding for the present propranolol-imprinted polymers. It is also observed that at a polymer concentration of 2 mg/mL, imprinted poly(AnAcr-*co*-DVB) and poly(AnMcr-*co*-DVB) achieved up to 60% and 50% propranolol binding, much higher than the corresponding

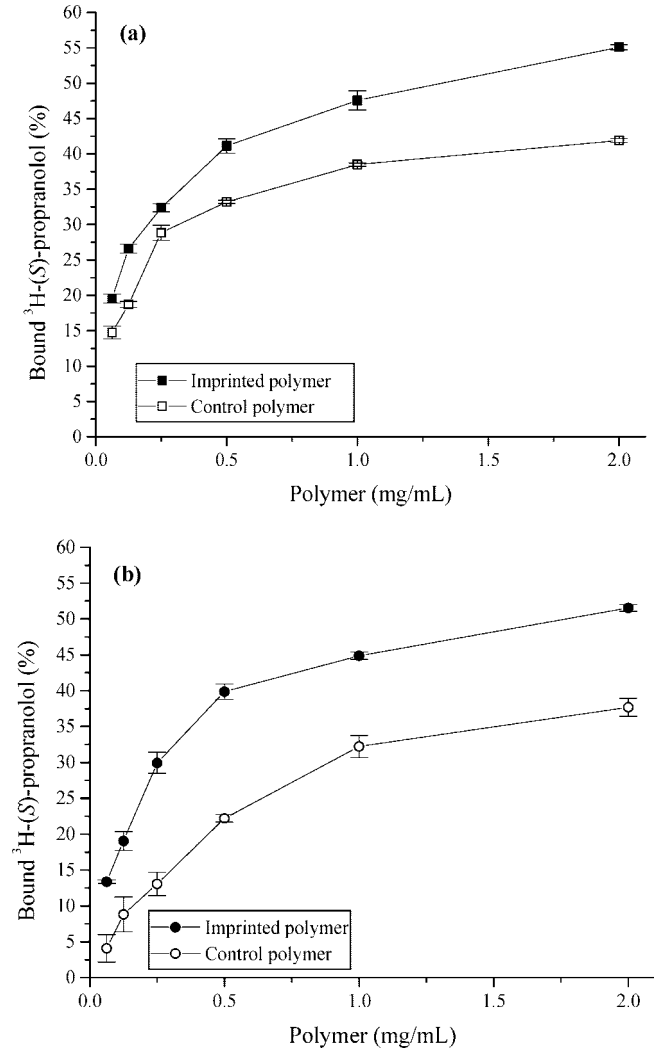


Figure 4. Uptake of (*S*)-[^{3}H]-propranolol as a function of polymer concentration: (**a**) poly(AnAcr-*co*-EGDMA), (**b**) poly(AnMcr-*co*-EGDMA).

imprinted poly(AnAc-*co*-EGDMA) (**Figure 3**). As has been suggested previously (*23*), the increased propranolol affinity for the imprinted poly(AnAcr-*co*-DVB) and poly(AnMcr-*co*-DVB) may be partially attributed to the contribution of the DVB moieties, which can provide additional π–π interaction with the naphthalene part of propranolol. The BET surface areas for the imprinted poly(AnAcr-*co*-DVB) and poly(AnMcr-*co*-DVB), as measured by nitrogen adsorption, were only 21.7 and 5.72 m^2/g, respectively. Obviously, the highly specific propranolol binding achieved with these polymers was not due to increased surface area but due to the successful formation of high fidelity recognition sites.

From **Figures 4** and **5**, it is obvious that for noncovalent molecular imprinting, AnAcr and AnMcr are better functional monomers than AnAc, because the former are more efficient in interacting with template rather than forming intramolecular hydrogen bond. In addition, the additional (meth)acryloyl C═C bonds in AnAcr and AnMcr can effectively increase the rigidity of the COOH within the binding sites (**Figure 6**), therefore providing much improved imprinting effect.

Binding Selectivity of Imprinted Poly(AnAcr-*co*-DVB) and Poly(AnMcr-*co*-DVB) Polymers. Selectivity of imprinted binding sites can be studied in competitive radioligand binding experiments, where increased nonlabeled test compounds are added to compete with a fixed amount of radioisotope-labeled template to bind to a limited number of recognition sites. The

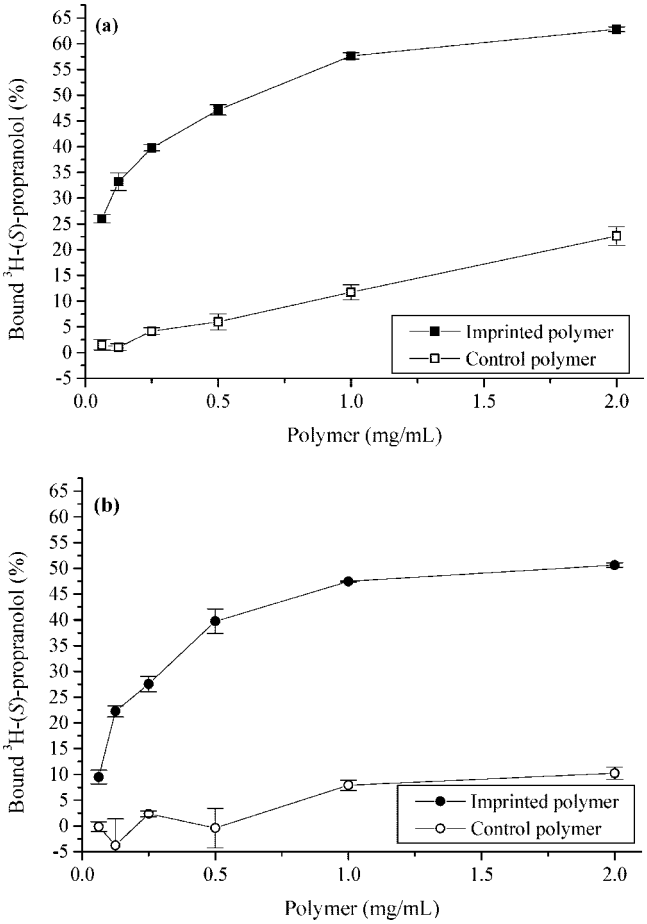
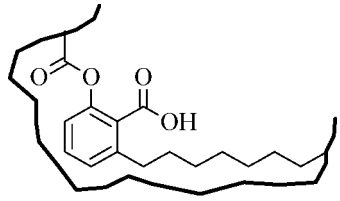


Figure 5. Adsorption of (*S*)-[^{3}H]-propranolol as a function of polymer concentration. (**a**) poly(AnAcr-*co*-DVB) (**b**) poly (AnMcr-*co*-DVB).



4

Figure 6. Proposed binding sites of imprinted polymers prepared from AnAcr and AnMcr (**4**).

IC_{50} value obtained from the dose-responsive curve of each test compound can be used to estimate the cross-reactivity of the imprinted sites for the given compound. When the radioligand is a pure enantiomer, the competitive binding experiments can also be used to study chiral selectivity of MIP even though a racemate template has been used during the imprinting reaction (*23*).

Figure 7 illustrates the displacement of (*S*)-[^{3}H]-propranolol from the imprinted poly(AnAcr-*co*-DVB) and poly(AnMcr-*co*-DVB) polymers when increased amounts of (*R*)-propranolol, (*S*)-propranolol, (*R*)-timolol, and (*R*,*S*)-metoprolol were added as competing ligands. As can be seen, for both the imprinted poly(AnAcr-*co*-DVB) and poly(AnMcr-*co*-DVB), the displacement curves for (*R*)-propranolol shifted to higher concentration range than those for (*S*)-propranolol, indicating clear chiral selectivity of individual binding sites. For the imprinted poly(AnAcr-*co*-DVB) and poly(AnMcr-*co*-DVB) polymers, the apparent affinity of the (*S*)-imprinted chiral sites for the correct enantiomer was approximately 20 and 10 times those of the

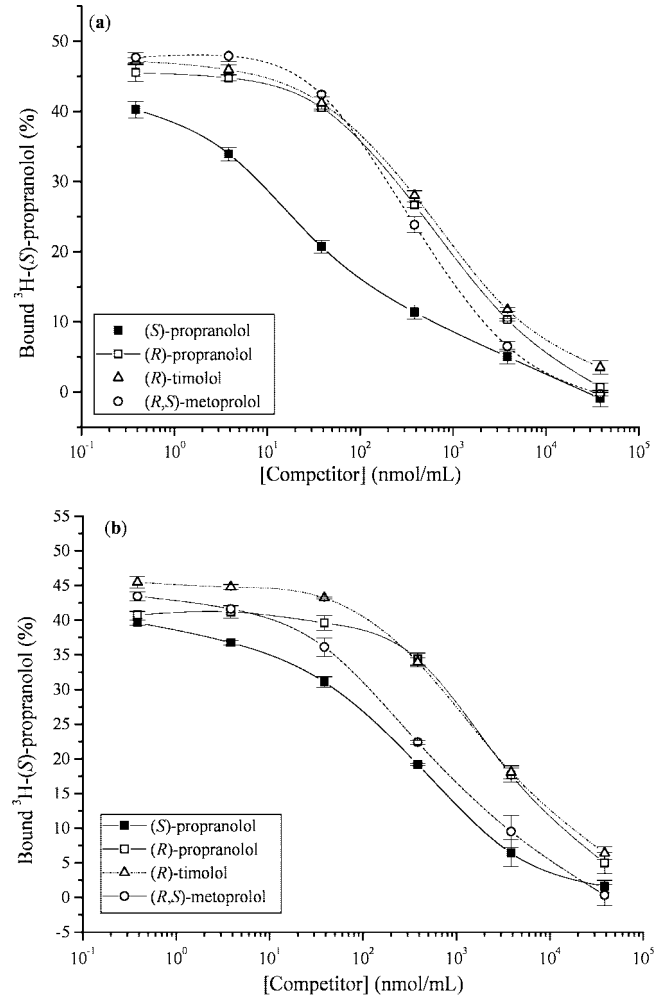


Figure 7. Displacement curves of (*S*)-propranolol, (*R*)-propranolol, (*R*)-timolol, and (*R*,*S*)-metoprolol obtained with 0.5 mg/mL of imprinted poly(AnAcr-*co*-DVB) (**a**) and imprinted poly(AnMcr-*co*-DVB) (**b**). The concentration of (*S*)-[^{3}H]-propranolol tracer was fixed at 1.2 nM in toluene containing 0.5% acetic acid.

Table 1. Results of Competitive Radioligand Binding Analysis with Imprinted Poly(AnAcr-*co*-DVB) and Poly(AnMcr-*co*-DVB)

imprinted polymer	competitor	IC_{50} (nM)[a]	cross-reactivity (%)[b]
poly(AnAcr-*co*-DVB)	(*S*)-propranolol	30	100
	(*R*)-propranolol	670	4.5
	(*R*)-timolol	800	3.8
	(*R*,*S*)-metoprolol	450	6.7
poly(AnMcr-*co*-DVB)	(*S*)-propranolol	220	100
	(*R*)-propranolol	2000	11
	(*R*)-timolol	2000	11
	(*R*,*S*)-metoprolol	380	58

[a] IC_{50} is defined as the concentration of analyte that displaces 50% of the labeled (*S*)-[^{3}H]-propranolol bound to the MIP. [b] Cross-reactivity of the (*S*)-imprinted sites is defined as the percentage of the IC_{50} of (*S*)-propranolol divided by the IC_{50} of the test compounds.

mismatched (*R*)-propranolol, respectively (**Table 1**). Interestingly, the structural analogue of (*R*)-propranolol, (*R*)-timolol generated a displacement curve almost identical to that of (*R*)-propranolol. For more, when the racemate (*R*,*S*)-metoprolol was used as competing ligand, its displacement curve was found to locate between those of (*S*)- and (*R*)-propranolol. Obviously, the presence of (*S*)-metoprolol made the racemate mixture a more potent competitor than (*R*)-propranolol, because (*S*)-metoprolol has the same chiral configuration as the radioactive

tracer (*S*)-[^{3}H]-propranolol, therefore giving a better fit into the (*S*)-imprinted sites.

Anacardic acid isolated from CNSL can be easily copolymerized with standard cross-linker EGDMA to produce cross-linked polymers. Due to the presence of the neighboring phenolic OH that forms an intramolecular hydrogen bond with the benzoic COOH, anacardic acid itself could not provide a high molecular imprinting effect when tested in the model system using propranolol as template. Blocking the phenol group of anacardic acid by acrylation and methacrylation allowed the resulting monomers to act as good functional monomers for propranolol noncovalent imprinting. While EGDMA-based MIPs of these new monomers displayed high background binding, the DVB-based MIPs showed much improved propranolol affinity and binding specificity. With the chiral radioisotope labeled (*S*)-propranolol as a tracer, competitive binding experiments indicated that the individual binding sites have high chiral recognition capability; e.g., the cross-reactivity of (*S*)-imprinted sites in the DVB-based polymers for (*R*)-propranolol is below 11%. The two DVB-based imprinted polymers however can effectively bind structurally related β-blockers, suggesting that these MIPs may be used as group-specific affinity adsorbents for direct measurement of total drug concentration or as solid phase extraction materials to afford one-step enrichment and clarification of propranolol-related β-blockers. These outcomes signify an imperative progress toward exploiting renewable sources from cashew nut shells to produce value-added new polymer products including MIPs. Furthermore, it is predicted that the amphiphilic nature of the new monomers studied in this work may be used to produce novel COOH-containing functional polymers for a series of new applications.

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