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Chiral chromatographic separation of β-blockers

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Abstract

A novel amide based chiral stationary phase *m*-[(+)-α-methyl benzyl carboxamide] XAD-4 has been synthesized by covalently linking *R*(+)-1-phenylethylamine to chloroformoyl Amberlite XAD-4 under weak alkaline conditions. The synthesized resin has been primarily characterized by m.p., elemental analysis and FT-IR and ^{13}C NMR spectra. β-Blockers viz. atenolol, metoprolol, and propranolol were successfully separated into their enantiomers using a mixture of sodium acetate–acetic acid buffer (pH 4.1):acetonitrile (4:6, v/v) solution using the synthesized resin. Hydrogen bonding and π–π interactions are supposed to be the major analyte–chiral stationary phase interactions.

Keywords: Chiral stationary phase; XAD-4; β-Blocker

1. Introduction

Atenolol, 2-(*p*-(2-hydroxy-3-isopropyl amino) propoxy) phenyl acetamide; metoprolol, isopropylamino 3-*p*-(2-methoxy ethyl) phenoxy propan-2-ol; and propranolol, 1-isopropylamino 3 (1-napthyloxy) propan-2-ol are cardioselective β-adrenoreceptor blocking agents that block adrenergic stimuli, and are responsible for the stimulation of heart and inhibition of several types of smooth muscles. They are used for the treatment of disorders such as hypertension, anginapectoris, cardiac arrhythmias, glaucoma, supraventricular and ventricular arrhythmias and are also known to reduce the intensity of migraine headache [1]. Each of these drugs possesses at least one chiral centre and are marketed as racemic mixture, although, it has been demonstrated that the (*S*)-isomers possesses much greater affinity (50–500 folds) for binding to the β-adrenergic receptors than the antipode which shows the sign of toxicity. Thus, the two enantiomers should be considered as different drugs and a clear picture of their pharmacodynamic and pharmacokinetic profile cannot emerge until the fate of each enantiomer is established. Hence, there is a need to develop a rapid and selective method for their enantiomeric resolution.

A literature survey showed enantiomeric resolution of β-blockers using an direct method on polysaccharide based chiral stationary phase (CSP) viz. Chiralcel OD [2–7], cyclodextrin bonded column [8–11], and Pirkle–1J column [12]. Aboul-Enein et al. have used a different approach by synthesizing molecularly imprinted polymers (MIP) of (*S*)-timolol and using this CSP in thin layer chromatography (TLC) method for the separation of β-blockers like propranolol, atenolol, timolol, nadolol with calcium channel blocker nifedipine and verapamil [13]. Bhusan et al. have carried out direct enantiomeric resolution of atenolol, metoprolol and propranolol by impregnating TLC using L-lysine, L-arginine [14] and L-aspartic acid [15] as chiral selectors.

The separation of metoprolol enantiomers has been carried out on Chiralcel OD column [16]; Chiralpak AD [17]; Chiralcel OD [18] and Chirobiotic-T [19,20] column. Mislanova et al. have reported direct HPLC determination of (*R*)- and (*S*)-propranolol in rat microdialysate using on-line two column switching procedures [21]. Protein based cellobiohydrolase I immobilized on silica gel has been used for separation of β-blockers (metoprolol, alprenolol and propranolol) [22,23] (Fig. 1). Coupled achiral and chiral column method has been employed for the separation of enantiomers of atenolol [24] and metoprolol [25] using Lichrospher ADS, Chirobiotic-T column; and silica column, Chiralcel OD column, respectively.

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During the last decade, a variety of CSPs for the direct resolution of enantiomers have been developed and applied for the enantioseparation. Nowadays, various CSPs are available and the liquid chromatographic separation of enantiomers on CSPs is known to be the most accurate and convenient means in determining the enantiomeric composition of optically active compounds [26]. Agrawal et al. have reviewed broadly various CSPs with respect to their general structure type [27]. Amberlite XAD-4, a nonionic polymeric resin composed of polystyrene chains cross-linked with divinylbenzene has become very popular due to their macroporous structure, excellent size, high rigidity, good hydraulic properties and low cost. These sorbents proved to be predominant support materials due to their excellent stability towards extreme pH conditions [28–30] and their wealth of surface chemistry makes them well suited for the preparation of separation media. Apart from this, cluster of functional groups can be introduced at or near the surface of polymers with the aim of resolving the compounds of interest, since chemically modified polymeric resins have a higher surface polarity than underivatized resins, and for this reason polar analytes are retained more in these sorbents (π–π and polar interaction) [31].

The present communication reports the synthesis of CSP having amide linkage derived by covalently binding *R*(+)-1-phenylethylamine to functionalized Amberlite XAD-4. Here, we mimic the multiple weak interactions of biospecific affinity using atenolol, propranolol and metoprolol as model sorbates to illustrate the design of sorbents with sorption capacity and selectivity. This design idea has not yet been reported and has proved to be highly selective for the chiral separation of β-blockers with improved performance due to its functionality and attractive properties of polymer.

2. Experimental

2.1. Materials

Amberlite XAD-4 (nonionic polymeric adsorbent, surface area 725 m^2/g, wet mesh size 20–60) was procured from Sigma. *R*(+)-1-phenyl ethyl amine was purchased from Merck. All other chemicals used in the resin synthesis, including solvents were carefully purified and dried before use. Quartz distilled deionized water, which was further purified by a Millipore Milli-Q water purification system was used. HPLC grade solvents and their mixtures were filtered and degassed before use. (±)-Atenolol was obtained from Zydus Cadila, Ahmedabad, while (±)-metoprolol and (±)-propranolol were procured from Cipla (Mumbai, India). These samples were examined for their optical rotation, to ensure their racemic nature, before subjecting them to resolution. The optically pure enantiomers of (+)- and (−)-atenolol; and (+)- and (−)-propranolol were from Aldrich, while the pure enantiomers of (+)- and (−)-metoprolol were from Sigma.

2.2. Equipments and measurements

The resolution of the enantiomers was performed with a Perkin-Elmer Binary LC pump model 250, a Perkin-Elmer Bip UV–Vis spectrophotometric detector model LC 290 and a CR-6A integrator. The injection loop has a 20 μL capacity. Detection was carried out at 254 nm. The CSP was packed into the stainless steel HPLC column (250 mm × 4.6 mm I.D.), by a conventional high-pressure slurry packing procedure. All the chromatograms were obtained at room temperature with a constant flow rate of 0.8 mL/min. pH measurements were made on a Systronics digital pH meter 335. A digital polarimeter from Equiptronics, Model No. EQ-800 was used. Scanning electron micrographs were obtained on Philips XL-30 ESEM. IR spectra were recorded on an FT-IR/410, Jasco Spectrometer as KBr pellets. Solid phase ^{13}C NMR spectra were obtained with a Chemagenitcs Inc. M-100 instrument, at 25.1 MHz.

2.3. Preparation of stationary phase

The synthetic scheme to the stationary phase is depicted in Fig. 2. Amberlite XAD-4 was first acetylated by Friedal–Crafts aceylation and then oxidized by sodium hypochlorite. Thereafter, it was chloroformoylated using thionyl chloride which was further coupled with *R*(+)-1-phenylethylamine in weakly basic condition to yield the required *m*-[(+)-α-methyl benzyl carboxamide] XAD-4 resin.

2.3.1. Synthesis of acetyl XAD-4 [32]

Into a 250-mL three-necked flask, acetylation of 15 g XAD-4 with 16.5 mL (0.232 mol) acetyl chloride was carried out by the Friedal–Crafts method, using 17.2 g (0.129 mol) anhydrous $AlCl_3$ in 150 mL 1,2-dichloromethane. The reaction mixture was stirred for 2 h at 35 °C, quenched by pouring it into a mixture of crushed ice, and was acidified with concentrated hydrochloric acid. After 8 h, the resin was filtered and rinsed with methanol, water and finally with concentrated hydrochloric acid. It was again rinsed with water until neutral to litmus and methanol, and dried. Yield 94.48%, m.p. 186 °C, d. The FT-IR spectrum of acetylated resin shows a strong peak at 1698 cm^{-1}, which is attributed to the carbonyl stretching frequency ($\nu_{C=O}$), indicating acetyl groups are successfully introduced into the resin. Actually conjugation of C=O with a C=C or an aryl group leads to the delocalisation of the π electrons on both the unsaturated groups and hence C=O stretching in a conjugated ketone occurs at lower frequencies. Calculated C 85%, H 7.54%; Found C 85.03%, H 7.52%s.

2.3.2. Synthesis of carboxy XAD-4

Into a 250-mL round bottom flask, 10 g acetylated XAD-4 was refluxed with 150 mL of 12% aqueous solution of sodium hypochlorite for 12 h. The reaction mixture was further acidified to give dark yellow coloured resin, which was filtered and thoroughly rinsed with water until free from chloride ions. It

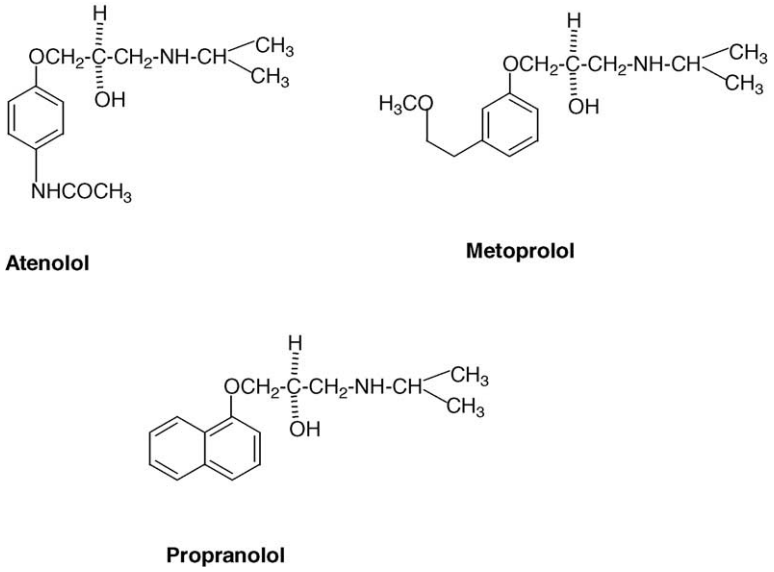


Fig. 1. Structures of β-adrenergic blockers.

was further washed with methanol and dried overnight. Yield 86%, m.p. 203 °C, d.

The FT-IR spectrum of *p*-carboxy XAD-4 showed a distinct band at 1716 and 3648 cm^{-1} which accounts for $\nu_{C=O}$, ν_{O-H} stretching vibrations, respectively. Further ν_{C-O} stretching and ν_{O-H} bending appears at 1325 and 1456 cm^{-1} in the spectra of carboxy XAD-4 resin.

Calculated C 76.89%, H 6.00%; Found C 76.93%, H 5.97%.

2.3.3. Synthesis of chloroformoyl XAD-4

Into a three-necked flask, 8 g carboxy XAD-4 was refluxed with 8 mL (0.110 mol) thionyl chloride at 70–75 °C for 4 h with intermittent stirring. The chloroformoyl XAD-4 was isolated, washed with diethylether and dried and further taken in situ for the coupling with *R*(+)-1-phenyl ethyl amine, since the chloroformoylated resin was unstable. Yield 86%, m.p. 170 °C, d.

The FT-IR spectrum of *p*-chloroformoyl XAD-4 showed a distinct band at 1780 cm^{-1} which accounts for $\nu_{C=O}$ with disappearance of broad band of –OH.

Calculated C 69.58%, H 4.82%, Cl 15.85; Found C 69.47%, H 4.80%, Cl 15.65s.

2.3.4. Coupling of R(+)-1-phenyl ethyl amine with chloroformoyl XAD-4 to give m-(α-methyl benzyl carboxamide) XAD-4

A 6.05 g aliquot (0.05 mol) of *R*(+)-1-phenyl ethyl amine was dissolved in 35 mL of dichloromethane at room temperature in a 100 mL three necked flask equipped with a mechanical stirrer. The temperature of above solution was kept at 0–5 °C. 12 g of solid chloroformoyl XAD-4 resin was added in small amounts over a period of 30 min. An aqueous suspension of sodium bicarbonate (0.075 mol) was added to the reaction mixture to keep weakly alkaline condition and to neutralize the hydrogen chloride being liberated during the reaction. After addition of chloroformoylated resin, the temperature of the reaction mixture was raised to room temperature and stirring was further continued for 2 h. The product was, washed with water until neutral and finally with methanol. Yield 70.37%, m.p. 220 °C, d.

A band at ν_{O-H} at 3648 cm^{-1} of carboxy XAD-4 is shifted to the ν_{N-H} stretching vibrations at 3421 cm^{-1}, which is due to secondary amides. The carbonyl absorption of amides $\nu_{C=O}$ known as amide I band of a pure sample shows a band at 1652 cm^{-1} which is at low frequency and not as strong as $\nu_{C=O}$ band in simple ketone or carboxylic acid due to H-bonding and resonance. The amide II band of ν_{N-H} bending of secondary amides appears in the region 1520 cm^{-1}. Further, in secondary amides, ν_{C-N} stretching vibrations is of similar frequency to that of ν_{N-H} bending mode and interaction of these two vibrations give rise to ν_{C-N} stretching which is amide III band at 1288 cm^{-1}. A band at 3050 and 3030 cm^{-1} which accounts for aromatic ν_{C-C} and ν_{C-H} stretching absorptions while a band at 2970 and 2929 cm^{-1} appears for asymmetric ν_{asCH_3} and ν_{sCH_3} absorptions. Further, the absorption band near 1374 cm^{-1} is assigned for symmetrical bending of methyl ν_{C-H} bands, which is very stable in position when the methyl group is attached to another carbon atom while a band at 1455 cm^{-1} occurs for the asymmetrical bending vibration ν_{asCH_3}. ^{13}C NMR, 24 ($C-CH_3$), 30(CH_2), 41 (XAD, CH_2), 51 (NH–C), 114 (XAD, $CH=CH_2$ adjoining phenyl), 129(CH of phenyl back bone), and δ 168 (C=O) Calculated C 80.37%, H 6.52%, N 3.55%; Found C 80.40%, H 6.53%, N 3.55%.

2.4. Preparation of mobile phase and samples

Sodium acetate–acetic acid buffer pH 4.1 was prepared as described, elsewhere [33]. The mobile phase, comprising

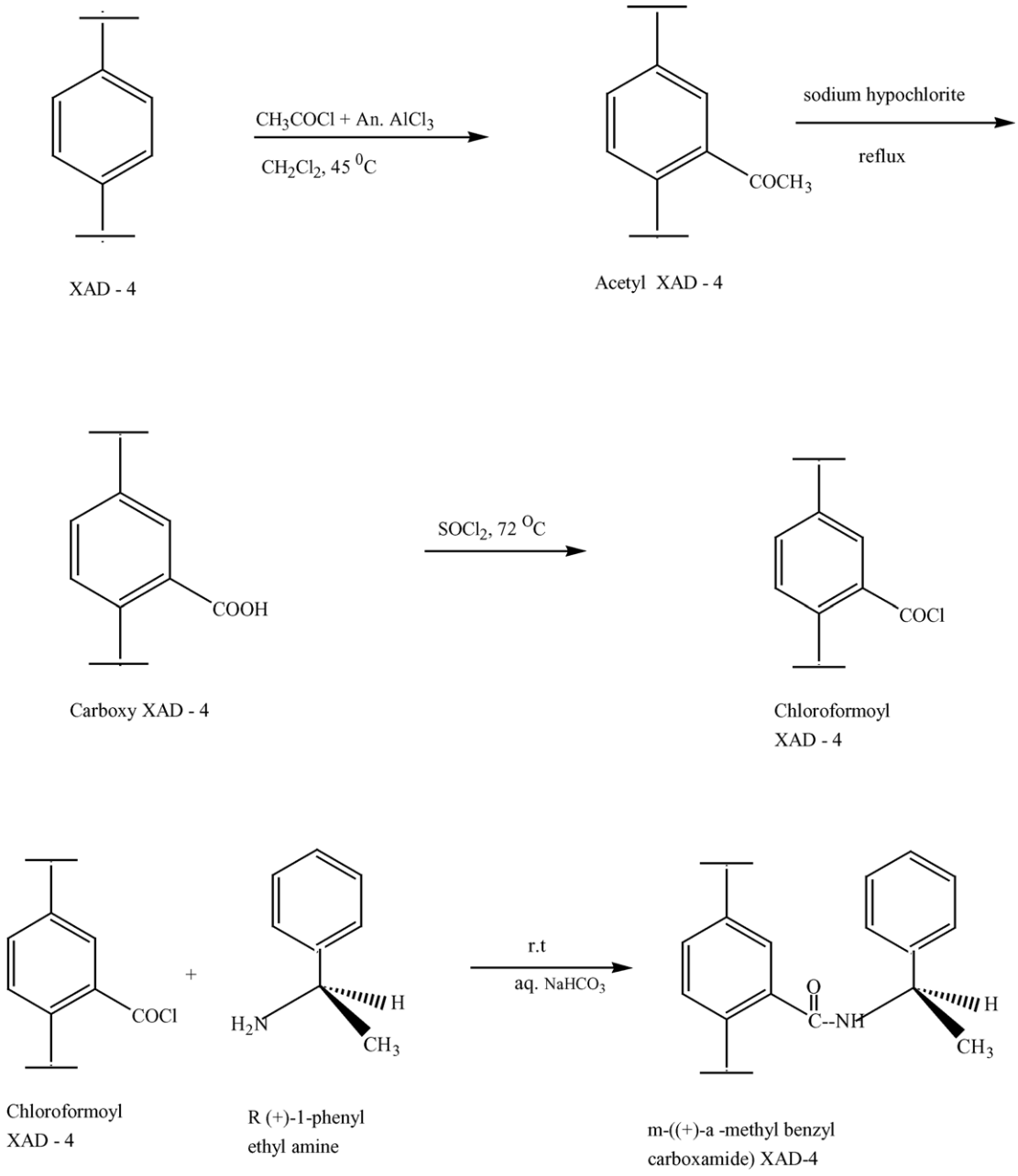


Fig. 2. Synthetic scheme for the preparation of CSP *m*-[(+)-α-methyl benzyl carboxamide] XAD-4.

of acetate buffer and the appropriate amount of the organic modifier, were freshly prepared, filtered, and were degassed under vacuum. A period of 1–2 h of equilibration after a pH change of the mobile phase was allowed in order to obtain reproducible results.

Standard sample stock solution of drugs namely atenolol, metoprolol and propranolol were prepared by dissolving 100 mg of respective drugs in 100 mL of methanol and then serially diluting in 10-fold stages to the required concentrations. All the experiments were carried out at room temperature (ca. 28 °C).

2.5. Chromatographic evaluation

The drug samples were loaded on the column and were further eluted by mixture sodium acetate–acetic acid buffer (pH 4.1):acetonitrile (4:6, v/v) solution as a mobile phase. The dependence of drug sorption at varying flow rate was investigated in the column procedure. The studies revealed that these drugs could be sorbed quantitatively by the resin at a flow rate of 0.8 mL/min at 25 °C by the fact that equilibria is better established. However, an increase in flow-rate results in the incomplete sorption due to the insuffi-

cient contact period between the polymer matrix and the drug solutions.

3. Results and discussion

3.1. Structural characterization of synthesized resin

The proposed structure of the *m*-[(+)-α-methyl benzyl carboxamide] XAD-4 was corroborated by FT-IR spectra and elemental analysis. The percentage of nitrogen measured by elemental analysis as well as the appearance of FT-IR vibrational bands attributable to amide-I ($\nu_{C=O}$ stretching), amide-II (ν_{N-H} bending) and amide-III (ν_{C-N} stretching) at 1652 cm^{-1}, 1520 cm^{-1} and 1288 cm^{-1}, respectively in *m*-[(+)-α-methyl benzyl carboxamide] XAD-4 resin provides for corroborative evidence that *R*(+)-1-phenylethylamine has been successfully bonded onto the surface of the functionalized Amberlite XAD-4 resin. The morphology of the functionalized polymer beads is shown by scanning electron micrographs in Fig. 3. SEM images of these polymers show that functionalization is not confined to pores, since functionalization is on the surface of spherical beads. Further, these functionalized beads does not show any cracks even after functionalization and have a perfect spherical shape which assisted to give improved peak shape along with higher flow rates. Moreover, the porous structure of the beads facilitates intraparticle convention, which reduces the mass transport resistance and improves the resolution. Thus, low backpressure and high flow-through characteristics showed that such functionalized polymer beads are an ideal material for rapid separation.

3.2. Enantioseparation of β-adrenergic blockers

β-Adrenergic blockers are hydroxylamines with the functional groups bearing secondary amines or *N*-isopropyl amines. These drugs also contain aromatic rings with different substituent moieties. β-Adrenergic blockers viz. atenolol, metoprolol and propranolol were separated on this column and results are summarized in Table 1. The pK_a values of these drugs are around 6.0; hence, it is expected that they are separated in media with the pH value below 6.0, which will suppress ionization of these drugs which may otherwise weaken the interaction with stationary phase and allow for a more facile elution from stationary phase. Separation was achieved in the mixture as shown in their separation patterns in Figs. 4–6.

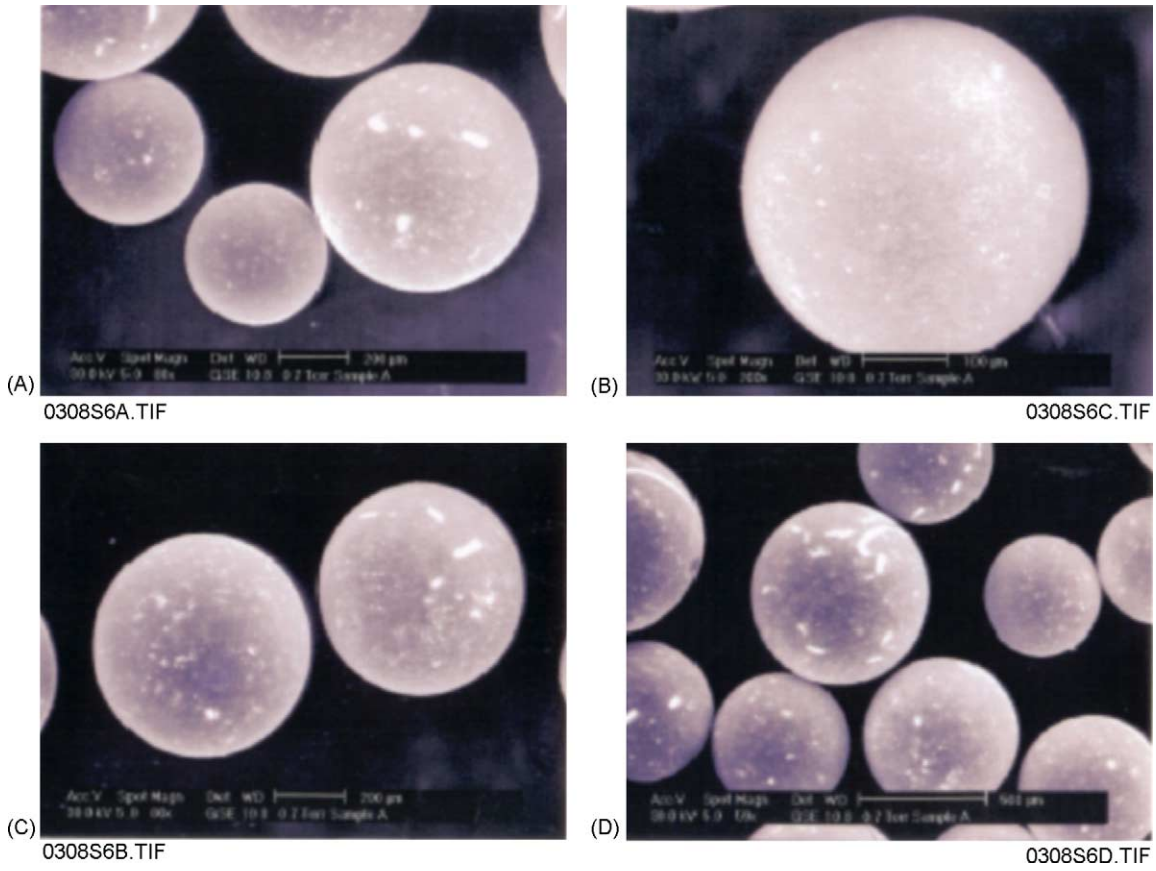
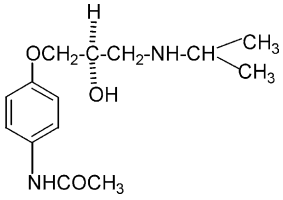
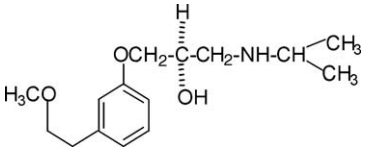
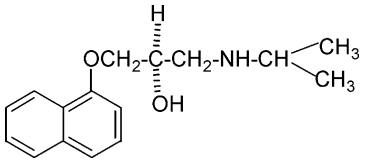


Fig. 3. Scanning electron microscopy of *m*-[(+)-α-methyl benzyl carboxamide] XAD-4 resin at the following original magnification: (A) top left: 200×; (B) top right: 59×; (C) bottom left: 80×; (D) bottom right: 80×.

Table 1
Enantioseparation of β-adrenergic blockers

Sr. no.	Structure of the drugs	Drugs	K_1	α	R_s
1		Atenolol	1.39	1.58	2.28
2		Metoprolol	2.16	1.34	1.83
3		Propranolol	3.58	1.49	3.00

Conditions: flow rate 0.8 mL/min; detection 254 nm; mobile phase sodium acetate–acetic acid buffer (pH 4.1):acetonitrile (4:6, v/v).

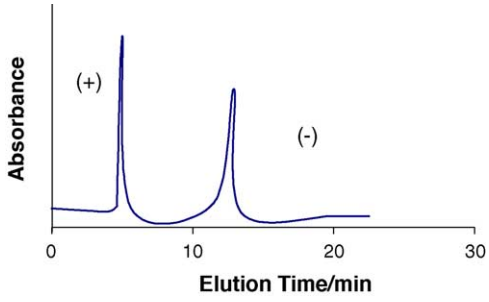


Fig. 4. Chromatogram of atenolol.

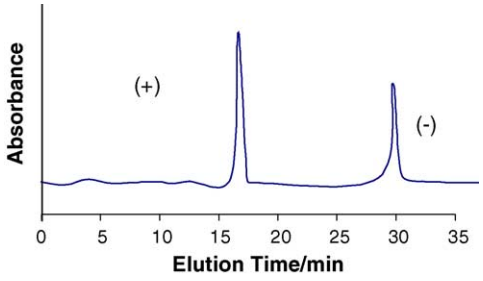


Fig. 6. Chromatogram of propranolol.

3.3. Stability of column/resin properties

The physico-chemical properties of resin is shown in Table 2 and were determined by methods described elsewhere [29]. These values suggest that resin structure remains rigid and can be used for column studies. The stability of synthesized *m*-[(+)-α-methyl benzyl carboxamide] XAD-4 was tested with acids (hydrochloric acid, sulphuric acid and nitric acid) and resin was found to be chemically stable and up to 4 M concentration with regard to acids. Since resin has amide functionality, it tends to hydrolyze at higher concentration of alkali (>5N NaOH). Also, it enables to have a high stability in a mobile phase with high aqueous phase content. To check the regenerating capacity of the resin, several loading and elution operations were subjected at optimum conditions for the given drugs. The resin showed good stability up to 65–70 replicate injections. Here, a loss in selectivity is less a question than a problem of irreversible adsorption of impurities blocking the active sites in the stationary

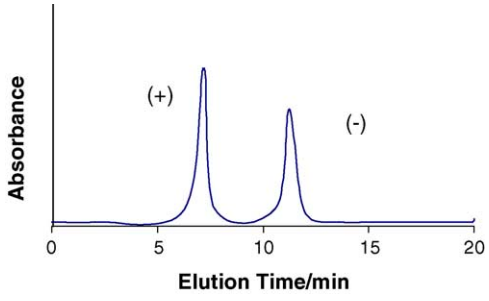


Fig. 5. Chromatogram of metoprolol.

Table 2
Physico-chemical properties of *m*-[(+)-α-methyl benzyl carboxamide] XAD-4 resin

Sr. no.	Parameters	Values
1	Moisture (%)	2.5
2	Swelling (W R/g)	0.506
3	Bulk density of dry resin (g cm^{-3})	0.225
4	Bulk density of swollen resin (g cm^{-3})	0.200
5	Void volume	0.60

Table 3
Accuracy and precision of the method for the determination of (*R*)- and (*S*)-atenolol ($n = 10$)

Concentrtion (mg mL^{-1})	(*R*)-atenolol			(*S*)-atenolol		
	Mean (mg mL^{-1})	RSD (%)	Accuracy (%)	Mean (mg mL^{-1})	RSD (%)	Accuracy (%)
Intra-assay precision						
45.00	45.08	1.19	100.17	45.03	1.10	100.06
30.00	29.96	1.85	99.86	29.94	1.95	99.80
20.00	19.94	1.91	99.70	19.94	1.70	99.70
10.00	10.03	1.60	100.30	9.97	1.93	99.70
2.00	1.96	1.75	98.00	1.97	1.20	98.50
Inter-assay precision						
45.00	44.96	1.85	99.91	44.92	2.85	99.82
30.00	29.08	2.25	96.93	29.24	1.99	97.46
20.00	19.79	2.40	98.95	19.94	2.64	99.70
10.00	9.96	2.91	99.60	9.92	1.79	99.20
2.00	1.92	2.60	96.00	1.94	2.53	97.00

phase. However, such a contaminated column can be washed with an acidic methanolic solution and regenerated for several times.

3.4. Effect of mobile phase

In this study, the influence of mobile phase composition on the separation of given drugs was investigated, since selectivity of the separation, retention time and solubility of the racemate often are very sensitive to changes of the composition of the mobile phase. It was observed that good separation was obtained by using a critical content of acetonitrile modifier, which reduced the tailing factor along with acetic acid–sodium acetate buffer which not only weakened the interaction of substrates with the polymer but also improved the efficiency by smoothing the surface of the stationary phase and modifying it by masking strong adsorption sites on the stationary phase, thus giving uniform separation. Hence, for these drugs, good separations were achieved using mixtures of aqueous sodium acetate–acetic acid buffer (pH 4.1):acetonitrile (4:6, v/v), which greatly improved the peak symmetry and eliminated the tailing problem.

3.5. Solute–sorbent interactions

Literature has revealed that enantioseparation may be possible via π–π complexation, hydrogen bonding, inclusion in hydrophobic pocket, dipole stacking, steric interactions or combinations thereof [34–36]. In the present study, the possibility of forming inclusion complexes is negligible compared to other decisive interactions such as hydrophobic, hydrogen bonding. In our case, the main adsorbing site in polymer is considered to be the amide group which has the ability to serve as either a donor or acceptor in the H-bonding and/or dipole stacking and hydroxy group of β-adrenergic blockers. Thus, enantioselective interactions may be due to charge-charge interaction, hydrogen bonding between hydroxy group of β-adrenergic blockers and -NH group of amide moiety of CSP, and steric interactions.

3.6. Accuracy and precision

Accuracy is defined as the ratio of the mean assayed concentration to that of the spiked concentration, expressed as a percentage. Tables 3–5 summarizes the mean accuracy data

Table 4
Accuracy and precision of the method for the determination of (*R*)- and (*S*)-metoprolol ($n = 10$)

Concentration (mg mL^{-1})	(*R*)-metoprolol			(*S*)-metoprolol		
	Mean (mg mL^{-1})	RSD (%)	Accuracy (%)	Mean (mg mL^{-1})	RSD (%)	Accuracy (%)
Intra-assay precision						
50.00	49.56	1.80	99.20	49.52	1.19	99.04
35.00	64.85	1.59	99.42	34.68	1.35	99.42
20.00	20.05	1.65	100.50	20.03	1.20	100.15
10.00	9.98	2.28	99.80	9.94	1.39	99.40
5.00	4.95	2.15	99.80	4.98	1.45	99.60
1.00	1.03	1.89	103.00	1.00	1.90	100.00
Inter-assay precision						
45.00	49.50	2.85	97.00	49.10	2.50	98.18
30.00	24094	2.54	99.14	24.62	2.48	98.88
20.00	19.89	2.95	98.65	19.85	2.65	98.90
10.00	10.10	1.98	101.00	10.10	1.99	101.00
2.00	9.92	1.99	98.40	4.75	1.85	99.00
1.00	0.98	2.98	99.00	1.01	2.07	101.00

Table 5
Accuracy and precision of the method for the determination of (*R*)- and (*S*)-propranolol ($n = 10$)

Concentr-ation (mg mL^{-1})	(*R*)-propranolol			(*S*)-propranolol		
	Mean (mg mL^{-1})	RSD (%)	Accuracy (%)	Mean (mg mL^{-1})	RSD (%)	Accuracy (%)
Intra-assay precision						
60.00	59.97	1.10	99.95	59.94	1.25	99.90
50.00	49.93	1.31	99.86	49.97	1.80	99.94
30.00	30.08	1.81	100.20	30.04	1.68	100.13
15.00	14.95	1.29	99.66	14.96	1.95	99.73
0.50	0.51	1.45	98.07	0.48	2.01	96.00
Inter-assay precision						
60.00	58.88	1.98	98.13	58.93	2.85	98.21
50.00	49.78	2.45	99.56	49.81	2.78	99.62
30.00	29.94	2.98	99.80	29.91	1.99	99.70
15.00	15.03	2.54	100.20	15.01	1.86	100.06
0.50	0.47	2.09	94.00	0.50	2.33	100.00

for (*R*)-atenolol and (*S*)-atenolol; (*R*)-metoprolol and (*S*)-metoprolol; and (*R*)-propranolol and (*S*)-propranolol, respectively. Inter and Intra run precision (Tables 3–5) was assessed by calculating the relative standard deviation of the control sample concentration measured in each validation run. The relative standard deviation for (*R*)- and (*S*)-atenolol varied from 1.19 to 1.91% and 1.10 to 1.95% for intra-assay precision and 1.85 to 2.91% and 1.79 to 2.85% for inter-assay precision, respectively. The relative standard deviation for (*R*)- and (*S*)-metoprolol varied from 1.80 to 2.28% and 1.19 to 1.90% for intra-assay precision and 1.99 to 2.98% and 1.99 to 2.65% for inter-assay precision, respectively. The relative standard deviation for (*R*)- and (*S*)-propranolol varied from 1.10 to1.81% and 1.25 to 2.01% for intra-assay precision and 1.98 to 2.98% and 1.99 to 2.85% for inter-assay precision, respectively.

4. Conclusions

A new platform based on nonionic polymeric beads endowed with homogenous surface chemistry is well suited for the engineering of a new generation of separation media. We have demonstrated an example that stationary phase prepared by attachment of a chiral selector to polymeric support gave excellent column stability with greatly improved chiral separation and afforded shorter retention times in the separation of given models. However, mobile phase constitution played a very important role in the separation under reversed phase condition.

Results have suggested that many inherent merits of synthetic polymer beads in general, and in particular, deserve more attention in the development of novel and more efficient stationary phases for chromatographic separation.

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