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Fast enantioseparations of basic analytes by high-performance liquid chromatography using cellulose *tris*(3,5-dimethylphenylcarbamate)-coated zirconia stationary phases

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Abstract

In this work, we study the influence of the mobile phase and column temperature on the enantioresolution of basic compounds on microparticulate porous zirconia coated with cellulose *tris*(3,5-dimethylphenylcarbamate) (CDMPC). The chiral analytes are amino compounds, including a number of β-blockers. Analytes are eluted with hexane–alcohol mobile phases. We investigated the effect of alcohol (type and concentration), basic eluent additives, and column temperature on the parameters that control resolution (column efficiency, retention and selectivity). Conditions for achieving an adequate separation in the least time have been determined for numerous racemic mixtures. For most solutes, baseline resolution of the enantiomeric pair was achieved in less than 1 min; 12 of 13 pairs were separated in less than 2 min.

Keywords: Chiral stationary phases, LC; Enantiomer separation; Mobile-phase composition; β-Blockers; Basic compounds; Cellulose *tris*(3,5-dimethylphenylcarbamate)

1. Introduction

It is widely known that individual enantiomers of chiral drugs can have very different pharmacological and toxicological activities. This has driven the promulgation of new and more stringent rules for chiral analysis by regulatory agencies for new chiral drugs [1,2]. Simultaneously, separation scientists have developed novel enantioselective analytical and preparative techniques to deal with the analysis of an increasing number of chiral compounds. As a result, there now exist many chiral stationary phases (CSPs) that have been developed for liquid [3,4], gas [5], and sub- and supercritical fluid chromatography [6–8]. Despite the variety of CSPs, fast, reproducible and efficient separations of enantiomers still constitute a challenge, due to the high degree of specificity of most CSPs. Of chiral HPLC methods, polysaccharide derivatives adsorbed on, or chemically bonded to, solid particles (usually aminopropylsilica) are perhaps the most widely used CSPs, due to their versatility in successfully separating chemically very diverse compounds [9,10], and due to their ap-

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plicability to both analytical- and preparative-scale separations.

Clearly, the main criteria in method development with CSPs is baseline resolution of enantiomers in the shortest possible analysis time, reproducibility in the analytical results (retention, selectivity and quantitative parameters) and overall method ruggedness. The most significant variables that can be used to reduce the analysis time are particle diameter and column length [11]. Since optimized analysis time is proportional to the square of the diameter of the particles that constitute the packing, and column efficiency is inversely proportional to the particle size, the use of short columns packed with smaller particles allows a given separation to be carried out in much less time [12]. Furthermore, the linear velocity at which column efficiency is maximized is inversely proportional to the particle diameter. As a result, analysis time can be greatly reduced by decreasing the particle size, shortening the column length and employing a relatively high eluent velocity. Finally, by taking advantage of the fact that the solvents usually employed as eluents under normal-phase conditions have relatively low viscosities, we are somewhat less constrained by column pump pressure-drop limitations.

In previous work [13], we described the preparation of cellulose *tris*-(3,5-dimethylphenylcarbamate) adsorbed onto 2.5 μm porous zirconia particles (CDMPC-coated zirconia). The optimum amount of chiral polymer that should be coated on the particles is about 3–4% (w/w) CDMPC. Higher loadings did not show any significant improvement in selectivity. On the other hand, according to nitrogen adsorption (BET) and column dynamics studies (plate height at different mobile-phase velocities), lower efficiencies are encountered above a 3% (w/w) polymer load. Furthermore, the zirconia columns proved to be very stable, even when a flow-rate as high as 4 ml/min of 2-propanol–*n*-hexane was employed as the eluent. Such relatively high pressure drops are far higher than those recommended for preserving the performance of silica-based polysaccharide columns [14]. Here, our main objective is to develop chiral analytical techniques for the fast separation of optically pure solutes and high-sensitivity enantiomeric purity determinations.

In the present work, we explore the separation of a group of basic compounds, including several β-blocking agents. Most β-blockers are administered as racemates, even though, in some cases, the relevant pharmacological activities of the individual enantiomers are not equally effective. Chemically, they all have, at least, one chiral center at the hydroxy-substituted carbon of the β-amino-alcohol function. Several strategies that allow the separation of β-blockers by HPLC methods have been proposed [15–21]. Vandenbosch et al. [22] published a very interesting and comprehensive study of resolution of several enantiomeric drugs, including a large number of amino-alcohols, by using HPLC techniques employing several chiral stationary phases and eluent compositions. Most of these studies focused on the variables (mobile and stationary phases) affecting the enantioseparation and resolution, but less importance has been paid to the separation time, which, in some cases, was quite long. An exception is the study carried out by Grieb et al. [23]. We first investigated several conditions that affect the chiral resolution of a number of basic compounds, including twelve β-blockers, on CDMPC-coated zirconia columns. The influence of mobile-phase conditions on the optimization of resolution and analysis time will be discussed. Finally, the very fast separation of the β-blockers and a number of other basic solutes is presented.

2. Experimental

2.1. Reagents

All chemicals used were obtained from commercial sources and were reagent-grade or better. *n*-Hexane, tetrahydrofuran (EM Sciences, Gibbstown, NJ, USA) and 2-propanol (Mallinckrodt, ChromAR, Paris, KY, USA) were of HPLC grade. *n*-Propanol, ethanol, *n*-butanol, *tert*.-butanol, diethylamine, triethanolamine, 2-(ethyl)-aminoethanol, 3,5-dimethylphenyl isocyanate, and pyridine were purchased from Aldrich (Milwaukee, WI, USA), and ethanolamine was obtained from Acros (Fisher, Pittsburgh, PA, USA). Avicel microcrystalline cellulose was obtained from Merck (Darmstadt, Germany). The molecular structures of the racemic compounds investigated in this study are shown in Fig. 1. All

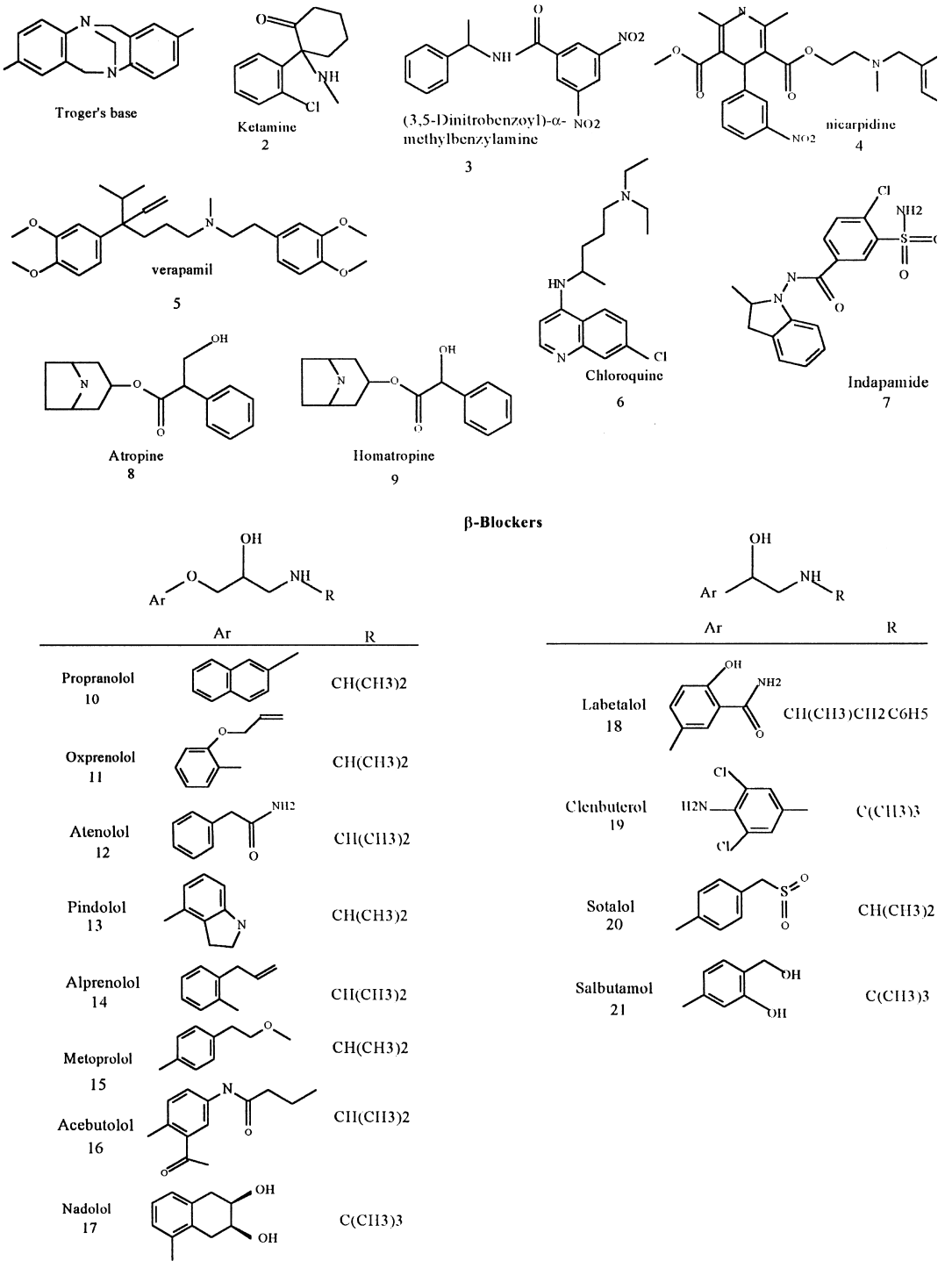

Fig. 1. Structures of the chiral compounds.

Table 1
Chromatographic results for chiral amino compounds on CDMPC-coated zirconia[a]

Solute	k_1'[b]		α[c]		R_s[d]		N[e]	
	NA[f]	A[f]	NA[f]	A[f]	NA[f]	A[f]	NA[f]	A[f]
1. Troger's base	0.69	0.63	1.19	1.17	1.2	1.0	4500	4500
2. Ketamine	0.78	0.67	1.14	1.18	0.8	1.2	3500	4700
3. DNBMB[a]	11.5	10.8	1.33	1.36	2.7	2.9	1700	1700
4. Nicarpidine	4.2	3.56	1.0	1.0	0	0	900	1100
5. Verapamil	2.4	1.65	1.0	1.0	0	0	800	2200
6. Chloroquine	7.91[g]	0.73	1.18	1.17	<1	<1	–	–
7. Indapamide	46.3[g]	–[h]	1.53	–[h]	<1	–[h]	–[g]	–[h]
8. Atropine	n.e.[i]	2.08	n.e.[i]	2.04	n.e.[i]	5.5	n.e.[i]	1700
9. Homatropine	n.e.	1.69	n.e.	1.37	n.e.	2.6	n.e.	2700
10. Propranolol	n.e.	1.98	n.e.	1.94	n.e.	5.7	n.e.	2500
11. Oxprenolol	n.e.	1.39	n.e.	4.63	n.e.	12.3	n.e.	2600
12. Atenolol	n.e.	37.8	n.e.	1.17	n.e.	1.0	n.e.	600
13. Pindolol	n.e.	11.4	n.e.	6.52	n.e.	15.5	n.e.	1700
14. Alprenolol	n.e.	0.58	n.e.	2.47	n.e.	5.7	n.e.	3300
15. Metoprolol	n.e.	1.00	n.e.	2.23	n.e.	6.1	n.e.	3300
16. Acebutolol	n.e.	5.41	n.e.	1.0	n.e.	0	n.e.	500[g]
17. Nadolol	n.e.	28.8	n.e.	1.23	n.e.	<1	n.e.	–[g]
18. Labetalol	n.e.	10.0	n.e.	1.0	n.e.	0	n.e.	1700
19. Clenbuterol	n.e.	0.89	n.e.	1.0	n.e.	0	n.e.	2100
20. Sotalol	n.e.	38.0	n.e.	1.0	n.e.	0	n.e.	–[g]
21. Salbutamol	n.e.	32.3	n.e.	1.0	n.e.	0	n.e.	–[g]

[a] Conditions: 7.5×0.46 cm column. Mobile phase, 2-propanol–*n*-hexane (10:90, v/v); flow-rate, 1 ml/min; temperature, 25°C. (a) (3,5-Dinitrobenzoyl)-α-methylbenzylamine. (b) Retention factor of the first-eluted enantiomer. (c) Separation factor. (d) Resolution factor. (e) Plate counts, computed as $5.54(t_r/w)^2$, where t_r and w represent retention time and the peak width at half-height, respectively. (f) NA and A denote eluent with no basic additive and with 0.05% (v/v) ethanolamine, respectively. (g) Highly tailed peaks. (h) Not tested under this condition. (i) n.e. denotes solute not eluted.

were purchased from Sigma (Saint Louis, MO, USA). Each enantiomeric mixture was prepared at a concentration of 0.5–1.0 mg/ml in *n*-hexane–2-propanol (8:2, v/v), and 1–2 μl of each solution were injected.

2.2. CDMPC–zirconia particles and chromatographic conditions

CDMPC-coated zirconia was prepared as reported previously [13]. Briefly, the chiral polymer was synthesized by reaction of an excess of 3,5-dimethylphenyl isocyanate in pyridine at 110°C. The carbamate derivative was isolated as the methanol-insoluble fraction [24]. The degree of substitution of cellulosic hydroxyl groups was estimated as 2.8 by elemental analysis. The chiral polymer, dissolved in tetrahydrofuran (THF), was added very slowly to a suspension of zirconia particles (particle diameter, 2.5 μm; surface area, 25 m^2/g; average pore diameter, 300 Å) in THF, with magnetic stirring of the suspension. The solvent was evaporated in a rotary vaporator and the particles were slurry packed at high-pressure (5000 p.s.i.) in 5×0.46 and 7.5×0.46 cm I.D. stainless-steel columns (Alltech Associates, Deerfield, IL, USA). Plate counts estimated with *trans*-stilbene oxide as a solute were 3800 and 6000 for the 5 and 7.5 cm columns, respectively. Several mobile phases were tested. Solutions were prepared by weighing the corresponding amine and the alcohol into a 1000-ml volumetric flask and filling to volume with filtered HPLC-grade *n*-hexane. Unless otherwise mentioned, the flow-rate was 1 ml/min. Dead volumes were measured by using 1,3,5-tri-*tert*.-butylbenzene as the marker [25].

2.3. Instrumentation

Chromatographic measurements were performed on a HP 1100 liquid chromatograph (Hewlett Pac-

kard, Palo Alto, CA, USA) equipped with a vacuum degasser, quaternary pump, autosampler, variable-wavelength UV detector and a computer-based HP Chemstation. The column was thermostated by the instrument and, typically, the temperature was set at 30°C. Detection was carried out at 254 nm.

3. Results and discussion

A systematic study of all of the variables that influence the resolution of several amino compounds using CDMPC-coated zirconia as the stationary phase was conducted. The type of eluent alcohol and its concentration in the mobile phase were varied, different basic mobile-phase additives were tested, and the influence of temperature on the separation was also explored. However, some preliminary screening experiments allowed us to focus better on the important optimization parameters and procedures. The results of these preliminary experiments led to some general observations, which are summarized in Table 1. First, the β-blockers, atropine and homatropine could not be eluted from the column unless a basic additive was present in the mobile phase. This indicates strong, and perhaps irreversible, adsorption of these analytes on the solid support. The adsorption of amino compounds in hydro-organic solvents on zirconia has been studied [26]; however, here we are dealing with a new situation due to the weakly polar nature of the eluent. Since zirconia's surface had been dehydroxylated before coating with the chiral polymer, the concentration of hydroxy-groups available for Brönsted acid–base interaction was very low. Zirconia's surface, due to the existence of unsatisfied coordination valences located at the zirconium centers, is also a strong, hard Lewis acid. We postulated that, under anhydrous conditions, molecules containing amino alcohol functionalities, and species containing only an amine group interact with these Lewis acid sites. This hypothesis was confirmed by injection of some racemic analytes (propranolol, labetalol and atropine) onto a column packed with bare zirconia and using only 2-propanol–*n*-hexane as the eluent. As expected, the peaks did not elute from this column (5×0.46 cm) after 60 min; however, they were eluted with virtually no retention when an eluent containing 0.05% (v/v) ethanolamine was used. Even, verapamil and nicarpidine, which can be eluted from CDMPC-coated zirconia in the absence of a basic additive in the mobile phase, were strongly adsorbed onto the bare support. On the other hand, indapamide was strongly retained on both CDMPC-coated zirconia and the bare support ($k'=43$), even when ethanolamine was present in the eluent, indicating that the amines used as additives are not able to completely block the interaction between this analyte and the Lewis-acid sites on zirconia's surface. This solute, which strongly adsorbs onto the support, is an example of retention on the achiral non-stereoselective sites of the column. The presence of the chiral polymer (CDMPC) provides sites for enantiorecognition, and, in this particular case, any mobile phase that decreases the strong interactions with the 'inert' support will induce an increase in the selectivity factor.

Second, Table 1 gives the retention and separation factors obtained by using a CDMPC-coated zirconia column and a mixture of 2-propanol–*n*-hexane–ethanolamine (10:90:0.05, v/v) as the eluent. In Table 1, we only show the results under a single mobile-phase condition; however, six racemates could not be resolved under any conditions tested in this work. Two of these analytes (verapamil and nicarpidine) could not been resolved using CDMPC coated on aminopropylsilica [15]. Interestingly, those β-blockers that could not be separated here (solutes 18 to 21) have a common chemical characteristic, i.e., they lack the ether function between the hydroxyl and the aryl groups. Bargmann-Leyder et al. [27] previously stated that, under typical LC conditions, the hydroxyl and the ether, but not the amino group, of the β-amino-alcohols are needed for chiral recognition. An exhaustive search of the literature on enantioseparations of β-blockers on CDMPC indicates that the basic oxygen atom is necessary (but not sufficient) for successful resolution with this type of CSP. This suggests that the ether oxygen is an active participant in the chiral recognition process with this CSP, probably through a hydrogen-bonding interaction between the ether oxygen of the analyte and the N–H carbamate group of the CDMPC. We should understand that the mere presence of the functional groups in the analyte and in the CSP is not sufficient to enable enantiorecognition. Given that

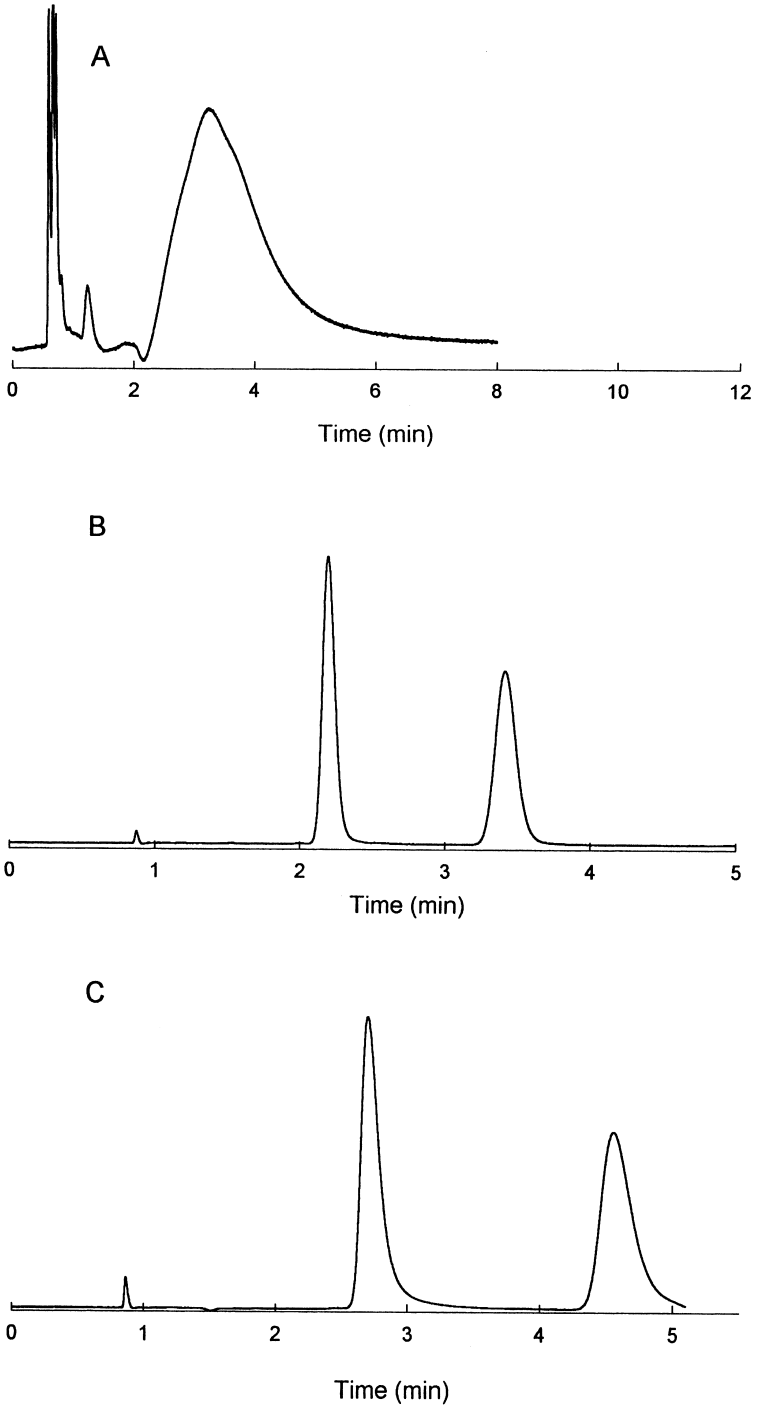

Fig. 2. Chromatogram of propranolol eluted from CDMPC-coated zirconia. Column dimension: 7.5×0.46 cm I.D.; mobile phase, 2-propanol–*n*-hexane (10:90, v/v) mixture containing 0.2% (v/v) of a basic additive; flow-rate, 1 ml/min; Column temperature, 25°C. Type of additive: (A) diethylamine; (B) ethanolamine and (C) 2-(ethyl)aminoethanol.

both amylose and cellulose carbamates have the same chemical composition, if the mere presence of the ether function were the only requirement, both should provide similar enantioselectivities. However, the α-values of most β-blockers are significantly lower on the amylose-based CSP than on the cellulose-based CSP. In contrast, both the sotalol [15] and labetalol [14] racemates are examples of β-blockers that lack the ether groups and are well separated on amylose carbamate. This provides strong confirmation of the importance of the higher-order structure of these two carbohydrate polymers in the enantiorecognition mechanism of β-blocker drugs.

3.1. Effect of the additive type

As mentioned above, the addition of a small amount of amine as the eluent additive is critical for the elution of many analytes. Consequently, we first tested the effect of a variety of amine additives [at a fixed concentration of 0.2% (v/v)] on elution. Highly distorted peaks were observed when diethylamine, triethylamine and triethanolamine were used as the additive. Fig. 2 shows chromatograms of propranolol obtained in a mixture of 2-propanol–*n*-hexane (20:80, v/v) with diethylamine. These anomalous elution profiles must be attributed to the interactions of the solutes with the zirconia support, which are not completely suppressed when diethylamine is added to the solvent. However, peak shapes and overall efficiency were drastically improved when the above amines were replaced with ethanolamine or 2-(ethyl)aminoethanol, as shown in Fig. 2B and C. Similar results were obtained with the remaining solutes. It is difficult to establish an explanation for this difference; however, we believe it is very probable that both the amine functionality and the adjacent hydroxyl group of the amino-alcohol simultaneously interact with the Lewis-acid sites, and, as a consequence, strongly anchor the analyte to the surface, thereby preventing the separation. Only the presence of an additive, which is chemically highly analogous to the solutes, can compete for adsorption on the support, and thus suppress the interaction of amino-alcohols with the zirconia's surface.

A comparison between separations obtained with ethanolamine and 2-(ethyl)aminoethanol as additives is given in Fig. 3. As can be observed, despite the slightly larger selectivities for some analytes achieved when 2-(ethyl)aminoethanol was used as the additive, all solutes showed improved peak symmetries and efficiencies when the mobile phase

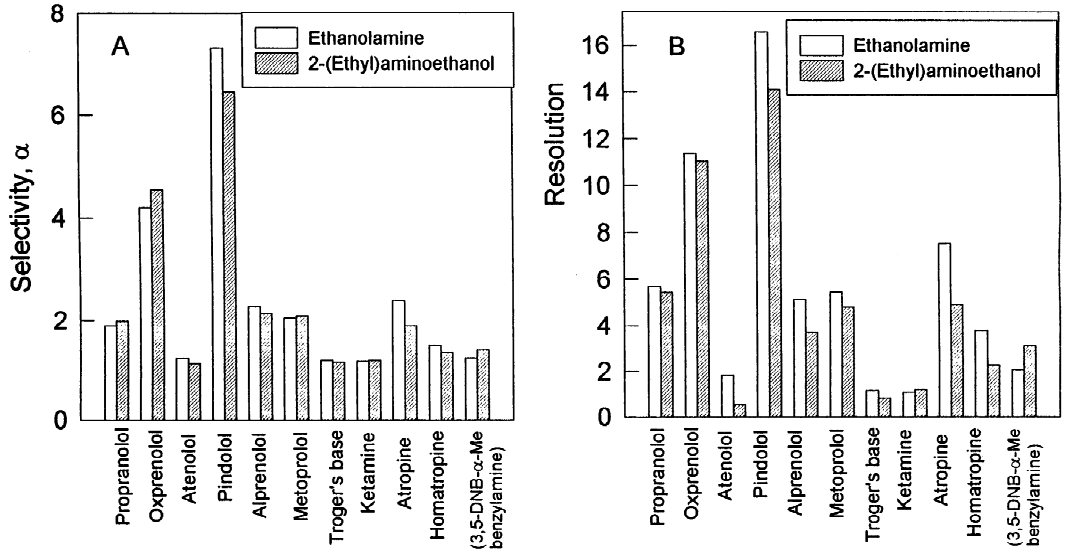

Fig. 3. Comparison between enantioseparations obtained with ethanolamine and with 2-(ethyl)aminoethanol as the basic additive. Chromatographic conditions: 7.5×0.46 cm CDMPC-coated zirconia column; mobile phase, 2-propanol–*n*-hexane–basic additive (10:90:0.2, v/v); flow-rate, 1 ml/min; column temperature, 25°C. (A) Separation factors and (B) resolution factors.

contained some ethanolamine and, consequently, the resolution of practically all racemic mixtures are better with ethanolamine than with 2-(ethyl)aminoethanol. Thus, ethanolamine was chosen as the additive for all subsequent work.

3.2. Effect of the amount of additive

We next examined the effect of the amount of ethanolamine on the enantioseparations. An increase in the volume fraction of ethanolamine monotonically decreased the retention factors of both forms of all analytes. Fig. 4 shows the selectivity and resolution factors as a function of the amount of ethanolamine in the eluent. For clarity, we divided the solutes into two groups. An increase in the ethanolamine concentration from 0.05 to 0.2–0.3% led to a reduction in peak tailing, enhancement in the symmetry and, consequently, in the resolution. Except for atenolol and nadolol, ethanolamine levels higher than 0.3% (up to 0.5%) had no additional effect on either peak shape or resolution. The influence of increasing amounts of ethanolamine is better seen in Fig. 5, which shows the resolution of the enantiomers of

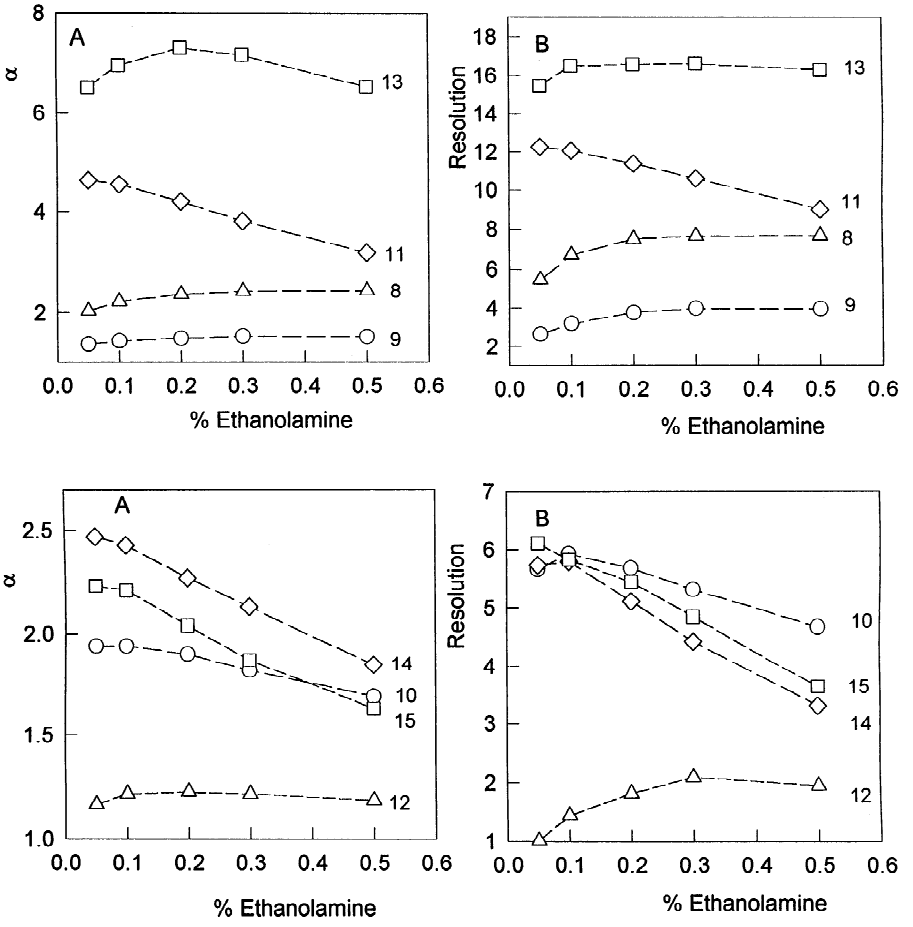

Fig. 4. Enantioseparation of basic compounds versus the amount of ethanolamine. Chromatographic conditions: 7.5×0.46 cm CDMPC-coated zirconia column. For solute number, see Fig. 1. Mobile phase, 2-propanol–*n*-hexane (10:90)% with the indicated volume % of ethanolamine; flow-rate, 1 ml/min; column temperature, 25°C.

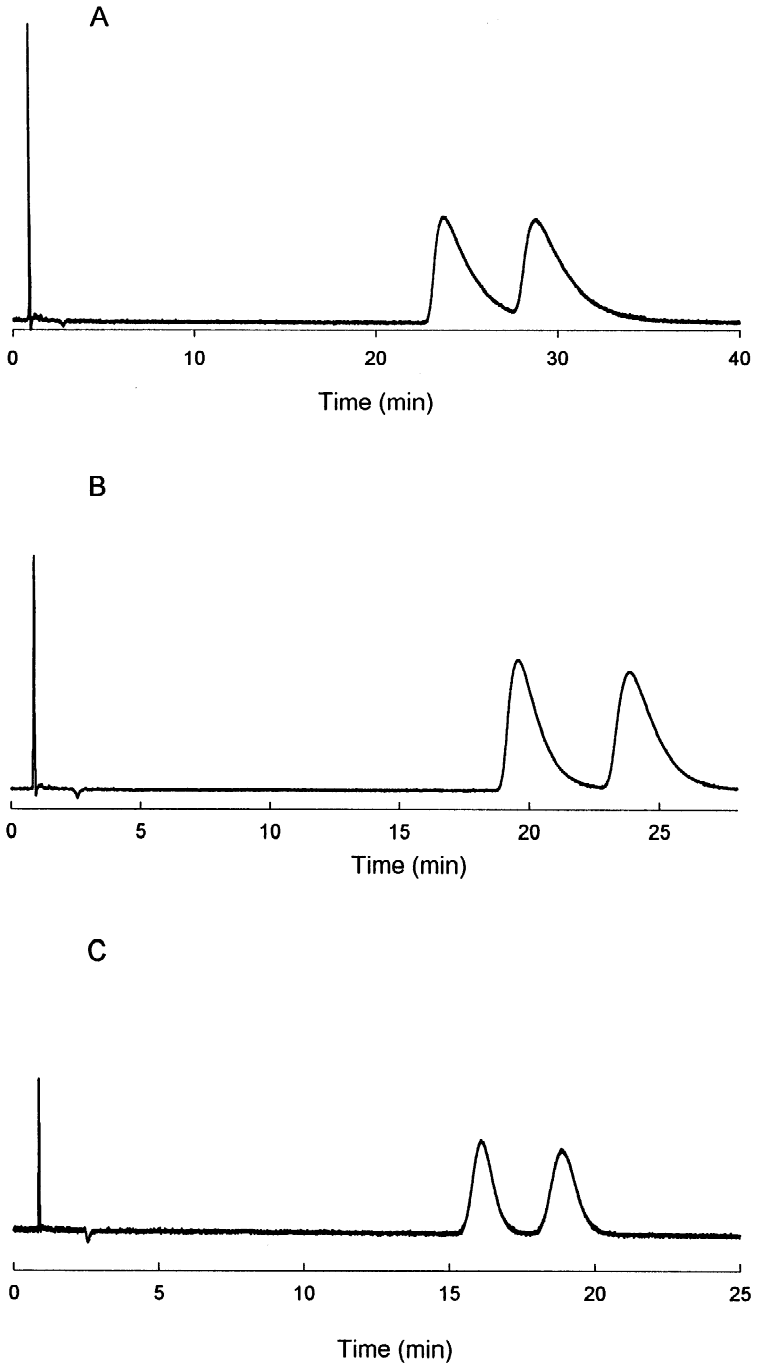

Fig. 5. Chromatograms of atenolol eluted from CDMPC-coated zirconia. Column dimensions: 7.5×0.46 cm I.D. Mobile phase, 2-propanol–*n*-hexane (10:90, v/v) mixture containing increasing amounts of ethanolamine; flow-rate, 1 ml/min; column temperature, 25°C. (A) 0.1%; (B) 0.2% and (C): 0.5% (v/v).

Table 2
Influence of the type of eluent alcohol on the retention and separation of amino compounds on CDMPC-coated zirconia[a]

Solute		Eluent alcohol				
		Ethanol	*n*-Propanol	*n*-Butanol	2-Propanol	*t*-Butanol
Troger's base	k_1'[b]	0.49	0.50	0.52	0.55	0.66
	α[c]	1.27	1.28	1.14	1.19	1.0
	R_s[d]	1.5	1.5	<1	1.2	0
	N[e]	5100	4900	–[f]	4700	–[f]
Ketamine	k_1'	0.59	0.55	0.64	0.65	0.81
	α	1.1	1.2	1.0	1.2	1.0
	R_s	<1	1.0	0	1.07	0
	N	–[f]	4700	2100	4700	2800
DNBMB[a]	k_1'	4.84	5.66	7.31	10.9	25.14
	α	1.32	1.15	1.0	1.23	1.0
	R_s	3.1	1.4	0	2.1	0
	N	2800	2200	1900	1900	2200
Chloroquine	k_1'	0.55	0.50	0.63	0.62	1.04
	α	1.3	1.0	1.0	1.3	1.0
	R_s	1.45	0	0	<1	0
	N	3600	–[f]	–[f]	–[f]	2100
Indapamide	k_1'	35.4	53.7	n.e.[g]	n.e.[g]	n.e.[g]
	α	1.56	1.27	–	–	–
	R_s	4.0	2.0	–	–	–
	N	2100	1400	–	–	–
Atropine	k_1'	1.46	1.48	1.97	1.87	2.89
	α	1.95	2.22	2.01	2.38	2.11
	R_s	6.4	7.1	6.4	7.5	7.1
	N	3500	3100	3200	2200	2600
Homatropine	k_1'	1.21	1.19	1.78	1.56	2.61
	α	1.34	1.40	1.52	1.49	1.36
	R_s	2.7	2.9	4.1	3.8	3.1
	N	4400	3800	3600	3600	3200
Propanolol	k_1'	1.17	1.49	1.99	1.62	3.12
	α	1.30	1.66	1.68	1.90	1.91
	R_s	2.2	4.2	4.2	5.7	5.9
	N	3800	2700	2400	3000	2200
Oxprenolol	k_1'	0.80	0.98	1.02	1.14	1.97
	α	2.17	3.44	3.92	4.20	3.87
	R_s	6.9	9.4	9.9	11.4	10.6
	N	4000	3000	2600	3000	2000
Atenolol	k_1'	10.1	13.1	21.4	22.5	56.8
	α	1.13	1.19	1.10	1.23	1.15
	R_s	1.3	1.8	1.3	1.8	1.9
	N	2400	1900	3300	1400	2900
Pindolol	k_1'	4.93	6.91	12.0	9.88	26.0
	α	3.16	5.87	8.39	7.31	5.41
	R_s	12.2	15.3	11.2	16.6	9.4
	N	3400	2500	2300	2500	2300
Alprenolol	k_1'	0.50	0.44	0.47	0.49	0.52
	α	1.20	1.89	1.97	2.27	2.05
	R_s	1.6	4.5	3.4	5.1	4.4
	N	5200	3600	2200	3900	2100

Table 2. Continued

Solute		Eluent alcohol				
		Ethanol	*n*-Propanol	*n*-Butanol	2-Propanol	*t*-Butanol
Metoprolol	k_1'	0.69	0.67	0.86	0.85	1.40
	α	1.49	1.92	1.38	2.04	1.53
	R_s	2.9	4.5	2.4	5.4	3.6
	N	4500	3800	3800	3700	3400
Acebutolol	$k_1'^{h}$	20.9	4.56	5.34	5.12	7.32
	α	1.09	1.00	1.00	1.00	1.00
	R_s	<1	0	0	0	0
	N	–[f]	–[f]	–[f]	–[f]	–[f]
Nadolol	k_1'	5.52	7.58	11.4	13.6	31.1
	α^{i}	1.05	1.11	1.11	1.0	1.21
	R_s^{i}	<1	<1	1.1	0	1.8
	N	–[f]	1800	2200	–[f]	1600

[a] Conditions: 7.5×0.46 cm column; mobile phase, alcohol–*n*-hexane–ethanolamine (10:90:0.2, v/v); flow-rate, 1 ml/min; temperature, 25°C. (a) (3,5-Dinitrobenzoyl)-α-methylbenzylamine. (b) Retention factor of the first eluted enantiomer. (c) Separation factor. (d) Resolution factor. (e) Plate counts, computed as $5.54(t_r/w)^2$, where t_r and w represent retention time and half-height peak width, respectively. (f) Not computed (g) n.e. denotes not eluted; (h) mobile-phase composition, hexane–ethanol–ethanolamine (97:3:0.2, v/v). (i) Separation factor and resolution of the more difficult pair.

atenolol in mobile phases containing 0.1, 0.2 and 0.5% ethanolamine in 2-propanol–*n*-hexane (10:90, v/v) mixtures.

3.3. Influence of the strong solvent type

Table 2 presents the results of separations obtained by changing the type of alcohol in the eluent. As the polarity of the strong solvent was decreased, the retention times of all analytes increased. However, a plot of retention factor versus polarity (expressed as the alcohol's solubility parameter) is far from linear (not shown). In principle, the reduction in the retention factors due to the presence of alcohol in the mobile phase could be due to: (a) competitive interactions of the strong solvent with the analyte for the polar chiral sites; (b) increases in the solubility of solutes in the mobile phase; (c) changes in the structures of the chiral cavities between the polysaccharide chains, due to different degrees of swelling in the different strong solvents; (d) a mixture of (a), (b) and/or (c). We believe that hypothesis (b) is not correct, or at least is not dominant. This is based on the fact that, while 2-propanol and *tert*.-butanol have very similar solubility parameters (23.5 and 21.7 $J^{1/2}$ $cm^{-3/2}$, respectively [28]), the retention factors of most analytes in *tert*.-butanol are more than twice as large as those observed in 2-propanol at the same concentration in the eluent.

Rather, we believe that, compared to 2-propanol, the access of the sterically more hindered *tert*.-butanol to the chiral cavities in the CSP is more limited, differentially affecting the enantioseparation process [29]. In general, β-blockers, atropine, and homatropine show higher selectivities and better resolution when either 2-propanol or *tert*.-butanol is used as the strong solvent. On the other hand, the separation factors of Troger's base, indapamide, and (3,5-dinitrobenzoyl)-α-methylbenzyl amine were all higher using ethanol as the strong eluent. The comparison also shows that, in all cases, the use of ethanol improved the column's efficiency (plate counts are from 10 to 80% higher in hexane–ethanol mixtures).

More interesting is the fact that, with 1-butanol and *tert*.-butanol, this column is able to baseline resolve the four enantiomers of nadolol. The chromatograms obtained with 1-butanol, 2-propanol and *tert*.-butanol as strong solvents are shown in Fig. 6. Solute 17, commercially known as nadolol, has three asymmetric carbons with two adjacent ring hydroxy groups in a *cis*-configuration, hence, the mixture consists of equal amounts of the four enantiomers. To the best of our knowledge, only

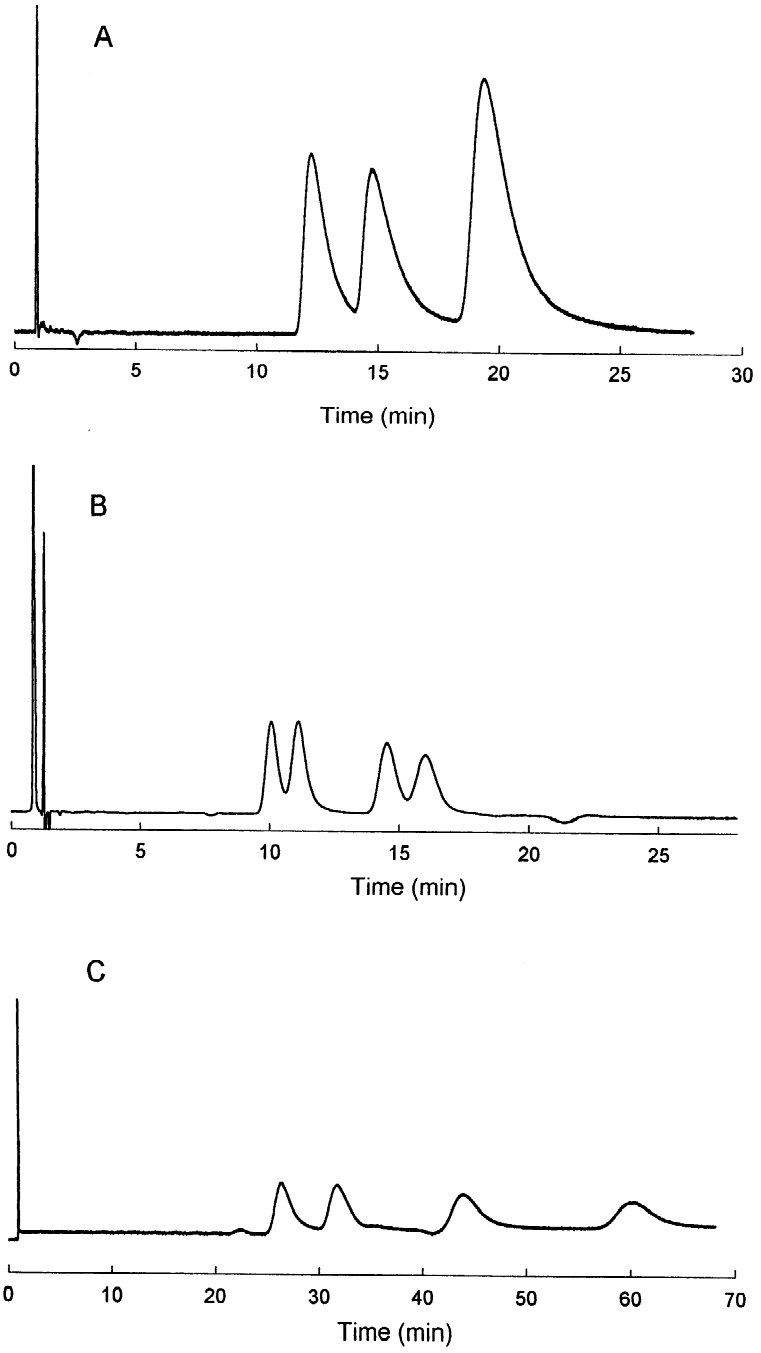

Fig. 6. Chromatogram of nadolol eluted from CDMPC-coated zirconia. Column dimensions: 7.5×0.46 cm I.D. Mobile phase: alcohol–*n*-hexane–ethanolamine (10:90:0.2) v/v mixture; flow-rate, 1 ml/min; column temperature, 25°C. Alcohols: (A) 2-propanol; (B) 1-butanol and (C) *tert*.-butanol.

three of these four species had been resolved previously with cellulose *tris*-(3,5-dimethylphenylcarbamate) [30,31]. McCarthy reported the separation of the four enantiomers of nadolol by using amylose *tris*-(3,5-dimethylphenylcarbamate) [32].

3.4. Effect of the amount of 2-propanol

Concentrations from 5 to 30% (v/v) 2-propanol in *n*-hexane were studied. As expected, a decrease in the 2-propanol concentration increased the retention factors of both enantiomers. However, as is shown in Fig. 7, these changes did not always improve the selectivity and resolution. The selectivity of the less retained pairs (alprenolol, for instance) is practically independent of the amount of 2-propanol. However, for most pairs, as the amount of alcohol is increased, the retention of the more retained enantiomer decreases more than that of the less retained enantiomer and, thus, selectivity decreases. Most of the decrease in resolution comes from the decreases in retention factors as the concentration of 2-propanol is increased, and not from the decrease in selectivity.

3.5. Influence of column temperature

An increase in column temperature had a negative effect on the separation of all racemates studied. In Table 3, retention, enantioselectivity and the resolution factor of some selected solutes at several

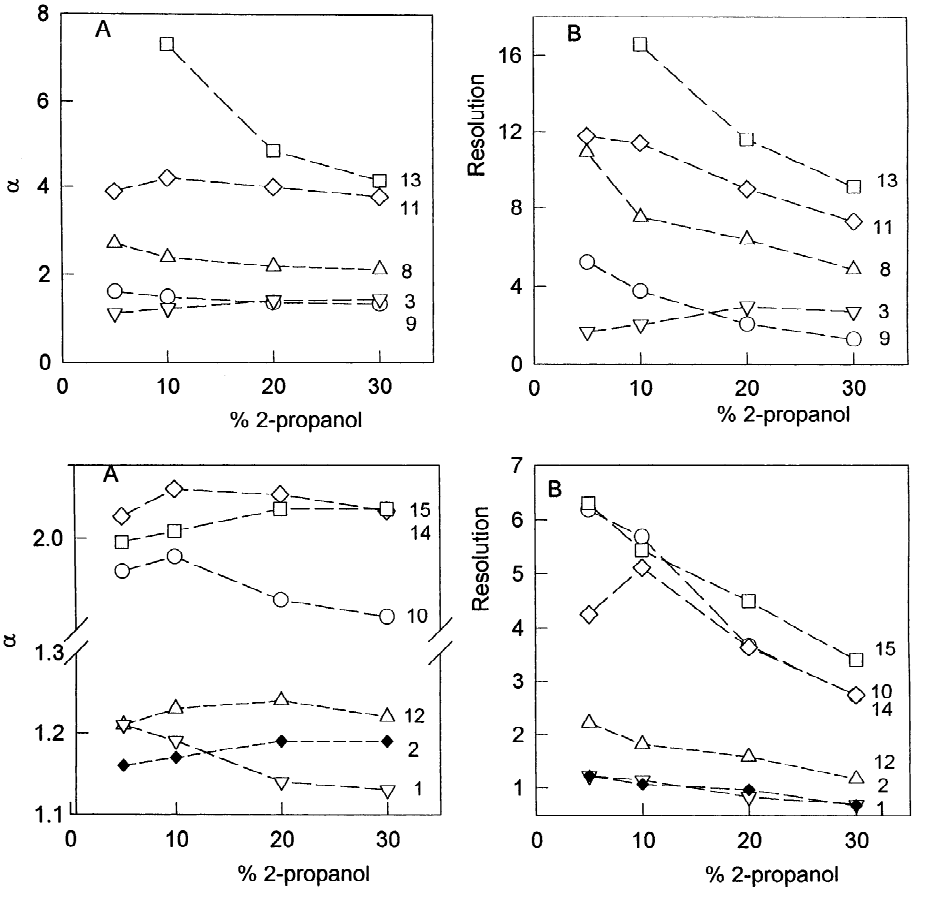

Fig. 7. Enantioseparation of basic compounds versus the amount of 2-propanol. Chromatographic conditions: 7.5×0.46 cm I.D. CDMPC-coated zirconia column. For solute number, see Fig. 1. Mobile phase, 2-propanol–*n*-hexane mixtures containing 0.2% (v/v) ethanolamine; flow-rate, 1 ml/min; column temperature, 25°C. (A) Separation factors; (B) resolution factors.

Table 3
Influence of temperature on retention and separation of amino compounds on CDMPC-coated zirconia[a]

Solute		Temperature (°C)				
		30	40	50	60	70
Atropine	k_1'[a]	1.80	1.31	1.19	1.05	0.93
	α[b]	2.30	1.89	1.71	1.56	1.45
	R_s[c]	4.1	3.2	2.8	1.9	1.1
Homatropine	k_1'	1.30	1.08	1.02	0.96	–[d]
	α	1.51	1.36	1.26	1.17	–
	R_s	2.4	1.4	<1	<1	–
Propranolol	k_1'	1.41	1.23	1.15	1.05	0.96
	α	1.84	1.74	1.58	1.54	1.38
	R_s	3.9	3.2	2.6	2.1	1.1
Oxprenolol	k_1'	0.98	0.77	0.57	0.43	–[d]
	α	4.29	3.33	2.92	1.78	–
	R_s	5.3	4.7	4.0	2.9	–
Atenolol	k_1'	18.20	15.14	14.0	12.82	11.93
	α	1.26	1.21	1.20	1.18	1.15
	R_s	1.6	1.4	1.4	1.2	<1
Pindolol	k_1'	8.13	7.00	6.20	5.52	4.97
	α	7.18	5.90	4.43	3.59	2.99
	R_s	12.4	11.9	10.9	9.9	8.7
Nadolol	k_1'	11.21	10.39	10.28	9.99	–[d]
	α[e]	1	1.0	1.12	1.0	–
	R_s[e]	0	0	<1	0	–

[a] Conditions: 7.5×0.46 cm column; mobile phase, 2-propanol–*n*-hexane–ethanolamine (10:90:0.2, v/v); flow-rate, 1 ml/min. Plate counts were practically constant for all analytes in this range of temperature. (a) Retention factor of the first-eluted enantiomer. (b) Separation factor. (c) Resolution factor. (d) Solute not tested under this condition. (e) Separation factor and resolution of the more difficult pair.

temperatures within the range of 30 to 70°C are presented. Both retention and selectivity decreased as the temperature was raised; however, the column efficiency did not significantly improve upon increasing the temperature. This unexpected behavior is probably due to the fact that the experimental flow-rate (1 ml/min) corresponds approximately to the minimum in the Knox plot. At this mobile-phase velocity, the band-broadening process is mainly dominated by eddy dispersion. Although the effect of temperature on the Knox *A*-coefficient is still controversial, it is accepted that its influence is not significant; therefore, column efficiency is only slightly affected by column temperature. Table 3 shows that, at 50°C, the four enantiomers of nadolol can also be separated; however, in this case, the worst resolution (corresponding to the second- and third-eluted peaks) was slightly lower than one. A longer column should improve the resolution of the four peaks under these conditions. Finally, it should be mentioned that no changes in solute retention were detected after operation of the column up to 70°C.

3.6. Fast resolution of amino compounds

In principle, higher resolution can always be achieved using a longer column, but the cost is a proportional increase in analysis time. Another criterion for optimization suggests that, if resolution is already adequate, optimization implies making the separation as fast as possible. The optimization criteria followed in this study were to set a threshold value for resolution, above which, the results are deemed acceptable, and then minimize the analysis time. Usually, a resolution factor of 1.5 is enough to

baseline-resolve equal size Gaussian peaks; however, a more conservative criterion indicates the choice of a resolution factor of two. This also allows the quantification of enantiomeric purity, in which one enantiomer is at a trace levels and the other is very often above the linear range of the isotherm. For most enantiomeric pairs studied here, the application of this criterion was possible since there exists at least one set of conditions under which the resolution was higher than two. Moreover, providing that the viscosity of the mixtures used as eluents is very low [estimated as 0.40 cP for 2-propanol–*n*-hexane

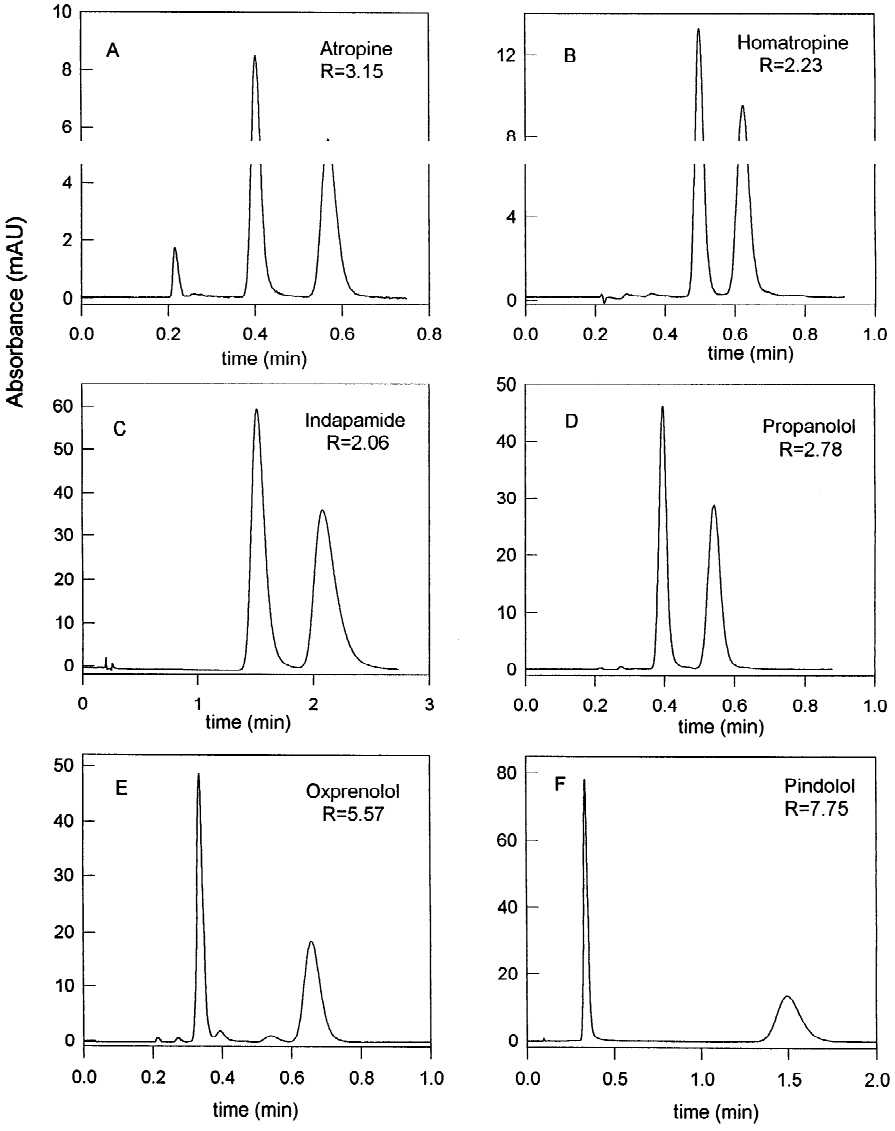

Fig. 8. Fast enantioseparation of basic compounds on CDMPC-coated zirconia. Conditions: flow-rate, 4 ml/min; column temperature, 25°C (unless otherwise mentioned). (A), (D), (E), (G) and (I) *n*-hexane–2-propanol–ethanolamine (80:20:0.2) % (v/v); (B) *n*-hexane–2-propanol–ethanolamine (90:10:0.3) % (v/v); (C) *n*-hexane–ethanol–ethanolamine (80:20:0.4) % (v/v), temperature, 50°C; (F) *n*-hexane–2-propanol–ethanolamine (70:30:0.2) % (v/v); (H) *n*-hexane–2-propanol–ethanolamine (90:10:0.3) % (v/v); (J) *n*-hexane–*tert*.-butanol–ethanolamine (90:10:0.2) % (v/v).

(10:90, v/v)], and that the stationary-phase coating is extremely stable at 4 ml/min, we are able to increase the eluent flow-rate to speed up the analysis.

In previous work, we carried out a dynamic study on columns packed with this chiral polymer and estimated the Knox coefficients. That study indicated that the optimum reduced plate height corresponded to a flow-rate of about 0.8 ml/min. A single calculation with the previously determined Knox coefficients indicated that an increase in the flow-rate from the optimum to 4 ml/min would decrease the estimated plate counts by about 40% of that obtained at the optimum reduced velocity and, hence, only decrease the resolution by only about 25%. Provided that the resolution stays above two, the analysis time can be substantially reduced by increasing the flow-rate. Fig. 8 shows chromatograms of several β-blockers and other amino compounds collected under the optimum mobile-phase conditions in each case, and then at a flow-rate of 3 or 4 ml/min. In most cases, the resolution factor was higher than two and the chromatographic analysis time was under 1 min, which is, in most cases, more than one order of magnitude faster than previously reported results.

4. Conclusions

The general conclusions obtained from this study are as follows:

1. Enantioresolution of numerous amino compounds from a column packed with 2.5 μm CDMPC-coated zirconia particles was achieved by optimizing the type and amount of strong solvent and the type and amount of basic additive. In all cases, resolution was better at room temperature than at higher temperatures.
2. The separation of the β-blockers, which possess an ether function adjacent to the aryl group, was feasible using CDMPC, whereas those amino-alcohols that do not have the ether bridge could not be resolved. This observation suggests that the

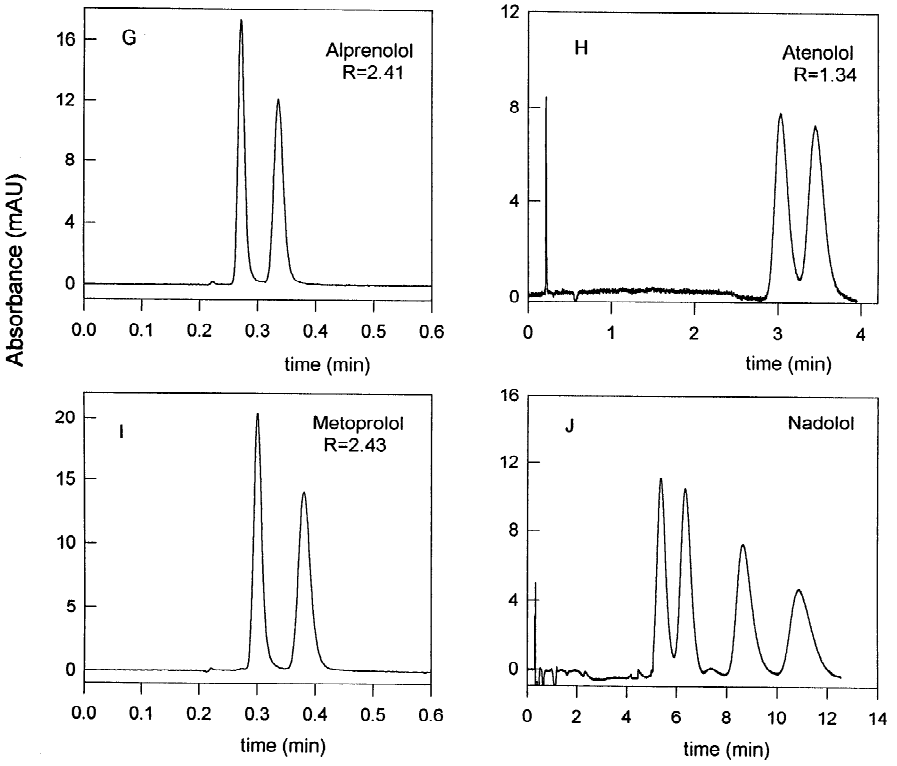

Fig. 8. (*continued*)

ether oxygen atom is an active participant in the enantiorecognition process.
3. The four enantiomers of nadolol were successfully baseline-separated by using 1-butanol or *tert*.-butanol as the strong solvent and a 7.5-cm column packed with CDMPC-coated zirconia.
4. The combination of small particle-diameter and short column-length with higher linear velocities of the mobile phase allowed us to achieve resolution factors higher than two in less than 1 min for most amino compounds studied.

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