Notes

Effects of Diethylamine on Capillary Chromatographic Enantioseparation of Some Chiral Analytes Using Polysaccharide Stationary Phases with Pure Polar Solvents as Mobile Phases

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Polysaccharide derivatives are most widely used chiral stationary phases for the HPLC separation of enantiomers in both analytical and preparative scale. Originally, polysaccharide-type stationary phases were developed for normal phase HPLC with mobile phase consisting of hexane and alcohol, usually 2-propanol or ethanol.[1-8] However, some polar organic solvents, such as methanol, ethanol or acetonitrile have been used effectively on these stationary phases in recent days.[5,9-12] Polar organic solvents may offer the advantages of alternative chiral recognition mechanisms, higher solubility of some analytes and having less environmental problems.[11] Though the separation mechanisms for polysaccharide-type stationary phases are not completely known, the contribution of hydrogen bonding and hydrophobic interaction to retention is commonly accepted.[13-17] A number of studies dealt with the effects of acidic and basic additives to improve the enantioseparation by changing the interaction with derivatized polysaccharide stationary

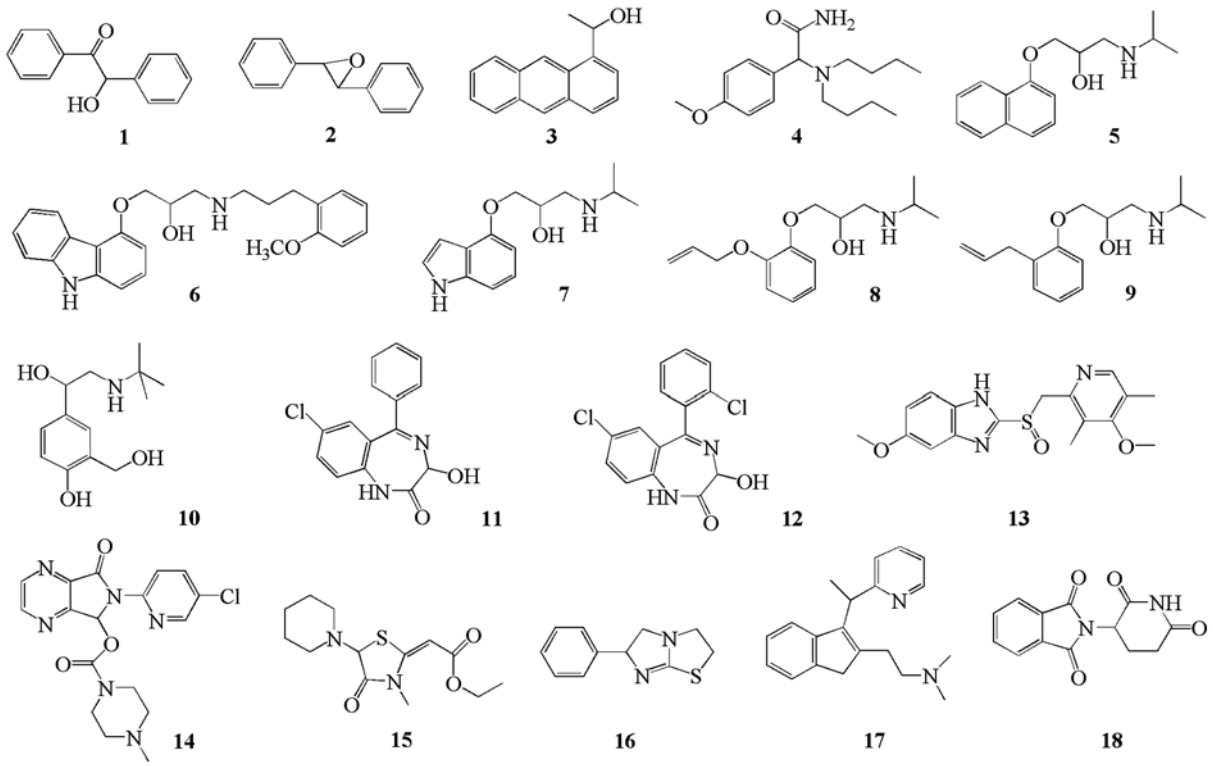

Figure 1. Structure of chiral analytes used in this study: 1. benzoin, 2. *t*-stilbene oxide, 3. anthryl ethanol, 4. ambucetamide, 5. propranolol, 6. carvedilol, 7. pindolol, 8. oxprenolol, 9. alprenolol, 10. salbutamol, 11. oxazepam, 12. lorazepam, 13. omeprazole, 14. zopiclone, 15. etozolin, 16. tetramisole, 17. dimetinden, 18. thalidomide.

phases.[2,5,8,18-23] Most of these studies focused on the effects of additives when polysaccharide-type stationary phases were used in normal mode. Hence, the effects of additives in hexane with various kinds of alcohols were well established. However, there has been no report describing the effects of diethylamine as an additive on the enantioseparation in the pure organic mobile phases, such as methanol or acetonitrile.

In the present study, the effects of diethylamine as an additive in the pure polar mobile phases were evaluated on the separation of some chiral analytes by capillary chromatography used cellulose tris(3,5-dimethylphenylcarbamate) (CDMPC) and amylose tris(3,5-dimethylphenylcarbamate) (ADMPC) as chiral stationary phases.

Experimental Section

Materials and reagents. Chiral analytes (Fig. 1) were from various commercial sources and used without any further purification. HPLC grade methanol, acetonitrile and water were from Roth GmbH (Germany). Tetrahydrofuran, diethylamine (DEA) and pyridine were from J. T. Baker (The Netherlands). 3,5-Dimethylphenylisocyanate was supplied from Aldrich (Germany). Microcrystalline cellulose (Avicel) and silica gel (LiChrospher 1000, 5 μm) were from E. Merck (Germany). Amylose B (MR ≈ 16,000) was purchased from Nacalai Tesque (Japan).

Preparation of capillary columns. CDMPC and ADMPC were prepared and isolated as methanol insoluble fractions as described.[24,25] The polysaccharide derivatives were dissolved in tetrahydrofuran and coated on aminopropylsilanized silica gel by a static technique. Fused-silica capillaries of 100 μm ID from Polymicro Technologies (Phoenix, AZ, USA) were used for the preparation of packed capillaries. The inlet-end of the capillary was connected to a HPLC-precolumn (4.6 × 50 mm) which served as a reservoir for the slurry of the packing material. A commercially available HPLC column frit was connected to the outlet-end of the capillary in order to retain the packing material. The slurry of the packing material (1 mg/mL in methanol) was transferred into the reservoir. The system was closed tightly, a pressure up to 400 bar was applied using a Knauer pneumatic pump (Knauer, Berlin, Germany) and maintained until the capillary was packed about 10 cm long. The capillary was washed with water for 1 h and the outlet and inlet frits were sintered by local heating of the capillaries at 600-700 °C for 40 s. A window for detection was provided by removing the polyimide coating of capillary by heating 2 or 3 seconds.

Capillary chromatography. Capillary chromatography was carried out on the system consisted of constant pressure pump, a variable wavelength UV detector and a micro-injector (CLC-1, Sepaserve, Germany). The signal was monitored at 220 nm with the software Eurochrom 2000. Pure methanol or acetonitrile with or without diethylamine (0.1%) was used as mobile phase and pressure for pumping the mobile phase was adjusted to 30-40 psi to show the same void time for solvent.

Table 1. The enantioseparation of selected chiral analytes on CDMPC capillary column using pure methanol or acetonitrile with or without diethylamine (DEA) as a mobile phase

Analytes	Methanol			Methanol + 0.1% DEA			Inf [d]
	k_1 [a]	α [b]	Rs [c]	k_1	α	Rs	
2	0.35	1.36	1.42	0.35	1.38	1.47	0
3	0.15	1.78	1.41	0.14	1.85	1.34	0
4	0.07	2.82	1.56	0.08	2.51	1.45	0
6	1.29	1.21	1.00	1.32	1.24	1.00	0
7	0.06	2.91	1.44	0.07	2.57	1.43	0
8	0.10	1.46	0.32	0.08	1.67	0.37	0
11	0.11	2.67	1.90	0.14	2.35	1.72	0
14	1.11	1.35	1.46	1.13	1.31	1.54	0
15	0.37	1.38	1.45	0.40	1.35	1.27	0
17	0.19	2.34	2.32	0.10	3.43	2.40	0
Analytes	Acetonitrile			Acetonitrile + 0.1% DEA			Inf
	k_1	α	Rs	k_1	α	Rs	
1	0.05	1.71	0.42	0.06	1.52	0.44	0
2	0.12	1.33	0.49	0.12	1.25	0.33	–
4	0.23	3.91	6.13	0.18	4.11	4.83	0
5	1.29	1.10	0.12	0.57	1.29	1.16	++
6	3.23	1.23	1.03	2.79	1.25	1.32	+
7	0.80	1.45	0.67	0.37	2.05	2.77	++
8	0.98	1.00	0.00	0.37	1.24	0.84	++
9	0.58	1.19	0.22	0.15	1.23	0.39	++
10	2.41	1.66	0.41	0.71	2.42	0.91	++
12	0.30	1.75	1.45	0.46	1.51	1.32	0
14	0.45	1.22	0.80	0.41	1.23	0.80	0
15	0.12	1.38	0.57	0.07	1.48	0.44	0

[a]Capacity factor of first eluted enantiomer. [b]Selectivity. [c]Resolution. [d]Change of the resolution by adding 0.1% DEA to pure solvent. –; –50~–25%, 0; –25~25%, +; 25~50%, ++; >50%.

Results and Discussion

Three compounds (**1**-**3**) among the used 18 analytes do not contain nitrogen atoms in their structures, while the other compounds contain nitrogen atoms as primary amine (**4**) or secondary hydroxylamine at side chain (**5**-**10**; β-blockers), or as heterocyclic compounds (**11**-**18**). The capillary chromatographic runs of these compounds were carried out on CDMPC and ADMPC stationary phases. The analytes were fully or partially separated when pure methanol and acetonitrile with or without DEA were used as mobile phases and the effects of DEA in mobile phase was evaluated.

Chiral separation on CDMPC. Fifteen racemate were fully or partially separated on CDMPC with methanol or acetonitrile as eluent (Table 1). The compounds **3**, **8**, **11** and **17** were separated only with methanol, while **1**, **5**, **9**, **10** and **12** only with acetonitrile. There was no structural analogy of the compounds separated with methanol or acetonitrile. It is generally known that acetonitrile is less suitable compared with methanol as a mobile phase on CDMPC stationary phase.[11] As shown in Table 1, pure methanol showed slightly better results in the enantioresolution than pure acetonitrile,

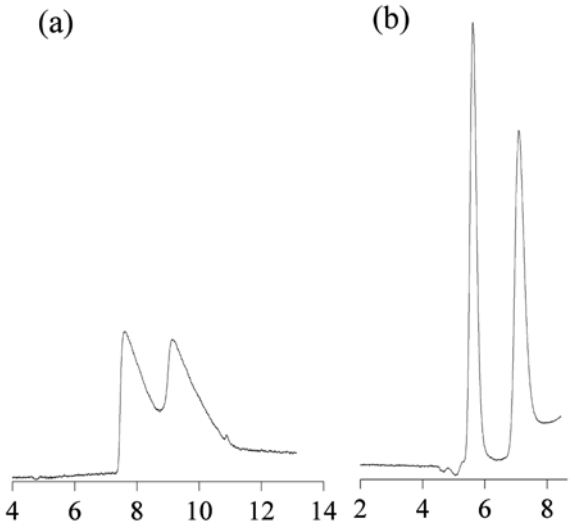

Figure 2. Enantioseparation of pindolol (**7**) on CDMPC using (a) acetonitrile or (b) acetonitrile + 0.1% DEA as mobile phase.

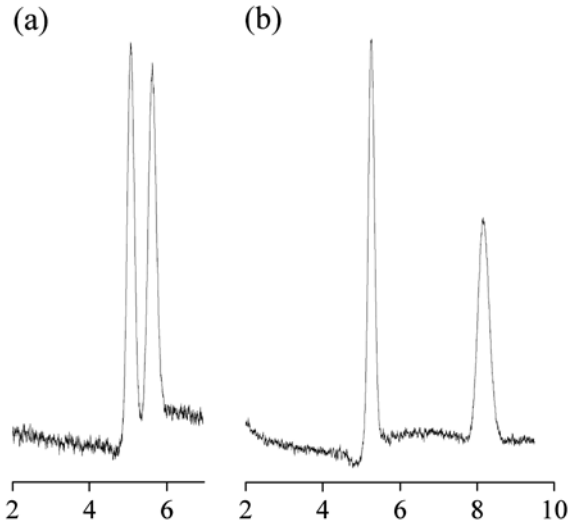

Figure 3. Enantioseparation of ambucetamide (**4**) on CDMPC using (a) methanol or (b) acetonitrile as mobile phase.

however, some complementary separation results could be observed with these two mobile phases. The effect of DEA on the resolution of enantiomers was practically not significant when methanol was used as mobile phase. When acetonitrile was used, the enantioresolution of the β-blockers increased significantly by adding 0.1% DEA in mobile phase through diminishing the tailing effects of some β-blockers (Fig. 2). The enantiomers of compound **4** were well separated with both pure methanol and pure acetonitrile (Fig. 3) indicating a good consistency with the separation results on pre-packed analytical column,[11] however, the effect of DEA on the resolution of this compound was negligible.

Chiral separation on ADMPC. ADMPC stationary phase containing the same phenylcarbamate moiety as CDMPC but attached to the amylose backbone instead of cellulose, exhibited rather low enantiomer resolving ability compared with its celluosic analog. When ADMPC was used as stationary phase, only seven racemate, including **13**, **16** and **18** which could not be separated on CDMPC, were fully or partially separated with methanol or acetonitrile as eluent (Table 2). For this stationary phase, methanol appeared to be more suitable mobile phase compared with acetonitrile. No analytes were base line separated using acetonitrile. The effect of DEA on the resolution of enantiomers on ADMPC was practically not significant or even negative for both methanol and acetonitrile as mobile phases. β-Blockers, separated well on CDMPC using acetonitrile containing 0.1% DEA, could not be separated on ADMPC using same mobile phase.

Table 2. The enantioseparation of selected chiral analytes on ADMPC capillary column using pure methanol or acetonitrile with or without diethylamine (DEA) as a mobile phase

Analytes	Methanol			Methanol + 0.1% DEA			Inf[d]
	k_1[a]	α[b]	Rs[c]	k_1	α	Rs	
2	0.56	1.37	0.88	0.54	1.40	0.95	0
13	0.97	1.63	0.50	0.34	1.81	0.34	–
14	1.08	1.54	0.50	1.90	1.19	0.19	––
15	0.83	2.20	1.46	0.87	1.79	1.41	0
18	1.23	2.13	1.16	1.17	2.04	0.98	0
Analytes	Acetonitrile			Acetonitrile + 0.1% DEA			Inf
	k_1	α	Rs	k_1	α	Rs	
4	1.53	1.30	0.26	1.55	1.32	0.27	0
14	0.63	1.43	0.40	0.59	1.51	0.55	+
15	0.23	1.33	0.38	0.21	1.27	0.28	–
16	0.61	1.22	0.40	0.79	1.18	0.49	0

[a]Capacity factor of first eluted enantiomer. [b]Selectivity. [c]Resolution. [d]Change of the resolution by adding 0.1% DEA to pure solvent. ––; <– 50%, –; –50~–25%, 0; –25~25%, +; 25~50%.

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