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Chiral high-performance liquid chromatography with cellulose carbamate-coated phases
Influence of support surface chemistry on enantioselectivity

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Abstract

The influence of support surface chemistry on the enantioselectivity and column performance of cellulose tris(3,5-dimethylphenyl carbamate)-coated chiral HPLC phases was investigated. Stable coated phases were produced using underivatised, aminopropylated and octadecylated silica as the support media. For many racemates, underivatised silica at a 20% (w/w) loading was found to be the most efficient phase.

1. Introduction

Polysaccharide tris(aryl carbamate) derivatives coated onto large pore (1000–4000 Å) 7–10 μm aminopropylated spherical silica (APS) have proved to be extremely useful stationary phases. Developed by Okamoto and co-workers [1–3] and marketed by Daicel as Chiralcel and Chiralpak columns, they are renowned for the separation of a wide range of neutral, basic and acidic chiral compounds [1–10]. Recently, we have shown that use of smaller-particle APS (< 5 μm) affords significant advantages, including high resolution and rapid separations [11].

APS is widely accepted as a suitable support for the polysaccharide tris(aryl carbamate)s. It is reportedly used [12] to (i) decrease non-stereoselective interactions by deactivating the acidic silanol groups and (ii) increase the stability of the coating by providing sites for hydrogen bond formation with the carbamate. However, to our knowledge, there have been no reported data substantiating either of these claims.

In this paper we report the results of a study using small-particle (3 μm) underivatised (SI), aminopropylated (APS) and octadecylated (ODS) spherical silicas from the Hypersil range to support cellulose tris(3,5-dimethylphenyl carbamate). This work has indicated that both polar (underivatised silica) and non-polar (octadecylated) supports can be used and in many cases, may offer some advantages.

2. Experimental

2.1. Chemicals and solvents

Underivatised, aminopropylated and octadecylated silicas (Shandon HPLC, UK)

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which had the following properties were used: particle size, 3 μm; pore size, 120 Å; surface area, 170–180 m^2/g; pore volume 0.6 ml/g. Elemental analyses, APS: C 1.67, H 0.48, N 0.57; ODS: C 9.50, H 1.84. The cellulose (Sigmacel, degree of polymerisation 400–500) was purchased from Sigma (UK) and 3,5-dimethylphenyl isocyanate from Lancaster (UK). Racemic mixtures and enantiomers were purchased from either Sigma or Aldrich (UK). Solvents (HPLC grade) were obtained from Rathburn (UK).

2.2. Apparatus

Separations were carried out on an HPLC system comprising a Beckman 114 M pump, a Beckman System Gold 166 UV detector and a Promise autosampler. Data were acquired using a Waters 860 Expertease package. A high-pressure slurry packer fitted with a Haskel 780-3 pump was used for column packing. Gel permeation chromatography (GPC) was carried out on a PL Modular System with Caliber software using refractive index (RI) detection. Particle size distributions were determined as dispersions in methanol using a Malvern Mastersizer X.

2.3. Chromatographic conditions

Chromatography was performed at ambient temperature using the mobile phase compositions listed along with the results at the foot of Tables 1–3. Typically, 10-μl samples of 1% (w/v) solutions dissolved in the mobile phase were injected. A flow-rate of 0.5 ml/min was maintained throughout the study and the racemates detected using a suitable wavelength. The dead time (t_0) of each column was determined by injection of 1,3,5-tri-*tert.*-butylbenzene.

2.4. Chromatographic calculations

The separation factor (α) was calculated as $\alpha = k'_2/k'_1$ and capacity factor (k') as $k'_1 = (t_1 - t_0)/t_0$ and $k'_2 = (t_2 - t_0)/t_0$, where t_1 and t_2 refer to the retention times for the first and second eluting enantiomers, respectively.

The resolution factor (R_s) was calculated by the formula: $R_s = 2(t_2 - t_1)/(w_1 + w_2)$ where w_1 and w_2 are the peak widths for the first and second eluting enantiomer peaks, respectively.

2.5. Preparation of the chiral columns

Cellulose tris(3,5-dimethylphenyl carbamate) (CDMPC) was prepared as previously reported [2]. The dried carbohydrate was refluxed for 24 h in dry pyridine. After cooling 3.5 equivalents of 3,5-dimethylphenyl isocyanate was added and the mixture refluxed for a further 72 h. The cooled solution was poured into 1.5 l methanol and stirred for 1 h. The white solid was filtered, washed well with methanol and dried under vacuum at 50°C to constant mass. Elemental analysis $(C_{33}H_{37}N_3O_3)_n$, calculated: C 65.66, H 6.18, N 6.98; found: C 64.94, H 6.07, N 6.81. GPC analysis [two PL Mixed-C columns in series, elution with tetrahydrofuran (THF)] showed product to have $M_n = 1.01 \cdot 10^5$ and $M_w/M_n = 5.46$, where M_n is number-average molecular mass and M_w is weight-average molecular mass.

Silica, APS or ODS (3 g) was refluxed in THF (30 ml) for 30 min and allowed to cool. An appropriate amount of CDMPC [0.529 g for 15% (w/w), 0.75 g for 20% (w/w)] was dissolved in 20 ml THF–N,N-dimethylacetamide (80:20, v/v) and added to the refluxed silica. The solvent was removed under vacuum to dryness and the material sieved (38 μm) to ensure a free flowing powder suitable for packing. Mean particle size distributions were essentially unchanged from corresponding starting supports. The phase was high-pressure slurry packed (6000 p.s.i.; 1 p.s.i. = 6894.76 Pa) into a stainless-steel column (10 cm × 4.6 mm I.D.) in hexane–2-propanol (50:50, v/v) and equilibrated with hexane–2-propanol (80:20, v/v).

The preparation of columns of this type has been shown to be very reproducible from one batch to another, e.g. several batches of CDMPC-coated APS supports gave very similar α and R_s values for a series of test analytes.

3. Results and discussion

3.1. Influence of support chemistry

Previous studies with small-particle APS [11] have shown that a coating of 15% (w/w) cellulose tris(aryl carbamate) is optimum for the 120 Å pore phase. Therefore, in order to compare their behaviour, each support was coated with 15% (w/w) CDMPC and the influence of support surface chemistry on column performance was investigated.

The enantiomeric resolution of 16 chiral analytes (6 neutral, 5 basic and 5 acidic) can be seen in Tables 1–3.

3.2. Neutral analytes

For all the neutral analytes examined, capacity factors are highest on the coated ODS phase and lowest on the coated SI phase (Table 1). This result may seem surprising, since the test analytes are all relatively polar in nature and would not be expected to interact strongly with the non-polar ODS surface. However, the separation factors also show the same trend, thus there must be more CDMPC interaction sites on the ODS phase than there are on the SI phase. An explanation for this, along with a possible reason for the lower than expected resolution observed on the coated ODS phase, will be given later.

3.3. Basic analytes

A marked difference in chromatographic behaviour can be seen (Table 2) between analytes with a sterically hindered basic group (Trogers base and homatropine) and those in which the basic group is more accessible (orphenadrine, alprenolol and oxprenolol).

The analytes which have the more accessible basic group have high capacity factors on the coated SI phase and low capacity factors on the coated ODS phase. This is consistent with previously reported studies [6,7] suggesting that there are strong interactions between accessible basic amino groups in the analyte and any exposed acidic silanol groups. In the case of the coated SI phase, interactions with the support surface are so strong that the chromatography is extremely poor. The analytes in which the basic group is more sterically hindered are not able interact as strongly with the surface groups and the capacity factors for the three phases are more similar.

For all the basic analytes, the separation factors are highest on the coated ODS phase and, in general, lowest on the coated SI phase. This is consistent with the suggestion that there are more CDMPC interaction sites on the coated ODS phase.

3.4. Acidic analytes

In general, capacity factors are largest when APS is the support (Table 3). This is the counterpart of the effect seen with the basic compounds on the coated SI phase, ie the acidic group appears to be interacting with exposed APS groups. For the majority of analytes, separation factors are slightly higher on the coated ODS phase, but in general are lower than have been described in the literature [8]. Reasons for this are as yet unclear. Suprofen shows a somewhat different pattern of behaviour to the other acidic analytes, presumably as a result of its polyfunctional nature.

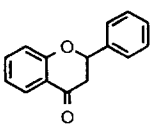
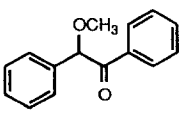
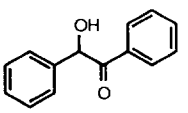
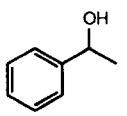
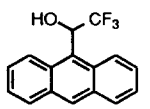
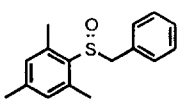
3.5. Stability

The stability of each 15% coated column was examined for 170 h under a flow-rate of 1 ml/min (total volume was approx. 10 l) at ambient temperature using hexane–2-propanol (80:20, v/v) as mobile phase. There was no deterioration in column performance, as judged by the lack of change in k', N or R_s for a test mixture.

3.6. General discussion

In order to explain the chromatographic trends seen for the three phases, the effects of introducing a bonded group onto the surface of a small-pored phase need to be considered. A bonded group will not only change the chemical nature of the surface (examples of non-stereospecific interactions between the analyte and the

Table 1
Resolution of neutral racemates on CDMPC-coated supports

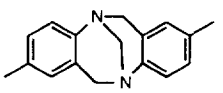
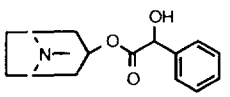
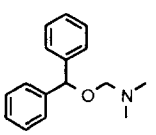
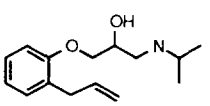
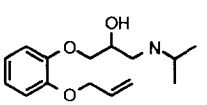
Sample	Mobile phase		APS, 15%	ODS, 15%	SI	
					15%	20%
 Flavanone	A	k'_1	1.78	2.49	1.62	2.12
		k'_2	2.30	3.15	1.96	2.78
		α	1.29	1.26	1.21	1.31
		R_s	2.46	2.14	1.80	2.53
 Benzoin methyl ether	A	k'_1	1.49	1.74	1.31	1.59
		k'_2	1.96	2.52	1.68	2.24
		α	1.32	1.44	1.28	1.41
		R_s	2.47	2.91	2.06	2.86
 Benzoin	A	k'_1	3.50	4.35	3.17	3.75
		k'_2	4.67	5.97	3.99	5.24
		α	1.33	1.37	1.26	1.40
		R_s	3.04	2.97	2.40	3.27
 Phenethyl alcohol	B	k'_1	5.04	5.21	4.81	5.80
		k'_2	5.85	6.16	5.25	6.85
		α	1.16	1.19	1.09	1.18
		R_s	1.89	1.65	1.03	1.99
 1-(9-Anthryl)-2,2,2-trifluoroethanol	A	k'_1	3.25	3.49	2.22	3.96
		k'_2	7.30	9.09	4.91	9.14
		α	2.25	2.61	2.21	2.31
		R_s	6.89	6.00	7.63	6.56
 Benzyl mesityl sulphoxide	A	k'_1	3.50	3.93	3.25	3.76
		k'_2	6.65	8.36	5.55	8.17
		α	1.90	2.13	1.71	2.17
		R_s	5.56	4.77	4.96	6.04

Mobile phase: hexane–2-propanol [A = 90:10 (v/v); B = 98:2 (v/v)].

surface group have already been discussed) but will also affect the pore structure.

An aminopropyl or octadecyl group will reduce the pore diameter and thus the pore volume, compared with the underivatised silica. This should lead to an increase in the amount of CDMPC which is prevented from entering the pores, the magnitude of this effect depending to some extent on the spatial arrangement and conformation of the bonded group. In addition,

Table 2
Resolution of basic racemates on CDMPC-coated supports

Sample	Mobile phase		APS, 15%	ODS, 15%	SI	
					15%	20%
 Trogers base	C	k'_1	1.39	1.62	1.59	2.01
		k'_2	1.75	2.27	2.10	2.46
		α	1.25	1.40	1.32	1.22
		R_s	1.71	2.21	2.24	1.49
 Homatropine	D	k'_1	1.52	1.37	1.27	1.41
		k'_2	2.10	2.21	1.84	2.17
		α	1.38	1.62	1.45	1.54
		R_s	2.65	3.04	2.49	3.20
 Orphenadrine	C	k'_1	0.90	0.63	2.16	2.00
		k'_2	1.23	1.05	2.49	2.44
		α	1.37	1.67	1.15	1.22
		R_s	2.02	2.18	1.07	1.40
 Alprenolol	D	k'_1	1.11	0.41	4.23	3.52
		k'_2	1.70	0.88	4.54	4.27
		α	1.53	2.14	1.07	1.21
		R_s	2.48	2.42	P.R.	1.21
 Oxprenolol	D	k'_1	2.26	1.11	6.56	5.27
		k'_2	5.43	3.61	8.54	9.38
		α	2.40	3.26	1.30	1.78
		R_s	5.48	5.40	1.50	3.69

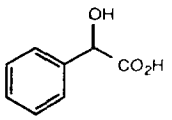
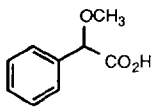
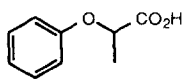
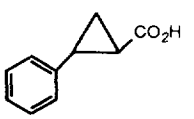
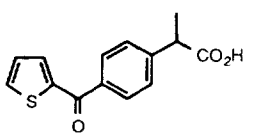
P.R. = Partial resolution (< 0.5). Mobile phases: C = hexane–2-propanol–diethylamine (90:10:0.1, v/v/v); D = hexane–2-propanol–diethylamine (80:20:0.1, v/v/v).

whilst the polar SI and APS supports may have an attractive or stabilising effect on the relatively polar CDMPC coating, via hydrogen bonding or dipole interactions, the more lipophilic ODS group may have a repelling or destabilising effect which will further deter the CDMPC from entering the pores.

An insight into how the chromatography is affected when there is a large amount of chiral phase on the outside of the particle was obtained during recent studies to determine the optimum carbamate loading for our small-pored APS supports [11]. At low levels ($< 15\%$, w/w), an increase in the carbamate loading was accompanied by an increase in the capacity, separation and resolution factors. This was assumed to be due to an increase in the number of carbamate interaction sites. However, as the loading was further increased, a loading threshold was eventually reached, above which resolution deteriorated and eventually the particles became aggregated and would not pack satisfactorily.

Taking account of the chromatographic results, the support pore model and the observa-

Table 3
Resolution of acidic racemates on CDMPC-coated supports

Sample		APS, 15%	ODS, 15%	SI	
				15%	20%
 Mandelic acid	k'_1	8.93	5.67	5.46	6.94
	k'_2	9.52	6.94	6.09	7.85
	α	1.06	1.11	1.12	1.13
	R_s	0.72	0.73	1.08	1.29
 2-Methoxyphenylacetic acid	k'_1	4.82	3.26	3.66	5.09
	k'_2	5.33	3.74	4.05	5.82
	α	1.11	1.15	1.11	1.14
	R_s	1.32	1.17	1.19	1.67
 2-Phenoxypropionic acid	k'_1	3.14	2.48	2.67	3.17
	k'_2	4.52	4.51	4.12	4.99
	α	1.44	1.82	1.54	1.57
	R_s	3.89	4.42	4.10	4.34
 2-Phenyl-1-cyclo-propane carboxylic acid	k'_1	5.48	4.78	4.67	6.45
	k'_2	6.25	5.13	5.35	7.49
	α	1.14	1.07	1.14	1.16
	R_s	1.78	P.R.	1.73	1.93
 Suprofen	k'_1	11.78	12.34	11.64	12.60
	k'_2	12.83	14.22	12.83	13.91
	α	1.09	1.15	1.11	1.10
	R_s	1.07	1.23	1.56	1.14

P.R. = Partial resolution (< 0.5). Mobile phase: hexane–ethanol–trifluoroacetic acid (96:4:1, v/v/v).

tions from the loading experiments, the following phase descriptions are proposed;

(i) *Coated SI phase*. Owing to its polar nature and relatively large pore volume, the underivatised silica support is able to accommodate the polar CDMPC coating more easily than the other two supports. Therefore it has the least amount of CDMPC coating on the outside of the particles, which reduces the number of chiral interaction sites, leading to the lowest degree of separations. The strong interactions seen with some basic analytes indicate that there are regions where the coverage of accessible silica surface by CDMPC is low.

(ii) *Coated APS phase*. The aminopropyl support appears to have slightly more CDMPC coating on the outside of the particles, demonstrated by higher capacity and separation factors for neutral analytes compared to the coated SI phase. High resolution is observed for many of the analytes and loading experiments confirm that 15% (w/w) CDMPC is close to optimum for

this support. Higher than average interactions with some acidic analytes suggest that, as with the SI support, there are regions where the density of the CDMPC phase is low.

(iii) *Coated ODS phase.* This support, due to its large non-polar bonded group, does not readily accommodate CDMPC into its pore volume. Therefore it has the largest amount of CDMPC on the outside of the particles, which provides the largest number of chiral interaction sites, as demonstrated by the high separation factors observed for the majority of analytes. However, in contrast to the APS coated phase, the amount of CDMPC on the outside of the ODS particles appears to have exceeded the optimum. Whilst the column has still packed satisfactorily, the resolution factors are not as high as might be predicted.

3.7. The use of underivatised silica

It was of particular interest that, contrary to popular belief, the coated SI phase showed good chromatography for many test analytes. It was noted that (i) SI appears to most readily accept the CDMPC coating, due to its polar nature and larger pore volume; and (ii) in previous studies [1–3], wider-pore APS was able to accept loadings of 20–25% (w/w). Consequently, the behaviour of a 20% (w/w) CDMPC coating on SI was investigated.

The underivatised silica readily accepted this higher loading and the results in Tables 1–3 show that the 20% (w/w) coated SI column was significantly more efficient than the 15% (w/w) coated SI column for the majority of racemates tested. The resolution of benzoin methyl ether on the 15 and 20% (w/w) coated SI phases is shown in Fig. 1.

For many analytes the 20% (w/w) coated SI column also gave superior performance to either the ODS or APS 15% (w/w) coated phases. Of remaining concern were the poor results seen with some basic analytes, due to interactions with exposed surface silanol groups. Compared to the 15% (w/w) coated SI phase, an improvement was seen in the capacity and separation factors on the 20% (w/w) coated SI phase, indicating that interactions with Si–OH groups had been reduced by the heavier coating. However, a substantial improvement was made by increasing the amount of diethylamine, a silanol

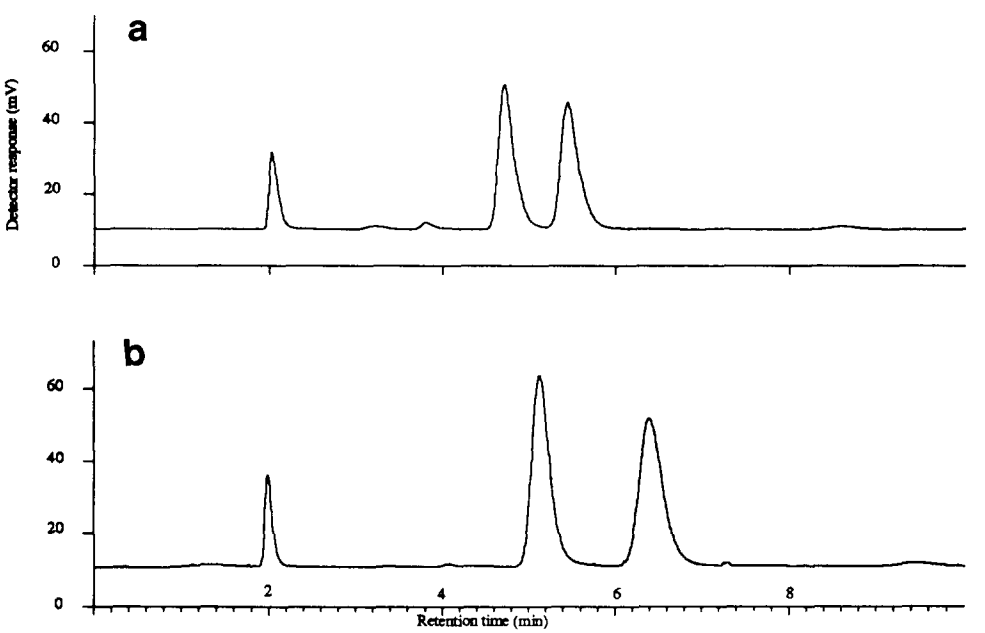


Fig. 1. Resolution of benzoin methyl ether at two loading levels of CDMPC on SI: (a) 15%, (b) 20%.

suppressor, in the mobile phase from 0.1 to 1.0%. For example, for oxprenolol on the 20% (w/w) coated SI phase, the k_1' value decreased from 5.27 to 2.86 and the α and R_s factors increased from 1.78 to 2.48 and 3.69 to 5.30, respectively. The column continued to remain stable with this mobile phase composition for the duration of the study and showed no deterioration in performance after return to standard test conditions.

4. Conclusions

It is possible to produce stable cellulose carbamate-coated phases using supports other than APS. For many compounds, 3 μm Hypersil SI coated with 20% (w/w) CDMPC was the most efficient phase. Further investigations of these phenomena are in progress.

Acknowledgements

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References

[1] Y. Okamoto, M. Kawashima and K. Hatada, *J. Am. Chem. Soc.*, 106 (1984) 5357.
[2] Y. Okamoto, M. Kawashima and K. Hatada, *J. Liq. Chromatogr.*, 363 (1986) 173.
[3] Y. Okamoto, R. Aburatani, T. Fukumoto and K. Hatada, *Chem. Lett.*, (1987) 1857.
[4] T. Shibata, I. Okamoto and K. Ishii, *J. Liq. Chromatogr.*, 9 (1986) 313.
[5] Y. Okamoto, R. Aburatani, K. Hatano and K. Hatada, *J. Liq. Chromatogr.*, 11 (1988) 2147.
[6] Y. Okamoto, M. Kawashima, R. Aburatani, K. Hatada, T. Nishiyama and M. Masuda, *Chem. Lett.*, (1986) 1237.
[7] H.Y. Aboul-Enein and V. Serignese, *J. Liq. Chromatogr.*, 16 (1993) 197.
[8] Y. Okamoto, R. Aburatani, Y. Kaida and K. Hatada, *Chem. Lett.*, (1988) 1125.
[9] Y. Okamoto, R. Aburatani, Y. Kaida, K. Hatada, N. Inotsume and M. Nakano, *Chirality*, 1 (1989) 239.
[10] S.A. Matlin, E. Tiritan, A.J. Crawford, Q.B. Cass and D. Boyd, *Chirality*, 6 (1994) 135.
[11] S.J. Grieb, S.A. Matlin, J.G Phillips A.M. Belenguer and H.J. Ritchie, *Chirality*, 6 (1994) 129.
[12] G. Felix and T. Zhang, *J. Chromatogr.*, 639 (1993) 141.