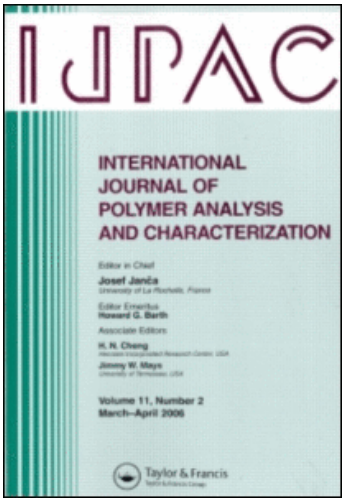

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The Influence of Coating Thickness of Polymer on Chiral Discrimination of Cellulose Tris(3,5-Dimethyl Phenylcarbamate) Chiral Stationary Phase

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Abstract: The influence of the coating thickness of the polymer on the chiral discrimination of cellulose tris(3,5-dimethyl phenylcarbamate) (CDMPC) chiral stationary phase (CSP) is investigated. CDMPC-CSPs with different thicknesses of polymer were prepared, and their chromatographic performances were investigated. Thinner coat of polymer will increase the interactions between the samples and the support of CSPs and decrease the selectivity of CSPs. Too thick coat of polymer will not increase the performance of CSPs significantly but will decrease the performance of the column. The suited thickness of polymer is very important for the selectivity of the chiral stationary phase.

Keywords: CDMPC; Chiral stationary phase; Chiral recognition; HPLC; Thickness of polymer

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INTRODUCTION

Polysaccharide-based chiral stationary phase was developed by Okamoto and coworkers[1,2] and marketed by Daicel as Chiralcel and Chiralpak columns. Okamoto[3] reported 80% of 483 racemic mixtures were successfully separated on either the cellulose or the corresponding amylose carbamate. Among the derivatives of cellulose, cellulose tris(3,5-dimethyl phenylcarbamate) (CDMPC) is the most attractive stationary phase for its remarkable chiral recognition ability and can be used for both normal and reversed phase.[4,5]

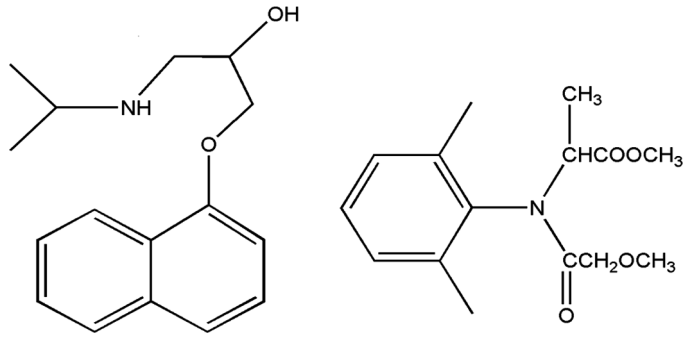
There are some reports about the preparation conditions of CDMPC-chiral stationary phase (CSP). Previous studies have shown that polysaccharide tris (aryl carbamate) derivatives coated onto large pore (100–400 nm, 7–10 μm) silica gel have the best separation performance with the large coating amount of 20% (w/w).[6] Grieb[7] investigated the influence of the support structure on the enantio-selectivity. They found that the loading amount of CDMPC should be below 20% (w/w) on 5 and 2.5 μm silica gels with 12 nm diameter pores, and they[8] also investigated the influence of the support surface chemistry on enantio-selectivity using silica gels, aminopropylated silica gels, and octadecylated silica gels. Yashima[6] investigated the influence of the pore size of silica gel (7 μm), coating solvents, loading amount of CDMPC, and column temperature on chiral discrimination. They found that CSPs prepared with large-pore (80–160 nm) silica gels having small surface areas showed higher chiral recognition, and the loading capacity of racemates increased with an increase of the coating amount of CDMPC supported on the silica gel.

In the present study, we investigated the influence of the coating thickness of polymer on the chiral discrimination of the cellulose tris(3,5-dimethyl phenylcarbamate) chiral stationary phase. We coated CDMPC on APS supports with different surface areas and smaller pore sizes (8–27 nm), then compared the chromatographic performance of these chiral stationary phases. The chromatographic performances were evaluated by measuring the column efficiency and chiral discrimination of the two racemates (Figure I) on all chromatographic columns.

EXPERIMENTAL SECTION

Instrumentation and Materials

The high-performance liquid chromatography (HPLC) system consists of a Waters 441 HPLC pump, an UVIDEC-100-V detector (Wescan, Japan), and a 7125 syringe loading sample injector (Rheodyne, USA)



(a) (b)

Figure 1. Structures of rac-propranolol (a) and rac-metalaxyl (b).

equipped with 20 μL loop. The chromatographic data were acquired and processed by HW-2000 chromatography manager software model (Qianpu, China). The elemental analyses were performed on a Vario EL (Elementar, Germany).

Microcrystalline cellulose was purchased from the Fourth Reagent Factory of Shanghai (China) and dried at 120°C before using. The 3, 5-dimethyl phenylisocyanate was obtained from Aldrich (99%), and the 3-aminopropyltriethoxysilane is a product of Liaoning Chemical Plant (China). Porous silica gels with particle size of 5 μm were made in our laboratory and dried in vacuum at 120°C; characteristics of the silica gels are shown in Table I. All the reagents used were analytical grade from Tianjin Second Chemical Reagent Plant (China). The reaction solvent, pyridine, was dried over calcium hydroxide and distilled before use.

Chromatographic Conditions

The mobile phase was hexane: 2-propanol (90:10, V:V), and the flow rate was 1.0 mL/min. The detection (UV) was performed at 230 nm for rac-metalaxyl and at 290 nm for rac-propranolol. The column was kept at ambient temperature. The samples were prepared by dissolving them in

Table I. Characteristics of silica gels

	Silica gels		
	A	B	C
Pore size (nm)	27	22	8.0
Surface area (m^2/g)	59	70	400

Table II. Coating amount of the chiral stationary phase

	Number				
	1	2	3	4	5
Kind of APS	A	A	B	C	B
Coating amount %(w/w)[a]	10.6	15.0	10.6	10.6	15.0
Coating amount (mg/m^2)[b]	2.0	3.0	1.7	0.27	2.0

[a]Calculated from the amount of CDMPC that was deposited on the APS.
[b]Calculated from the coating amount of the CSP and the surface areas of the support.

methanol and diluting them by the mobile phase. The dead time (t_0) was estimated by the first deviation of the baseline after injection.

Preparation of Chiral Stationary Phase

The aminopropylated silica gels (APS) were prepared as described by Qiu.[9] The silane used was 3-aminopropylsilane. Cellulose tris(3,5-dimethyl phenylcarbamate) was prepared as described in a previous study.[7] Elemental analyses (%) C: 64.55, N: 6.950, H: 5.790 (calculated %: C: 65.66, N: 6.960, H: 6.180).

CSPs are prepared by depositing different amounts of CDMPC on APS; the coating amounts of CSP are shown in Table II. Prepared CSPs were packed into a stainless steel column 50×4.6 mm by the conventional high-pressure slurry-packing procedure.

RESULT AND DISCUSSION

Optimum Load Amount of CDMPC on APS

In the case of APS, a polysaccharide coating of 15% has been cited in the literature.[7,8,10–12] However, in view of the significant changes in surface area and pore size in the present study, an investigation was carried out to determine the optimum level of the cellulose carbamate coating on APS with a pore size of 27 nm.

The chiral stationary phases were prepared with increasing amounts of polymer deposited on APS (pore size 27 nm). The results are shown in Figure 2. An increase in the enantio-selectivity (and as a consequence increase in Rs) at low levels (<8.13% (w/w)) was accompanied by an increase of the polymer load (Figure 2A). The rac-propanolol was not

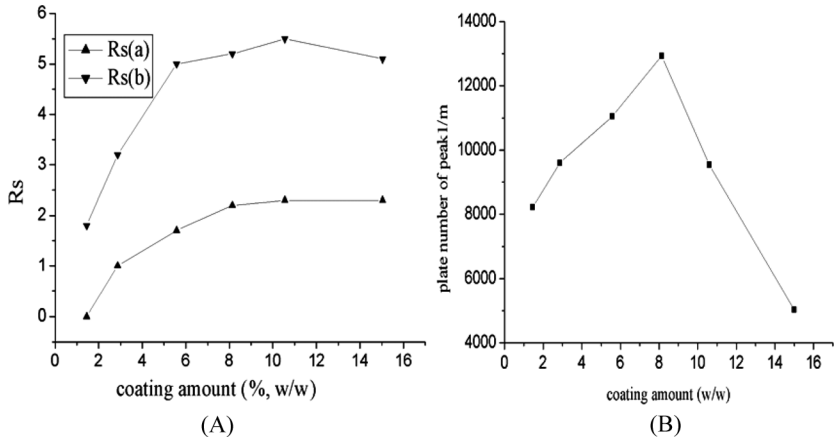


Figure 2. Effect of the coating amount of CDMPC on the performance of CSP: (A) Rs of rac-propranolol (curve a) and rac-metalaxyl (curve b) and (B) the plate numbers of rac-metalaxyl (the enantiomer 1).

separated when the coating amount was less than 2.87% (Figure 2A). This indicates that there is different minimum amount of CDMPC needed to achieve enantio-recognition when the samples are different. As the CDMPC load was further increased (>8.31%), the resolution and selectivity factor changes were no longer significant (Figure 2A). The tendency of the plots is similar to the results of previous studies.[6,7,13] An excessive amount of CDMPC affects the intraparticle diffusion of the samples, lowering performance of the columns (Figure 2B). When the coating amount of CDMPC reaches 19%, the stationary phases have aggregated and have not been packed well. So we did not make further investigations of the stationary phase with the coating amount of the CDMPC more than 19%. The appropriate coating amount of CDMPC is 8.31–15% by weight on APS with pore size of 27 nm.

Chromatographic Behavior of CSPs With Different Surface Areas

The same amount of polymer was coated on APS with different surface areas. Two coating amounts (10.6% and 15.0%) were selected to compare the chromatographic behavior of CDMPC-CSP with different thicknesses. The coating amount of 10.6% is close to the low point of the appropriate coating amount of supports with pore size of 27 nm; the coating amount of 15.0 % is close to the high point of the appropriate coating amount of supports with pore size of 27 nm. The chiral stationary phases we used are shown in Table II.

Chromatographic Behavior of CSPs with Coating Amount of 10.6% (w/w)

The chromatograms of both samples on the CSPs with different surface areas are shown in Figure 3. The weight ratio of CDMPC to CDMPC-CSP is 10.6% (w/w). Okamoto[6] investigated the effect of big pore size (80–160 nm) of silica gel on the chiral separation. Our study focuses on the meso-pore (8–27 nm) of support.

It can be seen clearly that the retention factor and the selectivity of both racemates are different when the same amount of CDMPC is coated on APS with different surface areas (Figure 3). The selectivity factors of rac-propranolol are decreased with the increase of the surface areas of the supports (Figure 3). If the amount of CDMPC is not enough to coat APS completely, the non-stereo-selective interaction between the support (-NH_2 or Si-OH) and the functional groups of the sample would decrease the probability of the stereo-selective interaction between CDMPC and the samples. That will reduce the resolution of the samples. Likely there is a minimum polymer thickness that may form a homogeneous coating onto the whole APS surface. That thickness of CDMPC could inhibit the sorption of solutes to non-stereo-selective sites present on the support surface.

The functional groups of the rac-metalaxyl did not have strong interaction with the support, so the resolution of rac-metalaxyl did not change much on the CSP with pore size of 22 nm and 27 nm (Figure 3). However, the performance of the column is higher when the same coating amount of CDMPC is coated on APS with larger surface area. This is because the

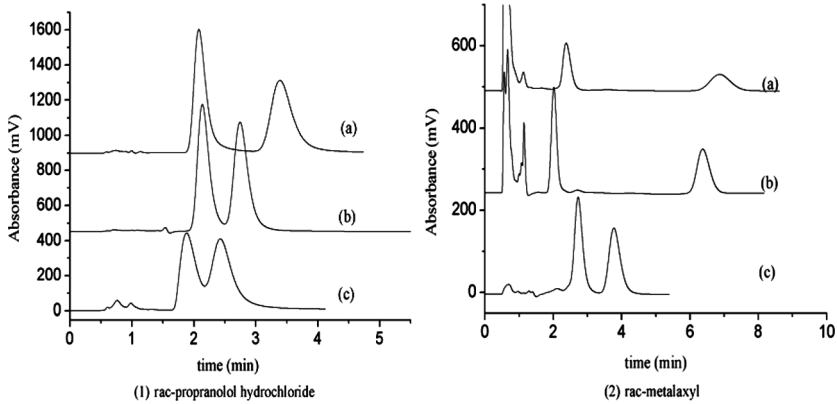


Figure 3. Chromatogram of the chiral separation of rac-propranolol (1) and rac-metalaxyl (2) on CDMPC-CSP with different pore diameters: (a) 27 nm, 59 m^2/g, (b) 22 nm, 72 m^2/g, (c) 8.0 nm, 400 m^2/g.

thickness of the CDMPC on the APS with larger surface areas is thinner than that on the smaller surface areas. Consequently, the diffusion of the samples between the stationary phase and the mobile phase became faster, and the performance of the column became higher.

The resolution of both samples on the support with pore size 8 nm decreased significantly in comparison with pore sizes 22 and 27 nm (Figure 3).

From the results of the elemental analysis, the polymer thicknesses of the two CSPs no.1 and no. 4 were calculated from the amount of polymer and the surface areas (Table III).

If CDMPC is deposited homogeneously on APS with pore size 8 nm, the thickness of the coat should be 0.24 nm (Table III). The entry of the samples to the pore of the support would not be blocked and the resolution of the samples would not decrease as significantly. CDMPC is a polymer of large size (length 200 nm × width 7 nm). In the process of coating, CDMPC may block the small pore (8 nm) of APS, and other CDMPC could not enter into the pore of APS. Thus the CDMPC is not coated homogeneously, and the surface areas within the pore of the support cannot be used effectively. This is the possible reason that the retention times and the Rs both decreased when the CDMPC was coated on the support with pore size of 8 nm.

Chromatographic Behavior of CSPs with Coating Amount of 15% (w/w)

Since the small pore size (8 nm) of the support could be blocked by CDMPC, we used only APS with larger pore size in this study.

CDMPC was coated on APS with coating amount 15%. The chromatographic results are shown in Table IV. The two stationary phases no. 1 and no. 5 have similar thicknesses of coating, and the two stationary phases no. 2 and no. 5 have a similar coating amount and different thicknesses of coating.

Table III. Calculation of the thickness of CDMPC

	Element analysis of CSP			CDMPC on CSP	
Number of CSP	C%	N%	H%	C%	Thickness (nm)[a]
1	8.773	0.853	1.103	7.476	1.85
4	14.67	3.333	2.556	6.678	0.24

[a]Calculated according to $s = 1000\frac{x}{y}/(1-\frac{x}{y}) \cdot O_{sp} \cdot d$,[14] x is the C% of the CDMPC on CSP, y is the C% of the cellulose derivatives, O_{sp} is the surface area of the APS, and d is the polymer density (1.2 g/cm^3).[13]

Table IV. Chromatographic performance of the CSP with greater thickness of polymer

	Samples					
	Rac-propranolol			Rac-metalaxyl		
Column no.	2	5	1	2	5	1
k_2	8.67	7.64	4.92	21.56	23.54	11.06
Rs	2.30	3.40	2.30	5.10	6.10	5.60
a	1.98	2.72	1.86	3.73	4.37	3.49

Although the selectivity of CSP no. 2 is better than that of CSP no. 1, the Rs of CSP no. 2 is similar to that of CSP no. 1 because the thicker CDMPC on APS decreases the performance of the column. However, the selectivity and the resolution of the samples on CSP no. 5 are both much higher than on CSP no. 1 and CSP no. 2. There may be two reasons for the results in Table IV.

Whenever the thickness of CDMPC on APS is higher, CDMPC is coated according to the interactions between CDMPC chains. The interaction sites between CDMPC chains are different than the interaction sites between the CDMPC and APS. The interaction sites on CSP, which are important for the chiral discrimination of the stationary phases, are reduced. The chiral discrimination ability of CDMPC, which is near to the support, is lowered. This may be the reason why the selectivity of the CSP will be reduced when the thickness of the CDMPC is increased.

Another possible reason we considered is the diffusion of the samples. The thickness of the coating increases with the increase of coating amount, and the sample cannot easily diffuse to the chiral cavities of CDMPC, which is near the APS. Thus, the contact of the sample with CDMPC is not sufficient to accomplish the recognition.

Nevertheless, if the same amount of CDMPC (15%, w/w) is coated on the support with larger surface area ($70\,m^2/g$), the thickness of the coat will decrease and the effects of the absorbance between the CDMPC chains will decrease. As a result, there will be more effective surface areas on CSP, and the selectivity of the chiral stationary phase would obviously be improved. Consequently, the samples will obtain better diffusion on the CSP with smaller thickness of CDMPC, which will result in better performance of the column.

From the above study, we can infer that likely an appropriate polymer thickness is needed to form a homogeneous coating onto the whole APS surface that could inhibit the sorption of solutes to non-stereo-selective sites on the support surface and provide higher performance of the columns and more effective surface areas.

CONCLUSION

In this article, we discussed the effect of the thickness of the CDMPC on the ability of chiral separation of CDMPC-CSP. CSPs that have better ability of chiral separation should have an appropriate thickness of CDMPC. If the thickness of CSP is too low, there will be non-stereo-selective interaction between the support (-NH_2 or Si-OH) and the functional groups of the sample and the probability of stereo-selective interaction between CDMPC and the samples will be decreased. If the thickness of CSP is too large, the important interaction sites of the chiral discrimination on CSP will be occupied by the interaction between the CDMPC chains. Too thick CDMPC will also decrease the performance of the column and the effective contact surface of CSPs. Thus the effective method that could increase the selectivity of chiral stationary significantly is to coat the appropriate thickness of CDMPC on APS with bigger surface areas.

REFERENCES

[1] Okamoto, M., M. Kawashima, and K. Hatada. (1986). Chromatographic resolution. XI. Controlled chiral recognition of cellulose triphenyl carbamate derivative supported on silica gel. *J. Chromatogr. A* **363**, 173–186.

[2] Okamoto, Y., M. Kawashima, and K. Hatada. (1984). Useful chiral packing materials for high-performance liquid chromatographic resolution of enantiomers: Phenylcarbamates of polysaccharides coated on silica gel. *J. Am. Chem. Soc.* **106**, 5357–5359.

[3] Okamoto, Y., Y. Kaida, R. Aburatani, and K. Hatada. (1991). In *Chiral Separations by Liquid Chromatography*, ed. S. Ahuja, 101–113. Washington, D.C.: American Chemical Society.

[4] Yashima, E. (2001). Polysaccharide-based chiral stationary phases for high-performance liquid chromatographic enantioseparation. *J. Chromatogr. A.* **906**, 105–125.

[5] Tachibana, K., and A. Ohnishi. (2001). Reversed-phase liquid chromatographic separation of enantiomers on polysaccharide type chiral stationary phases. *J. Chromatogr. A* **906**, 127–154.

[6] Yashima, E., A. Pennaf, and Y. Okamoto. (1996). Enantioseparation on cellulose tris (3,5-dimethylphenylcarbamate) as a chiral stationary phase: Influences of pore size of silica gel, coating amount, coating solvent, and column temperature on chiral discrimination. *Chirality* **8**, 446–451.

[7] Grieb, S. J., S. A. Matlin, J. G. Phillips, A. M. Belenguer, and H. J. Ritchie. (1994). Chiral HPLC with carbohydrate carbamates: Influence of support structure on enantioselectivity. *Chirality* **6**, 129–134.

[8] Grieb, S. J., S. A. Matlin, A. M. Belenguer, and H. J. Ritchie. (1995). Chiral high-performance liquid chromatography with cellulose carbamate-coated phases: Influence of support surface chemistry on enantioselectivity. *J. Chromatogr. A* **697**, 271–278.

[9] Qiu, H., Q. Jiang, Z. Wei, X. Wang, X. Liu, and S. Jiang. (2007). Preparation and evaluation of a silica-based 1-alkyl-3-(propyl-3-sulfonate) imidazolium zwitterionic stationary phase for high-performance liquid chromatography. *J. Chromatogr. A* **1163**, 63–69.

[10] Zhai, Z., Y. Shi, and T. Wang. (2005). Development and validation of HPLC methods for enantioseparation of mirtazapine enantiomers at analytical and semipreparative scale using polysaccharide chiral stationary phases. *Anal. Chim. Acta* **550**, 123–129.

[11] Zhai, Z., X. Luo, X. Wu, H. Zhang, and Y. Shi. (2005). Analytical and semipreparative resolution of enatiomers of albendazole sulfoxide by HPLC on amylose tris (3,5-dimethylphenylcarbamate) chiral stationary phase. *J. Biochem. Biophys. Methods* **62**, 69–79.

[12] Wang, P., S. Jiang, D. Liu, W. Shan, H. Zhang, and Z. Zhou. (2006). Chiral separation of pesticide enantiomers by high-performance liquid chromatography using cellulose triphenylcarbamate chiral stationary phase. *J. Chromatogr. Sci.* **144**, 602–606.

[13] Castells, C. B., and P. W. Carr. (1999). Cellulose tris(3,5-dimethylphenylcarbamate)-coated zirconia as a chiral stationary phase for HPLC. *Anal. Chem.* **71**, 3013–3021.

[14] Liu, G., and Z. Yu. (2006). *The Technology of the Chromatography Column*. Beijing: Chemical Industry Publishing Co, p. 140.