Received: 5 September 2018 | Revised: 4 December 2018 | Accepted: 12 December 2018

DOI: 10.1002/chir.23044

REGULAR ARTICLE

WILEY

HPLC with cellulose Tris (3,5-DimethylPhenylcarbamate) chiral stationary phase: Influence of coating times and coating amount on chiral discrimination

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Abstract

Coating cellulose tris (3,5-dimethylphenylcarbamate) (CDMPC) on silica gels with large pores have been demonstrated as an efficient way for the preparation of chiral stationary phase (CSP) for high-performance liquid chromatography (HPLC). During the process, a number of parameters, including the type of coating solvent, amount of coating, and the method for subsequent solvent removing, have been proved to affect the performance of the resultant CSPs. Coating times and the concentration of coating solution, however, also makes a difference to CSPs' performance by changing the arrangement of cellulose derivatives while remaining the coating amount constant, have much less been studied before, and thereby, were systematically investigated in this work. Results showed that CSPs with more coating times exhibited higher chiral recognition and column efficiency, suggesting that resolution was determined by column efficiency herein. Afterwards, we also investigated the effect of coating amount on the performance of CSPs, and it was shown that the ability of enantio-recognition did not increase all the time as the coating amount; and four of seven racemates achieved best resolution when the coating amount reached to 18.37%. At the end, the reproducibility of CDMPC-coated CSPs were further confirmed by two methods, ie, reprepared the CSP-0.15-3 and reevaluated the effect of coating times.

KEYWORDS

cellulose tris (3,5-dimethylphenylcarbamate), chiral recognition, chiral stationary phase, enantioseparation, high-performance liquid chromatography, resolution

1 | INTRODUCTION

Optical resolution of racemic compounds by high-performance liquid chromatography (HPLC) using chiral stationary phases (CSPs) is one of the most popular methods for determining optical purity and obtaining optically pure enantiomers.[1-3] Polysaccharide-based CSP developed by Okamoto and co-workers[4,5] had achieved a great reputation in the area of chiral resolution because of their high selectivity, sensitivity, and reproducibility. Among the derivatives of polysaccharide, cellulose tris (3,5-dimethylphenylcarbamate) (CDMPC), with two electron-donating groups at 3- and 5-position, shows very high chiral recognition abilities to many racemates.[6-8]

The CDMPC-CSP was typically prepared by coating CDMPC onto large pore silica gel with the coating amount of 20 wt%.[4] The influences of silica gel, CDMPC, and coating process on chiral discrimination have been

investigated by many researchers. Much work so far has focused on the effect of the physical and chemical property of silica, including particle size,[9] pore size,[5,10-12] and functional groups on the silica gel surface.[13] Okamoto[14] and Zhang[15] have reported the influence of the properties of CDMPC, for instance, the degree of polymerization or the molecular weight of the cellulose used for preparing CDMPC and the substitution degree of the prepared CDMPC. Considerable research efforts have also been devoted to the influence of the parameters of coating process, such as the type of coating solvent,[5,16] coating amount,[5,11] and the method of removing solvent.[17] However, few studies have been reported about the influence of coating times or concentration of coating solution on the performance of the CDMPC-CSP. According to the mechanism of CDMPC-CSP for chiral separation proposed by Okamoto et al,[18] whether the arrangement of cellulose derivatives was ordered may affect the separation performance, and the coating times and the concentration of the coating solution might affect the arrangement of cellulose derivatives. On the other hand, the CDMPC-CSP had been commercially available from Daicel Chemical Industries, Ltd. and Chiral Technologies. Since the expiration of the patents covering these CSPs, generic materials have become available from a number of suppliers under different tradenames. However, the overall performance of these generics often differ significantly from the original products in terms of chiral recognition capability, retention behavior, and column efficiency, because distinct supports and coating protocols are used for their fabrication (just as all C18 RPC columns are not equivalent).[19]

In the present study, the influence of the coating times and coating amount on the chiral discrimination of the CDMPC-CSP was investigated. We prepared CDMPC-CSP with different coating times and different concentration of coating solution, and compared the chromatographic performance of these chiral stationary phases. The CSP with excellent performance was prepared through optimization, and the plate numbers for (±) trans-stilbene oxide exceed 40 000/m. This indicates coating times or concentration of coating solution should be taken into account in the preparation process of CDMPC-CSP.

2 | MATERIALS AND METHODS

2.1 | Chemicals and apparatuses

Spherical silica gel, with a particle diameter of 7 μm and a pore size of 80 nm, was prepared by our group in the laboratory. Specifically, the silica gel with a mean pore size of 10 nm was prepared by agglutination of silica sols in presence of a polymer, followed by calcination of the beads. Then, the silica gel with a mean pore size of 80 nm was gained by a certain concentration of NaCl solution impregnation of small pore silica gel and calcination of the beads, followed by pickling with hydrochloric acid. Cellulose was purchased from Sigma-Aldrich (Shanghai, China). 3,5-dimethylphenyl isocyanate was purchased from InnoChem (Beijing, China). Pyridine, methanol, tetrahydrofuran (THF), and xylene were analytical grade, and were purchased from Sinopharm Chemical Reagent (Shanghai, China). N-hexane and isopropanol were HPLC grade, and were purchased from Spectrum (Shanghai, China) and Sinopharm Chemical Reagent (Shanghai, China), respectively.

IR spectra were recorded on a Nicolet-6700 instrument (Thermo Fisher Scientific, USA). The morphology analysis of the samples was obtained by using a JEOL JSM-7800F scanning electron microscope (SEM). The particle size distribution was determined using the Malvern Mastersizer 2000 (Malvern Instruments Ltd., UK). The pore size distribution was conducted on an AutoPore IV 9500 (Micromeritics Instrument Corporation, USA). The specific surface areas were measured using a BET surface area analyzer (NOVA-2000, Quantachrome, USA). Elemental analysis measurement was conducted on a CHNS-analyzer (Elementar Vario III, Elementar Analysensysteme GmbH, Hanau, Germany). The ^{1}H NMR spectra (700 MHz) were recorded in tetrahydrofuran-d8 using a Bruker-700 spectrometer (Bruker, USA). Molecular weight was determined by a Viscotek GPC $_{MAX}$ liquid chromatograph. THF was used as the eluent. Polystyrene standards (Aldrich) were used for calibration. Chromatographic experiments were performed using a SHIMADZU HPLC system consisting of a UV detector, a quaternary pump, a column oven, and an auto sampler.

2.2 | Preparation of CDMPC-coated CSP

Tris (3,5-dimethylphenylcarbamate) cellulose was prepared according to the reported method.[4]

The different amount of CDMPC (i = 0.05, 0.1, 0.15, 0.2, 0.25 g) was dissolved in 3.5 mL THF. The solution was added to the silica gel (2 g), and the wetted silica gel was dried under vacuum after it was dried at ambient temperature. This coating process was repeated for j (j = 1, 2, 3, 4, 5) times to get the achieved amount. According to the above coating method, a series of CSP-i-j samples were prepared when the amount of CDMPC and coating times were different. The theoretical coating amount was shown in Table 1

TABLE 1 The coating times and amount per coating of chiral stationary phases (CSP)-*i*-*j* and their total coating amount

Amount per coating *i*/g	Coating times *j*				
	1	2	3	4	5
0.05	-[a]	4.76	6.98	9.09	11.11
0.1	4.76	9.09	13.04	16.67	20
0.15	6.98	13.04	18.37	23.08	b
0.2	9.09	16.67	23.08	-[b]	-[c]
0.25	11.11	20	-[b]	-[c]	-[c]

[a]CSP with too low coating amount has almost no chiral recognition ability.

[b]When the coating amount of CDMPC exceeds 25%, the stationary phases aggregated and could not be packed well.

[c]CSPs with too high coating amount were also not prepared.

2.3 | Column packing and chromatography

CSP-i-j was suspended in a solution consisting of n-hexane and isopropanol (n-hexane/isopropanol, 90/10, *v*/v). The suspension was sonicated to form slurries, and then was packed into empty stainless-steel column (150 × 4.6 mm ID) at 40 MPa pressure, with n-hexane as the displacing solvent. All the columns were packed with the same method. Enantioseparation evaluations were performed in the mobile phases of n-hexane/isopropanol (90/10), and the dead times (t_0) were determined with 1, 3, 5-tris- (tert-butyl) benzene in the same mobile phase. All of the mobile phases were filtered and degassed before use. The chiral sample (as shown in Figure 1) solutions were prepared by dissolving the analytes in mobile phase(C, D, E, and G) or isopropanol (A, B, and F) (1 mg mL^{-1}). All the samples were filtered through 0.45 μm filters. The injection volume was set as 10 μL. The column temperature was set at 30°C, and the flow rate was 0.5 mL min^{-1} for the detection of all analytes. All analytes were measured at 254 nm. Retention factor (k′), separation factor (α), resolution (Rs), and column efficiency (N) were calculated in the same way as previous literature.[20]

3 | RESULTS AND DISCUSSION

3.1 | Characterizations of prepared CSP

As described in Section 2, the wide-pore silica microspheres were prepared by calcinating silica after NaCl impregnation. Figure 2A-C showed the pore size distribution, particle size distribution, and SEM images of silica gel, respectively. It was shown that the silica gel had a pore size of 80 nm and a particle diameter size of 7 μm, and they were consistent with the results shown in the SEM image. In addition, it can be seen that the pore size distribution and particle size distribution of silica gel used in this study were relatively narrow.

The cellulose derivative plays important roles in realizing the chiral discrimination of enantiomers. The successful synthesis of CDMPC in this study was confirmed by Fourier-transform infrared (FT-IR), element analysis and ^1H NMR. As shown in Figure 3, the peaks of 3500-3200 cm^{-1} in the line of cellulose was attributed to the stretching vibration of the hydroxyl group of cellulose, and the peaks of 3000-2800 cm^{-1} in the same line represented the antisymmetric stretching peak of methylene. The peaks of 3500-3200 cm^{-1} in the line of CDMPC was narrowed, and the strength was weakened because the hydroxyl group of cellulose was reacted and a secondary amide appeared. The presence of new peaks at 1750 cm^{-1} and 1600-1450 cm^{-1} in the line of CDMPC can be attributed to the carbonyl group and frame vibration of benzene, respectively, which confirmed the successful addition of 3,5-dimethylphenyl isocyanate.

Elemental analysis $(C_{33}H_{37}N_3O_8)_n$ found: C 64.04, H 6.28, N 6.75 and calculated: C 65.66, H 6.18, N 6.98. According to this result, the degree of substitution (DS) of the hydroxyl group in the cellulose skeleton was 2.68.

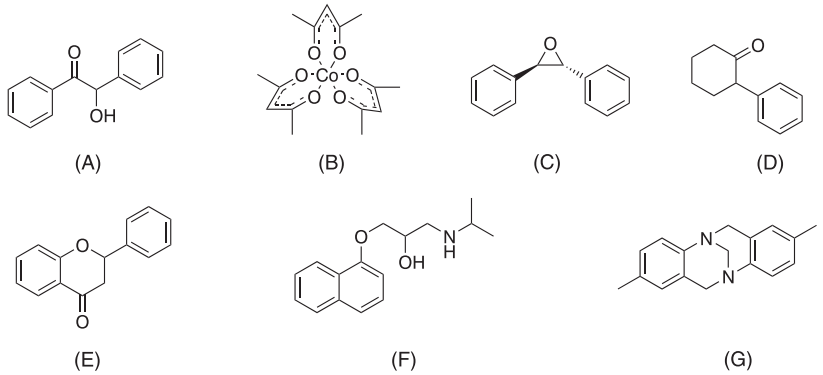

FIGURE 1 Structures of racemates

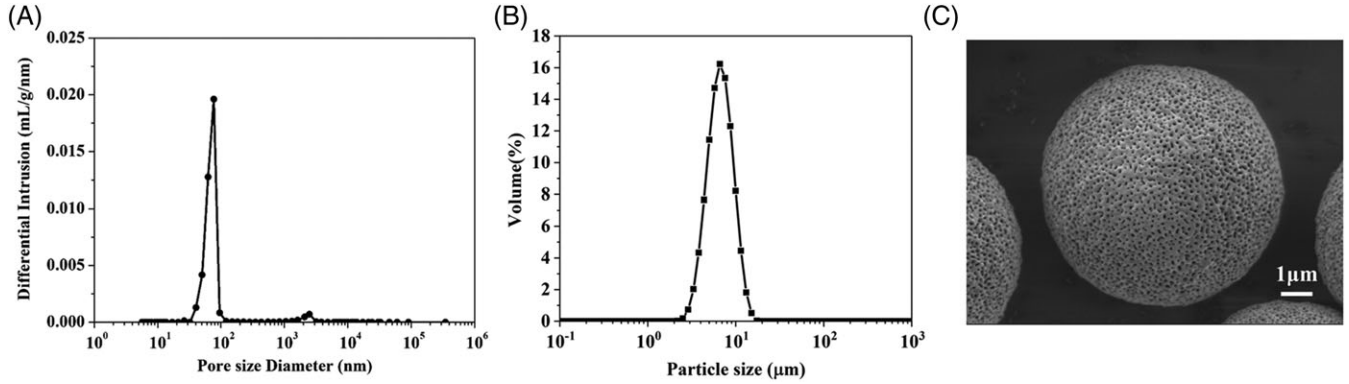

FIGURE 2 (A) pore size distribution, (B) particle size distribution, and (C) SEM image of the wide pore silica

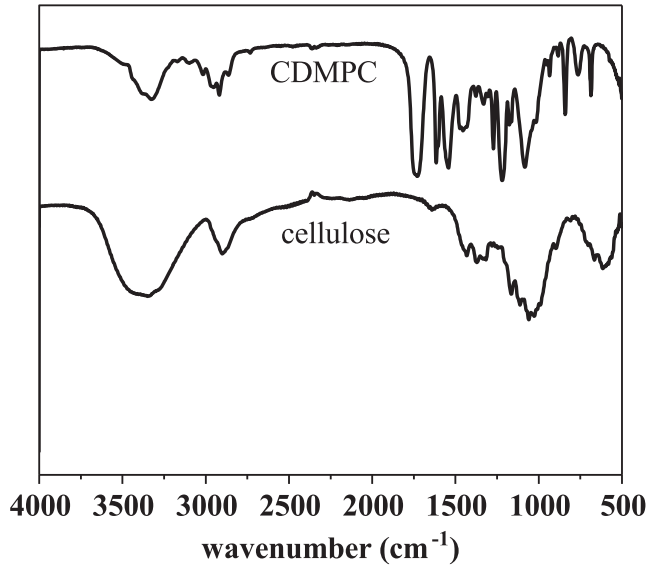

FIGURE 3 Fourier-transform infrared (FT-IR) spectra of cellulose and CDMPC

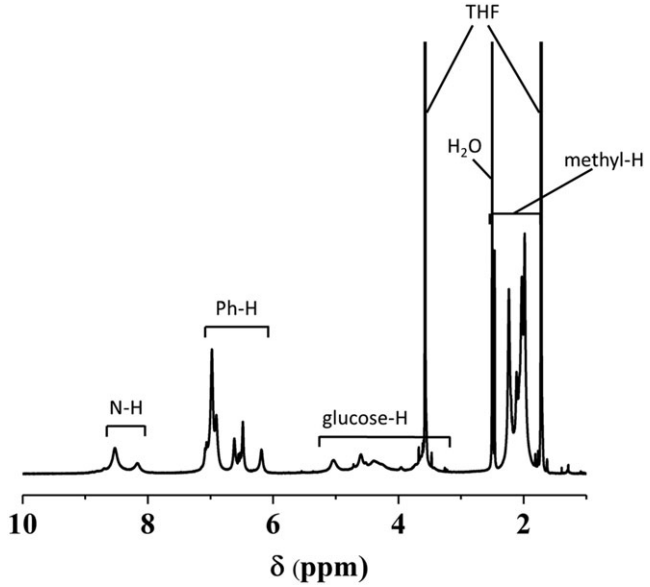

FIGURE 4 ^{1}H NMR spectrum of CDMPC in tetrahydrofuran-d8 (700 MHz)

The ^{1}H NMR spectrum of the obtained cellulose derivative was shown in Figure 4. The peaks in the range of δ = 8.0-8.8, 6.1-7.2, and 1.8-2.4 were attributed to the amino protons, aromatic protons, and methyl protons of 3,5-dimethylphenylcarbamate, respectively. The peaks in the range of δ = 2.8-5.5 were attributed to the protons of cellulose backbone. Those peaks proved that the carbanilation of cellulose was achieved successfully. The peak shape was similar with that cellulose derivative with DS of 3.0 in previous study.[21]

The molecular weight of the obtained cellulose derivative was measured by the gel permeation chromatography (GPC), and the result was shown in Table 2.

In order to characterize CSPs, the specific surface area (S_{BET}), particle size distribution, pore size distribution, and SEM of CSPs were investigated for selected CSPs. It was shown in Table 3 that the uncoated silica gel had a specific surface area of 29.86 m^2 g^{-1}, and the CSPs had a specific surface area of 25.37 m^2 g^{-1} and 24.69 m^2 g^{-1}, respectively. The particle diameter of the uncoated silica gel was 7.13 μm, and the particle diameters of the two CSPs

TABLE 2 The molecular weight of the cellulose derivative measured by gel permeation chromatography (GPC)

Mn-(Daltons)	Mw-(Daltons)	Mz-(Daltons)	Mp-(Daltons)	Mw/Mn
172 067	254 679	534 127	161 878	1.480

TABLE 3 The specific surface area and particle size distribution of chiral stationary phases (CSPs) and uncoated silica

	S_{BET}(m^2 g^{-1})	Particle size (μm)
Uncoated silica	29.86	7.13
CSP-0.20-3	25.37	7.89
CSP-0.15-4	24.69	7.80

were 7.89 μm and 7.80 μm, respectively. The pore size distribution and particle size distribution of the CSP-0.15-4 and naked silica were shown in Figure 5. The particle size of CSP increased slightly, and a new peak of less than 80 nm appeared in the pore size distribution of the prepared CSP. Changes in specific surface area, particle size distribution, and pore size distribution confirmed that the CDMPC was coated on silica gel. However, there was less obvious difference from the SEM images (shown in Figure 6), and it was not found in the particle agglomeration or the particles with a size of tens of microns from the SEM images and the patterns of particle size distribution, which indicated the dispersion of CSPs was very well.

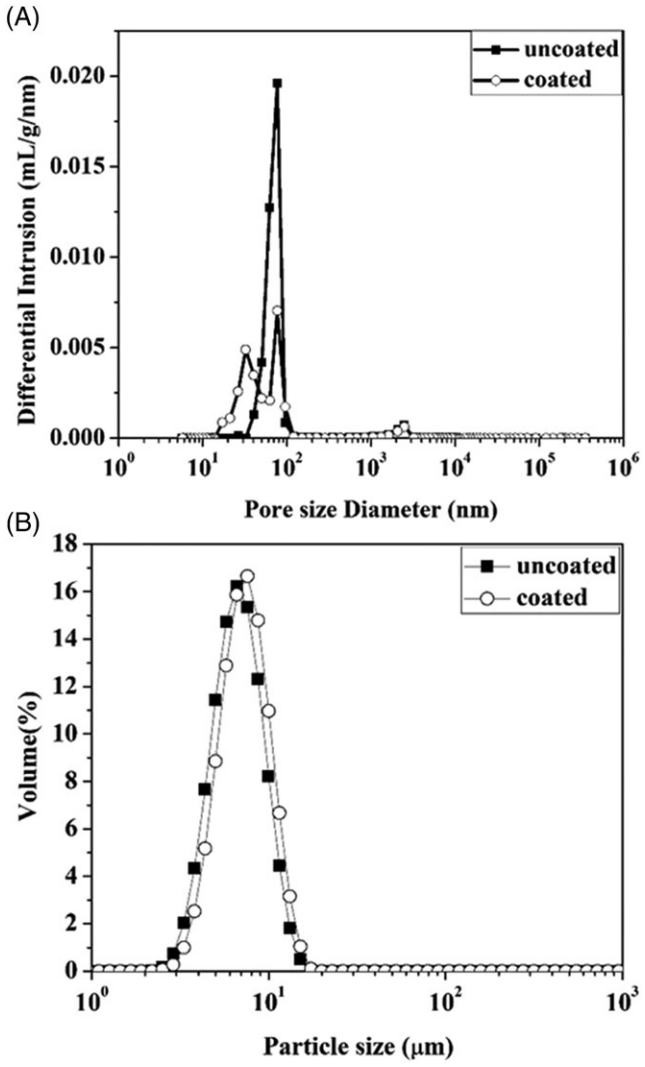

FIGURE 5 The pore size distribution (A) and particle distribution (B) of uncoated silica and the CSP-0.15-4

3.2 | Separation properties of prepared columns

The 18 CSPs prepared in Table 1 were evaluated with racemates in Figure 1, and the results obtained were shown in Table S1.

3.3 | Influence of coating times and each coating amount

Eight groups of chiral stationary phase with different total coating amount were compared in this section, these CSPs in each group had the same total coating amount but different coating times. The influence of coating times on resolution Rs was illustrated in Figure 7. The separation results of seven racemates on each CSP in the eight groups were shown in Figure 7A-H, respectively. To gain more insight, Figure 7I counts the racemates number of achieving better resolution on the two or three CSPs in each group. For example, in Figure 7B, five of seven racemates get better resolution on CSP-0.05-3 than in CSP-0.15-1. It was indicated that the CSPs with more coating times showed higher chiral recognition in most groups in Figure 7I, and the reason for this tendency will be explained later.

Figure 8 showed the effect of coating times on separation factor α, and the data processing method of separation factor was similar to that of resolution. Different from the resolution, the CSPs with less coating times showed higher selectivity in most groups. The concentration of coating solution decreased with the increase of coating times when the total coating amount was the same. As we all know, CDMPC forms a lyotropic liquid crystal phase at the high solution concentrations.

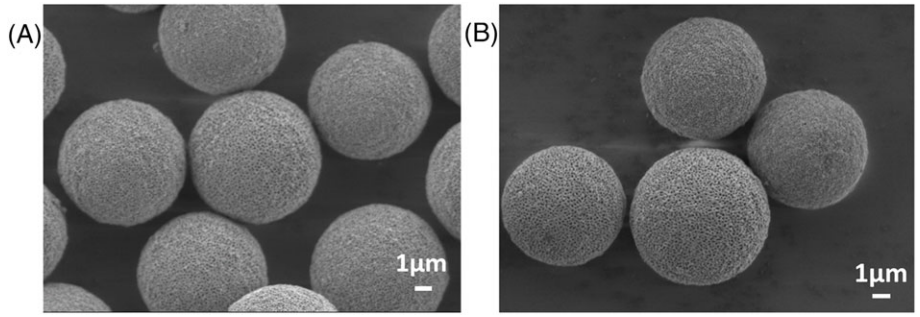

FIGURE 6 SEM image of (A) uncoated silica and (B) CSP-0.20-3

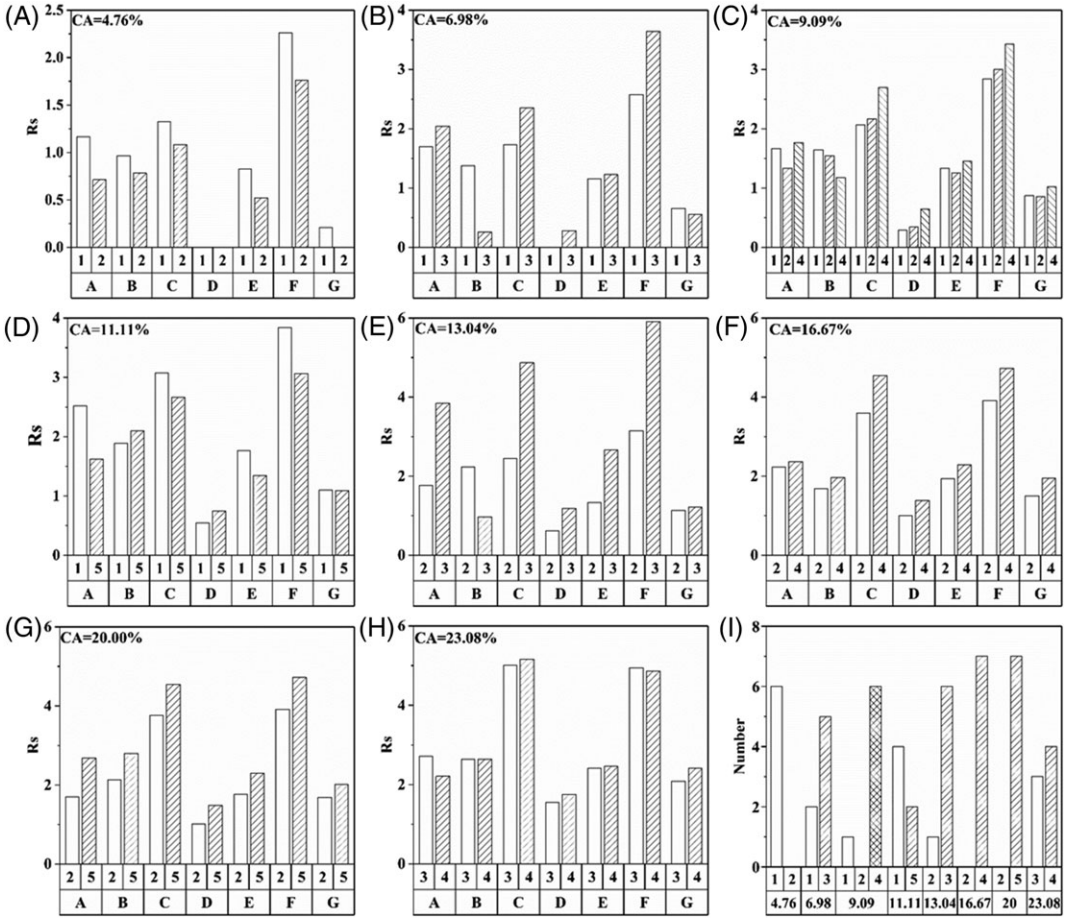

FIGURE 7 Resolution of seven racemates on chiral stationary phases (CSPs) in eight groups (A-H), and the racemates number of achieving better resolution on the two or three CSPs in each group (I). CA is the coating amount. The first line of the abscissa in each figure represents the coating times, and the second line represents the racemates to be separated (A-H) or the total coating amount (I)

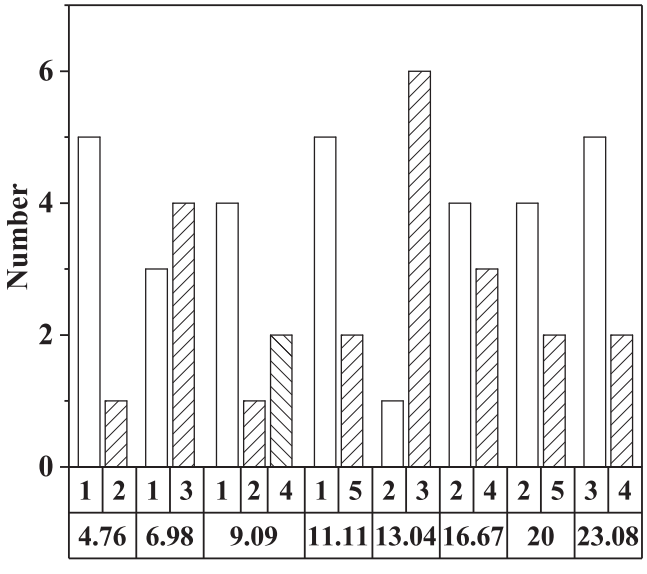

FIGURE 8 The racemates number of achieving better separation factor on the two or three chiral stationary phases (CSPs) in each group

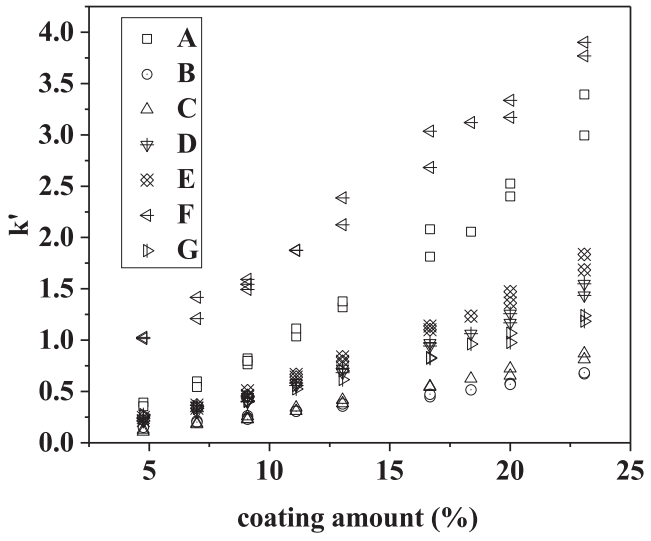

FIGURE 9 The retention factors of seven racemates

However, when the dilute solution was used, it would turn to be more and more denser during the evaporation of the solvent. Ultimately, all of the coating solution was at high concentration before it was coated on silica, so all of the CDMPC solution could form an ordered structure. The decrease of separation selectivity with coating times may result from the different orientation of the CDMPC coated by every time.

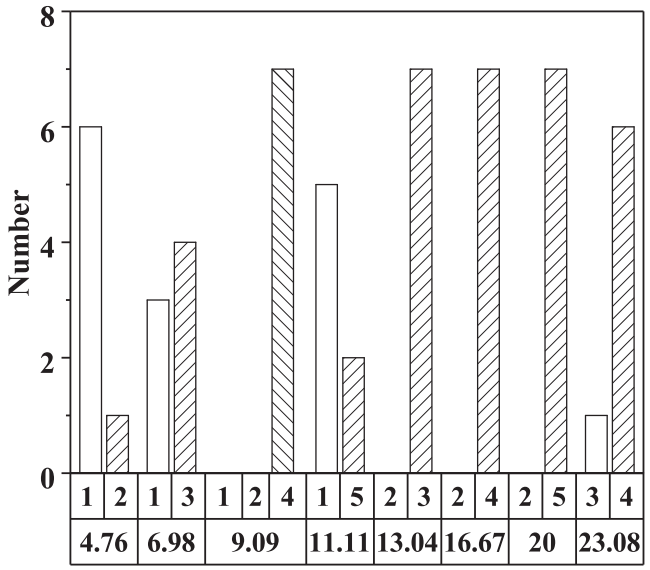

FIGURE 10 The racemates number of achieving higher column efficiency on the two or three chiral stationary phases (CSPs) in each group

The influence of coating times on retention factors k_1' was shown in Figure 9. Almost no change of k_1' value was observed in each group. The total coating amount of every CSP in each group was the same, so the amount of adsorption site was the same, thus they had similar retention factor. The influence of coating times on column efficiency was shown in Figure 10. It can be seen that the CSPs with more coating times showed higher column efficiency in most groups. It is easy to form a homogeneous coating on the surface of silica with increasing the coating times and decreasing the concentration of coating solution. Then, it was in favor of the diffusion of the samples between the stationary phase and the mobile phase, and the efficiency of the column became higher.

The influence of coating times on resolution, separation factor, retention factor, and column efficiency were summarized in Table 4. It was worthwhile mentioning that the column efficiency and resolution displayed the same tendency. It is well-known that resolution can be expressed as a function of the retention factor k_1' (term a), the separation factor α (term b), and column efficiency or the plate number N (term c), as shown in Equation 1. In the present work, from the results we have obtained, it is concluded that the resolution was determined by column efficiency when the coating times of these CSPs were different.

$$R_s = \underbrace{\left(\frac{1}{4}\right)\left[\frac{k}{k+1}\right]}_{(a)} \quad \underbrace{(\alpha - 1)}_{(b)} \quad \underbrace{N^{0.5}}_{(c)}. \tag{1}$$

TABLE 4 The influence of coating times on resolution, separation factor, and column efficiency

	Coating Amount %	Coating Times	Resolution	Separation Factor	Column Efficiency
CSP-0.10-1	4.76	1	√	√	√
CSP-0.05-2		2			
CSP-0.15-1	6.98	1			
CSP-0.05-3		3	√	√	√
CSP-0.20-1	9.09	1		√	
CSP-0.10-2		2			
CSP-0.05-4		4	√		√
CSP-0.25-1	11.11	1	√	√	√
CSP-0.05-5		5			
CSP-0.15-2	13.04	2			
CSP-0.10-3		3	√	√	√
CSP-0.20-2	16.67	2		√	
CSP-0.10-4		4	√		√
CSP-0.25-2	20	2		√	
CSP-0.10-5		5	√		√
CSP-0.20-3	23.08	3		√	
CSP-0.15-4		4	√		√

3.4 | Influence of total coating amount

Overall, it was revealed that columns with low coating amount (less than 12%) showed very poor chiral recognition, and the highest resolution for all of the racemates never appeared in these columns. The reason for this low resolution was that the amount of the chiral selector was too low to offer sufficient energy difference for the two enantiomers. On the other hand, when the coating amount was low, the silanol group of the bare silica gel might provide an achiral effect. However, the ability of enantio-recognition did not increase all the time as the coating amount, and four of seven racemates achieved best resolution when the coating amount was 18.37%.

To investigate the influence of coating amount on each parameters (t_0, k_1', α, and N), CSPs with the same coating times need to be selected. In this study, the CSPs with the coating times of three were taken as an example. As it was plotted in Figure 11A, the retention time of 1, 3, 5-tris-(tert-butyl) benzene noted as dead time t_0 on these column decreased as the coating amount increased. This was because the coating of CDMPC resulted in a smaller pore size and a shorter diffusion distance. The influence of coating amount on retention factor k_1' could be seen in Figure 9. The amount of adsorption site increased with coating amount, so the retention factor increased. In terms of separation factor α (shown in Figure 11B), except for B, they hardly changed with the change of coating amount, which indicated that the coating amount had little effect on the separation factor. As shown in Figure 12, the effect of coating amount on column efficiency N was the same as that of previous studies,[22] and an excessive amount of CDMPC affected the intra-particle diffusion of the samples, lowering performance of the columns.

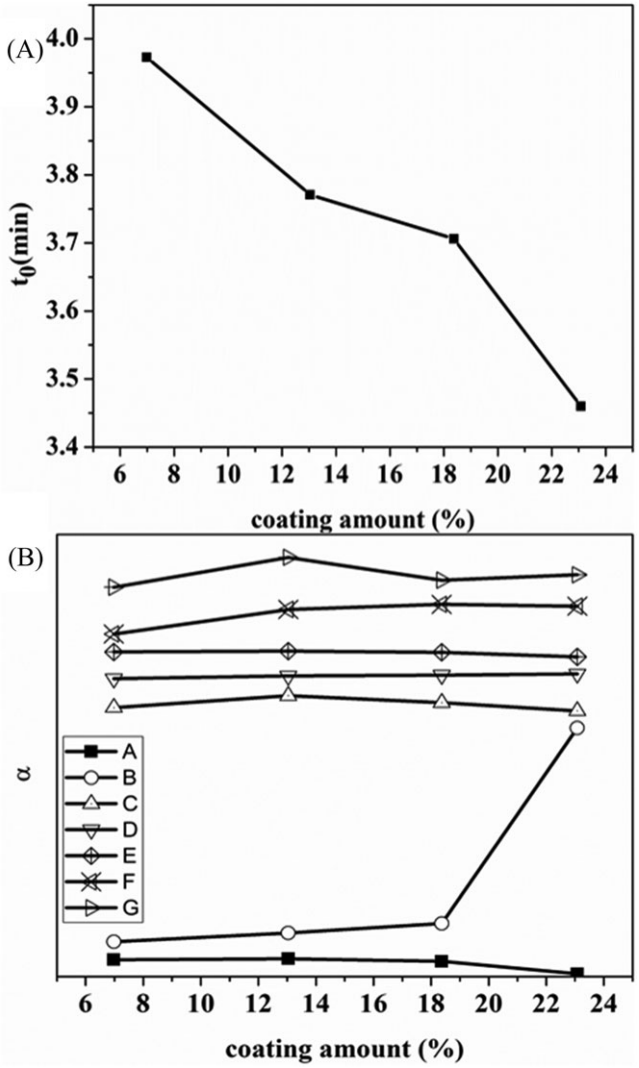

FIGURE 11 The influence of coating amount on dead time (A) and separation factor (B)

3.5 | Reproducibility of CDMPC-coated CSPs

The CSP-0.15-3 with the best separation performance was reprepared and evaluated, and the evaluation parameters were compared with the original one in the previous section. Figure 13 showed the results of reprepared CSP-0.15-3 with the resolution of the seven substances on the left and the column efficiency on the right. It can be seen from the figure that the reproducibility was good whether it was resolution or column efficiency.

CSPs with a total coating amount of 15% and coating times of 1, 2, 3, and 4 were prepared and evaluated, and their separation performance was compared according to the processing method mentioned before. Table S2

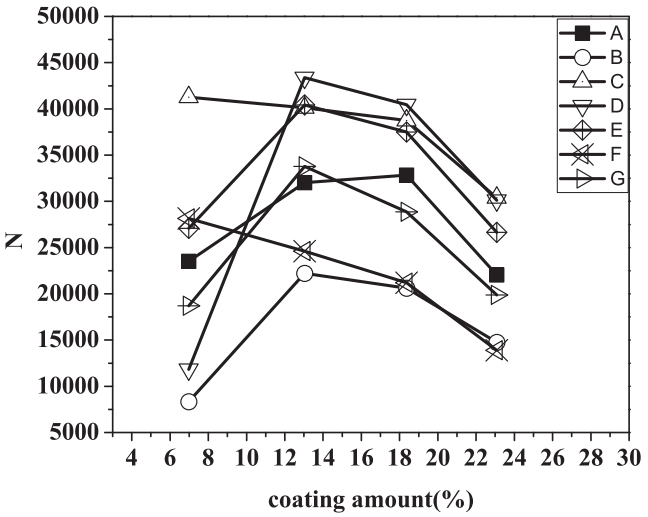

FIGURE 12 The influence of coating amount on column efficiency (N)

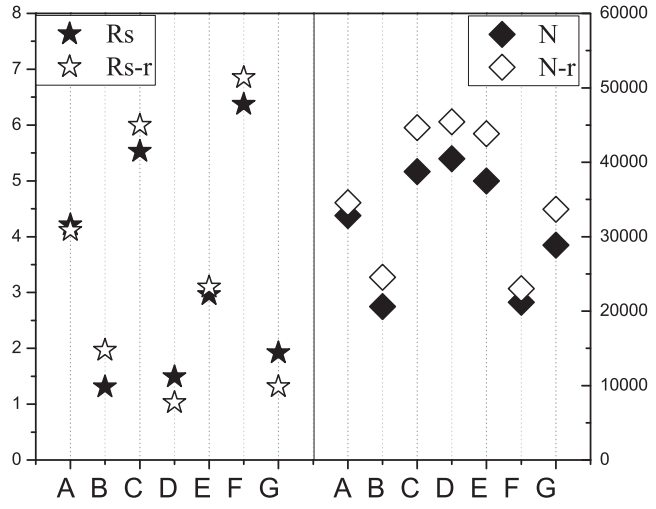

FIGURE 13 The results of reprepared CSP-0.15-3

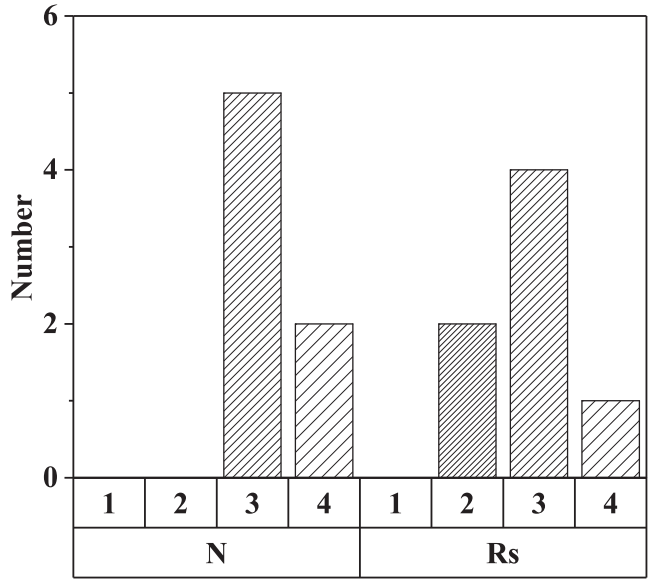

FIGURE 14 The racemates number of achieving better resolution (Rs) or column efficiency (N) on the 4 CSPs

showed the evaluation results of CSPs with a total coating amount of 15% and different coating times. The racemates number of achieving better resolution or column efficiency on the four CSPs was shown in Figure 14. It was indicated that the CSPs with three coating times showed the best chiral recognition, and it was consistent with the conclusion before. This result also showed that the before experiment results were relatively reproducible.

4 | CONCLUSION

In this work, the influence of the coating times and coating amount on the chiral discrimination of the CDMPC-CSP was investigated. The coating times had great influence on the chiral separation performance under the same coating amount. Resolution and column efficiency increased with the increase of coating times, while separation factor decreased with the increase of coating times. Resolution was determined by column efficiency. The CSPs with the coating times of three were selected to investigate the influence of coating amount. It was found that the coating amount had a significant effect on retention factor and column efficiency. The CSP with excellent performance was prepared through optimization. When the coating amount was 18.37%, the plate numbers for (±) trans-stilbene oxide exceed 40 000/m, and four of seven racemates achieved the best resolution. The reproducibility of CDMPC-coated CSPs were confirmed by repreparing the CSP-0.15-3 and reevaluating the effect of coating times.

ACKNOWLEDGMENT

We gratefully acknowledge support from the Youth Innovation Promotion Association of CAS.

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REFERENCES AND NOTES

1. Lorenz H, Seidel-Morgenstern A. Processes to separate enantiomers. *Angew Chem Int Ed Engl*. 2014;53(5):1218-1250.
2. Okamoto Y, Ikai T. Chiral HPLC for efficient resolution of enantiomers. *Chem Soc Rev*. 2008;37(12):2593-2608.
3. Okamoto Y, Kaida Y. Resolution by high-performance liquid chromatography using polysaccharide carbamates and benzoates as chiral stationary phases. *J Chromatogr a*. 1994; 666(1–2):403-419.
4. Okamoto Y, Kawashima M, Hatada K. Chromatographic resolution: XI. Controlled chiral recognition of cellulose triphenylcarbamate derivatives supported on silica gel. *J Chromatogr a*. 1986;363(2):173-186.
5. Yashima E, Sahavattanapong P, Okamoto Y. HPLC 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*. 1996;8(6):446-451.
6. Shen J, Okamoto Y. Efficient separation of enantiomers using stereoregular chiral polymers. *Chem Rev*. 2016;116(3):1094-1138.
7. Tachibana K, Ohnishi A. Reversed-phase liquid chromatographic separation of enantiomers on polysaccharide type chiral stationary phases. *J Chromatogr a*. 2001;906(1):127-154.
8. Yashima E. Polysaccharide-based chiral stationary phases for high-performance liquid chromatographic enantioseparation. *J Chromatogr a*. 2001;906(1–2):105-125.

9. Grieb SJ, Matlin SA, Phillips JG, Belenguer AM, Ritchie HJ. Chiral HPLC with carbohydrate carbamates: influence of support structure on enantioselectivity. *Chirality*. 1994;6(2): 129-134.
10. Minguillon C, Franco P, Oliveros L. Bonded cellulose-derived high-performance liquid chromatography chiral stationary phases. II. Influence of the porosity of the silica gel matrix on performance. *J Chromatogr a*. 1996;728(1 + 2):415-422.
11. Félix G, Zhang T. Chiral packing materials for high-performance liquid chromatographic resolution of enantiomers based on substituted branched polysaccharides coated on silica gel. *J Chromatogr a*. 1993;639(2):141-149.
12. Bezhitashvili L, Bardavelidze A, Ordjonikidze T, et al. Effect of pore-size optimization on the performance of polysaccharide-based superficially porous chiral stationary phases for the separation of enantiomers in high-performance liquid chromatography. *J Chromatogr a*. 2017;1482:32-38.
13. Grieb S, Matlin S, Belenguer A, Ritchie H. Chiral high-performance liquid chromatography with cellulose carbamate-coated phases influence of support surface chemistry on enantioselectivity. *J Chromatogr a*. 1995;697(1):271-278.
14. Okada Y, Yamamoto C, Kamigaito M, Gao Y, Shen J, Okamoto Y. Enantioseparation using cellulose Tris(3,5-dimethylphenylcarbamate) as chiral stationary phase for HPLC: influence of molecular weight of cellulose. *Molecules*. 2016;21(11).
15. Zhang X, Wang L, Dong S, et al. Nanocellulose 3, 5-Dimethylphenylcarbamate derivative coated chiral stationary phase: preparation and Enantioseparation performance. *Chirality*. 2016;28(5):376-381.
16. Liu Y, Zou H. High-performance liquid chromatographic evaluation of a coated cellulose tris(3,5-dimethylphenylcarbamate) chiral stationary phase having a small-pore silica support. *J Chromatogr a*. 2008;1178(1–2):118-125.
17. Vinkovic V, Stucchi L, Navarini L, Sunjic V. Comparison of two methods of preparation of the stationary phase for HPLC chiral columns based on tris(3,5-dimethylphenylcarbamoyl) cellulose. *J Liq Chromatogr Relat Technol*. 1999;22(7): 1041-1053.
18. Okamoto Y, Yashima E. Polysaccharide derivatives for chromatographic separation of enantiomers. *Angew Chem Int Ed*. 1998;37(8):1020-1043.
19. Snyder LR, Kirkland JJ, Dolan JW. Enantiomer Separations. *Introduction to Modern Liquid Chromatography, Third Edition*. 665-724.
20. Tang S, Bin Q, Chen W, Bai ZW, Huang SH. Chiral stationary phases based on chitosan bis (methylphenylcarbamate)-(isobutyrylamide) for high-performance liquid chromatography. *J Chromatogr a*. 2016;1440:112-122.
21. W-w C, M-c D, Zhang M, Gao X, He J-s. Chiral separation abilities of homogeneously synthesized cellulose 3, 5-dimethylphenylcarbamates: influences of degree of substitution and molecular weight. *Chin J Polym Sci*. 2015;33(12):1633-1639.
22. Chen LM, Wang XS, Liu X, Jiang SX. The influence of coating thickness of polymer on chiral discrimination of cellulose Tris(3,5-dimethyl Phenylcarbamate) chiral stationary phase. *International Journal of Polymer Analysis and Characterization*. 2009;14(2):160-169.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

How to cite this article: Wei Q, Su H, Gao D, Wang S. HPLC with cellulose Tris (3,5-DimethylPhenylcarbamate) chiral stationary phase: Influence of coating times and coating amount on chiral discrimination. *Chirality*. 2019;1–10. https://doi.org/10.1002/chir.23044