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Efficient Immobilization of Polysaccharide Derivatives as Chiral Stationary Phases via Copolymerization with Vinyl Monomers

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Abstract: The direct chromatographic separation of enantiomers by chiral stationary phases (CSPs) has been extensively developed over the past two decades, and has now become the most popular method for the analytical and preparative separations of enantiomers. Polysaccharide derivatives coated onto silica gel, as CSPs, play a significantly important role in the enantioseparations of a wide range of chiral compounds using high-performance liquid chromatography (HPLC). Unfortunately, the strict solvent limitation of the mobile phases is the main defect in the method developments of these types of coated CSPs. Therefore, the immobilization of polysaccharide derivatives onto silica gel, via chemical bonding, to obtain a new generation of CSPs compatible with the universal solvents used in HPLC is increasingly important. In this article, our recent studies on the immobilization of polysaccharide derivatives onto the silica gel, as CSPs, through radical copolymerization with various vinyl monomers are reported. Polysaccharide derivatives, with low vinyl content, can be efficiently fixed onto silica gel with high chiral recognition.

Keywords: polysaccharide derivative, radical copolymerization, chiral stationary phase, immobilization, HPLC.

Introduction

In recent years, it has been widely accepted that a pair of optically pure enantiomers may exhibit different bioactivities, pharmacological and toxicological behaviors, etc., and therefore, their preparation and analysis have been becoming increasingly important.[1] The economic interests are obvious and essential driving forces in the development of advanced technologies for chiral separations. During the past two decades, chromatographic techniques, such as gas chromatography (GC),[2,3] high-performance liquid chromatography (HPLC),[4-7] supercritical fluid chromatography (SFC)[8,9] and capillary electrochromatography (CEC)[10] have been extensively developed for the separation of enantiomers. In particular, the direct HPLC separation of enantiomers by chiral stationary phases (CSPs) has been recognized to be the most advantageous one and has become one of the most useful methods in many fields dealing with drugs, natural products, intermediates, agrochemicals, etc., not only for determining their optical purity, but also for obtaining optical isomers on a large scale. Until now, many chiral selectors have also been reported along with the constant development in the methodology and applications of chromatographic enantioseparations, such as proteins,[11] oligosaccharides,[12] polysaccharides,[5-7,13-16] antibiotics[17] and other low molecular weight compounds.[18] Among them, polysaccharide derivatives, such as cellulose 3,5-dimethylphenylcarbamate (CDMPC) and amylose 3,5-dimethylphenylcarbamate (ADMPC) as shown in Figure 1, exhibit a unique chiral recognition to a broad range of chiral compounds and have been widely used as

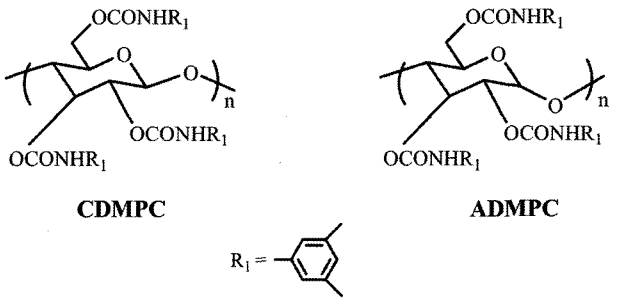

Figure 1. Structures of the CDMPC and ADMPC.

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CSPs for HPLC.[5-7] More than 80% of the tested racemates have been successfully resolved on two kinds of polysaccharide derivative-based CSPs[16] and 70% of the CSPs used for preparative purposes are derived from polysaccharide derivatives.[19]

These polysaccharide-based CSPs have been traditionally prepared by coating the derivatives on the macroporous silica gel.[20,21] Due to their coated nature, they can be only used with a limited range of solvents as the mobile phases, such as alkanes, alcohols, acetonitrile and their mixtures, or aqueous solvents including alcohols or acetonitrile. The solvents not listed may damage or destroy the coated CSPs, which is a serious defect since the suitable selection of various solvents is very important for attaining an efficient resolution. For a preparative large-scale separation, the sufficient solubility of a target sample is essential for achieving a high productivity. Thus, versatility in the solvent selection is highly desirable. Therefore, the preparation of chemically immobilized CSPs with polysaccharide derivatives has been considered as a direct approach to confer a universal solvent compatibility on these kinds of CSPs. This flexibility significantly broadens the choice of solvents as mobile phases or sample dissolving reagents, such as tetrahydrofuran, chloroform and many others, which cannot usually be applied on the coated types of CSPs.

Some interesting methods have been developed for the immobilization of the polysaccharide derivatives as CSPs, such as the bifunctional reagent method by diisocyanates,[22,23] the radical polymerization,[24-28] photoirradiation,[29-32] enzyme-catalyzed polymerization,[33] and some others.[34-36] These immobilization methods have been described in two review articles.[37,38] Basically, the previous methods showed a somewhat low efficiency for the immobilization of polysaccharide derivatives, or the immobilized CSPs exhibited a low chiral resolving power except for amylose 3,5-dimethylphenylcarbamate immobilized through a chain end method.[33]

Recently, we developed a method for the immobilization of polysaccharide derivatives on silica gel as CSPs through the radical copolymerization with vinyl monomers.[39-44] It was found that the chiral selectors can be efficiently fixed with a high chiral recognition over the previously reported radical polymerization methods without the addition of vinyl monomers.[24-28] The schemes for the immobilization approaches are shown in Figure 2. In this method, a vinyl group was regioselectively or randomly introduced on the polysaccharide derivatives, and the vinylated polymers were then coated onto the aminated or vinylated silica gel.[41] The radical copolymerization was thus initiated by AIBN with the addition of a vinyl monomer in solution. According to the positions of the placed vinyl groups on the glucose units, the approaches can be classified as regioselective and non-regioselective immobilizations. The influences of the copolymerization conditions, such as the used vinyl monomers, the employed temperature and the structures and content of the vinyl groups introduced on the polysaccharide, on the immobilization efficiency and enantioseparations are described. The solvent versatility of the immobilized CSPs over the coated CSPs is also briefly discussed.

Regioselective Immobilization

In our previous studies, we have reported the first immobilization of polysaccharide derivatives on silica gel as CSPs using the bifunctional reagents of diisocyanates.[22,23] It was found that the immobilization efficiency and the chiral recognitions of the immobilized derivatives were different depending on the positions of the placed covalent bonds on the glucose units, in particular, for cellulose derivatives. The regioselectively prepared CSPs generally exhibited a higher chiral resolution power than the non-regioselectively prepared ones, and the chemical bond at the 6-positions was superior to that at the 2, 3-positions. Based on these facts, we also tried to immobilize the derivatives on silica gel via the 6-positions to ensure a higher chiral recognition of the immobilized chiral selectors when we started to prepare the immobilized CSPs via radical copolymerization.

With this objective, the 2-methacryloyloxyethylcarbamate group (R_2) was regioselectively introduced at the 6-positions on CDMPC according to Figure 3. A protecting group was first placed on the hydroxyl groups at the 6-position, and the

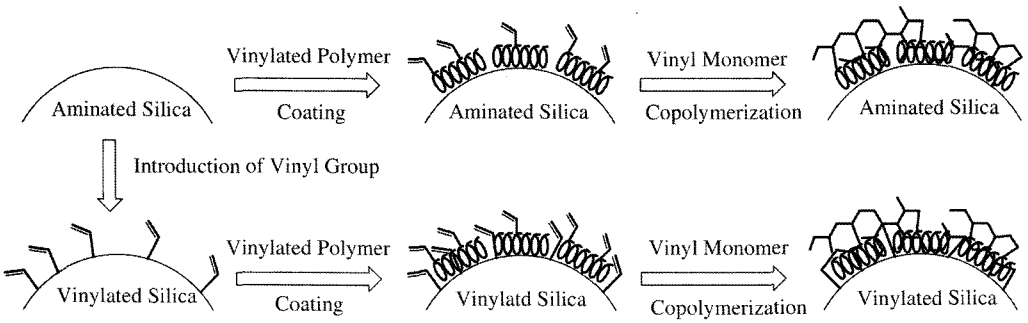

Figure 2. Procedures for the immobilization of the vinylated polysaccharides on the aminated and vinylated silica gel through the radical copolymerizations.

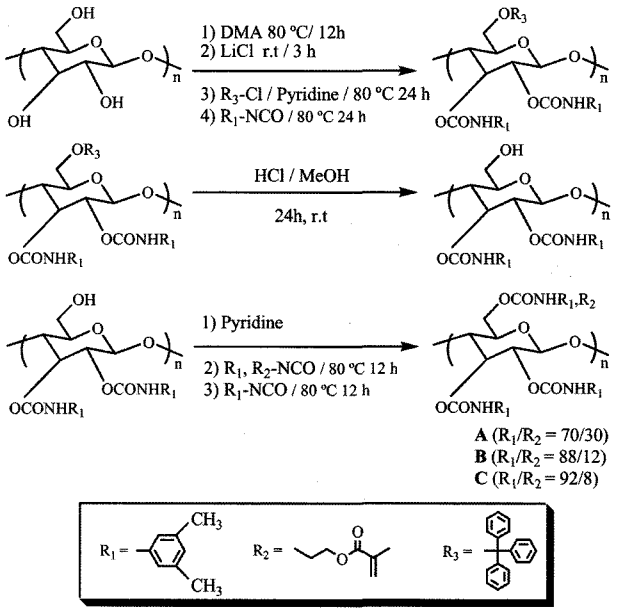

Figure 3. Schemes for the synthesis of the regioselectively vinylated polysaccharide derivatives.

hydroxyl groups at the 2,3-positions were then converted to 3,5-dimethylphenylcarbamate by the reaction with 3,5-dimethylphenyl isocyanate. Thereafter, the hydroxyl groups at the 6-position were recovered by cleaving the protecting group and thus were reacted with a mixture of 3,5-dimethylphenyl isocyanate and 2-methacryloyloxyethyl isocyanate to give the vinylated polysaccharide. By using the same procedures, A, B, and C having different R_2 contents were obtained. Similarly, R_2 was introduced on ADMPC and cellulose 3,5-dichlorophenylcarbamate to give D and E, and another vinyl group of 4-vinylphenylcarbamate (R_5) was also introduced on CDMPC to give F, respectively, as shown in Figure 4.

We have used various vinyl monomers for the copolymerization of the derivatives as shown in Figure 5. We found that A, D, E, and F having 30% vinyl groups can be efficiently immobilized onto the aminated silica gel through the copolymerization with styrene.[40-42] For example, 99% of A can be fixed on the aminated silica gel with 10 wt% styrene. However, the styrene unites in CSP may generate non-selective interactions with the enantiomers and lead to a decrease in the chiral recognition particularly when a large amount of styrene was applied. To attain effective immobilization with a smaller amount of styrene, a vinyl group was introduced on the aminated silica gel to give a vinylated silica gel, and it was found that the immobilization efficiency was improved from 86% (aminated silica) to 97% (vinylated silica) with 5% styrene, and thus reducing the influence of the vinyl monomer on the chiral recognition.[41]

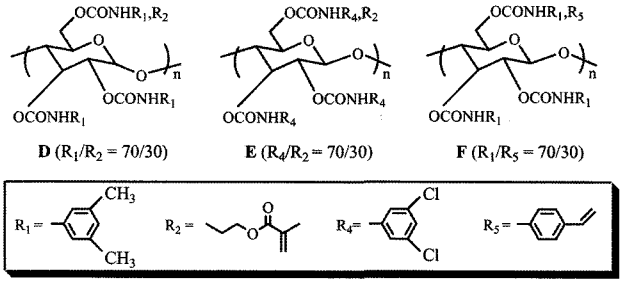

Figure 4. Structures of the regioselectively vinylated polysaccharide derivatives.

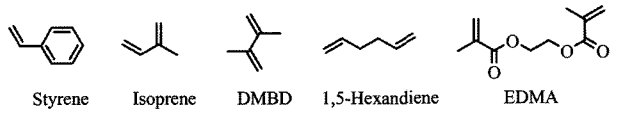

Figure 5. Structures of the used vinyl monomers for the radical copolymerizations (DMBD: 2,3-dimethyl-1,3-butadiene; EDMA: ethylene glycol dimethacrylate).

However, these derivatives still exhibited somewhat lower chiral resolving abilities than the coated CDMPC or ADMPC due to the significant amounts of vinyl groups introduced on the derivatives.[40-42] To further improve the chiral recognitions of the immobilized derivatives, one of the preferable approaches is to reduce the vinyl content introduced on the polysaccharide derivatives. For this, we have synthesized derivatives B and C bearing 12 and 8% R_2, respectively, although the immobilization efficiency might be decreased with the reduction of the vinyl content on the polysaccharide. We optimized the copolymerization conditions to maintain a high immobilization efficiency.

Table I lists the influence of the vinyl monomers on the immobilization and chiral recognitions of B. The prepared CSPs were evaluated using ten test racemates as shown in Figure 6. We can clearly see that both the immobilization efficiency and the chiral recognitions (α values) of B (under the same mobile phase I) were very similar when using isoprene and DMBD as the vinyl monomers. However, the immobilization efficiency can be significantly improved when it came to EDMA. However, the chiral recognitions were reduced in this case probably due to the polar groups on the EDMA that caused non-stereoselective interactions with the enantiomers. This defect can be partially eliminated using a smaller amount of EDMA as the monomer.[43]

We also found that the temperature applied to the radical copolymerization has a significant effect on the immobilization of the chiral selector. The immobilization efficiency of B could be significantly improved from 55 to 92% when heated from 60 to 80 °C even without the addition of any vinyl monomers,[43] which suddenly provides an alternative approach for enhancing the immobilization efficiency. For this reason, the introduced vinyl content was reduced to a level at 8% on C and found that 89% of C can be fixed by copolymerizing with 1,5-hexadiene at 80 °C as listed in Table I. The non-polar nature of 1,5-hexadiene makes it valuable

Table I. Influence of Vinyl Monomers on Immobilization and Enantioseparations[a]

i	B		B			B		C	
ii	DMBD[b]		Isoprene			EDMA[c]		1,5-Hexadiene	
iii	78%		77%			90%		89%	
iv	I		I		II	I		I	
Racemates									
	k'_1	α	k'_1	α	k'_1	α	k'_1	α	k'_1	α
1	0.81(+)	1.64	0.84(+)	1.59	0.58(+)	1.64	1.08(+)	1.67	0.90(+)	1.58
2	0.75(-)	1.49	0.75(-)	1.54	0.72(-)	2.19	1.01(-)	1.22	0.81(-)	1.46
3	2.56(+)	1.34	2.53(+)	1.36	2.46(+)	1.40	3.61(+)	1.24	2.80(+)	1.33
4	1.59(-)	1.17	1.58(-)	1.18	1.61(-)	1.30	2.35(-)	1.02	1.70(-)	1.15
5	1.23(-)	1.27	1.23(-)	1.26	0.90(-)	1.30	1.61(-)	1.25	1.31(-)	1.25
6	1.82(+)	2.42	1.82(+)	2.46	2.89(+)	2.69	2.70(+)	1.91	2.06(+)	2.27
7	0.42(-)	1.13	0.43(-)	1.13	0.12(-)	~1	0.78(-)	~1	0.55(-)	~1
8	1.46(-)	1.23	1.46(-)	1.25	1.06(-)	1.29	2.11(-)	1.15	1.61(-)	1.18
9	1.51(+)	1.40	1.49(+)	1.42	3.12(+)	1.44	1.97(+)	1.14	1.46(+)	1.32
10	2.05(-)	3.83	1.99(-)	3.71	2.73(-)	2.85	2.48(-)	3.32	2.01(-)	3.21

i)Vinylated derivatives; ii)Vinyl monomers (monomer/polymer=30 wt%); iii) Immobilization efficiency calculated from the recovered derivatives; iv) Mobile phases: (I) *n*-hexane/2-propanol (90/10); (II) *n*-hexane/chloroform/2-propanol (85/15/1) for racemates 1-5 and 7-8 and *n*-hexane/chloroform/2-propanol (90/5/5) for racemates 6, 9, and 10. [a]The signs in parentheses represent the CD detection of the first eluted enantiomer; Radical copolymerization: [Vinyl group]/[AIBN]=30; Temp.=60 °C (B) and 80°C (C); Solvent=toluene; Column size: 250 × 2 mm; Flow rate: 0.1 mL/min. [b]2,3-Dimethyl-1,3-butadiene. [c]Ethylene glycol dimethacrylate.

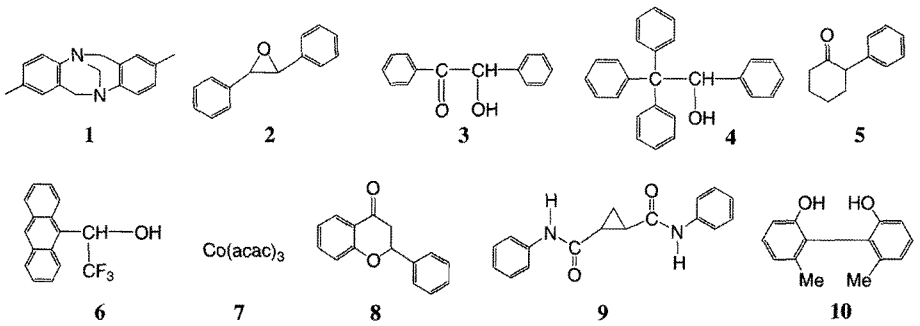

Figure 6. Structures of the ten test racemates for evaluation of the prepared CSPs.

as the vinyl monomer for the efficient immobilization of the vinylated polysaccharides while without an obvious negative influence on the chiral separations even if used with a large amount.

We have compared the chiral recognitions on the immobilized A and B using the same mobile phase.[43] Indeed, the enantioseparations for most of the test racemates were improved to various degrees on B, which confirms our hypothesis that the reduction of the vinyl content on the polysaccharide derivatives really enhances the chiral recognition. The immobilization efficiency could then be improved by the vinyl monomers and temperature during the radical copolymerizations.

Although the polysaccharide derivatives could be effectively immobilized on the silica gel as CSPs using the regioselective method, the vinyl groups were regioselectively introduced at the 6-position on the glucose units by a rather complicated process as already described. Therefore, the time-consumed procedures are not very perfect for practical purposes or scale-up production. However, the random introduction of vinyl groups on the polysaccharide is significantly simplified without the protection and de-protection steps, and thus becomes preferably attractive. This obvious advantage encourages us to develop a practical non-regioselective method for the immobilization of the chiral selector as CSPs.[44]

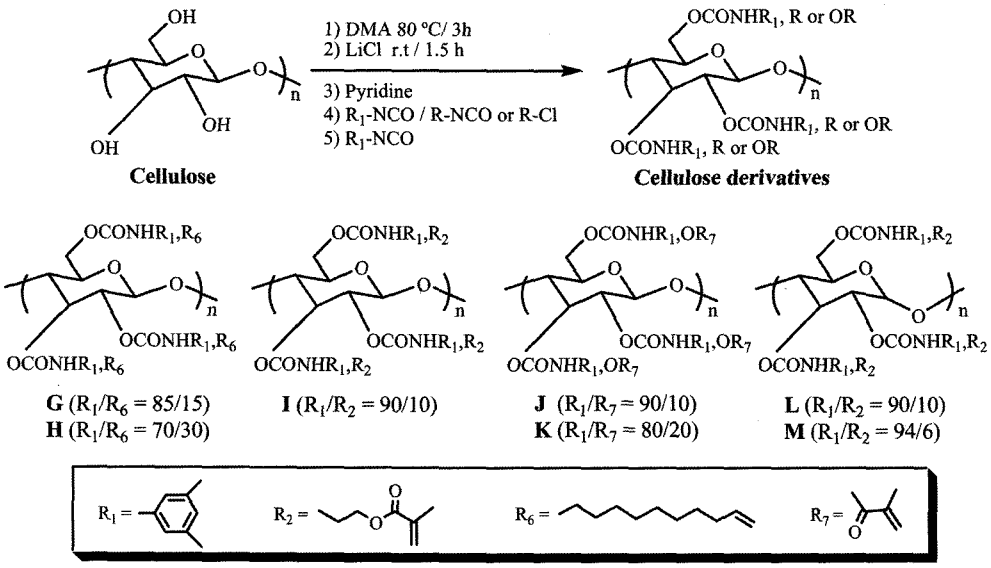

Figure 7. Synthesis of the randomly vinylated polysaccharide derivatives.

Non-regioselective Immobilization

A typical example for the random introduction of vinyl groups on CDMPC is presented in Figure 7. In this case, the cellulose was first dissolved in a mixture of DMA/LiCl/Pyridine, then a small amount of 3,5-dimethylphenyl isocyanate and the designed amount of the vinyl reagent were simultaneously reacted with the polysaccharide. Thereafter, an excessive 3,5-dimethylphenyl isocyanate was added to completely react with the residue hydroxyl groups to give the vinylated polysaccharide derivative. The derivatives G to M with different vinyl groups or contents randomly placed at the 2, 3, or 6-positions were readily prepared using this method, as also shown in Figure 7. The simplicity of the non-regioselective method vs. the regioselective one is obvious.

As is well known, CDMPC coated onto silica gel exhibits high chiral recognitions to a variety of enantiomers.[5-7] However, its chiral recognition abilities might be influenced by the placed vinyl spacers since the helix structure of the polymer may be changed. Therefore, different vinyl reagents were selected for comparison, such as the methacrylate group (R_2) from 2-methacryloyloxyethyl isocyanate, the typical olefin group (R_6) from 9-decenyl isocyanate, which was previously reported by Minguillón *et al.*, but as an ester moiety,[25-27] and the methacryloyl group (R_7) from methacryloyl chloride. The prepared derivatives G, I, and J with three different kinds of vinyl spacers were separately coated on the aminopropyl silica gel using a coating method,[20,21] and their chiral resolution powers were compared with the commercially available Chiralcel OD consisting of the coated CDMPC.

As listed in Table II, the coated derivatives G, I, and J showed some different recognition properties for the test racemates, although the elution order for all the eluted enantiomers was the same. The chiral recognitions on G and I were more or less similar, while J generally presented slightly higher recognitions especially for the racemates 2, 6, 9, and 10, which makes J even more comparable to the coated CDMPC. The k'_1 values on G were much lower than those on I and J, which may indicate that the longer spacer R_6 might hinder the interactions between the racemates and the CSP.

Based on these results, it appears that the smaller molecular size of the vinyl spacer like R_7 may affect the helix structure of the CDMPC less, thus maintaining a higher recognition. In addition, the polar groups on the vinyl spacer near the adhered chiral glucose unit are superior to those that are farer way from the glucose units since the enantiomers may be absorbed by the polar groups that cause a negative effect on the chiral discriminations. The higher k'_1 values observed on I than on J as described in Table II may also support the stronger adsorptions between the enantiomers and the vinyl spacer R_2.

During our experiments, we also found that the immobilization efficiency depended on the types of introduced vinyl groups. As shown in Table III, the immobilization efficiency on G and H was markedly lower than that on I, J, and K. As more detail, only 33% G and 60% H were fixed, while 87% I, 78% J, and 91% K were immobilized. These results indicate that the typical olefin group is significantly less effective than the methacrylate and methacryloyl groups for the radical copolymerization reaction.

The effect of the vinyl content on the α values was also investigated. As shown in Figure 7, G and H had 15% and 30% R_6, and J and K contained 10% and 20% R_7, respectively. From Table III, it can be seen that the α values on G and H were slightly changed as the vinyl content increased.

Table II. Influence of Vinyl Spacers on Chiral Recognitions on CDMPC[a]

Racemates \ Derivatives	G^b		I^b		J^b		CDMPC[c] (Chiralcel OD)	
	k'_1	α	k'_1	α	k'_1	α	k'_1	α
1	0.38(+)	1.44	0.67(+)	1.51	0.68(+)	1.19	1.05(+)	1.26
2	0.30(-)	1.83	0.57(-)	1.70	0.45(-)	2.03	0.79(-)	2.11
3	1.02(+)	1.34	1.93(+)	1.38	1.73(+)	1.30	2.44(+)	1.57
4	0.61(-)	1.20	1.34(-)	~1	0.96(-)	1.22	1.50(-)	1.27
5	0.48(-)	1.20	0.93(-)	1.20	0.74(-)	1.21	1.22(-)	1.14
6	0.90(-)	2.26	1.67(-)	2.33	1.37(-)	2.90	2.29(-)	2.87
7	0.18(-)	~1	0.34(-)	~1	0.24(-)	~1	0.42(-)	1.13
8	0.57(-)	1.25	1.14(-)	1.25	0.88(-)	1.33	1.46(-)	1.40
9	0.46(+)	1.77	0.84(+)	1.55	0.59(+)	2.11	0.91(+)	2.63
10	2.25(-)	1.57	3.07(-)	1.78	1.47(-)	2.30	3.74(-)	1.47

[a]The signs in parentheses represent the CD detection of the first eluted enantiomer. [b]Column size: 250 × 2 mm; Flow rate: 0.1 mL/min; Mobile phase: *n*-hexane/2-propanol (90/10). [c]Column size: 250 × 4.6 mm; Flow rate: 0.5 mL/min; Mobile phase: *n*-hexane/2-propanol (90/10).

Table III. Immobilization and Chiral Recognitions of the Randomly Vinylated Polysaccharide Derivatives[a]

Racemates	$G^{b,c}$ 33%[e]	$H^{b,c}$ 60%[e]	$I^{b,c}$ 87%[e]	$J^{b,c}$ 78%[e]	$K^{b,c}$ 91%[e]	$L^{b,c}$ 96%[e]	$M^{b,c}$ 92%[e]	ADMPC (Chiralpak AD)[d]
1	1.47(+)	1.57(+)	1.52(+)	1.34(+)	1.60(+)	1.48(+)	1.48(+)	1.70(+)
2	1.75(-)	1.45(-)	1.53(-)	1.66(-)	1.24(-)	2.20(+)	2.52(+)	2.81(+)
3	1.33(+)	1.26(+)	1.37(+)	1.43(+)	1.29(+)	1.10(-)	1.14(-)	1.31(-)
4	1.06(-)	1.15(-)	~1 (-)	1.23(-)	1.17(-)	1.86(-)	2.04(-)	2.24(-)
5	1.17(-)	1.22(-)	1.23(-)	1.24(-)	1.29(-)	~1(-)	~1(-)	1.02(-)
6	2.13(-)	2.00(-)	2.24(-)	2.61(-)	2.06(-)	~1(-)	~1(-)	1.39(-)
7	~1 (-)	~1 (-)	1.10(-)	~1 (-)	1.10(-)	~1(+)	~1(+)	~1 (+)
8	1.21(-)	1.16(-)	1.22(-)	1.27(-)	1.13(-)	1.14(+)	~1(+)	1.04(+)
9	1.52(+)	1.27(+)	1.39(+)	1.96(+)	1.21(+)	3.30(+)	2.69(+)	1.59(+)
10	2.30(-)	2.36(-)	2.75(-)	3.72(-)	3.49(-)	2.07(-)	2.15(-)	2.22(-)

[a]The signs in parentheses represent the CD detection of the first eluted enantiomer; Radical copolymerization: [Vinyl group]/[AIBN] = 30; Temp.= 80 °C; Solvent=toluene; Monomer=1,5-hexadiene (monomer/polymer=45 wt%); Mobile phase: *n*-hexane/2-propanol (90/10). [b]Column size: 250 × 2 mm; Flow rate: 0.1 mL/min. [c]Derivatives. [d]Column size: 250 × 4.6 mm; Flow rate: 0.5 mL/min. [e]Immobilization efficiency calculated from the recovered derivatives.

However, for J and K, a sharper reduction in the α values was observed for most of the test racemates as the vinyl content increased. These results suggest that the methacryloyl content is more sensitive than the typical olefin content on the chiral recognitions of CDMPC. As a result, the chiral recognitions on H bearing 30% R_6 can be comparable to those on K bearing 20% R_7 as shown in Table III.

This non-regioselective method was also extended to amylose to give L and M bearing 10% and 6% R_2, respectively, as shown in Figure 7. It can be seen from Table III that 96% L and 92% M were successfully fixed on the silica gel through the copolymerization with the vinyl monomer of 1,5-hexadiene. The higher immobilization efficiency observed on amylose suggests that the chain of the amylose derivatives might be more flexible than that of the cellulose derivatives. The immobilized L and M only showed slightly lower chiral recognitions than the coated ADMPC as shown in Table III.

Thus we have determined a simpler method for the sufficient immobilization of polysaccharide derivatives as CSPs, and the immobilized chiral selectors showed more or less similar chiral recognitions to the regioselectively immobilized

ones.

Solvent Versatility on Immobilized Polysaccharide Derivatives

As already mentioned, the immobilized CSPs overcome the defect of solvent limitations than the coated CSPs, thus the solvent versatility of the immobilized CSPs significantly broadens the selection of solvents used as the mobile phase components or sample dissolving reagents during the HPLC. Therefore, the immobilized CSPs may enhance the chiral separations or even provide a totally new selectivity profile for some racemates with the addition of these solvents in the mobile phase, which cannot be used on the coated CSPs, such as chloroform, dichloromethane, THF, and so on.

We investigated the effect of chloroform on the chiral separations on immobilized B, and the results are shown in Table I. The α values for most of the racemates were improved to different degrees with the addition of 5-15% chloroform to the mobile phases (mobile phase II). Under this case, the immobilized B exhibited more or less similar chiral recognitions compared to the coated CDMPC on Chiralcel OD (in Table II). A typical example is presented in Figure 8 for the resolution of racemate 2, *trans*-stilbene oxide. The α value on the immobilized B was 1.54 using the typical mobile phase of *n*-hexane/2-propanol (90/10) (I). It was then increased up to 2.19 using the mobile phase of *n*-hexane/chloroform/2-propanol (85/15/1) (III). In this case, its α value was even comparable to that on Chiralcel OD using the mobile phase of *n*-hexane/2-propanol (90/10) (α=2.11) (II).

Recently, we also separated some compounds on the immobilized 3,5-dimethylphenylcarbamates of cellulose and amylose with the addition of chloroform or dichloromethane into the mobile phases, such as the homochirally substituted bis(dipyrromethene) zinc(II) helicates,[45] long chain-substituted molecular knots[46] and cyclochiral Bonnane.[47] These compounds cannot be eluted or separated on the coated CSPs using the compatible mobile phases.

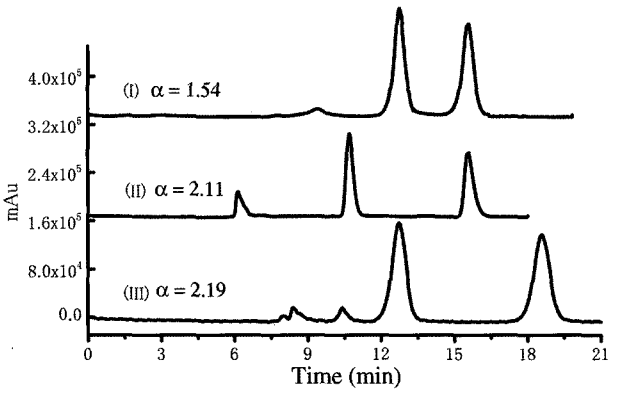

Figure 8. Chromatograms of *trans*-stilbene oxide on the immobilized B and coated CDMPC on Chiralcel OD. (I) Derivative: B; Column size: 250×2 mm; Flow rate: 0.1 mL/min; Mobile phase: *n*-hexane/2-propanol (90/10); (II) Derivative: CDMPC; Column size: 250×4.6 mm; Flow rate: 0.5 mL/min; Mobile phase: *n*-hexane/2-propanol (90/10); (III) Derivative: B; Column size: 250×2 mm; Flow rate: 0.1 mL/min; Mobile phase: *n*-hexane/chloroform/2-propanol (85/15/1).

Conclusions

In conclusion, we have shown that the 3,5-dimethylphenylcarbamates of cellulose and amylose can be efficiently immobilized on silica gel as CSPs through the copolymerizations with vinyl monomers. The immobilization efficiency and chiral recognitions of the polysaccharide derivatives can be readily tunable by the introduced vinyl groups or the employed vinyl monomers. This method provides a wide applicability for the preparation of CSPs immobilized with different kinds of polysaccharide derivatives with a high chiral recognition ability. The solvent versatility of the immobilized CSPs may enhance the success of chiral separations, in particular, for those that cannot be resolved on the coated CSPs due to the solubility problem. Recently, the immobilized 3,5-dimethylphenylcarbamates of amylose and cellulose (Chiralpak IA and Chiralpak IB) have commercially available.[48,49] We believe that the immobilized polysaccharide-based CSPs will become more and more popular in the future.

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References

(1) N. M. Maier, P. Franco, and W. Linder, *J. Chromatogr. A*, **906**, 3 (2001).
(2) V. Schurig, *J. Chromatogr. A*, **906**, 275 (2001).
(3) V. Schurig, *Trends Anal. Chem.*, **21**, 647 (2002).
(4) D. W. Armstrong, *LC.GC Int.*, **11**, 22 (1998).
(5) E. Yashima, C. Yamamoto, and Y. Okamoto, *Synlett*, 344 (1998).
(6) Y. Okamoto and E. Yashima, *Angew. Chem. Int. Ed.*, **37**, 1021 (1998).
(7) E. Yashima, *J. Chromatogr. A*, **906**, 105 (2001).
(8) K.W. Phinney, *Anal. Chem.*, **72**, 204A (2000).
(9) G. Terfloth, *J. Chromatogr. A*, **906**, 301 (2001).
(10) J. Kang, D. Wistuba, and V. Schurig, *Electrophoresis*, **23**, 4005 (2002).
(11) J. Haginaka, *J. Chromatogr. A*, **906**, 253 (2001).
(12) Z. Juvancz and J. Szejtli, *Trends Anal. Chem.*, **21**, 379, (2002).
(13) G. Felix, *J. Chromatogr. A*, **906**, 171 (2001).
(14) K. Tachibana and A. Ohnishi, *J. Chromatogr. A*, **906**, 127 (2001).
(15) H. Y. Aboul-Enein, *J. Chromatogr. A*, **906**, 185 (2001).
(16) C. Yamamoto and Y. Okamoto, *Bull. Chem. Soc. Jpn.*, **77**, 227 (2004).
(17) J. W. Timothy and B. F. Alton III, *J. Chromatogr. A*, **906**, 73

(2001).
(18) F. Gasparrini, D. Misiti, and C. Villani, *J. Chromatogr. A*, **906**, 35 (2001).
(19) E. Francotte, in *The Impact of Stereochemistry on Drug Development and Use*, H. Y. Aboul-Enein and I. W. Wainer, Eds., Wiley, New York, 1997, chap. 23, pp. 633-683.
(20) Y. Okamoto, M. Kawashima, and K. Hatada, *J. Am. Chem. Soc.*,**106**, 5357 (1984).
(21) Y. Okamoto, M. Kawashima, and K. Hatada, *J. Chromatogr.*, **363**, 173 (1986).
(22) Y. Okamoto, R. Aburatani, S. Miura, and K. Hatada, *J. Liq. Chromatogr.*, **10**, 1613 (1987).
(23) E. Yashima, H. Fukaya, and Y. Okamoto, *J. Chromatogr. A*, **667**, 11 (1994).
(24) K. Kimata, R. Tsuboi, K. Hosoya, and N. Tanaka, *Anal. Methods Instrum.*, **1**, 23 (1993).
(25) C. Minguillón, P. Franco, L. Oliveros, and P. Lopez, *J. Chromatogr. A*, **728**, 407 (1996).
(26) P. Franco, C. Minguillón, L. Oliveros, and P. Lopez, *J. Chromatogr. A*, **791**, 37 (1997).
(27) P. Franco, A. Senso, C. Minguillón, and L. Oliveros, *J. Chromatogr. A*, **796**, 265 (1998).
(28) X. Chen, F. Qin, Y. Liu, X. Huang, and H. Zou, *J. Chromatogr. A*, **1034**, 109 (2004).
(29) E. Francotte, PCT WO Patent 96/27615 (1996).
(30) E. Francotte and T. Zhang, PCT WO Patent 97/044011 (1997).
(31) E. Francotte, PCT WO Patent 97/49733 (1997).
(32) E. Francotte, and D. Huynh, *J. Pharm. Biomed. Anal.*, **27**, 421 (2002).
(33) N. Enomoto, S. Furukawa, Y. Ogasawara, H. Akano, Y. Kawamura, E. Yashima, and Y. Okamoto, *Anal. Chem.*, **68**, 2798 (1996).
(34) X. Chen, Y. Liu, F. Qin, L. Kong, and H. Zou, *J. Chromatogr. A*, **1010**, 185 (2003).
(35) B. Chankvetadze, T. Ikai, C. Yamamoto, and Y. Okamoto, *J. Chromatogr. A*, **1042**, 55 (2004).
(36) T. Ikai, R. Muraki, C. Yamamoto, M. Kamigaito, and Y. Okamoto, *Chem. Lett.*, **33**, 1188 (2004).
(37) P. Franco, A. Senso, L.Oliveros, and C. Minguillón, *J. Chromatogr. A*, **906**, 155 (2001).
(38) T. Ikai, C. Yamamoto, M. Kamigaito, and Y. Okamoto, *Polym. J.*, **38**, 91 (2006).
(39) T. Kubota, T. Kusano, C. Yamamoto, E. Yashima, and Y. Okamoto, *Chem. Lett.*, **7**, 724 (2001).
(40) T. Kubota, C. Yamamoto, and Y. Okamoto, *Chirality*, **15**, 77 (2003).
(41) T. Kubota, C. Yamamoto, and Y. Okamoto, *J. Polym. Sci.; Part A: Polym. Chem.*, **41**, 3703 (2003).
(42) T. Kubota, C. Yamamoto, and Y. Okamoto, *J. Polym. Sci.; Part A: Polym. Chem.*, **42**, 4704 (2004).
(43) X. Chen, C. Yamamoto, and Y. Okamoto, *J. Chromatogr. A*, **1104**, 62 (2006).
(44) X. Chen, C. Yamamoto, and Y. Okamoto, *J. Sep. Sci.*, **29**, 1432 (2006).
(45) T. E. Wood, A. C. Ross. N. D. Dalgleish, E. D. Power, A. Thompson, X. Chen, and Y. Okamoto, *J. Org. Chem.*, **70**, 9967 (2005).
(46) M. R. Kishan, A. Parham, F. Schelhase, A. Yoneva, G. Silva, X. Chen, Y. Okamoto, and F. Vögtle, *Angew. Chem. Int. Ed.*, **45**, 7296 (2007).
(47) A. Böhmer, J. Brüggemann, A. Kaufmann, A. Yoneva, S. Müller, W. M. Müller, U. Müller, F. W. Vergeer, L. F. Chi, L. DeCola, H. Fuchs, X. Chen, T. Kubota, Y. Okamoto, and F. Vögtle, *Eur. J. Org. Chem.*, **1**, 45 (2007).
(48) T. Zhang, C. Kientzy, P. Franco, A. Ohnishi, Y. Kagamihara, and H. Kurosawa, *J. Chromatogr. A*, **1075**, 65 (2005).
(49) T. Zhang, D. Nguyen, P. Franco, T. Murakami, A. Ohnishi, and H. Kurosawa, *Anal. Chim. Acta*, **557**, 221 (2006).