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Review

Immobilized-type chiral packing materials for HPLC based on polysaccharide derivatives☆

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Dedicated to Professor W. Lindner on the occasion of his 65th birthday.

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ABSTRACT

The polysaccharide-based chiral packing materials (CPMs) for high-performance liquid chromatography (HPLC) have been recognized as the most powerful ones for the analyzing and preparative separating of the chiral compounds. These CPMs have been conventionally prepared by coating polysaccharide derivatives on a silica gel support. This means that the solvents, which swell or dissolve the derivatives on the silica gel and reduce the performance of the chiral columns, do not allow to be applied as components of the eluents. Therefore, the polysaccharide-based CPMs can be used with a rather limited number of eluents. In order to enhance the versatility of the eluent selection for more practical and economical chromatographic enantioseparations, the polysaccharide derivatives must be immobilized onto the silica gel. This review summarizes our latest studies on the development of the immobilized-type CPMs via the radical copolymerization and the polycondensation of the polysaccharide derivatives bearing small amounts of vinyl groups and alkoxysilyl groups, respectively.

Contents

1. Introduction

Most biologically active compounds including drugs, agrochemicals and foods are chiral and their pharmacologic, toxic and metabolic activities are often different between enantiomers. Therefore, the systematic investigation of their biological properties of individual enantiomers has become indispensable, particularly for the development of new chiral drugs [1–4]. In this way, the efficient preparation of both optically pure isomers and the precise determination of the enantiomeric excess of chiral compounds are becoming increasingly important. Today, many top selling drugs around the world have been used as single enantiomers with the desired physiological effect [2].

During the past few decades, the direct enantioseparation by high-performance liquid chromatography (HPLC) has significantly advanced, and definitely contributed to the progress in many fields dealing with chiral compounds [5–17]. The resolution by chiral HPLC can be achieved on the basis of the different adsorption behaviors of two enantiomers on chiral selectors of chiral packing materials (CPMs). Therefore, the design and preparation of chiral selectors are key points. The number of commercial CPMs has greatly increased, and today, more than 100 CPMs are commercially available. The chiral selectors of the CPMs can be classified into two types.

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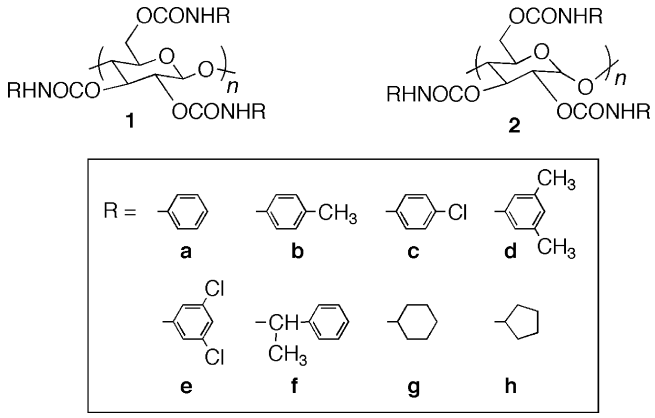

Fig. 1. Structure of cellulose (**1**) and amylose (**2**) derivatives with high recognition abilities.

The first type of chiral selectors consists of optically active small molecules, which are usually linked to a silica gel or organic polymer gel as supports [15–17]. Since Davankov initially reported this type of CPMs in the early 1970s [18,19], which was prepared by bonding cyclic amino acids onto poly(styrene-*co*-divinylbenzene) beads, a wide range of optically active small molecules has been applied as chiral selectors for CPMs [20–33]. Recently, the cinchona alkaloid-based CPMs developed by Lindner et al. have been commercialized under the trade names Chiralpak QN-AX and Chiralpak QD-AX, which are prepared from the 9*O*-*tert*-butylcarbamate derivatives of quinine and quinidine as chiral selectors, respectively [30,31]. These CPMs show excellent chiral recognitions for a wide range of chiral acids including important intermediates in pharmaceuticals and efficient chiral auxiliaries and ligands for asymmetric synthesis [34–38].

The second type of chiral selectors consists of optically active polymers including polysaccharide derivatives [8–12,39,40], proteins [41,42] and synthetic polymers, such as polyacrylamides with optically active side chains [43–45] and polymethacrylates with one-handed helical structure [46–49]. The chiral recognition on the optically active polymers often depends on their higher order structure in addition to the structure of their monomeric units, which control in the first small molecule-based phases.

Among the large variety of CPMs, the polysaccharide-based CPMs, which are derived from the carbamate derivatives of cellulose (**1**) and amylose (**2**) (Fig. 1), have been recognized as the most useful ones for both analytical and preparative separations for a wide range of chiral compounds [8–12,39,40]. These CPMs have been conventionally prepared by coating the polysaccharide derivatives on macroporous silica gel without a chemical linkage. This means that the commonly used solvents, such as chloroform, tetrahydrofuran (THF), acetone, ethyl acetate, etc., cannot be applied as the eluents, because these solvents can swell or dissolve the polysaccharide derivatives on silica gel and cause fatal damage to the CPMs. Therefore, the conventional coated-type CPMs based on the polysaccharide derivatives can be used with a rather limited number of eluents, which consist of hexane–alcohol mixtures for normal-phase chromatography and water–acetonitrile mixtures for the reversed-phase chromatography.

The versatility of the eluent selection generates the possibility to improve the performance of both the analytical and preparative separations by HPLC. For analytical separation, a better enantioselectivity on the polysaccharide derivatives and a reversed elution order of enantiomers might be attained using the above-prohibited solvents [50–53]. For a large-scale preparative separation, the selection of a solvent with a good solubility for a sample is essential for high productivity [54–57]. Therefore, the enhancement of the solvent compatibility for the CPMs is strongly required.

One of the potential ways to realize this requirement is to immobilize the polysaccharide derivatives onto chromatographic supports, such as spherical silica gel [58–61], monolithic silica gel [62,63], fused-silica capillary wall [64] and organic polymer gel [65]. Since the first immobilized-type CPMs based on polysaccharide derivatives were reported in 1987 [66], several immobilization methods have been developed: the immobilization of the derivatives bearing hydroxy groups with a diisocyanate [66–68]; the chemical bonding of an amylose derivative at an activated chain end [69,70]; the immobilization of the derivatives bearing vinyl groups with or without a vinyl monomer [71–75]; the photochemical cross-linking [76–79]; the immobilization of the derivatives bearing azido groups [80]; and the immobilization of the derivatives bearing alkoxysilyl groups [81–83].

The immobilized-type CPMs based on the polysaccharide derivatives **2d**, **1d** and **1e** (Fig. 1) have recently been commercialized under the trade names Chiralpak IA, Chiralpak IB and Chiralpak IC (Daicel), respectively [50–52]. However, novel immobilization methods, which can achieve a simple processing, a high immobilization efficiency and a high chiral recognition ability, are still being actively explored. In this review, we describe our latest studies on the development of the immobilized-type CPMs based on the polysaccharide derivatives.

2. Immobilization of polysaccharide derivatives bearing a vinyl group via copolymerization with a vinyl monomer

The immobilization of the polysaccharide derivative bearing a vinyl group onto silica gel via radical copolymerization with a vinyl monomer was investigated by our group in 2001 (Fig. 2) [73]. Since then, we have been exploring the optimum preparation conditions for the immobilized-type CPM by changing the type and amount

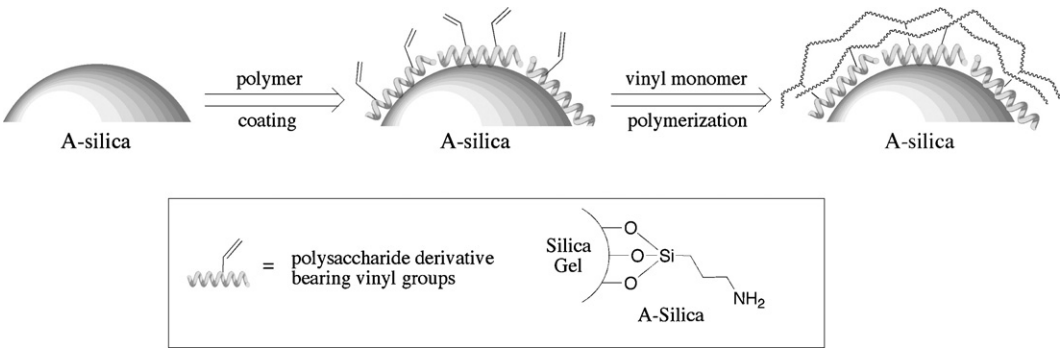

Fig. 2. Scheme of immobilization of polysaccharide derivatives onto A-silica by means of copolymerization with vinyl monomer.

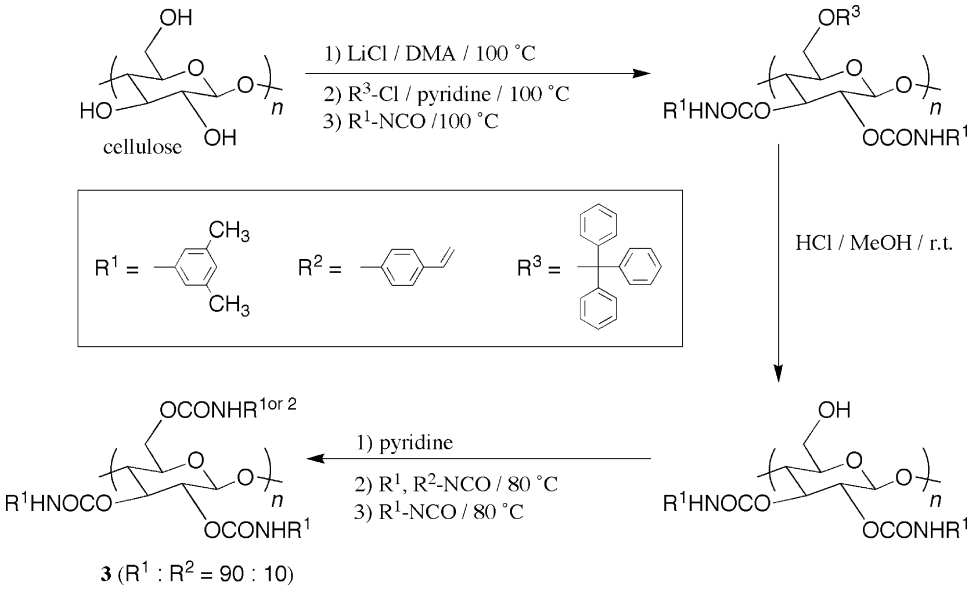

Fig. 3. Synthesis of cellulose derivative (**3**) bearing a 4-vinylphenyl group.

of the vinyl monomer or the vinyl group introduced to the polysaccharide derivatives and also by introducing a vinyl group on the silica surface as follows [72,74,84–89].

The cellulose derivative **3** bearing a vinyl group at the 6-position on a glucose unit was synthesized as shown in Fig. 3 [73,84]. The ratio of the (3,5-dimethylphenylcarbamate)/(4-vinylphenylcarbamate) residues in the derivative **3** was determined to be 90/10 from the ^{1}H NMR spectrum. The obtained cellulose derivative was coated on a 3-aminopropyl functionalized-silica gel (A-silica in Fig. 2), and styrene and α,α′-azobisisobutyronitrile (AIBN) dissolved in hexane were then added to the coated silica gel. The immobilization of the cellulose derivative was performed at 60 °C for 20 h, and the **3**-immobilized silica gel was fully washed with THF. The immobilization efficiency, which is defined as the ratio of the (immobilized polysaccharides)/(coated polysaccharides), was estimated by ^{1}H NMR analysis of the THF washing solution.

The results of the immobilization onto the A-silica using a different amount of styrene (0, 5, 10, 30 and 50 wt.% relative to the cellulose derivative) are shown in Table 1. Cellulose derivative **3** was quantitatively immobilized onto silica gel using greater than 10 wt.% styrene. However, when the styrene content was reduced to 5 wt.%, the immobilization efficiency was decreased to 86%. Without styrene, the immobilization efficiency was found to be only 50%. These results indicate that styrene as a comonomer can play a significant role in the immobilization of the cellulose derivative onto silica gel.

The obtained immobilized-type CPMs were packed into an HPLC column and their chiral recognition abilities for racemic compounds (**4**–**13** in Fig. 4) were evaluated using a hexane/2-propanol mixture as the eluent. As shown in Table 1, an increase in the styrene content caused a decrease in the chiral recognition. This decrease may be attributed to the following two reasons: (1) the higher order structure of the cellulose derivative is slightly changed during the immobilization process, especially at high styrene contents; (2) the styrene units in the CPMs non-enantioselectively interact with both enantiomers. These results suggest that the immobilization should be carried out under an optimized condition from the viewpoints of immobilization efficiency and chiral recognition.

In order to enhance the immobilization efficiency of the cellulose derivative with a small amount of styrene, the M-silica containing a vinyl group on the silica surface, has been used as a support (Fig. 5) [74]. The immobilization results onto the M-silica and A-silica under the same copolymerization condition are compared in Table 1. When 5 wt.% styrene was used in the immobilization process, the cellulose derivative was more efficiently

Table 1
Influence of styrene content on immobilization of **3** and separation factors (α) on CPMs-**1**–**6**[a]

CPMs	CPM-**1**	CPM-**2**	CPM-**3**	CPM-**4**	CPM-**5**	CPM-**6**
Styrene/cellulose derivative	(0 wt.%)	(5 wt.%)	(10 wt.%)	(30 wt.%)	(50 wt.%)	(5 wt.%)
Silica gel	A-silica	A-silica	A-silica	A-silica	A-silica	M-silica
Immobilization efficiency	50%	86%	99%	>99%	>99%	97%
4	1.26 (−)	1.22 (−)	1.32 (−)	1.31 (−)	1.31 (−)	1.32 (−)
5	1.45 (+)	1.45 (+)	1.68 (+)	1.60 (+)	1.53 (+)	1.73 (+)
6	~1 (−)	1.33 (−)	~1 (+)	1.17 (+)	1.23 (+)	1.0
7	~1 (+)	~1 (+)	1.12 (+)	~1 (+)	~1 (+)	1.09 (+)
8	2.74 (−)	2.57 (−)	3.20 (−)	2.76 (−)	2.63 (−)	4.27 (−)
9	1.25 (+)	1.34 (+)	1.18 (+)	1.16 (+)	1.14 (+)	1.19 (+)
10	1.23 (−)	1.22 (−)	1.13 (−)	1.08 (−)	~1 (−)	1.14 (−)
11	~1 (+)	1.17 (+)	1.32 (+)	~1 (+)	~1 (+)	1.23 (+)
12	1.92 (−)	2.18 (−)	1.96 (−)	1.81 (−)	1.73 (−)	1.90 (−)
13	1.40 (+)	1.42 (+)	~1 (+)	~1 (+)	~1 (+)	1.12 (+)

Column, 25 cm × 0.20 cm I.D. Eluent, hexane–2-propanol (90:10). Flow rate, 0.1 ml/min. [Vinyl group]/[AIBN] = 50. Solvent for polymerization; hexane.

[a] The signs in parentheses represent the optical rotation of the first-eluted enantiomer.

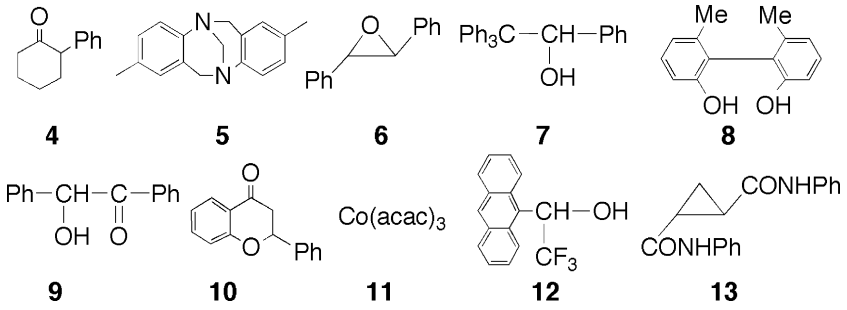

Fig. 4. Structures of racemates.

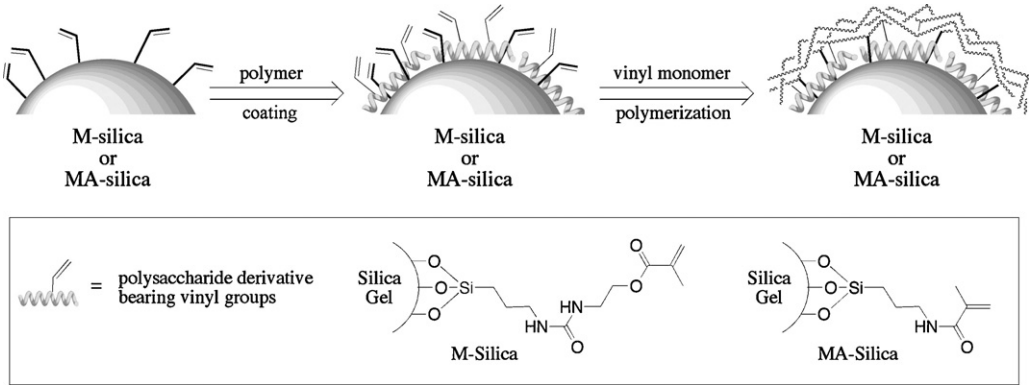

Fig. 5. Immobilization of polysaccharide derivatives onto M-silica or MA-silica by means of copolymerization with vinyl monomer.

immobilized onto the M-silica (97%) than onto the A-silica (86%). The introduction of a vinyl group on the silica surface seems to be valuable for the efficient immobilization. However, the chiral recognition on the CPM-**6** using the M-silica was slightly different from that on the CPM-**2** using the A-silica. The urea group of the M-silica may non-enantioselectively interact with the racemates and influence the chiral recognition to some extent.

Cellulose derivative (**14a** in Fig. 6) bearing 10% 2-methacryloyloxyethylcarbamate residue has also been prepared and immobilized using 10 wt.% styrene onto the A-silica [74]. The derivative **14a** has been quantitatively immobilized onto the A-silica, and the chiral recognition on the obtained immobilized-type CPM is similar to that on the CPM-**3** prepared from the derivative **3**. 2-Methacryloyloxyethyl isocyanate used for preparing the derivative **14** is commercially available and more stable and easier to handle than 4-vinylphenyl isocyanate used for the derivative **3**. Therefore, 2-methacryloyloxyethyl isocyanate seems to be the more useful reagent for the preparation of a cellulose derivative suitable for the HPLC resolution.

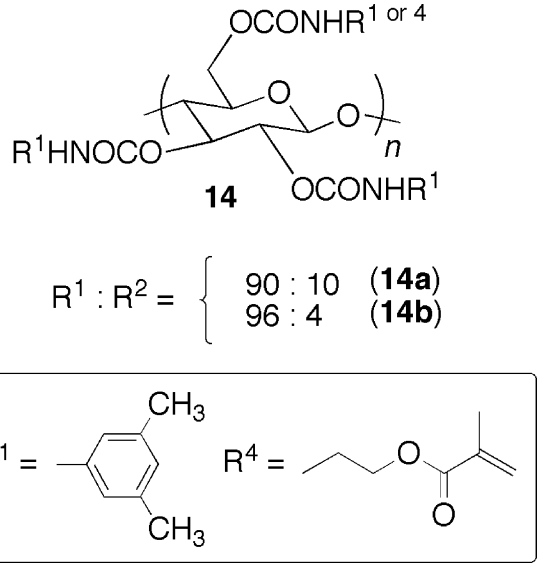

Fig. 6. Structures of cellulose derivative (**14**) bearing 2-methacryloyloxyethyl group.

The above immobilized-type CPMs exhibited somewhat different and lower chiral recognitions than the traditional coated-type CPM-**9** (Table 2), which is prepared by coating the cellulose derivative **1d** on the plain silica gel [82]. This decrease in chiral recognition is mainly caused by the rather high content of vinyl groups introduced on the polysaccharide (about 10% of the total OH groups). One of the preferable ways to improve the chiral recognition abilities on immobilized-type CPMs is to decrease the vinyl group content on the polysaccharide derivatives. Although this approach will reduce the immobilization efficiency, the chiral recognition on the obtained immobilized-type CPM is expected to be close to that on the coated-type CPM-**9**. Therefore, cellulose 3,5-dimethylphenylcarbamate **14b** bearing a lower content of the 2-methacryloyloxyethylcarbamate residue (4% of the total OH groups) has been prepared (Fig. 6) [86]. The derivative **14b** was immobilized onto M-silica via the copolymerization with 30 wt.% 2,3-dimethyl-1,3-butadiene (DMBD) as the vinyl monomer at 60 °C in toluene.

The resolution results of the obtained immobilized-type CPM-**8** were compared to those of CPM-**7**, which was prepared by the copolymerization of the derivative **4a** bearing 10% 2-methacryloyloxyethylcarbamate residue with 10 wt.% DMBD, and the coated-type CPM-**9**. As shown in Table 2, more than half of the racemates are better resolved on CPM-**8** than on CPM-**7**, and the chiral recognition on CPM-**8** was close to that on CPM-**9**. Based on these results, it is clear that the chiral recognition ability can be

Table 2
Influence of the content of vinyl group on immobilization of **14** and chiral recognitions on CPMs-**7**–**9** and Chiralpak IB[a]

CPMs	CPM-**7**[b]		CPM-**8**[c]		CPM-**9**		Chiralpak IB[d]	
Cellulose derivatives	**14a** (R^1/R^4 = 90/10)		**14b** (R^1/R^4 = 96/4)		**1d** (R^1/R^4 = 100/0)		–	
Immobilization efficiency	88%		78%		–		–	
	α		k_1'	α	k_1'	α	k_1'	α
4	1.30 (−)		1.23 (−)	1.27	0.76 (−)	1.17	1.00 (−)	1.14
5	1.71 (+)		0.81 (+)	1.64	0.75 (+)	1.31	0.96 (+)	1.22
6	1.20 (−)		0.75 (−)	1.49	0.48 (−)	1.96	0.55 (−)	1.77
7	1.15 (+)		1.59 (+)	1.17	1.06 (+)	1.12	1.12 (+)	1.22
8	3.80 (−)		2.05 (−)	3.83	1.15 (−)	2.40	1.48 (−)	2.72
9	1.27 (+)		2.56 (+)	1.34	1.50 (+)	1.50	2.00 (+)	1.33
10	1.18 (−)		1.46 (−)	1.23	0.96 (−)	1.34	1.13 (−)	1.26
11	1.20 (+)		0.42 (+)	1.13	0.42 (+)	∼1	3.15 (+)	∼1
12	2.16 (−)		1.82 (−)	2.42	1.38 (−)	2.77	1.54 (−)	2.42
13	∼1 (+)		1.51 (+)	1.40	0.61 (+)	1.99	0.86 (+)	1.89

[a] The signs in parentheses represent the optical rotation of the first-eluted enantiomer. Column: 25 cm × 0.20 cm I.D. Eluent: hexane–2-propanol (90:10). Flow rate: 0.1 ml/min.
[b] Silica gel: A-silica. Vinyl monomer: 2,3-dimethyl-1,3-butadiene (10 wt.%). [Vinyl group]/[AIBN] = 30. Solvent for polymerization: toluene.
[c] Silica gel: M-silica. Vinyl monomer: 2,3-dimethyl-1,3-butadiene (30 wt.%). [Vinyl group]/[AIBN] = 30. Solvent for polymerization: toluene.
[d] Column: 25 cm × 0.46 cm I.D. Flow rate: 0.5 ml/min.

improved by decreasing the vinyl group content on the cellulose derivative from 10% to 4%, although the immobilization efficiency decreases as expected.

The resolution results on the commercially available Chiralpak IB containing the immobilized cellulose 3,5-dimethylphenylcarbamate as the chiral selector are also listed in Table 2. Although the elution orders for the enantiomers were always the same on the two immobilized-type CPMs, the chiral recognitions are more or less different, depending on the structures of the racemates. It can be seen that the CPM-**8** exhibits the higher α values than Chiralpak IB for racemates **4**, **5**, **8** and **11**. On the other hand, the retention factors on the CPM-**8** are basically greater than those on Chiralpak IB except for racemates **5** and **11**. The content of the chiral selector may be greater on the CPM-**8** than on Chiralpak IB.

As described above, the cellulose derivatives bearing the vinyl group at the 6-position on the glucose unit can be efficiently immobilized onto silica gel by the radical polymerization with a vinyl monomer. However, the synthetic route of these derivatives is rather complicated and time-consuming because the protection and deprotection process at the 6-position on the glucose unit is required to regioselectively introduce the vinyl groups. This regioselective immobilization method may not be appropriate for the large-scale preparation of the CPMs. On the other hand, the simple preparation of immobilized-type CPMs can be achieved using the polysaccharide derivatives bearing vinyl groups non-regioselectively at the 2-, 3- and 6-positions [84,87].

The non-regioselectively substituted cellulose derivatives, **15**, **16** and **17**, which contain the methacrylate group (R^4), the methacryloyl group (R^5) or the aliphatic olefin group (R^6), respectively, have been easily prepared by a one-pot process (Fig. 7) and immobilized onto the MA-silica (see Fig. 5) via the copolymerization with 1,5-hexadiene in toluene at 80 °C [87]. The immobilization efficiencies and the chiral recognitions of the immobilized-type CPMs are listed in Table 3. The immobilization efficiency of the cellulose derivative **17** (33%) was significantly lower than those of **15** (87%) and **16** (78%). This is due to the lower polymerizability of the aliphatic olefin in **17** compared to the conjugated olefins in **15** and **16**.

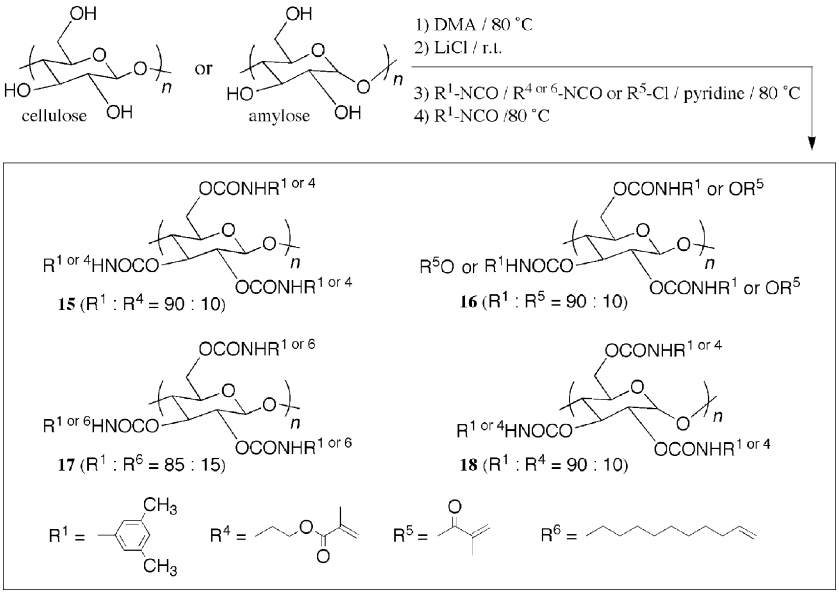

Fig. 7. Synthesis of cellulose (**15**–**17**) and amylose (**18**) derivatives bearing vinyl groups at 2-, 3- and 6-positions.

Table 3
Influence of structures of vinyl groups on immobilization efficiencies and chiral recognitions on CPM-**10**–**12**[a]

CPMs	CPM-**10**		CPM-**11**		CPM-**12**	
Cellulose derivatives	**15**		**16**		**17**	
Immobilization efficiency	87%		78%		33%	
	k_1'	α	k_1'	α	k_1'	α
4	1.34 (−)	1.23	0.95 (−)	1.24	0.30 (−)	1.17
5	0.97 (+)	1.52	0.77 (+)	1.34	0.23 (+)	1.47
6	0.82 (−)	1.53	0.59 (−)	1.66	0.18 (−)	1.75
7	1.89 (+)	~1	1.18 (+)	1.23	0.40 (+)	1.06
8	2.05 (−)	2.75	1.51 (−)	3.72	0.58 (−)	2.30
9	2.73 (+)	1.37	1.92 (+)	1.43	0.63 (+)	1.33
10	1.65 (−)	1.22	1.12 (−)	1.27	0.37 (−)	1.21
11	0.54 (+)	1.10	0.38 (+)	~1	0.14 (+)	~1
12	2.30 (−)	2.24	1.54 (−)	2.61	0.58 (−)	2.13
13	1.35 (+)	1.39	0.96 (+)	1.96	0.34 (+)	1.52

Column: 25 cm × 0.20 cm I.D. Eluent: hexane–2-propanol (90:10). Flow rate: 0.1 ml/min. Silica gel: MA-silica. Vinyl monomer: 1,5-hexadiene (45 wt.%). [Vinyl group]/[AIBN] = 30. Solvent for polymerization: toluene.

[a] The signs in parentheses represent the optical rotation of the first-eluted enantiomer.

The immobilized-type CPM-**11** prepared from the derivative **16** exhibits a slightly higher chiral recognition than CPM-**10** and CPM-**12**, which were prepared from **15** and **17**, respectively, especially for racemates **7**, **8**, **12** and **13**. Because the polar carbonyl group on the vinyl spacer R^5 is located near the chiral glucose units, some racemates might be enantioselectively absorbed to the polar group on R^5, resulting in a positive effect on the chiral recognition on CPM-**11**. In addition, the higher order structure of the cellulose derivative **16** might not be significantly disturbed by the introduction of R^5 with a smaller molecular size.

The immobilization of amylose derivatives bearing a vinyl group onto the silica gel is also possible [85,87]. The amylose 3,5-dimethylphenylcarbamate (**18**) bearing the 10% methacrylate group (R^4) non-regioselectively at the 2-, 3- and 6-positions has been prepared by the one-pot method (Fig. 7) [87]. The derivative **18** was immobilized onto the MA-silica under the same radical polymerization condition as applied to the cellulose derivative **15**. As shown in Table 4, the amylose derivative containing a 10% vinyl content can be efficiently immobilized via radical polymerization. Compared to the cellulose derivative **15** bearing the same content of the methacrylate group, the amylose derivative **18** could be more efficiently immobilized. This may be associated with the fact that the amylose derivative has a more flexible structure than the cellulose derivative. The chiral recognition on the immobilized-type CPM-**13** derived from the amylose derivative **18** is compared to that on the commercially available Chiralpak IA, which is prepared by immobilization of the amylose 3,5-dimethylphenylcarbamate, under the same chromatographic condition (Table 4). Although the recognition abilities on both CPMs were basically similar, Chiralpak IA exhibits a comparable or slightly better recognition ability except for the racemate **13** than CPM-**13**.

Table 4
Immobilization of amylose derivative **18** and separation factors (α) on the CPM-**13** and Chiralpak IA

CPMs	CPM-**13**[a]	Chiralpak IA[b]
Immobilization efficiency	96%	–
4	~1 (−)	1.06 (−)
5	1.48 (+)	1.44 (+)
6	2.20 (+)	2.57 (+)
7	1.86 (+)	2.28 (+)
8	2.07 (−)	2.19 (−)
9	1.10 (−)	1.16 (−)
10	1.14 (+)	1.10 (+)
11	~1 (−)	~1 (−)
12	~1 (−)	1.07 (−)
13	3.30 (+)	1.89 (+)

The signs in parentheses represent the optical rotation of the first-eluted enantiomer. Eluent: hexane–2-propanol (90:10).

[a] Column: 25 cm × 0.20 cm I.D. Flow rate: 0.1 ml/min. Silica gel: MA-silica. Vinyl monomer: 1,5-hexadiene (45 wt.%). [Vinyl group]/[AIBN] = 30. Solvent for polymerization: toluene.

[b] Column: 25 cm × 0.46 cm I.D. Flow rate: 0.5 ml/min.

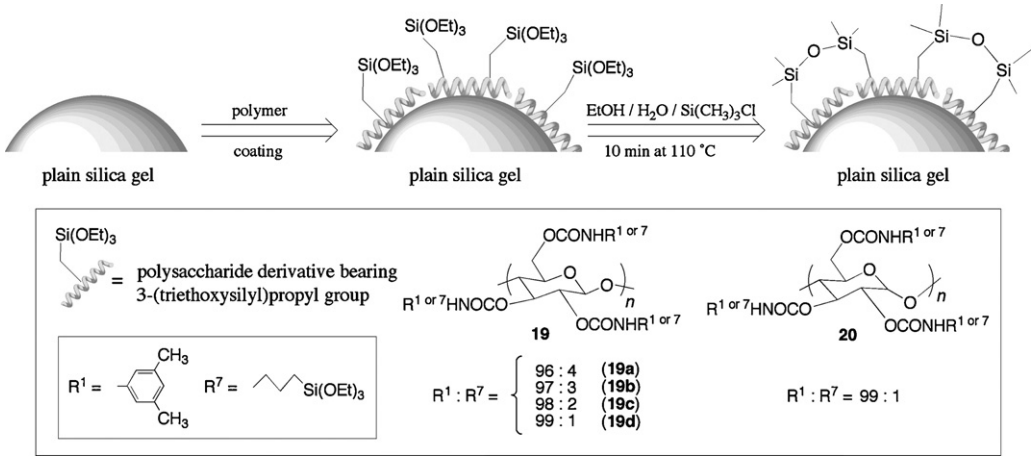

Fig. 8. Scheme of immobilization of cellulose (**19**) and amylose (**20**) derivatives bearing 3-(triethoxysilyl)propyl group via intermolecular polycondensation.

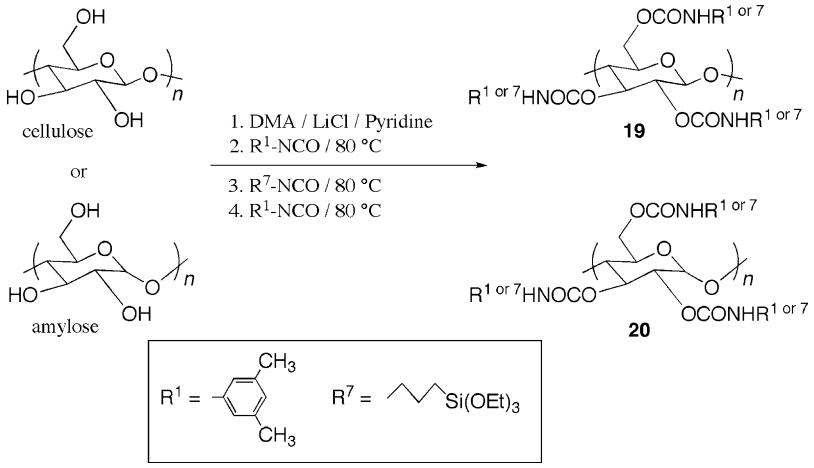

Fig. 9. Synthesis of cellulose (**19**) and amylose (**20**) derivatives bearing 3-(triethoxysilyl)propyl group.

3. Immobilization of polysaccharide derivatives bearing a triethoxysilyl group via intermolecular polycondensation

As another approach for the preparation of the immobilized-type CPM, we have recently developed a facile immobilization method via the intermolecular polycondensation of the triethoxysilyl groups introduced to the polysaccharide derivatives (Fig. 8) [81,82]. This immobilization method may satisfy all of the necessary requirements for the immobilized-type CPMs, i.e., simple processing, high immobilization efficiency, high chiral recognition and wide applicability to various polysaccharide derivatives.

The cellulose (**19**) and amylose (**20**) derivatives bearing the 3-(triethoxysilyl)propyl residues were synthesized by the sequential additions of 3,5-dimethylphenyl isocyanate and 3-(triethoxysilyl)propyl isocyanate as shown in Fig. 9.

In contrast to the previous study by Zou and co-workers [83], the introduction of the 3-(triethoxysilyl)propyl groups was clearly confirmed from the ^{1}H NMR spectra of the obtained polysaccharide derivatives (Fig. 10). The ratio of the (3,5-dimethylphenylcarbamate)/(3-(triethoxysilyl)propylcarbamate) could be determined from the ratio of (aromatic proton)/($SiCH_2$) in the ^{1}H NMR spectrum.

Fig. 8 shows the immobilization process via the intermolecular polycondensation of the triethoxysilyl groups. During the heat treatment under an acidic condition, the polysaccharide derivatives were immobilized onto the silica gel by the polycondensation of the triethoxysilyl groups. After the reaction, the **19**- and **20**-immobilized silica gels were thoroughly washed with THF and acetone, respectively, in order to remove the unimmobilized deriva-

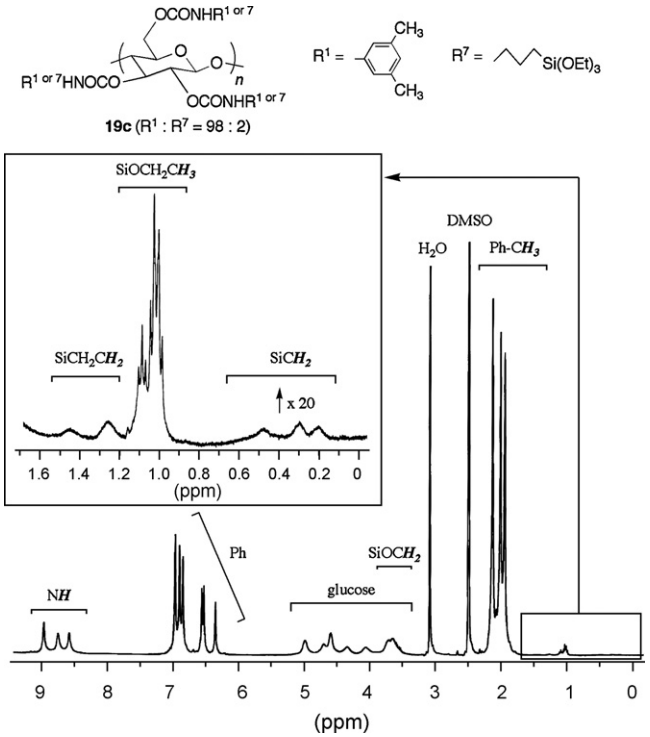

Fig. 10. ^{1}H NMR spectrum of the cellulose derivative **19c** in DMSO-d_6 at 80 °C.

Table 5
Immobilization of **19a**–**19d** and separation factors (α) on the CPMs-**14**–**17**

CPMs	CPM-**14**	CPM-**15**	CPM-**16**	CPM-**17**
Cellulose derivatives	**19a** (R^1/R^7 = 96/4)	**19b** (R^1/R^7 = 97/3)	**19c** (R^1/R^7 = 98/2)	**19d** (R^1/R^7 = 99/1)
Immobilization efficiency	99	97%	89%	72%
4	1.27 (−)	1.31 (−)	1.23 (−)	1.27 (−)
5	1.52 (+)	1.56 (+)	1.53 (+)	1.49 (+)
6	1.21 (−)	1.30 (−)	1.51 (−)	1.59 (−)
7	1.17 (+)	1.18 (+)	1.15 (+)	1.19 (+)
8	3.66 (−)	4.36 (−)	3.52 (−)	3.70 (−)
9	1.23 (+)	1.31 (+)	1.35 (+)	1.35 (+)
10	1.13 (−)	1.19 (−)	1.21 (−)	1.23 (−)
11	~1 (+)	~1 (+)	~1 (+)	~1 (+)
12	2.01 (−)	2.29 (−)	2.35 (−)	2.47 (−)
13	1.22 (+)	1.48 (+)	1.65 (+)	1.70 (+)

The signs in parentheses represent the optical rotation of the first-eluted enantiomer. Column: 25 cm × 0.20 cm I.D. Flow rate: 0.1 ml/min. Eluent: hexane–2-propanol (90:10).

Table 6
Separation factors (α) on the immobilized-type CPM-**16**, coated-type CPM-**9** and Chiralpak IB

CPMs	CPM-**16**[a]					CPM-**9**[a]	Chiralpak IB[b]
Eluents	H/I (90/10)	H/C/I (90/10/1)	H/T/I (90/10/1)	H/C (70/30)	H/T (70/30)	H/I (90/10)	H/I (90/10)
4	1.23 (−)	1.27 (−)	1.19 (−)	1.29 (−)	1.17 (−)	1.17 (−)	1.14 (−)
5	1.53 (+)	1.35 (+)	1.36 (+)	1.06 (+)	1.36 (+)	1.31 (+)	1.22 (+)
6	1.51 (−)	2.12 (−)	1.0	1.93 (−)	∼1 (+)	1.96 (−)	1.77 (−)
7	1.15 (+)	1.12 (+)	1.28 (+)	1.16 (+)	∼1 (+)	1.12 (+)	1.22 (+)
8	3.52 (−)	1.80 (−)	1.0	1.10 (+)	1.0	2.40 (−)	2.72 (−)
9	1.35 (+)	1.40 (+)	1.35 (+)	–	1.23 (+)	1.50 (+)	1.33 (+)
10	1.21 (−)	1.25 (−)	1.28 (−)	1.09 (−)	1.0	1.34 (−)	1.26 (−)
11	∼1 (+)	1.0	1.08 (+)	1.0	1.0	∼1 (+)	∼1 (+)
12	2.35 (−)	2.93 (−)	2.59 (−)	2.90 (−)	1.56 (−)	2.77 (−)	2.42 (−)
13	1.65 (+)	1.69 (+)	1.60 (+)	–	1.18 (+)	1.99 (+)	1.89 (+)

The signs in parentheses represent the optical rotation of the first-eluted enantiomer. Eluent: H: hexane, I: 2-propanol, C: chloroform, T: tetrahydrofuran.
[a] Column: 25 cm × 0.20 cm I.D. Flow rate: 0.1 ml/min.
[b] Column: 25 cm × 0.46 cm I.D. Flow rate: 0.5 ml/min.

tives. The immobilization efficiency was determined from the organic contents in the CPMs estimated by thermogravimetric analysis.

The immobilization efficiencies of the cellulose derivatives are shown in Table 5. As the content of the 3-(triethoxysilyl)propyl group in the cellulose derivatives was reduced, the immobilization efficiency decreased. However, even if the amount of the 3-(triethoxysilyl)propyl group was decreased to 1% (**19d**), 72% of the cellulose derivative could be immobilized.

When the immobilization of **19b** was also examined using the A-silica in place of the plain untreated silica gel, the immobilization efficiency was found to be the same 97% as that obtained with the plain silica gel. This result indicates that the immobilization mainly proceeds via the polycondensation of the triethoxysilyl groups of the polysaccharide derivative, and the bond formation between the silica gel and the polysaccharide derivative seems to be negligible.

The recognition abilities on the obtained immobilized-type CPMs were evaluated using racemates **4**–**13** as shown in Fig. 4. The resolution results on the CPMs based on the cellulose derivative **19** are summarized in Table 5.

The elution orders of the enantiomers were same on the four immobilized-type CPMs, but their chiral recognition abilities were slightly different depending on the CPMs. The immobilized-type CPM-**17** derived from **19d** with the lowest 3-(triethoxysilyl)propyl content showed the relatively high chiral recognition ability among the four CPMs, although it contained a lower degree of immobilization than those of the other immobilized-type CPMs. The derivatives **19a** and **19b** coated on the silica gel were almost quantitatively immobilized, but their chiral recognition abilities for the racemates except for **8** were similar or slightly lower compared to CPM-**17**. Consequently, the derivative **19c** bearing the 2% 3-(triethoxysilyl)propyl group seems to be preferable for the preparation of the immobilized-type CPM from the viewpoints of both the immobilization efficiency and the chiral recognition.

The various eluents containing any proportion of chloroform and THF can be used with these immobilized-type CPMs without any dissolution of the chiral selectors from the columns. The resolution results on the immobilized-type CPM-**16** with the eluents containing chloroform and THF are summarized in Table 6 together with the results on the coated-type CPM-**9** prepared from the cellulose derivative **1d**. Under the standard chromatographic conditions using an eluent consisting of the hexane/2-propanol mixture, the immobilized-type CPM-**16** showed a similar chiral recognition to the coated-type CPM-**9**. This result suggests that the higher order structure of **19c** seems to be similar to that of **1d** coated on the silica gel because of the low content of the 3-(triethoxysilyl)propyl group. The immobilization can be attained without changing its structure due to the low content of cross-linkable groups. In addition, the chiral recognition ability on CPM-**16** was improved for most racemates using the eluents containing chloroform and THF, which cannot be used for the coated-type CPM. Therefore, seven racemates, except for **9**, **10** and **13**, could be better resolved on the immobilized-type CPM-**16** than on the coated-type CPM-**9**.

The chiral recognition on the immobilized-type CPM-**16** based on the cellulose derivative **19c** was also compared to that on the commercial Chiralpak IB using the hexane/2-propanol (90/10) mixture as the eluent (Table 6). Although the elution orders for the enantiomers were the same on both CPMs, the chiral recognition abilities were slightly different depending on the types of racemates. For racemates **7**, **9**, **10**, **11** and **12**, the chiral recognition

Table 7
Separation factors (α) on the immobilized-type CPM-**18**, coated-type CPM-**19** and Chiralpak IA

CPMs	CPM-**18** (immobilization efficiency: 86%)[a]					CPM-**19**[a]	Chiralpak IA[b]
Eluents	H/I (90/10)	H/C/I (90/10/1)	H/T/I (90/10/1)	H/C (70/30)	H/T (70/30)	H/I (90/10)	H/I (90/10)
4	∼1 (−)	1.0	1.0	∼1 (+)	1.0	∼1 (−)	1.06 (−)
5	1.44 (+)	1.48 (+)	1.67 (+)	∼1 (+)	1.24 (+)	1.60 (+)	1.46 (+)
6	2.83 (+)	2.98 (+)	2.83 (+)	2.24 (+)	2.06 (+)	3.33 (+)	2.71 (+)
7	2.11 (+)	1.49 (+)	1.54 (+)	–	1.62 (+)	2.02 (+)	2.07 (+)
8	2.27 (−)	2.26 (−)	2.07 (−)	1.74 (−)	1.36 (−)	2.21 (−)	2.15 (−)
9	1.18 (−)	1.14 (−)	1.13 (+)	1.0	1.48 (+)	1.31 (−)	1.15 (−)
10	1.08 (+)	1.63 (+)	1.31 (+)	1.33 (+)	1.0	∼1 (+)	1.17 (+)
11	1.0	1.0	1.0	1.0	1.0	1.0	∼1 (−)
12	1.0	1.38 (−)	1.0	1.68 (−)	1.0	1.36 (+)	∼1 (+)
13	3.49 (+)	–	–	1.72 (−)	1.0	2.54 (+)	2.06 (+)

The signs in parentheses represent the optical rotation of the first-eluted enantiomer. Eluent: H, hexane; I, 2-propanol; C, chloroform; T, tetrahydrofuran.
[a] Column: 25 cm × 0.20 cm I.D. Flow rate: 0.1 ml/min.
[b] Column: 25 cm × 0.46 cm I.D. Flow rate: 0.5 ml/min.

abilities were close on the two CPMs, while for racemates **4**, **5** and **8**, the immobilized-type CPM-**16** showed a higher chiral recognition than Chiralpak IB, and vice versa for racemates **6** and **13**.

The immobilization result of the amylose derivative **20** bearing 1% 3-(triethoxysilyl)propyl group is given in Table 7. The immobilization efficiency of **20** (86%) seems to be higher than that of the cellulose derivative **19d** (72%) bearing the same amount of a 3-(triethoxysilyl)propyl group. This is in good agreement with the results from the above copolymerization method, in which the amylose derivative can also be efficiently immobilized compared to the corresponding cellulose derivative. As already mentioned, the high immobilization efficiency of the amylose derivatives probably results from their flexible structures.

The chiral recognition on the immobilized-type CPM-**18** derived from the amylose derivative **20** was evaluated using various eluents. The resolution results on CPM-**18** are shown in Table 7 together with the data on the coated-type CPM-**19**, which is prepared by coating the amylose derivative **2d** on the plain silica gel. As well as the cellulose derivatives, most racemates could be better resolved on the immobilized-type CPM than on the coated-type CPM by changing the eluents. Furthermore, the immobilized-type CPM-**18** showed a similar or higher chiral recognition for eight racemates, except for **4** and **10**, compared to the commercial Chiralpak IA (Table 7).

4. Conclusion

In this review, our latest studies on the immobilization of polysaccharide derivatives onto silica gel were outlined. From the viewpoints of the simplicity of processing, immobilization efficiency and chiral recognition, the immobilization via the intermolecular polycondensation of the alkoxysilyl groups seems to be more valuable than that via the copolymerization with a vinyl monomer. The immobilized-type CPMs based on polysaccharide derivatives possesses a universal solvent compatibility and open up the possibility to improve the performance of both the analytical and preparative resolutions of chiral compounds. We expect that the applications of the immobilized-type CPMs will become more and more widespread and will contribute to further progress in all fields of science linked to chirality.

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References

[1] N.M. Maier, P. Franco, W. Lindner, J. Chromatogr. A 906 (2001) 3.
[2] H. Caner, E. Groner, L. Levy, I. Agranat, Drug Discov. Today 9 (2004) 105.
[3] I. Agranat, H. Caner, J. Cadwell, Nat. Rev. Drug Discov. 1 (2002) 753.
[4] E. Francotte, W. Lindner (Eds.), Chirality in Drug Research, Wiley–VCH, Weinheim, 2006.
[5] G. Subramanian (Ed.), Chiral Separation Techniques: A Practical Approach, 3rd completely revised and updated ed., Wiley–VCH, Weinheim, 2007.
[6] G. Subramanian (Ed.), Chiral Separation Techniques: A Practical Approach, 2nd ed., Wiley–VCH, Weinheim, 2001.
[7] G. Subramanian (Ed.), A Practical Approach to Chiral Separations by Liquid Chromatography, Wiley–VCH, Weinheim, 1994.
[8] R.W. Stringham, Adv. Chromatogr. 44 (2006) 257.
[9] C. Yamamoto, Y. Okamoto, Bull. Chem. Soc. Jpn. 77 (2004) 227.
[10] E. Yashima, J. Chromatogr. A 906 (2001) 105.
[11] Y. Okamoto, Chemtech 17 (1987) 176.
[12] E. Yashima, C. Yamamoto, Y. Okamoto, Synlett (1998) 344.
[13] E. Francotte, J. Chromatogr. A 906 (2001) 379.
[14] S. Ahuja (Ed.), Chiral Separations: Applications and Technology, ACS, Washington, DC, 1997.
[15] D.R. Taylor, K. Maher, J. Chromatogr. Sci. 30 (1992) 67.
[16] W.H. Pirkle, T.C. Pochapsky, Chem. Rev. 89 (1989) 347.
[17] D.W. Armstrong, Anal. Chem. 59 (1987) 84A.
[18] S.V. Rogozhin, V.A. Davankov, Dokl. Akad. Nauk SSSR 192 (1970) 1288.
[19] V.A. Davankov, S.V. Rogozhin, J. Chromatogr. 60 (1971) 284.
[20] W.H. Pirkle, C.J. Welch, Tetrahedron Asym. 5 (1994) 777.
[21] A.M. Blum, K.G. Lynam, E.C. Nicolas, Chirality 6 (1994) 302.
[22] C.J. Welch, J. Chromatogr. A 666 (1994) 3.
[23] G.D.Y. Sogah, D.J. Cram, J. Am. Chem. Soc. 101 (1979) 3035.
[24] K. Hirose, T. Nakamura, R. Nishioka, T. Ueshige, Y. Tobe, Tetrahedron Lett. 44 (2003) 1549.
[25] M.H. Hyun, J. Sep. Sci. 29 (2006) 750.
[26] G. Gübitz, W. Jellenz, G. Löffler, W. Scanti, J. High Resolut. Chromatogr. 2 (1979) 145.
[27] G. Gübitz, W. Jellenz, W. Scanti, J. Chromatogr. 203 (1981) 377.
[28] E. Schneiderman, A.M. Stalcup, J. Chromatogr. B 745 (2000) 83.
[29] S.M. Han, Biomed. Chromatogr. 11 (1997) 259.
[30] M. Lämmerhofer, W. Lindner, J. Chromatogr. A 741 (1996) 33.
[31] N.M. Maier, L. Nicoletti, M. Lämmerhofer, W. Lindner, Chirality 11 (1999) 522.
[32] D.W. Armstrong, Y. Tang, S. Chen, Y. Zhou, C. Bagwill, J.R. Chen, Anal. Chem. 66 (1994) 1473.
[33] T.J. Ward, A.B. Farris, J. Chromatogr. A 906 (2001) 73.
[34] X. Xiong, W.R.G. Baeyens, H.Y. Aboul-Enein, J.R. Delanghe, T. Tu, J. Ouyang, Talanta 71 (2007) 573.
[35] K. Gyimesi-Forrás, J. Kökösi, G. Szász, A. Gergely, W. Lindner, J. Chromatogr. A 1047 (2004) 59.
[36] H. Gika, M. Lämmerhofer, I. Papadoyannis, W. Lindner, J. Chromatogr. B 800 (2004) 193.
[37] M. Lämmerhofer, O. Gyllenhaal, W. Lindner, J. Pharm. Biomed. Anal. 35 (2004) 259.
[38] K.H. Krawinkler, N.M. Maier, E. Sajovic, W. Lindner, J. Chromatogr. A 1053 (2004) 119.
[39] Y. Okamoto, E. Yashima, Angew. Chem. Int. Ed. 37 (1998) 1021.
[40] X.M. Chen, C. Yamamoto, Y. Okamoto, Pure Appl. Chem. 79 (2007) 1561.
[41] S.G. Allenmark, S. Andersson, J. Chromatogr. A 666 (1994) 167.
[42] J. Haginaka, J. Chromatogr. A 906 (2001) 253.
[43] G. Blaschke, Chem. Ber. 107 (1974) 232.
[44] G. Blaschke, Angew. Chem. Int. Ed. 19 (1980) 13.
[45] G. Blaschke, J. Liq. Chromatogr. 9 (1986) 341.
[46] H. Yuki, Y. Okamoto, I. Okamoto, J. Am. Chem. Soc. 102 (1980) 6356.
[47] Y. Okamoto, I. Okamoto, H. Yuki, S. Murata, R. Noyori, H. Takaya, J. Am. Chem. Soc. 103 (1981) 6971.
[48] Y. Okamoto, K. Hatada, J. Liq. Chromatogr. 9 (1986) 369.
[49] Y. Okamoto, T. Nakano, Chem. Rev. 94 (1994) 349.
[50] T. Zhang, C. Kientzy, P. Franco, A. Ohnishi, Y. Kagamihara, H. Kurosawa, J. Chromatogr. A 1075 (2005) 65.
[51] T. Zhang, D. Nguyen, P. Franco, T. Murakami, A. Ohnishi, H. Kurosawa, Anal. Chim. Acta 557 (2006) 221.
[52] T. Zhang, D. Nguyen, P. Franco, Y. Isobe, T. Michishita, T. Murakami, J. Pharm. Biomed. Anal. 46 (2008) 882.
[53] A. Ghanem, J. Chromatogr. A 1132 (2006) 329.
[54] G.B. Cox (Ed.), Preparative Enantioselective Chromatography, Blackwell Publishing, Oxford, 2005.
[55] E. Francotte, J. Chromatogr. A 666 (1994) 565.
[56] E. Francotte, in: H.Y. Aboul-Enein, I.W. Wainer (Eds.), The Impact of Stereochemistry on Drug Development and Use, Chemical Analysis Series, vol. 142, Wiley, New York, 1997, p. 633.
[57] E. Francotte, in: S. Ahuja (Ed.), Chiral Separations: Applications and Technology, ACS, Washington, DC, 1997, p. 271.
[58] T. Ikai, C. Yamamoto, M. Kamigaito, Y. Okamoto, Polym. J. 38 (2006) 91.
[59] T. Ikai, C. Yamamoto, M. Kamigaito, Y. Okamoto, Chem. Rec. 7 (2007) 91.
[60] I. Ali, H.Y. Aboul-Enein, J. Sep. Sci. 29 (2006) 762.
[61] P. Franco, A. Senso, L. Oliveros, C. Minguillón, J. Chromatogr. A 906 (2001) 155.
[62] B. Chankvetadze, T. Ikai, C. Yamamoto, Y. Okamoto, J. Chromatogr. A 1042 (2004) 55.
[63] B. Chankvetadze, T. Kubota, T. Ikai, C. Yamamoto, M. Kamigaito, N. Tanaka, K. Nakanishi, Y. Okamoto, J. Sep. Sci. 29 (2006) 1988.
[64] T. Wakita, B. Chankvetadze, C. Yamamoto, Y. Okamoto, J. Sep. Sci. 25 (2002) 167.
[65] F. Ling, E. Brahmachary, M. Xu, F. Svec, J.M.J. Fréchet, J. Sep. Sci. 26 (2003) 1337.
[66] Y. Okamoto, R. Aburatani, S. Miura, K. Hatada, J. Liq. Chromatogr. 10 (1987) 1613.
[67] E. Yashima, S. Fukaya, Y. Okamoto, J. Chromatogr. A 677 (1994) 11.
[68] X. Chen, H. Zou, Q. Zhang, J. Ni, Z. Zhang, J. Chromatogr. Sci. 40 (2002) 315.
[69] N. Enomoto, S. Furukawa, Y. Ogasawara, H. Akano, Y. Kawamura, E. Yashima, Y. Okamoto, Anal. Chem. 68 (1996) 2798.
[70] H.G. Breitinger, Tetrahedron Lett. 43 (2002) 6127.
[71] K. Kimata, R. Tsuboi, K. Hosoya, N. Tanaka, Anal. Methods Instrum. 1 (1993) 23.
[72] L. Oliveros, P. López, C. Minguillón, P. Franco, J. Liq. Chromatogr. 18 (1995) 1521.
[73] T. Kubota, T. Kusano, C. Yamamoto, E. Yashima, Y. Okamoto, Chem. Lett. 30 (2001) 724.
[74] T. Kubota, C. Yamamoto, Y. Okamoto, Chirality 15 (2003) 77.
[75] X. Chen, F. Qin, Y. Liu, X. Huang, H. Zou, J. Chromatogr. A 1034 (2004) 109.
[76] E. Francotte, PCT International Patent Application WO 96/27615.
[77] E. Francotte, PCT International Patent Application WO 97/044011.
[78] E. Francotte, PCT International Patent Application WO 97/49733.
[79] E. Francotte, D. Huynh, J. Pharm. Biomed. Anal. 27 (2002) 421.
[80] S. Zhang, T.-T. Ong, S.-C. Ng, H.S.O. Chan, Tetrahedron Lett. 48 (2007) 5487.
[81] T. Ikai, C. Yamamoto, M. Kamigaito, Y. Okamoto, Chem. Lett. 35 (2006) 1250.

[82] T. Ikai, C. Yamamoto, M. Kamigaito, Y. Okamoto, J. Chromatogr. A 1157 (2007) 151.
[83] X. Chen, Y. Liu, F. Qin, L. Kong, H. Zou, J. Chromatogr. A 1010 (2003) 185.
[84] T. Kubota, C. Yamamoto, Y. Okamoto, J. Polym. Sci. Part A: Polym. Chem. 41 (2003) 3703.
[85] T. Kubota, C. Yamamoto, Y. Okamoto, J. Polym. Sci. Part A: Polym. Chem. 42 (2004) 4704.
[86] X.M. Chen, C. Yamamoto, Y. Okamoto, J. Chromatogr. A 1104 (2006) 62.
[87] X.M. Chen, C. Yamamoto, Y. Okamoto, J. Sep. Sci. 29 (2006) 1432.
[88] C. Minguillón, P. Franco, L. Oliveros, P. López, J. Chromatogr. A 728 (1996) 407.
[89] P. Franco, C. Minguillón, L. Oliveros, J. Chromatogr. A 791 (1997) 37.