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Review

Covalently bonded polysaccharide derivatives as chiral stationary phases in high-performance liquid chromatography

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Abstract

Polysaccharide derivatives have been extensively used as chromatographic chiral selectors in chiral stationary phases (CSPs) for the separation of enantiomers by HPLC. When coated onto a silica matrix, they represent nowadays one of the most popular type of CSPs. However, they are only compatible with a limited choice of solvents. The main drawback of these CSPs is related to the solubility of the chiral selector in a number of solvents, which limits their applicability. The different attempts which have been described up to now to overcome this problem by covalently fixing the chiral selector to a matrix are reviewed in this paper.

Keywords: Reviews; Chiral stationary phases, LC; Enantiomer separations; Polysaccharide derivatives; Cellulose; Amylose; Chitosan

Contents

1. Introduction

Historically, natural polymers, such as cellulose or starch components, were the first to be used as chromatographic chiral selectors due to their inherent

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chiral nature and ready availability. The resolving ability of polysaccharides, in particular cellulose, was first observed in paper chromatography when a racemic amino acid gave two spots [1,2]. This led to further use of cellulose and other polysaccharides, mainly amylose, as the chiral starting material in the preparation of selectors to be used in chiral stationary phases (CSPs).

Despite the original chiral recognition ability shown by natural polysaccharides, their derivatives showed more interesting properties. They present the broadest application domain of all the existing chiral selectors, only surpassed by proteins, and also have a higher loadability than the latter. Both features make them especially suitable for preparative scale enantioseparations [3,4].

Since the description of the complete resolution of racemic Tröger's base by Hesse and Hagel in 1973 [5,6], microcrystalline triacetylcellulose (MCTA) was one of the most studied derivatives and it is still considered a highly versatile CSP. The broad applicability and the high loadability of this material make it very adequate for both analytical and preparative purposes. At preparative scale, it is still the most widely used chiral sorbent [4] together with cellulose tribenzoate (CTB) [7–9]. However, the limited mechanical and physical properties of the first columns of pure MCTA (low chromatographic efficiency, low resistance to pressure and swelling in certain solvents) were the main drawbacks for its use as a high-performance liquid chromatography (HPLC) support. These problems were overcome by Okamoto and co-workers [10,11] and Ichida et al. [12] in 1984. They developed CSPs in which the polysaccharide derivatives were adsorbed onto macroporous γ-aminopropylsilica. The existence of a chromatographic matrix provides an additional mechanical stability and a high efficiency to the resulting CSPs, while maintaining the advantages of the chiral selectors used.

Polysaccharide-derived CSPs supported on silica gel are classically prepared by reaction of the polysaccharide with a benzoyl chloride or a phenyl isocyanate in homogeneous conditions, to obtain the corresponding benzoates or carbamates [13]. These derivatives are coated from a solution onto macroporous γ-aminopropylsilica matrix, by evaporation of the solvent. The resulting CSPs are commercially available and can be mainly used with hydrocarbon/alcohol or hydrocarbon/ether mixtures, such as hexane/2-propanol or hexane/*tert*-butylmethyl ether, under normal-phase conditions and with aqueous eluents in reversed-phase mode, often containing perchlorate salts [14,15]. However, the solubility of the chiral selector prevents the use of other solvents, such as chloroform or tetrahydrofuran, in which the polysaccharides can be dissolved or swollen. Because of their high solubility some polysaccharide derivatives could not be properly tested even with the classical solvent mixtures [13,16]. These limitations in the mobile phase composition represent a disadvantage in the search for new applications of these kinds of chiral supports. This drawback is even more evident at preparative scale. The solubility of the sample in the mobile phase is an important parameter to be considered in order to increase the amount of racemate loaded in a single run [4,17]. Unfortunately, often the solubility of drugs, intermediates in their synthesis, and other interesting products is not high in the conditions compatible with the coated polysaccharide-derived CSPs. The fixation of the polysaccharide derivative to the chromatographic matrix can be the solution to this problem. However, with the important role of the secondary (conformation of each molecule) and supramolecular structures on the chiral recognition mechanism of polysaccharide derivatives [14] being generally accepted, the fixation process has to ensure a balance between the reduction in the enantioselectivity that it may produce and the advantages offered by the resulting materials.

2. Fixation by means of a bisfunctional reagent

In order to overcome the problems of solubility of polysaccharide derivatives as coated chiral selectors, Okamoto et al. described in 1987 the first preparation of CSPs in which the polysaccharide was bonded to a γ-aminopropylsilica gelmatrix [18]. The fixation method consisted in using a diisocyanate which was expected to react with the free amino groups on the matrix surface and the hydroxyl groups on the polysaccharide acting, therefore, as a covalently bond spacer.

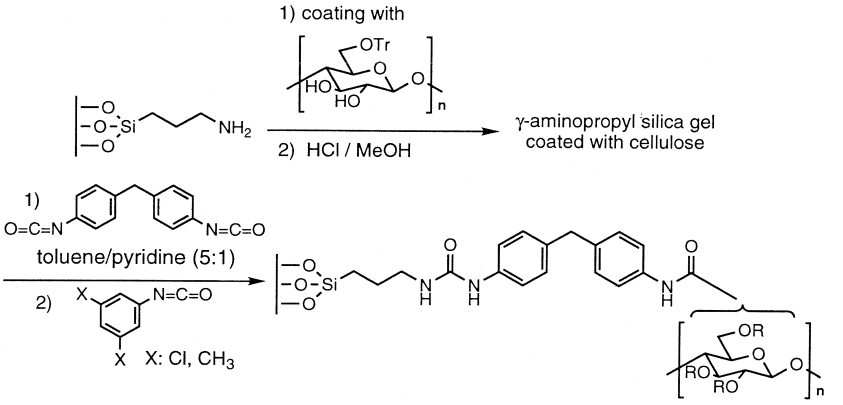

Fig. 1. Fixation of 3,5-dichloro- and 3,5-dimethylphenylcarbamate of cellulose onto γ-aminopropylsilica gel [18].

As it is shown in Fig. 1, firstly cellulose was coated onto γ-aminopropylsilica gel as 6-*O*-tritylcellulose. The solubility of the latter allows the coating of the modified silica gel in the usual way. Underivatized cellulose is regenerated from the 6-*O*-tritylcellulose on the surface of the matrix by acidic treatment. In the next step a certain amount of a diisocyanate was allowed to react with the resulting material, covering the remaining unreacted hydroxyl groups of the cellulose by derivatization with an excess of either 3,5-dichloro- or 3,5-dimethylphenyl isocyanate. Several diisocyanates (4,4′-methylenediphenyl diisocyanate, 1,6-hexamethylene diisocyanate and 1,4-phenylene diisocyanate), with different distances between the isocyanate functions, and various amounts of one of them (4,4′-methylenediphenyl diisocyanate) were used. The resulting CSPs were tested under classical conditions (hexane/2-propanol) in order to compare the results with those obtained with the correspondingly coated CSPs (Table 1) (Fig. 2). The stability of the 3,5-dichlorophenylaminocarbonyl-derived CSP was higher than the one of the correspondingly coated CSP, in which a gradual leak of this chiral selector occurred in the same conditions. However, a certain decrease in the enantioselectivity values was observed. The authors attributed this reduction in selectivity to the lack of ordered arrangement of the chiral selector bonded to the chromatographic matrix. This reduction was found to be dependent on the amount of diisocyanate used, but not on its nature. Thus, the higher the amount of this reagent, the lower the enantioselectivity for a given analyte.

Table 1
Selectivity factor values obtained with CSPs containing coated (A) and covalently bonded (B) cellulose tris(phenylcarbamate)s (CTPCs) [18][a]

Compound	CTPC-3,5-diCl		CTPC-3,5-diMe	
	A	B[b]	A	B[b]
1	1.84 (+)	1.54 (+)	1.68 (−)	1.18 (−)
3	1.11 (+)	1.20 (−)	1.83 (−)	4.02 (−)
4	1.38 (−)	1.38 (−)	2.59 (−)	1.19 (−)
5	1.26 (−)	1.24 (−)	1.15 (−)	1.26 (−)
7	–	–	1.58 (+)	1.24 (+)

[a] Mobile phase: hexane/2-propanol (90:10). Flow-rate: 0.5 ml/min. Signs in parentheses represent the optical rotation of the first eluted enantiomer.

[b] 3% of bonding agent.

It is worth noting the difference in the selectivity values for compound **3** on the columns containing coated and fixed cellulose 3,5-dimethylphenylcarbamate as chiral selector. However, this difference disappeared after a thermal treatment of the column containing the fixed selector, suggesting a metastable arrangement for the polysaccharide derivative in the fixed CSP. This may be the result of the derivatization of cellulose after the fixation step. At room temperature the cellulose already fixed may be unable to change conformation in accordance with its derivatization. In coated CSPs, the polysaccharide derivative is free to adopt the most stable disposition during the coating process.

A modification of this method was published by

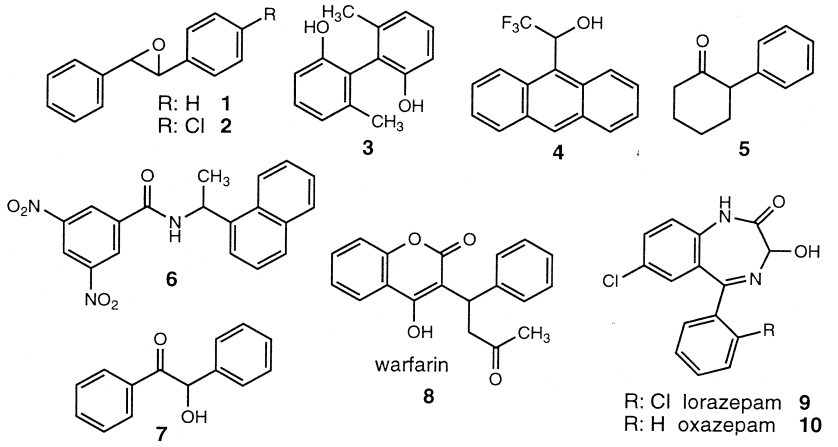

Fig. 2. Chemical structures of some of the racemic compounds used to test the CSPs.

the same research group in 1994 [19]. The modified method involves a regioselective fixation. Thus, 4,4′-methylenediphenyl diisocyanate was allowed to react with γ-aminopropylsilica gel coated with either a polysaccharide derivative in which the 6 positions were blocked with trityl groups (Fig. 3), or with a derivative in which the 2 and 3 positions were derivatized and only the 6 positions were free (Fig. 4). This latter derivative was also obtained from the 6-*O*-trityl polysaccharide. The fixing agent was used at 3% and 10% ratios, relating to the hydroxyl groups to be derivatized on the polysaccharide. Finally, a last derivatization step was carried out in both cases to ensure the complete reaction of all hydroxyl groups in the resulting CSPs.

The chromatographic behavior of the obtained

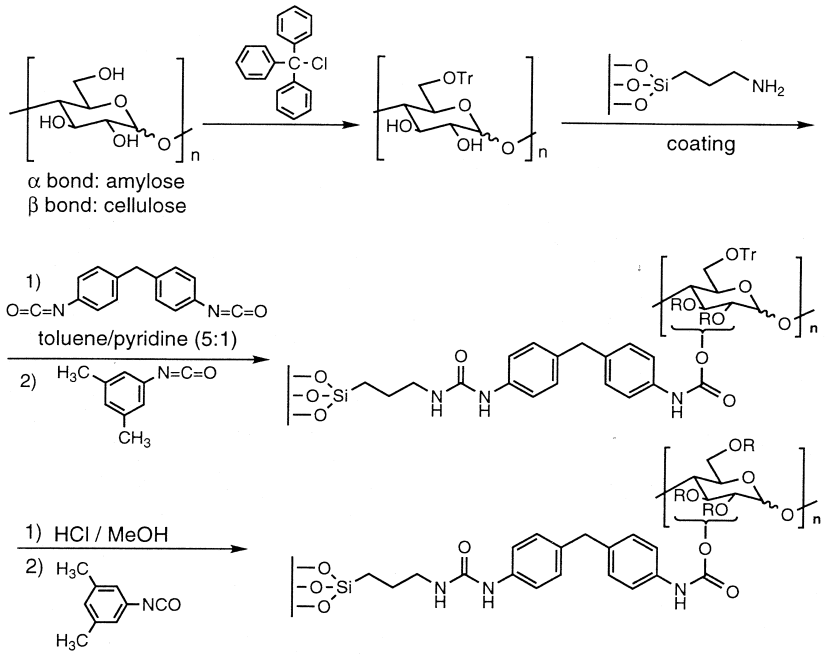

Fig. 3. Regioselective fixation of 3,5-dimethylphenylcarbamates of cellulose and amylose by positions 2 and 3 of the glucosidic rings [19].

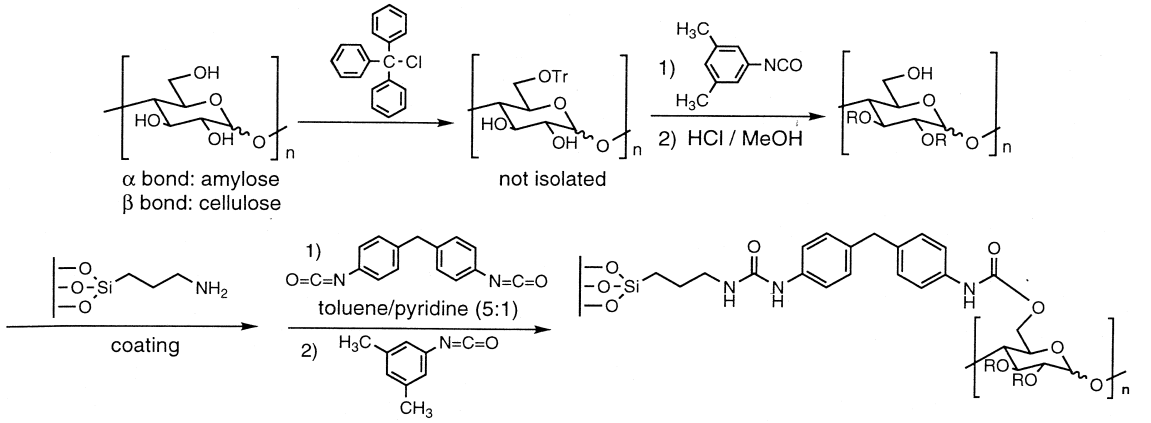

Fig. 4. Regioselective fixation of 3,5-dimethylphenylcarbamates of cellulose and amylose by position 6 of the glucosidic rings [19].

CSPs was compared to the coated CSPs and again a reduction in the enantioselectivity was observed (Table 2). As in the previous study, this reduction was more pronounced when a higher amount of fixing agent was used. The results obtained for analogous selectors fixed by different positions were also compared. While the enantioselectivity of the amylose derivative was higher when fixed through the 6 position, no significant difference was found for the cellulose derivatives.

Interesting results were obtained when a small amount of chloroform was used in the mobile phase. Some changes in the chiral discrimination ability of the chiral selectors were observed. Certain analytes, that were not resolved when the classical mobile phases were used, could be separated using mobile phases containing a 5% of chloroform. This effect was attributed to a change in the conformation of the chiral selector in the presence of this solvent.

It has to be taken into account that the fixing method here presented involves the addition of the diisocyanate to the coated material [18,19]. This reagent has to diffuse through the coated polysaccharide to reach the amino groups on the surface of the matrix. Therefore, even if the authors claim for a real bonding between the γ-aminopropylsilica gel and the polysaccharide derivatives [18], the most likely fixation mechanism would come from the reaction of two hydroxyl groups on the polysaccharide with the diisocyanate. This reaction will result in the cross-linking of the polysaccharide derivative and, therefore, in its insolubilization in solvents. This hypothesis is consistent with the similar enantioselectivity values obtained for all diisocyan-

Table 2
Selectivity factor values obtained with CSPs containing coated (A) and regioselectively bonded (B and C) tris(3,5-dimethylphenylcarbamate)s of cellulose and amylose [19][a]

Compound	Cellulose derivatives			Amylose derivatives		
	A	B[b]	C[c]	A	B[b]	C[c]
1	1.68 (−)	1.30 (−)	1.46 (−)	3.04 (+)	2.53 (+)	2.08 (+)
3	1.83 (−)	3.47 (−)	2.76 (−)	2.11 (−)	2.10 (−)	1.63 (−)
4	2.59 (−)	2.33 (−)	2.12 (−)	1.15 (+)	1.00	ca. 1
5	1.15 (−)	1.22 (−)	1.19 (−)	ca. 1	1.00	ca. 1 (−)
7	1.58 (+)	1.31 (+)	1.29 (+)	1.21 (−)	1.09 (−)	1.09 (+)

[a] Mobile phase: hexane/2-propanol (90:10). Flow-rate: 0.5 ml/min. Signs in parentheses represent the optical rotation of the first eluted enantiomer.

[b] Derivative fixed by the 6 positions of the glucose units using 3% of bonding agent.

[c] Derivative fixed by the 2,3 positions of the glucose units using 3% of bonding agent.

ates used, even the short and rigid 4,4′-phenylene diisocyanate [18].

Despite the reduction in their chiral discrimination ability in comparison with the corresponding coated CSPs, the enantioselectivity of the described fixed supports is still high enough to be considered useful, even more when they are resistant to a broader choice of solvents than the coated sorbents. Although these latter have been the object of a high number of studies and applications, no more studies appeared dealing with these bonded polysaccharide-derived CSPs.

Other methods of fixing polysaccharides to a chromatographic matrix by means of a bisfunctional reagent have been described [20]. However, the resulting materials were not applied to the resolution of enantiomeric mixtures.

3. Fixation of a polysaccharide derivatized with a polymerizable group

The fixation of a tris(4-vinylbenzoate) of cellulose on a modified silica, by means of a radical co-polymerization reaction, was described in 1993 [21]. In this case there was no spacer between the matrix and the polysaccharide derivative and both were bearing activated double bonds able to be polymerized. On the one hand, the γ-aminopropylsilica gel used as a matrix was treated with acryloyl chloride. On the other hand, cellulose was fully derivatized with 4-vinylbenzoyl chloride. After coating the cellulose derivative onto the modified silica, a suspension of the resulting material in heptane was heated in the presence of a radical initiator (Fig. 5). The CSP obtained from the above described process was stable in solvents such as dichloromethane or tetrahydrofuran.

A coated phase was prepared with the same selector in order to compare the chromatographic results with those of the bonded CSP. This latter showed slightly lower selectivity factors (a 10% of reduction is claimed by the authors) for the analytes tested, but its higher stability against solvents, compared to the coated material, was clearly proved. The method was patented [22] but no studies were published later.

Due to the now high number of double bonds on the polysaccharide, it is likely that the copolymerization between the modified silica gel and the polysaccharide derivative was accompanied by a homo-

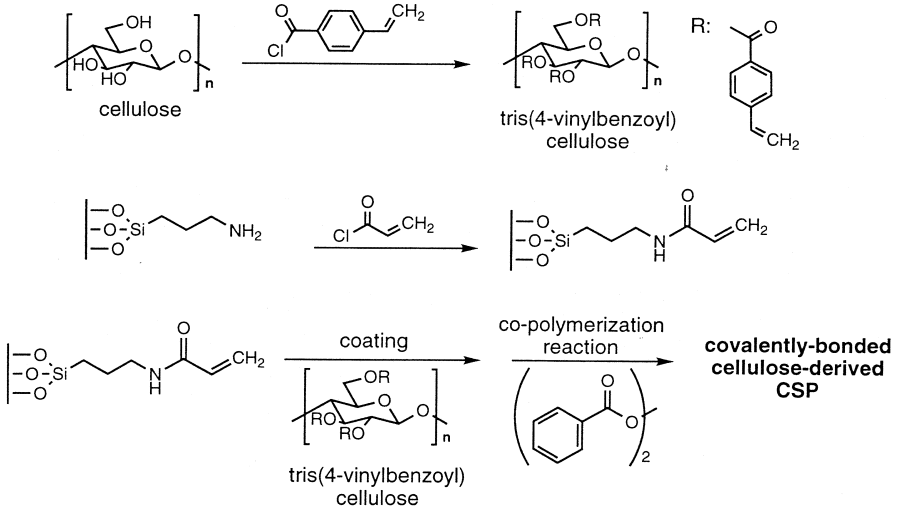

Fig. 5. Polymerization of tris(4-vinylbenzoate) of cellulose onto acrylamidopropylsilica gel [21].

polymerization or cross-linking, of the polysaccharide itself.

4. Fixation of mixed derivatives of polysaccharides

In 1994 the fixation of polysaccharide derivatives with two different substituents onto diverse chromatographic matrices was described [23,24]. The method consisted of the polymerization of a mixed polysaccharide derivative bearing, besides the aromatic substituents usual in these kind of chiral selectors, 10-undecenoyl groups. These latter groups were destined to fix the polysaccharide to the chromatographic matrix.

The ability of the unsaturated esters of cellulose, such as the tricrotonate, to easily polymerize, at high temperature or when irradiated with UV light, was already described in 1934 [25]. In mixed acetate/crotonate derivatives this transformation is more difficult but possible. In the case of 10-undecenoates of cellulose, although the double bonds are not activated, it has been described that they are highly reactive [26]. Moreover, these derivatives are readily prepared and very stable against hydrolysis [27]. In the present case, due to the application to which these compounds will be devoted, besides 10-undecenoyl groups, aromatic substituents were needed to ensure a high enantioselectivity as chiral selectors for the resulting polysaccharide derivatives.

The validity of the fixation method was proved with a 10-undecenoate/3,5-dimethylphenylcarbamate of cellulose having a ratio of 1:1.16 of both groups per glucose unit. This cellulose derivative was prepared in a single synthetic step by the successive addition of 10-undecenoyl chloride and 3,5-dimethylphenyl isocyanate to the reaction medium containing the cellulose. It was successfully fixed after coating onto allylsilica, end-capped silica, non treated silica, alumina and graphite [24] (Fig. 6). The fixation process consisted of a thermal treatment in the presence of α,α′-azobisisobutyronitrile (AIBN) and the absence of solvent. The amount of cellulose derivative fixed on the matrix was almost the same for all supports.

All obtained CSPs were tested using heptane/2-propanol and heptane/chloroform mixtures, with a high content of this latter solvent, as a mobile phase. They were also tested under reversed-phase conditions. Results were highly dependent on the matrix used. Thus, allylsilica gel originated the CSP with the best performance either in selectivity or resolution. The unmodified matrices (native silica, alumina and graphite) often originated long retention times and tailing peaks which did not improve under reversed-phase conditions. This phenomenon could be the result of a high cross-linking, or reticulation, of the polysaccharide on the surface of these supports, since cross-linking is the only possible mechanism of insolubilization for these matrices. On allylsilica gel reticulation of the same polysaccharide can be lower due to the contribution of the allyl groups on the matrix to an heterogeneous coupling.

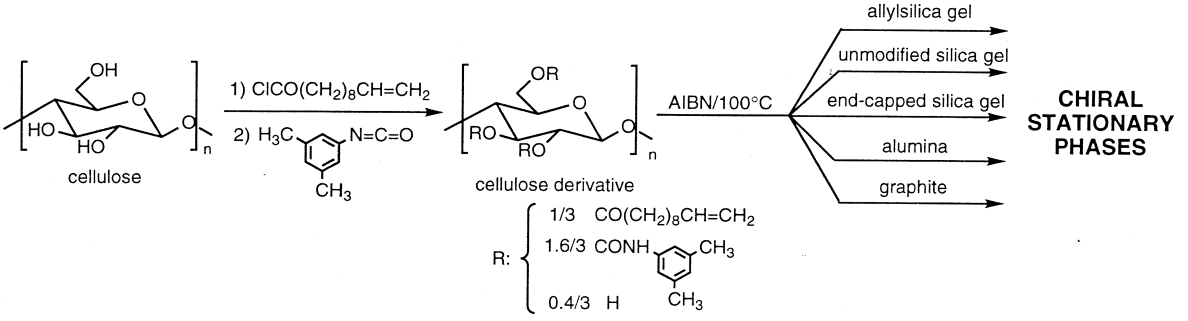

Fig. 6. Fixation of a 10-undecenoate/3,5-dimethylphenylcarbamate of cellulose onto several chromatographic matrices [24].

Therefore, allylsilica gel was used as a matrix in the following studies.

4.1. Optimization of the fixation process

In the search of an optimized CSP, the influence of the amount of 10-undecenoyl groups on the selectivity was studied [28]. As for the fixation methods previously discussed, here the fixation process takes place both by reaction of 10-undecenoyl groups on the cellulose with allyl groups on the silica gel (heterogeneous coupling) or by reticulation of the 10-undecenoyl groups themselves. In spite of the conformational flexibility of the 10-undecenoyl groups, both processes can affect the secondary helical structure of the cellulose derivative originating the already mentioned reduction in the enantioselectivity values. In order to minimize the effect of this reticulation while maintaining a proper fixation rate, four 10-undecenoate/3,5-dimethylphenylcarbamates of cellulose with different content of 10-undecenoyl groups (from less than 0.1 to 1.16 groups per glucose unit) were prepared and fixed on allylsilica gel. The derivative containing in the order of 0.3 undecenoyl groups per glucose unit afforded the best chromatographic results. The application domain of the resulting CSP was the broadest in the series, indicating the lowest effect on the secondary structure, while the fixation was still correct.

In the next study the influence of the porosity of the matrix on the performance of the resulting CSPs was taken into account [29]. In most of the studies related to coated polysaccharide-derived CSPs modified macroporous silica gel was used as a matrix in order to furnish an important surface where polysaccharides can be coated. However, the pore size of the matrix not only has an influence on the accessible surface, but also on the number of reactive groups that would be available for bonding (Fig. 7, I). Two cellulose derivatives, the best in the preceding study

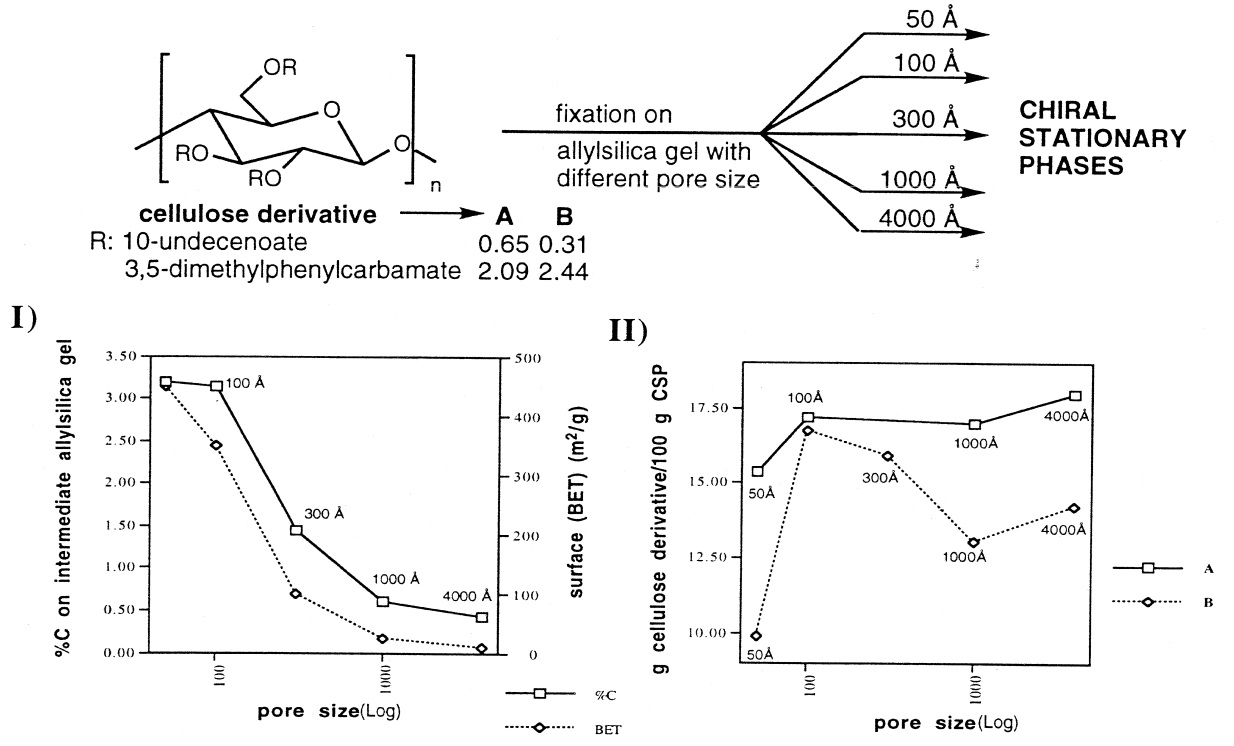

Fig. 7. Fixation of 10-undecenoate/3,5-dimethylphenylcarbamates of cellulose onto allylsilica gels of different pore size. Relationship between silica gel pore size (log scale) and (I) the carbon content on intermediate allylsilica gels and (II) the amount of cellulose derivative fixed on the CSP [29].

and one with a higher content in 10-undecenoyl groups (0.6 groups per glucose unit), were fixed onto allylsilica gels of different pore size (50, 100, 300, 1000 and 4000 Å) (Fig. 8).

When the cellulose derivative with a higher content of 10-undecenoyl groups was used (derivative **A** in Fig. 7), the content of chiral selector on the obtained CSPs was similar for all matrices (Fig. 7, II). In contrast, it appeared highly dependent on the matrix pore size when the derivative with a low content in fixing groups was utilized (derivative **B** in Fig. 7). Therefore, the pore size of the matrix has to be adapted to the fixing ability of the chiral selector used. The best results for the derivative optimized in the previous study [28] were obtained when 300 Å allylsilica gel was used as a matrix. It was not possible to properly pack the CSPs obtained by the fixation of this polysaccharide derivative onto macroporous allylsilica gels (1000 and 4000 Å), in which the content of allyl groups is also low. This was attributed to a deficient bonding. However, only slight differences in selectivity were observed for the rest of matrices when using the same derivative. Nevertheless, resolution clearly improved with the increase of the pore size.

When the derivative with a higher content in 10-undecenoyl groups was used, 50 Å allylsilica gel gave the best results in the series. The higher accessibility of the chiral selector by the analytes could account for this result. Thus, in macroporous matrices the selector can partially enter the pores. The effective pore size is reduced and, therefore, the accessibility of the analytes for the chiral selector may be lower. In 50 Å matrices the chiral selector is not able to enter the pores and all of it is accessible to the analytes on the external surface of the matrix.

The influence of reticulation or cross-linking on the selectivity was also the object of study [30]. A cellulose derivative containing 0.6 alkenoyl groups per glucose unit was fixed onto end-capped silica gels of different pore size. The results were compared with those of CSPs resulting from the fixation of the same derivative onto allylsilica gels of identical pore sizes. The CSPs in which end-capped silica gel was used as the matrix, showed either broader peaks or lower selectivity values than those prepared from allylsilica gel, suggesting again that reticulation strongly affects the conformation of the polysaccharide. The influence of the heterogeneous coupling allyl/10-undecenoyl groups cannot be further reduced without affecting the quality of bonding [28–30].

4.2. Derivatives of cellulose

Once the fixation was optimized for the 10-undecenoate/3,5-dimethylphenylcarbamate of cellulose, thanks to the ease of preparation of mixed derivatives of this polysaccharide and the fixation process itself, the method was successfully applied to other derivatives such as benzoates [31] and carbamates of cellulose [32]. All derivatives were systematically tested using heptane/2-propanol and heptane–chloroform mixtures as mobile phases (Table 3). All of them were successfully tested regardless the solubility of the chiral selector prior to fixation.

Among the different cellulose benzoate derivatives tested 10-undecenoate/3,5-dichlorobenzoate showed the widest application domain for the racemic compounds tested, followed by the 4-methylbenzoate and the benzoate. The 10-undecenoate/3,5-dichlorophenyl carbamate of cellulose was the best among the different cellulose carbamates, being only comparable to the 3,5-dimethylphenylcarbamate.

Some interesting changes in the selectivity of the stationary phases were observed when changing the mobile phase. Thus, a reduction in the number of racemic compounds resolved was generally observed when chloroform was used. The 10-undecenoate/4-chlorophenylcarbamate of cellulose constitutes an exception to this rule. The CSP containing this chiral selector was able to discriminate a higher number of racemic analytes when this solvent was used in the mobile phase than when using 2-propanol as a modifier. However, the incorporation of chloroform as a mobile phase modifier led to the resolution of some racemic compounds not separated using heptane/2-propanol mixtures (Fig. 9). This phenomenon implies an enlargement of the application domain for the considered CSPs.

4.3. Solvent effects on the obtained CSPs

Besides the above mentioned changes in enantioselectivity produced by the use of different mobile phase components, the most direct advantage added

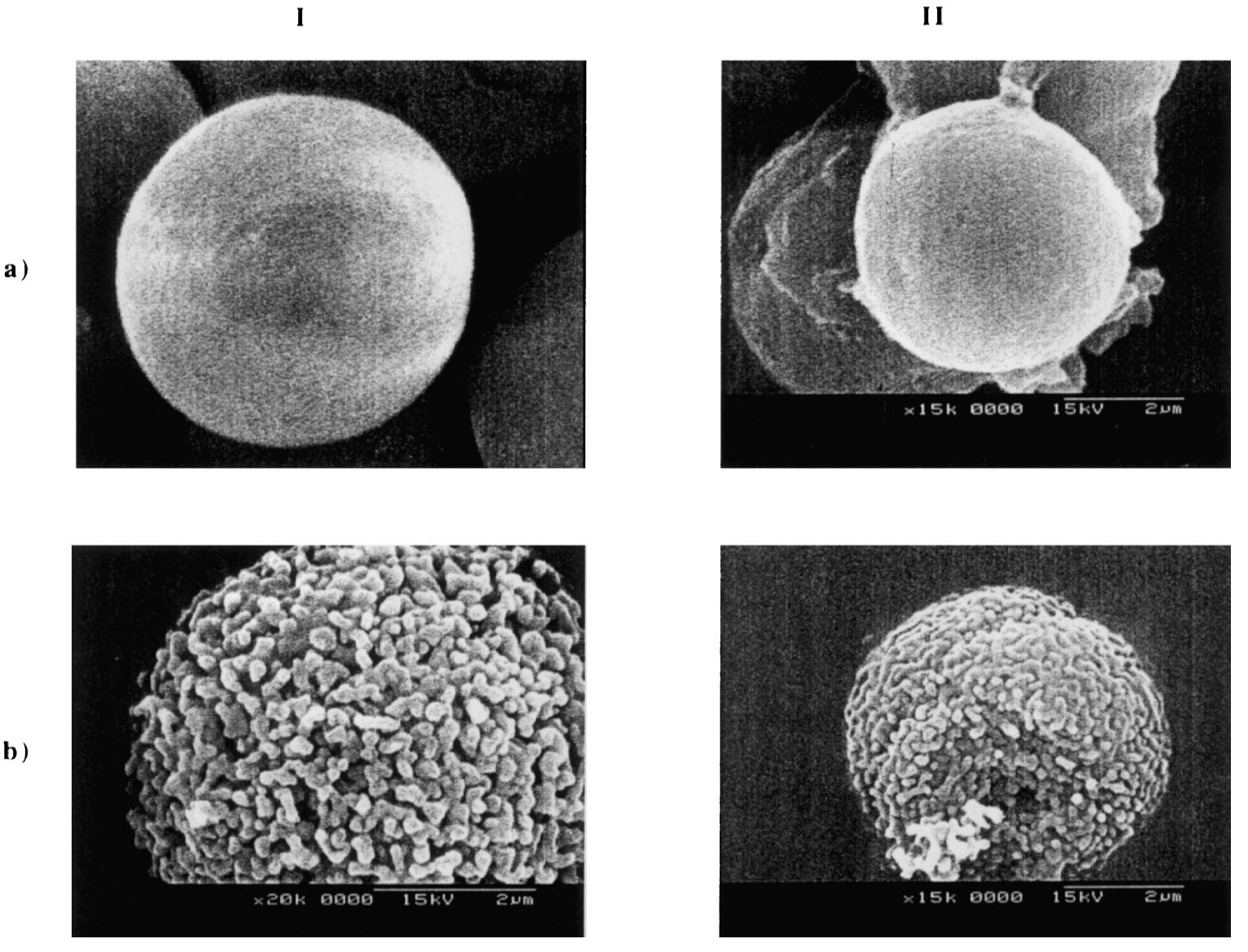

Fig. 8. Scanning electron micrographs of (I) 5 μm silica gel particles (a) pore size, 100 Å; (b) pore size, 4000 Å; (II) CSPs resulting from fixing 10-undecenoate/3,5-dimethylphenylcarbamate of cellulose on (a) and (b).

Table 3
Selectivity factor values obtained with CSPs containing immobilized mixed 10-undecenoate/benzoates or phenylcarbamates of cellulose [31,32][a]

Compound	Benzoates -CO-C6H4-X					Phenylcarbamates -CONH-C6H4-X					Mobile phase[b]
	X:					X:					
	3,5-diMeO	3,5-diMe	4-Me	H	3,5-diCl	3,5-diMe	4-Me	H	4-Cl	3,5-diCl	
1	1.23	1.25	1.31 (+)	1.21 (+)	1.38 (+)	1.49 (−)	1.49 (+)	1.25	1.80 (+)	1.50 (+)	**a**: 98:2
2	1.35	1.33	1.68 (+)	1.28 (+)	1.34 (+)	1.00	1.52 (+)	1.22	1.45 (+)	1.48 (+)	**a**: 98:2
4	1.00	1.00	1.00	ca. 1	1.00	2.44 (*R*)	1.59 (*R*)	1.38 (*R*)	1.21 (*R*)	1.48 (*R*)	**a**: 98:2
6	1.28	1.46	1.29	1.00	1.15[c]	1.46 (*R*)	1.22 (*R*)	1.39 (*R*)	1.12	1.27 (*R*)	**a**: 80:20
6	1.39	1.40	1.00	1.00	1.00	1.23 (*R*)[d]	1.20 (*R*)	1.28 (*R*)[d]	1.21 (*R*)	1.00	**b**: 50:50
7	1.00	1.00	1.22 (−)	1.00	1.12 (−)	1.33 (+)	1.00	1.00	1.13 (−)	1.13	**a**: 98:2
8	1.58	1.18	1.00[c]	1.22[c]	1.91[c]	1.28	1.42 (−)	1.31	1.28 (−)	–	**a**: 80:20
8	1.00	1.00	1.40	1.00	1.00	1.56 (+)[d]	1.27 (+)[d]	1.52 (+)[d]	1.91 (+)[d]	2.43 (+)[d]	**b**: 75:25
9	1.15[c]	1.00[c]	1.00[c]	1.40 (+)[c]	1.16[c]	1.56 (−)	2.40 (−)	2.70 (−)	1.42 (−)	1.83 (+)	**a**: 80:20
10	1.00[c]	1.00[c]	1.00[c]	1.24 (+)[c]	1.12[c]	1.00	1.23 (−)	1.24	1.30 (+)	1.37 (+)	**a**: 80:20

[a] Signs in parentheses represent the optical rotation or the absolute configuration of the first eluted enantiomer.
[b] Flow-rate: 1 ml/min. **a**: Heptane/2-propanol; **b**: heptane–chloroform.
[c] Heptane/2-propanol (90:10).
[d] Heptane/chloroform (25:75).

by the fixation of the chiral selector to the chromatographic matrix in this kind of CSPs is the resistance to solvents. This advantage was applied to the preparative separation of the eutomer of the diuretic drug cyclothiazide. The enantiomeric pair containing the eutomer of cyclothiazide can be resolved by HPLC on a CSP coated with 3,5-dimethylphenylcarbamate of cellulose. However, the poor solubility of cyclothiazide in solvents compatible with this kind of CSP makes this separation difficult to carry out at preparative scale. This resolution was achieved with a CSP constituted by a 10-undecenoate/3,5-dimethylphenylcarbamate of cellulose fixed on allylsilica gel. As cyclothiazide is highly soluble in acetone, the mobile phase used consisted of a mixture of toluene/acetone (2:1) [33].

The same CSP was also used with tetrahydrofuran, or ethyl acetate, besides the already mentioned chloroform and 2-propanol, in the mobile phase [34]. The effect of the different mobile phase modifiers on the loadability of several racemic compounds was studied. In Table 4 the results obtained for compound **4** are shown. The loadability for this racemate is highly dependent on the solvent used. Thus, it ranges from 0.35 mg under classical conditions (heptane/2-propanol) to 6 mg, on the same analytical column, when chloroform was the mobile phase modifier. Therefore, the loading capacity of the column depends, not only on the racemic compound to be resolved, but also on the eluent used for the separation.

4.4. Mixed derivatives of amylose and chitosan

The same fixation method has been applied to mixed derivatives of amylose [35] and chitosan [36] (Fig. 10). Some modifications in the preparation of chiral selectors from chitosan had been introduced with the aim to prepare derivatives comparable to those obtained with cellulose and amylose. Firstly a previous hydrolysis/purification treatment was applied in order to obtain an homogeneous and reactive starting product. Afterwards, due to differences in reactivity relating to cellulose or amylose, the reversed order in the addition of reagents was considered [37].

In contrast to the low enantioselectivity of benzoyl derivatives of amylose [35,16], benzoyl derivatives of chitosan, as those of cellulose, showed enan-

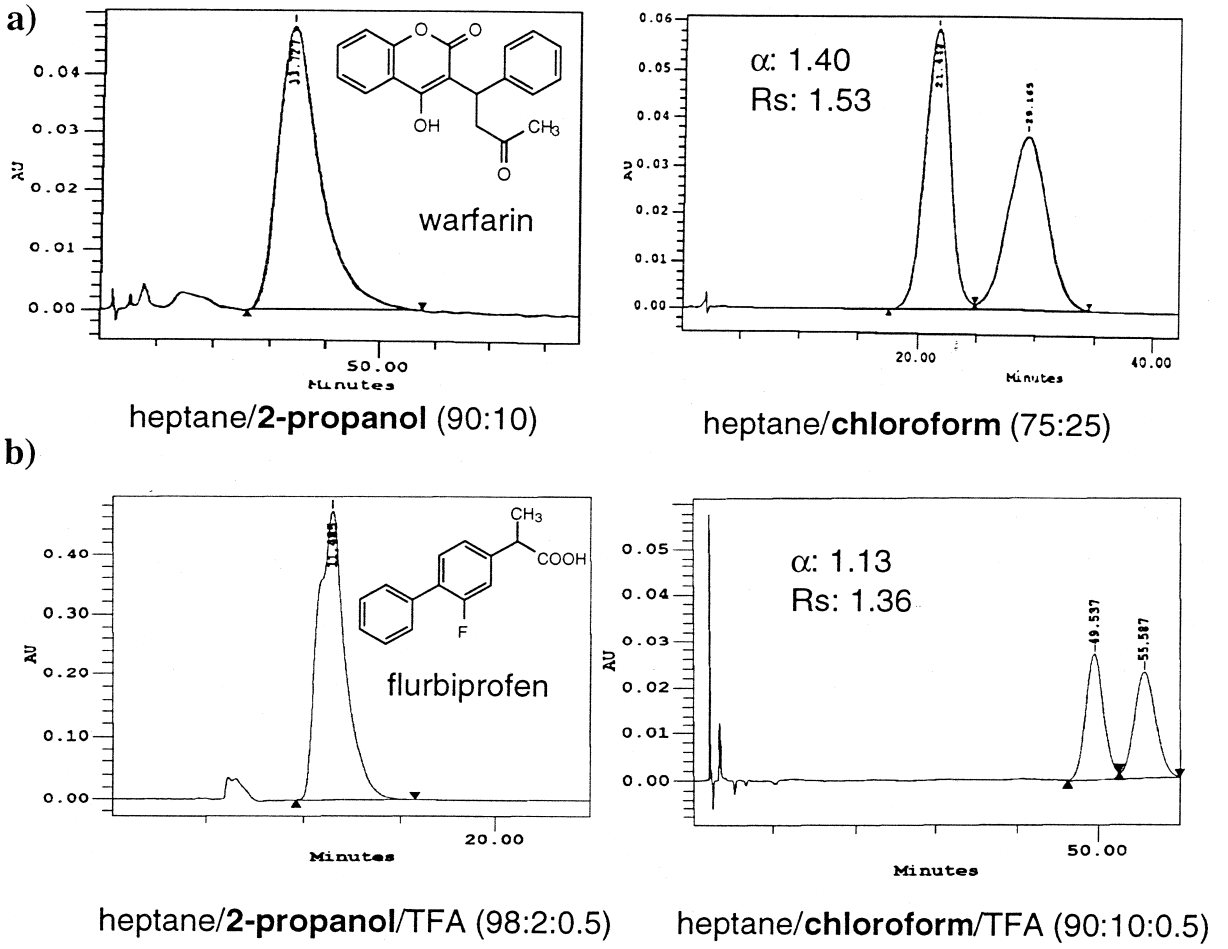

Fig. 9. Changes in selectivity produced by the change of mobile phase modifier (A) CSP: 10-undecenoate/4-methylbenzoate of cellulose; (b) CSP: 10-undecenoate/4-chlorophenylcarbamate of cellulose.

Table 4
Loadability for compound **4** on a 10-undecenoate/3,5-dimethylphenylcarbamate of cellulose CSP using different mobile phase modifiers[a]

	Mobile phase modifier			
	2-Propanol (10%)	Chloroform (50%)	Tetrahydrofuran (10%)	Ethyl acetate (20%)
α	2.20	2.29	2.02	2.04
Mass of injected solute (mg)	0.35	6.00	3.44	3.80
mmol of injected solute	1.3	21.7	12.5	13.8
Injected volume[b] (μl)	140	800	32	38
Time of analysis (min)	10	15	12	10
Productivity (mg/day)	50	576	413	547

[a] Flow-rate: 1 ml/min. UV detection at 280 nm (to avoid the UV saturation) and polarimetric detection at 546 nm.
[b] The sample was dissolved in the pure organic modifier.

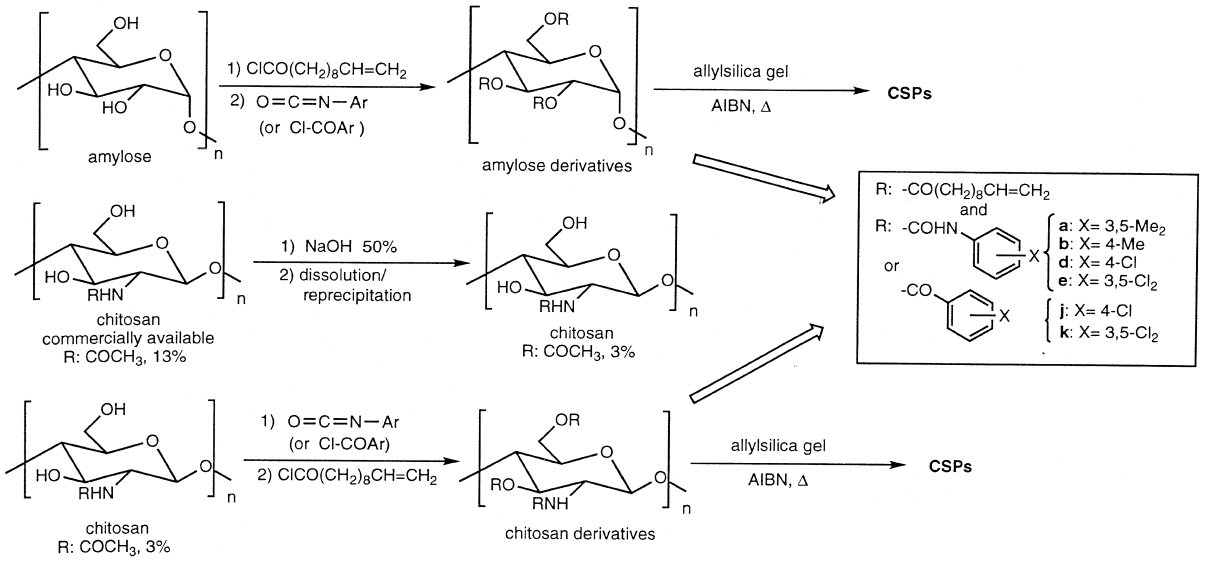

Fig. 10. Preparation of amylose- and chitosan-derived CSPs.

tioselective abilities for many racemic compounds (Table 5). However, contrarily to cellulose and amylose derivatives, a noticeable increase in the application domain for most of the chitosan-based CSPs was observed when chloroform was used in the mobile phase. Not only higher enantioselectivity values but also higher resolution factor values were obtained under these conditions. This effect was more noticeable for carbamate than for benzoate derivatives of chitosan and has been related to the behavior of the chiral selectors in both solvents. Carbamates of chitosan are more swollen by chloroform than by alcohols. Thus, in the former these chiral selectors may become more accessible to

Table 5
Selectivity factor values obtained with CSPs containing immobilized mixed 10-undecenoate/benzoates (BZs) or phenylcarbamates (PCs) of amylose and chitosan [35,36][a]

Compound	Amylose derivatives				Chitosan derivatives					Mobile phase[b]
	3,5-diMePC	4-MePC	4-ClPC	3,5-diClPC	4-ClBZ	3,5-diClBZ	4-ClPC	3,5-diClPC	4-MePC	
1	2.75 (+)	1.40 (+)	2.36 (+)	1.29	1.18 (+)	1.14	1.00	1.36 (+)	1.00	**a**: 98:2
1	1.68 (+)	1.45 (+)	2.68 (+)[c]	1.33 (+)[c]	1.19 (+)	1.20 (+)	1.25 (+)	1.00	1.18 (+)	**b**: 95:5
2	2.89 (+)	1.33 (+)	1.88 (+)	1.00	1.19 (+)	1.00	1.00	1.34 (+)	1.00	**a**: 98:2
2	1.47 (+)	1.34 (+)	2.20 (+)[c]	1.30 (+)[c]	1.18 (+)	1.23 (+)	1.17 (+)	1.00	1.27	**b**: 95:5
4	1.10	1.00	1.08	1.09	1.00	1.00	1.00	1.00	1.12	**a**: 98:2
7	1.08 (−)	1.00	1.00	1.00	1.16 (−)	1.08 (−)	1.24 (−)	1.00	1.00	**a**: 98:2
7	ca. 1	1.00	1.00	1.00	1.10 (−)	1.07	1.29 (−)	1.07	1.00	**b**: 90:10
8	2.63 (−)	1.31 (−)	1.29 (−)	1.37	1.00	1.22 (−)	1.00	1.72 (−)	1.00	**a**: 90:10
8	1.70 (+)	1.78	1.47 (+)	1.55	1.00	1.11 (−)	2.15[d]	3.47 (+)[d]	1.00	**b**: 50:50
9	1.72 (+)	1.17	1.32 (+)	1.83 (+)	1.34 (−)	1.19 (−)	1.00	1.26	1.00	**a**: 80:20
10	1.31 (+)	1.00	1.18 (+)	1.16	1.29 (−)	1.27 (−)	1.00	1.00	1.00	**a**: 80:20

[a] Signs in parentheses represent the optical rotation or the absolute configuration of the first eluted enantiomer.
[b] Flow-rate: 1 ml/min. **a**: Heptane/2-propanol; **b**: heptane–chloroform.
[c] Heptane/chloroform (90:10).
[d] Heptane/chloroform (0:100).

analytes. Benzoates showed a similar behavior in both solvents. Therefore, they may maintain a similar accessibility for analytes when these solvents are used.

Relating to amylose carbamates, the inversion of the elution order of enantiomers for some racemic compounds has been observed when the mobile phase modifier changes from 2-propanol to chloroform [35]. This effect suggested the existence of different recognition mechanisms for the same racemate depending on the solvent used.

To sum up, this method of fixing can be easily applied to any polysaccharide derivative and has the advantage to allow the characterization of the chiral selector prior to the fixation step [38].

5. Fixation of amylose by the reducing terminal residue

In 1996 Enomoto et al. described the fixation of amylose to the chromatographic matrix by the reducing terminal residue of each molecule [39]. The method is based in the enzymatic polymerization of α-D-glucose 1-phosphate. The reaction was catalyzed by a phosphorylase isolated from potato and it was carried out using two kinds of primers derived from maltopentaose. Once the amylose chains, with a desired chain length and a narrow molecular mass distribution, were constructed by enzymatic polymerization, they were bonded to silica gel to be used as CSPs. Two methods were used to carry out the fixation. In the first one (method I, Fig. 11) maltopentaose was lactonized after oxidation at the terminal residue and allowed to react with 3-aminopropyltriethoxysilane through the first glucosidic unit. The glucose polymerization was brought about on the obtained glucosilane. Then the resulting amylose, modified in the first residue, was allowed to react with silica gel to produce the immobilization. The second method (method II, Fig. 11) implies a different order in the synthetic steps. Thus, maltopentaose was first oxidized to form a gluconate at the reducing terminal residue. The enzymatic polymerization was then performed and afterwards the terminal residue was lactonized to be immobilized onto 3-aminopropylsilica gel. Both amylose conjugated silica gels were treated with a large excess of 3,5-dimethylphenyl isocyanate in order to derivatize the hydroxyl groups of amylose.

The main advantage of this fixation method over the previously presented methods is that it prevents the modification in the structure of amylose caused by the other fixing strategies described. Several CSPs derived from 3,5-dimethylphenylcarbamate of amylose were prepared by changing either the length of the polysaccharide or the spacer used to bond it to the matrix. Results showed that enantioselectivity depends on both the preparation method and the degree of polymerization (DP) of amylose. Method II originated CSPs with better performance than method I. Regarding to the length of the polysaccharide chain, the enantioselectivity of the CSPs increased with DP of the polysaccharide. Amylose with a DP of 120 originated enantioselectivity values in the same order or even higher than those of the correspondingly coated CSP. This result is probably due to the high conformational freedom of the chiral selector which is fixed to the matrix by only one bond placed on the terminal residue.

6. Other methods

Recently three different methods to fix polysaccharide derivatives on chromatographic supports have been patented. Two of them referred to the photochemical insolubilization of a polysaccharide derivative bearing [40] or not [41] photopolymerizable functional groups. The third of them [42] consisted of the thermal treatment (120°C) of a macroporous γ-aminopropylsilanized silica gel coated with a triscarbamate of cellulose in the presence of a high amount of AIBN. Even if no indication about the fixation mechanism is given by the authors, the insolubilization of the polysaccharide derivative may be the result of a cross-linking reaction.

Some other methods to bond polysaccharides onto chromatographic matrices exist [43,44]. However, only applications for the resulting CSPs have been published.

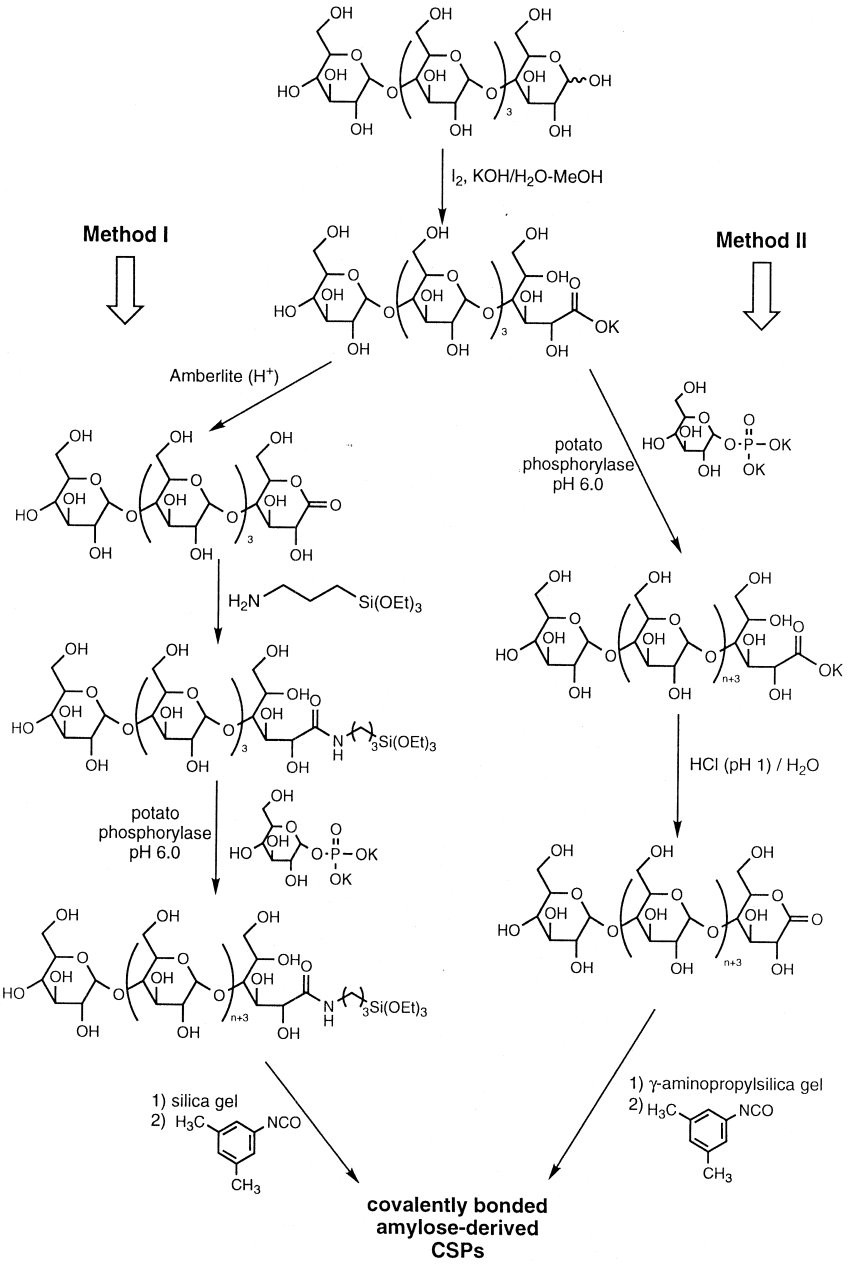

Fig. 11. Preparation of amylose-derived CSPs in which the chiral selector is fixed to the matrix by the reducing terminal residue [38].

7. Conclusions

All the presented methods have in common the ability to render polysaccharide-derived CSPs stable against solvents. This feature allows a broader choice of solvents in the mobile phase, which represents an advantage either for analytical or preparative purposes. Thus, the application domain of a CSP can be

broadened by the possibility to use different chromatographic conditions, due to changes in selectivity. Moreover, derivatives highly soluble when coated can be also used as chiral selectors, and therefore, the choice of polysaccharide derivatives to be used in CSPs is also extended.

At preparative scale, not only the above mentioned advantages are applicable. The use of certain solvents can have a strong effect on the loadability of certain enantiomeric mixtures and, therefore, on the cost of the separations. In this case, the mobile phase can be chosen in order to have the most advantageous separation.

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