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Chiral packing materials for high-performance liquid chromatographic resolution of enantiomers based on substituted branched polysaccharides coated on silica gel

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ABSTRACT

The preparation of amylopectin-based chiral stationary phases coated on an achiral support, according to a process similar to that reported for substituted cellulose or amylose carbamate derivatives, is reported. The influence of the different synthesis parameters on chiral discrimination is discussed.

INTRODUCTION

Resolution of enantiomers by liquid chromatography on chiral stationary phases (CSPs) has become a practically useful method for obtaining optical isomers and determining their purities [1]. Several different types of chiral stationary phases have been developed and a wide range of applications have been published during the last decade [2].

Among the numerous chiral stationary phases that have been investigated, polysaccharide-based phases have been identified as versatile and useful chiral sorbents for the separation of enantiomers [3]. Numerous applications have been reported on microcrystalline cellulose triacetate used in the pure polymer form [2,3]. A variety of cellulose and amylose derivatives have been introduced for the same purpose by Okamoto and co-workers as a coating of polymer on large-pore silica gel [2,3]. Most of these coated silica materials are commercially available and they show different selectivities depending on the type of polysaccharide and on the derivatizing groups on the polysaccharide. More recently, benzoylcellulose beads in the pure cellulosic form have been shown to have remarkable chiral recognition abilities [4,5].

All these polysaccharide CSPs are produced from linear polysaccharides. No work has been published on branched polysaccharides except the use of pure starch to resolve atropoisomers containing polar substituents [6–10] and soluble starch derivatized with 3,5-dimethylphenyl isocyanate and supported on silica gel, which exhibited similar but slightly lower recognition compared with amylose tris(3,5-dimethylphenylcarbamate) [11]. However, starch is in reality a mixture of polysaccharides containing, for example, 20% amylose and 80% amylopectin in the case of maize starch. Of these two polysaccharides, amylose CSPs are well known [11–17],

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but amylopectin CSPs have not been described and studied.

We report here the synthesis of and chromatographic studies on chiral discrimination of CSPs based on substituted amylopectin triphenylcarbamates.

EXPERIMENTAL

Chemicals and solvents

Amylopectins were commercially available under the trade name Glucidex, from Roquette (Lestrem, France). Silica gels were obtained from Macherey–Nagel (Strasbourg, France). The physical characteristics of the different silica gels (data from manufacturer) are reported in Table I.

Triaminosilane, N-aminoethyl-3-aminopropyltrimethoxysilane and 3-aminopropyl trimethoxysilane were obtained from Hüls (Paris, France). Isocyanates and racemates were purchased from Aldrich Chemicals (Strasbourg, France) and HPLC solvents from CIL (Sainte-Foy la Grande, France).

Apparatus

Separations were carried out on an HPLC system composed of a Philips Model 4015 pump, a Philips Model 4025 multi-wavelength UV detector (Philips Science et Industrie, Bobigny, France) and a Kipp & Zonen BD 40 recorder (Enraf-Nonius, Gagny, France). The columns (250 × 4.6 mm I.D.) were packed by Silichrom (Pessac, France). The prepared bonded materials were sent for carbon content analysis to the Service Central d'Analyses du CNRS (Vernaison, France).

TABLE I

PHYSICAL CHARACTERISTICS OF SILICA GEL

Silica gel	Pore size (Å)	Specific surface area (m^2/g)	Pore volume (ml/g)	Particle size (μm)
N-100-5	100	350	1.0	5
N-300-5	300	100	0.8	5
N-1000-5	1000	25	0.8	5
N-4000-5	4000	10	0.7	5

Chromatographic conditions

Chromatography was performed using the same mobile phase composition [hexane–2-propanol (90:10, v/v)] at a constant flow-rate of 0.5 ml/min. The dead time of the columns was determined by injection of 1,3,5-tri-*tert.*-butylbenzene used as non-retained compound. Typically, 10 μl of a 1% solution of racemate dissolved in hexane were injected.

In order to investigate the influence of the different parameters on the recognition ability, five racemates having the structures presented in Fig. 1 were chromatographed: *trans*-stilbene oxide (SO), Tröger base (TB), benzoin (Bz), benzoin methyl ether (BME) and flavanone (Fla).

Preparation of the chiral phases

General procedure for the silanization of silica gel. A 10-g amount of silica gel, dried under vacuum (0.1 Torr) (1 Torr = 133.322 Pa) at 180 °C, is made to react with the silane in 80 ml of xylene. The mixture is heated under reflux for 24 h and the product is filtered off and washed successively with xylene (50 ml), tetrahydrofuran (THF) (100 ml), THF–water (50:50) (50 ml), water (100 ml), THF (200 ml) and hexane (50 ml). The bonded material is dried at 80 °C under vacuum (0.1 Torr).

General procedure for the preparation of substituted amylopectin triphenylcarbamates. A 5-g amount of amylopectin dissolved in freshly distilled pyridine (50 ml) is dried by a Dean and Stark procedure. After removing water, 0.28 mol of isocyanate is added dropwise and refluxed at

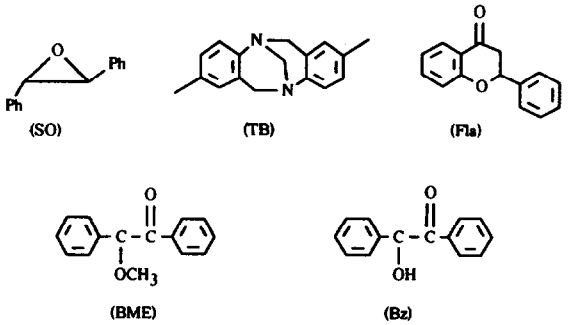

Fig. 1. Structures of test compounds.

110 °C for 24 h. After cooling, 30 ml of methanol are added and the resulting solution is poured into 400 ml of methanol with stirring. The precipitate is filtered and washed twice with methanol. The product is dissolved in methylene chloride and precipitated with 400 ml of methanol. This process is repeated three times, at room temperature, to give a brown solid.

IR (KBr): ν(NH) 3400, 3300, 1530 cm^{-1}; ν(CO) 1740 cm^{-1}; ν(C–O–C) 1030–1100 cm^{-1} (no adsorption at 3470 cm^{-1}, hydroxyl groups of polysaccharides). Elemental analyses were satisfactory.

General procedure for the coating. A 3.2-g amount of bonded silica gel is added to 0.8 g of amylopectin carbamate dissolved in dioxane (30 ml). The mixture is stirred for 15 min, then the solvent is removed with a rotary evaporator.

RESULTS AND DISCUSSION

Description of amylopectin

As for the cellulose CSPs, the polysaccharides cannot be used as they are because their molecular mass is too high for them to be soluble. Hence it is necessary to cut them into lower molecular mass soluble moieties [18]. The same applies to amylopectin but fortunately degraded amylopectin is readily available under the trade name Glucidex, used as a sugar food. Glucidex is a mixture of "nutritive saccharides" obtained by a sparing hydrolysis of starch maize, followed by purification and spray drying.

Starch maize is a mixture containing 78% amylopectin and 22% amylose [19]. Amylose is composed of about 250–300 glucose units linked by 1,4-α-disaccharide bridges [20]. The amylopectin chain composition is similar to that of amylose, but the molecular structure is more complex. Amylopectin is a branched structure constituted of about 1000 glucose units. Several hundred side-chains, having 20–25 glucose units each, are linked to the major chain by 1,6-α-disaccharide bridges (Fig. 2) [21].

During hydrolysis (acidic or enzymic) of starch, amylose is degraded to maltose and then to glucose [22,23] and amylopectin is slightly degraded giving a lower molecular mass ramified polysaccharide [21]. When the degradation of

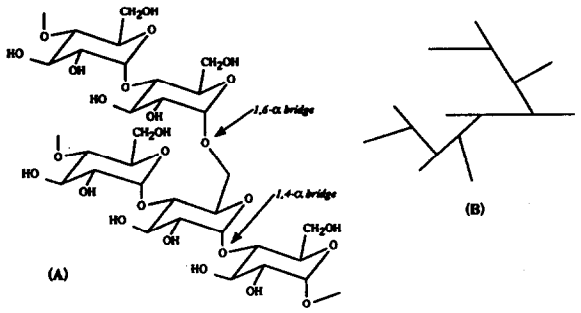

Fig. 2. (A) Structure of amylopectin; (B) simplified structure of amylopectin.

starch is substantial, the breakdown products are named dextrins.

The final product (Glucidex) used here is a mixture of glucose (1%), maltose (2%) and higher polysaccharides (97%) having a polydispersity of 8500, a mass-average molecular mass of 12 800 and a number-average molecular mass of 1500 [24].

Preparation of the chiral agent

The Glucidex is easily converted into carbamates by adding isocyanate derivatives under the usual conditions (Fig. 3). The crude carbamate is purified by a series of precipitations followed by solubilization to remove glucose, maltose and oligosaccharide carbamates, giving a purer amylopectin carbamate with a narrow molecular distribution, measured by gel permeation chromatography. For example, amylopectin triphenylcarbamate has a polydispersity of 2550 and a mass-average molecular mass of 9000, indicating loss of material. Then amylopectin carbamate is coated on an amino-bonded silica, packed in a 250 × 4.6 mm I.D. stainless-steel

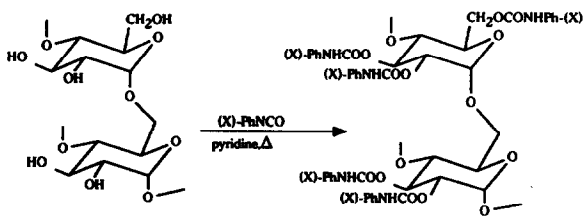

Fig. 3. Preparation of amylopectin carbamates.

column by a slurry method [18] and used as chiral support.

To have a large range of amylopectin CSPs, several substituents listed in Table II were introduced onto the phenyl group of derivatized amylopectin.

Before discussing the chiral behaviour of the different amylopectin CSPs, several achiral parameters such as the type of aminosilane, the pore size of the bonded aminosilica and the percentage of chiral agent coated have to be studied in order to establish their influence on chiral discrimination.

Influence of the aminosilane

Okamoto *et al.* [18] used γ-aminopropyltrimethoxysilane to deactivate silica gel because amino groups have the property of forming hydrogen bonds with carbamate functions, stabilizing the coating. In this way, to have a more stable coating, it would be of interest to increase the hydrogen bonds by using a di- or a triaminosilane for silanization. However, it is not certain that all amino groups form hydrogen bonds and as a consequence they can have undesirable effects on the elution.

Therefore, in order to establish the best aminosilane, silica gel (N-1000-5) was derivatized with 3-aminopropyltrimethoxysilane (1N), N - aminoethyl - 3 - aminopropyltrimethoxysilane (2N) and triaminosilane (3N) and coated with amylopectin triphenylcarbamate (PhAMY) as chiral agent using the procedure described Experimental. Their influences on chiral recognition are reported Fig. 4.

In general, the capacity factors are slightly better with monoaminosilane derivatization and

TABLE II

LIST OF DIFFERENT AMYLOPECTIN CSPs

Phase	Substituent
pMeOPhAMY	4-OCH_3
pMePhAMY	4-CH_3
3,5MePhAMY	3,5-$(CH_3)_2$
PhAMY	H
pFPhAMY	4-F
3,4ClPhAMY	3,4-Cl_2

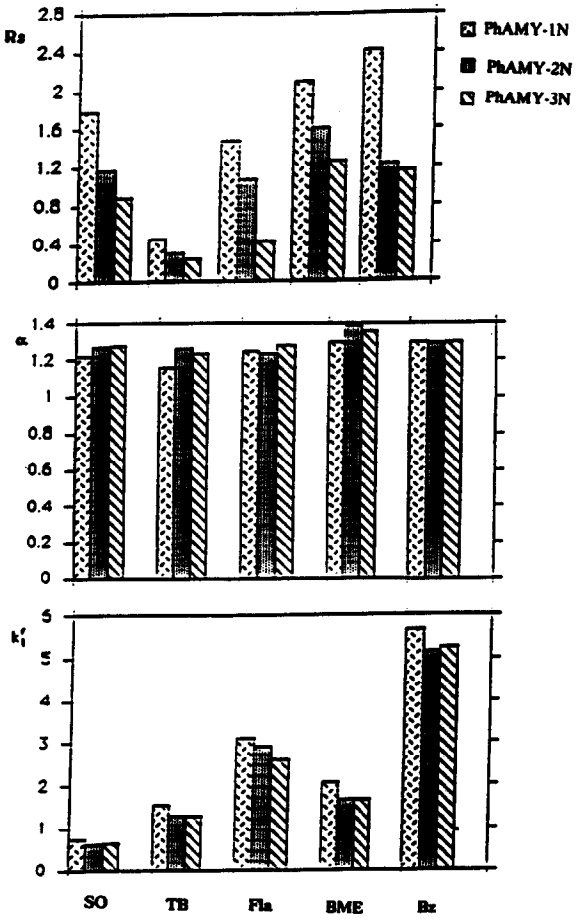

Fig. 4. Influence of the nature of aminosilane on the chiral discrimination.

the α values are almost the same for the three amino-bonded silicas, showing the weak influence of the type of amino chain on the discrimination power. However, for all racemates, the resolution is higher with monoamino-bonded silica gel and the decrease in chiral resolution is comparatively greater with increase in the number of amino groups and the longer the arm. Therefore, silica gel was subsequently derivatized with γ-aminopropyltrimethoxysilane.

Influence of the silica gel pore size

To prepare cellulose phases, Okamoto *et al.* [18] used large-pore silica gel with a diameter ≥1000 Å because celluloses have a molecular mass of about 32 000. As amylopectins have a lower molecular mass (about 10 000), the use of

large-pore silica gel is not obvious. Hence, to define the exact type of silica gel, PhAMY CSPs were made with four silica gels having pore sizes of 100, 300, 1000 and 4000 Å. The studies under the usual conditions are reported in Fig. 5.

All the CSPs show a chiral recognition power, but silica gel of 100 Å pore size has a poor ability. It seems to be difficult for the macromolecules of substituted amylopectin to penetrate the pores, giving a heterogeneous coating of the surface, closing more or less the pores. Considering the changes in enantioselectivities, resolutions and the specific surface areas, it appears that a 300 Å pore-size silica gel is the best compromise.

Percentage of chiral agent coated

The chiral ability of the CSP depends on the thickness of the coating. Usually, the greater the amount of chiral agent, the better is the chiral discrimination. For the coated cellulose CSP, Okamoto and co-workers [18,25,26] chose a rate of coating of about 20–25% by mass.

To have an exact view of the amount of chiral agent, several PhAMY CSPs coated with different percentages on a 300 Å pore-size aminosilica gel were studied (Figs. 6 and 7).

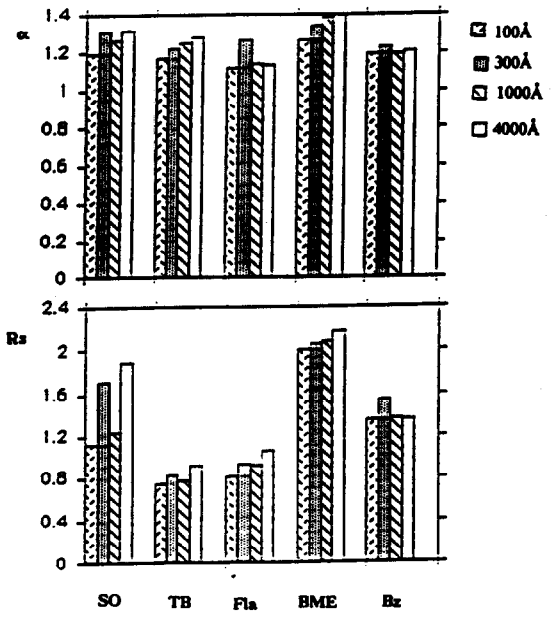

Fig. 5. Influence of silica gel pore size on the chiral discrimination.

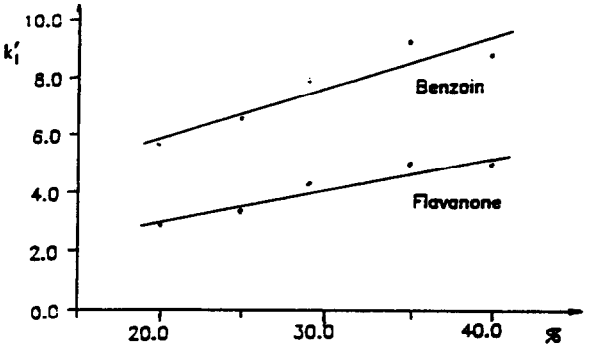

Fig. 6. Influence of percentage of PhAMY coated on the capacity factor.

As the percentage of PhAMY coated on silica gel increases, the capacity factors increase (Fig. 6) and do not affect the enantioselectivity (Fig. 7). This is a normal phenomenon in chromatography. However, the resolution increases rapidly with a coating rate between 20 and 25%, beyond which the effect is small. It appears that resolution increases when the capacity factors increase, but for values higher than 25% the efficiency of the column decreases because the substituted polysaccharides close the pores. Hence a coating of 25% by mass appears to be the best average.

Therefore, in subsequent work, a 25% coating of substituted amylopectin on 300 Å silica gel derivatized with 3-aminopropyltrimethoxysilane was used.

Influence of the substituent present on phenyl group

As for the other polysaccharide CSPs, the chiral recognition ability depends largely on the substituent attached to the phenyl moiety [11–17,25,26]. In order to investigate the influence on chiral discrimination, the racemates used above were chromatographed on the substituted amylopectin phenylcarbamates listed in Table II. (No discrimination ability was observed with benzolamylopectin like that for benzoylpullulan [27]. Amylopectin 4–nitrophenylcarbamate and 4-chlorophenylcarbamate were also synthesized, but they are soluble in hexane, as mobile phase,

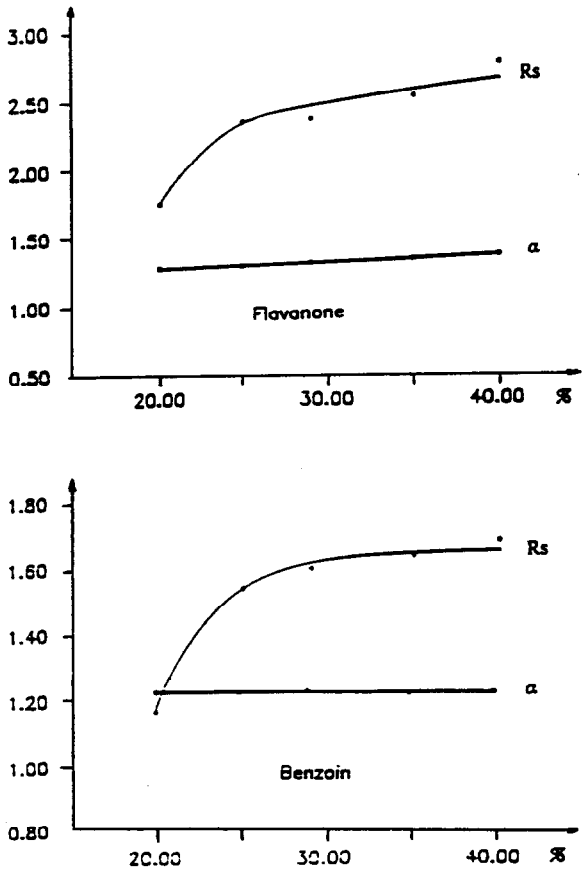

Fig. 7. Influence of the percentage of PhAMY coated on enantioselectivity.

containing 2% of 2-propanol.) The results are summarized in Table III.

With polysaccharide carbamate derivatives, the polar interaction centre of the CSP is the urethane group. The inductive effect of substituents on the phenyl group of the urethane moiety depends on the nature, position and number of substituents. Hence a chromatographic system with a non-polar eluent, hydrogen bond and dipole–dipole interactions between the urethane moiety of amylopectin triphenylcarbamate and the polar groups of racemic compounds are considered to be the dominant forces for chiral recognition. Both CO and NH of the carbamate moiety can interact with the solute through a hydrogen bond.

The introduction of an electron-withdrawing substituent such as a halogen increases the acidity of the NH proton [26]. On the one hand, the increase in the acidity of the NH proton should enhance the capability for hydrogen bond formation of this proton with an electron-donating group such as carbonyl. On the other hand, an electron-donating group such as an alkyl increases the electron density of the carbonyl group, strengthening the hydrogen bond. Hence the introduction of an alkyl or halogen group is expected to improve the chiral recognition ability of cellulose or amylose triphenylcarbamates [11–17,26].

TABLE III

CAPACITY FACTORS OF THE LESS STRONGLY RETAINED ENANTIOMER AND SELECTIVITIES

CSP	Parameter	Racemate				
		SO	TB	Fla	BME	Bz
pMeOPhAMY	k'_1	0.72	1.60	2.82	1.95	5.87
	α	1.18	1.00	1.08	1.18	1.19
pMePhAMY	k'_1	0.57	1.36	2.10	1.43	4.29
	α	1.30	1.00	1.18	1.47	1.19
3,5MePhAMY	k'_1	0.67	1.00	1.82	4.64	1.33
	α	1.46	1.38	1.33	1.18	2.47
PhAMY	k'_1	0.72	1.44	2.84	1.94	5.68
	α	1.31	1.22	1.27	1.34	1.23
pFPhAMY	k'_1	0.62	1.64	2.68	2.08	5.70
	α	1.13	1.14	1.22	1.11	1.00
3,4ClPhAMY	k'_1	0.61	2.70	3.50	3.20	7.11
	α	1.00	1.00	1.27	1.00	1.00

This mechanism, well established for cellulose and amylose CSP, is not completely valid for amylopectin triphenylcarbamates. For example, the 3,4ClPhAMY CSP shows no discriminating power whereas the similar cellulose type exhibits a high chiral recognition [26] and MeOPhAMY CSP gives separations of the racemates (except Tröger base) whereas the cellulose type does not improve chiral recognition [17]. The best separations are observed when the substituents are weak electron donors (PhAMY and 3,5MePhAMY), giving similar results to those obtained on the cellulose and amylose CSPs [11–17,26]. The ramified structure of amylopectin is probably responsible for the difference in behaviour, by increasing the inclusion phenomenon and changing the environment of the glucose unit. Examples of separations of racemic com-

pounds on 3,5MePhAMY CSP are given in Fig. 8.

Comparison of the chiral recognition abilities of cellulose, amylose and amylopectin CSPs

Capacity factors of the less strongly retained enantiomers and selectivities for four racemates (SO, TB, Fla and Bz) on cellulose, amylose and amylopectin CSPs are listed in Table IV. The data for cellulose and amylose CSPs were obtained from the literature [11, 26]. For racemates of *trans*-stilbene oxide and Tröger base, amylose and cellulose CSPs appear to have a better chiral recognition ability. For flavanone and benzoin, it is less evident. In general, AMY CSPs exhibit a better discriminating power than cellulose CSPs (Fla and Bz) and amylose CSPs (Bz). This ability is particularly true when the substituents of the phenyl group have a donor effect.

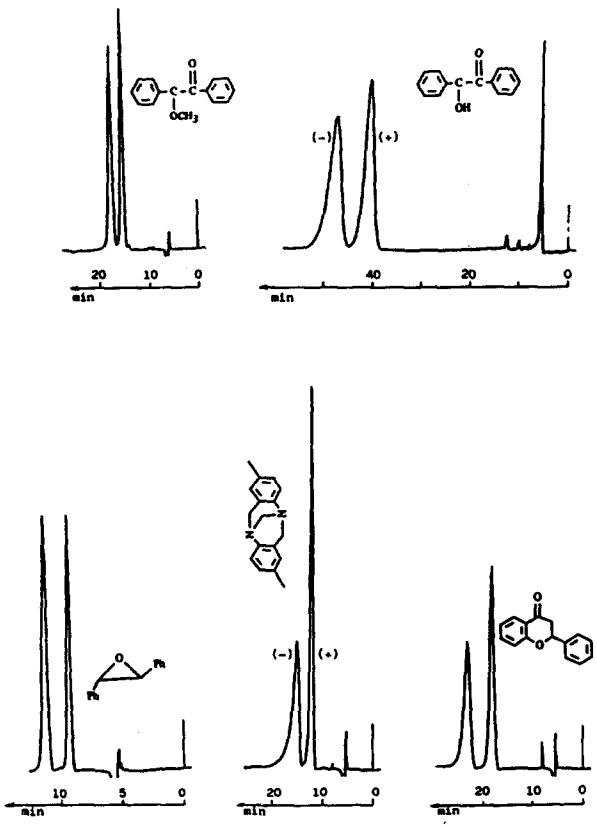

Fig. 8. Examples of separation of racemic compounds on 3,5MePhAMY CSP. Mobile phase, hexane–2-propanol (95:5); flow-rate, 0.5 ml/min.

In fact, each polysaccharide CSP has its own chiral properties; the discriminating power depends largely on the structure of the polysaccharide but also on the structure of the racemates.

Study of the separation mode

The cellulose CSP can be used in normal and reversed modes [28]. Effectively, the carbamate group gives polar interactions (hydrogen bonds and dipole–dipole interactions) and the phenyl moiety hydrophobic (apolar) and $\pi-\pi$ interactions. Hence, in the reversed-phase chromatographic mode hydrophobic interactions occurred and in the normal mode polar or $\pi-\pi$ interactions were responsible for the separations.

To examine the effect of the chromatographic mode on chiral discrimination, 3,5MePhAMY CSP was studied in normal and reversed modes. The results are given in Table V.

In the normal mode, racemates were eluted in the order Bz > Fla > BME > TB > SO, showing the relationship between enantiomer structures and polar strength intensities. *trans*-Stilbene oxide enantiomers are the least retained owing to the deficiency of polar interaction centres and benzoin enantiomers are the most retained because they have two polar groups (OH and C = O), able to form strong hydrogen bonds. However, the enantioselectivity values increase in the opposite order (Bz < BME < Fla < TB < SO), showing that a long retention time does not lead to the best chiral separation. The solute affinities for the CSP do not contribute to the chiral discrimination.

With the reversed mode, the polar interactions are destroyed and replaced by hydrophobic interactions, giving the order SO > Fla > TB > Bz > BME for capacity factors and Bz < BME < SO < TB < Fla for enantioselectivities. The AMY CSP can be used in both modes, but its behaviour is different.

CONCLUSIONS

Preparations of substituted amylopectin CSPs coated at 25% by mass on an achiral support of 300 Å pore size and derivatized with 3-aminopropyltrimethoxysilane were described. Chiral

TABLE IV

COMPARISON OF RECOGNITION ABILITIES OF CELLULOSE (Cel), AMYLOSE (Am) AND AMYLOPECTIN (AMY) CSPs

CSP		Racemate							
		SO		TB		Fla		Bz	
		k'_1	α	k'_1	α	k'_1	α	k'_1	α
p-MeO	Cel	0.56	1.34	1.09	1.01	2.23	1.01	0.48	1.01
	AMY	0.72	1.18	1.60	1.00	2.82	1.08	5.87	1.19
p-Me	Cel	0.51	1.55	0.75	1.48	1.57	1.16	3.00	1.12
	AMY	0.57	1.30	1.36	1.00	2.10	1.18	4.29	1.19
3,5-Me	Cel	0.74	1.68	0.97	1.32	1.47	1.41	2.43	1.58
	Am	0.42	3.04	0.53	1.58	0.93	1.12	3.14	1.21
	AMY	0.67	1.46	1.00	1.38	1.82	1.33	1.33	2.47
H	Cel	0.67	1.46	1.12	1.37	2.22	1.10	5.28	1.01
	Am	0.39	1.46	0.77	1.28	2.21	1.51	3.72	1.00
	AMY	0.72	1.31	1.44	1.22	2.84	1.27	5.68	1.23
p-F	Cel	0.52	1.38	1.00	1.14	1.89	1.13	4.26	1.14
	AMY	0.62	1.13	1.64	1.14	2.68	1.22	5.70	1.00
3,4-Cl	Cel	0.38	1.93	0.79	1.47	1.29	1.04	3.24	1.10
	AMY	0.61	1.00	2.70	1.00	3.50	1.27	7.11	1.00

TABLE V

EFFECT OF SEPARATION MODE WITH 3,5MePhAMY CSP

Mobile phase	Parameter	SO	TB	Fla	BME	BZ
Hexane–2-propanol (90:10), 1 ml/min	k'_1	0.67	1.00	1.82	1.24	4.64
	α	1.46	1.38	1.33	1.23	1.18
Ethanol–water (75:25), 1 ml/min	k'_1	1.44	0.57	1.34	0.19	0.30
	α	1.17	1.40	1.46	1.11	1.00

recognition of amylopectin triphenylcarbamate derivatives depended greatly on the type, position and number of substituents on the phenyl groups. Racemic compounds considered in this work are resolved with 3,5-dimethylphenylcarbamate; however, 3,4-dichlorophenylcarbamate has a very low chiral recognition whereas 4-methoxyphenylcarbamate exhibits a real discriminating power. The branched structure of amylopectin induces different mechanisms to those for cellulose or amylose, requiring further studies to explain the retention mechanisms.

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