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HPLC of Structural Isomers with Cyclodextrin–Poly(vinylamine)-Coated Silica Columns, Part I: Synthesis and Characterization of Cyclodextrin-Bonded Stationary Phases by NMR Solid State

G. Crini and **M. Morcellet***
Laboratoire de Chimie Macromoléculaire, U.R.A. 351 CNRS, Université des Sciences et Technologies de Lille,
59655 Villeneuve d'Ascq Cedex, France

G. Torri
Istituto Scientifico di Chimica e Biochimica "G. Ronzoni", Via G. Colombo, 81, 20133 Milano, Italy

Abstract

Different supports for high-performance liquid chromatography are prepared by using the coating technology. These supports are based on silica beads coated with a β-cyclodextrin-containing poly(vinylamine). Surface area, pore size distribution, and solid-state nuclear magnetic resonance are used for the characterization of these supports. The two different methods of preparation used lead to different arrangements of the polymer layers; hence, the supports have different mobility, rigidity, and accessibility of the cyclodextrin ring.

Introduction

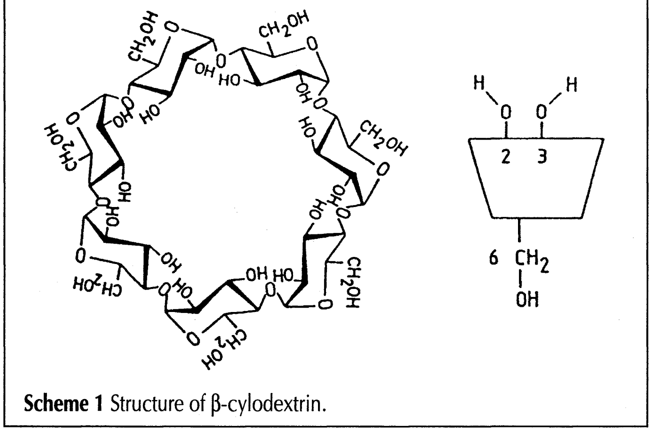
β-Cyclodextrins (β-CDs) are torus-shaped cyclic oligosaccharides made up of seven α-1,4-linked D-glycopyranose units (Scheme 1). The inside of the cavity is hydrophobic. These molecules have the ability to form specific inclusion complexes with organic compounds, especially aromatics. The stability of the complex depends on the size and polarity of the host molecule and its shape (1,2). The selective inclusion properties of β-CD have been used as an advantage in many separation techniques, including classical liquid chromatography (3–5). This property can be applied in two ways in high-performance liquid chromatography (HPLC). The first approach is to use β-CD as a selective component of the mobile phase in reversed-phase systems (6–8); the second approach is to use chemically bonded β-CD silica stationary phases (9–12). These two procedures have been used to separate structural isomers (e.g., phenolic compounds, aromatic acids).

More recently, another general method ("coating technology") has been used; it consists of coating and cross-linking a polymer onto the surface of silica beads. Such stationary phases can then be modified by grafting appropriate ligands (13,14).

We chose to work with poly(vinylamine) (PVA) as the polymer; it is part of the poly(alkylamine) family. Over the last 15 years, poly(alkylamines) have been the subject of much industrial and academic interest. This interest has focused mainly on the high reactivity of the amino groups that they possess. These amino functions allow the formation, by appropriate chemical reaction, of a wide range of extremely varied novel polymers that may be used in such areas as catalysis (15), metal chelation (16), liquid chromatography (17,18), or pollution cleanup (19,20).

Among the most well-known and studied polyamines are poly(allylamine) (PAA) and the linear and branched chains of poly(ethyleneimine) (PEI-l and PEI-b, respectively), all of which are commercially available. In comparison to these two polyamines, PVA has been the object of relatively few studies in the literature to our knowledge. This is undoubtedly because of the fact that it is not commercially available.

PVA is a linear, hydrosoluble polyelectrolyte of simple structure. It has two main properties: PVA, like other poly(alkylamines), is strongly adsorbed on silica and it has a reactive amine groups that allow relatively easy functionalization. PVA was functionalized by β-CD. This grafted polymer, which has never been used for HPLC to our knowledge, then served as the



Scheme 1 Structure of β-cylodextrin.

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* Author to whom correspondence should be addressed.

base for the preparation of supports for HPLC.

The aim of this work was to produce and characterize new supports for HPLC. We have chosen to modify silica by the adsorption of PVA by using the method known as coating. Thus, we have prepared several chemically bonded β-CD stationary phases that are based on silica beads coated with PVA. We used two different methods of synthesis which differed in that the β-CD coupling step (with the monotosyl derivative of β-CD as the intermediate) was either performed before (type I support) or after (type II support) coating. These methods were used successfully to immobilize β-CD on porous organic polymers that are employed in the treatment of water (20).

Experimental

Reagents and chemicals

β-CD, which was supplied by Janssen Chimica (Beerse, Belgium), was recrystallized from water and dried overnight in a vacuum at 110°C. Lichrospher Si-100 (particle diameter, 10 µm), was obtained from Merck (Nogent-sur-Marne, France). The surface area determined by the Brunauer-Emmet-Teller (BET) method (21) was 345 m^2/g. The pore size distribution is shown in Figure 1A (dv_p/dr_p is the differential of the porous volume, with the pore radius value plotted versus the pore radius, r_p). The average pore diameter of 100 Å has been measured. The concentration of silanol groups on the surface (0.25 SiO^-/nm^2 at pH 7) was determined by using the method of Frère and Gramain (22).

Poly(vinylamine) (PVA), which has an average molecular weight of 36,600 as determined with viscosimetric measurements, was synthesized from polymerization and acid hydrolysis of *N*-vinyl-*tert*-butylcarbamate according to the method described by Hart (23). Other compounds were of the highest quality available and were purchased from various suppliers. These reagents were used without further purification.

Preparation of β-CD-bonded phases

We used two methods to prepare the supports. In the first method, the β-CD was coupled to the amine groups of the polymer, and then the grafted polymer was

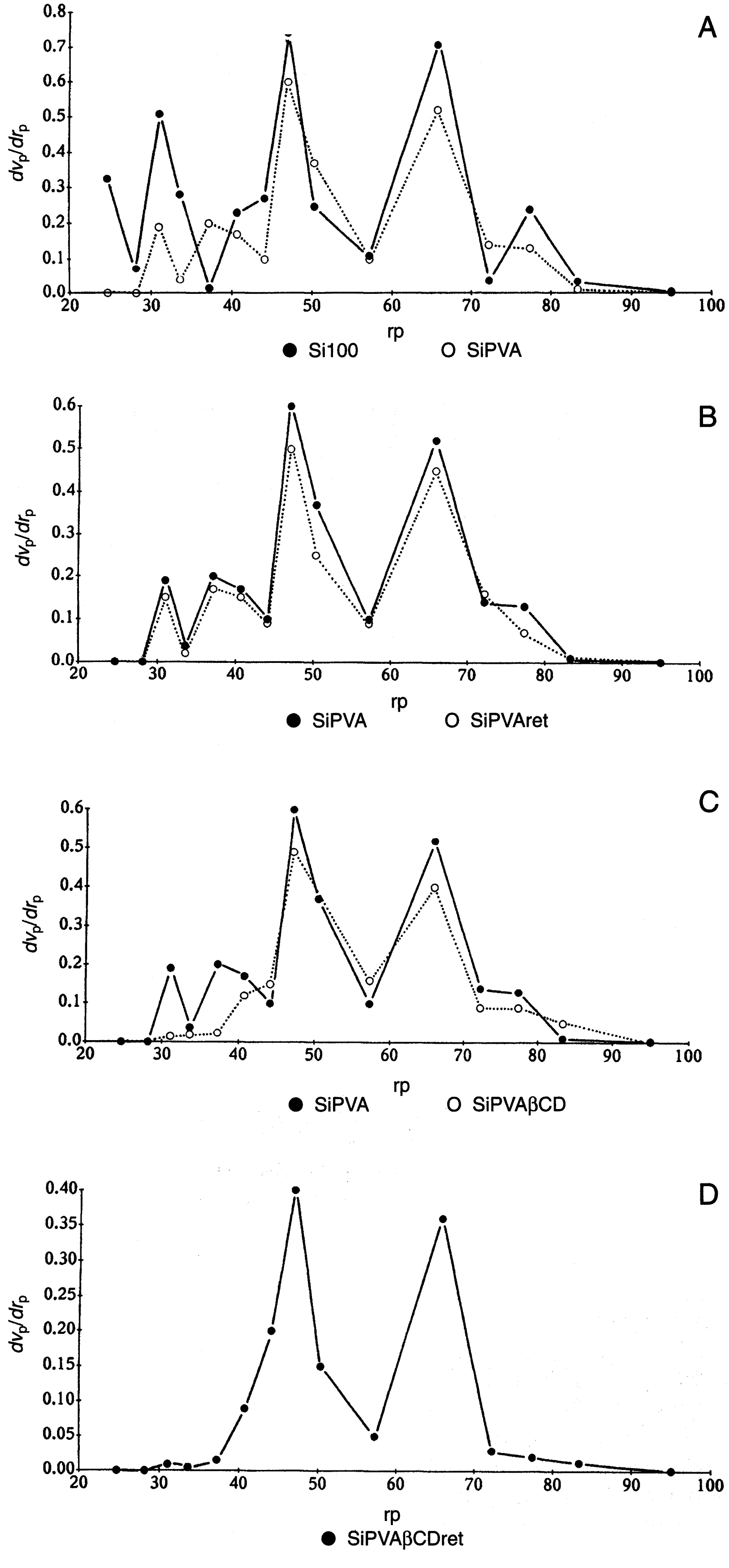

Figure 1. Pore size distribution obtained by nitrogen adsorption for the following supports: A, Si100 and SiPVA; B, SiPVA and SiPVAret; C, SiPVA and SiPVAβCD; and D, SiPVAβCDret. *ret* indicates cross-linking (reticulation).

Table I. Experimental Conditions and Results of the Coupling of β-CDOTS to PVA

							Degree of substitution[†]		
Reference*	Molar ratio PVA to CDOTS	PVA (g)	CDOTS (g)	Reaction time (h)	T (°C)	H_2O (mL)	SP	EA	P
PVAβCD7	7	2	4.6	24	60	100	0.8	0.9	1
PVAβCD5	5	2	6.4	24	60	50	1	1	1.5
PVAβCD4	4	1.5	6.09	30	60	75	2.4	2.2	2.3
PVAβCD3	3	1.5	8.1	24	70	75	3.6	3.1	3.8
PVAβCD2	2	1	8.1	48	70	100	5	4.6	5.9

* The polymers are referred to as PVAβCDx, which means that the polymer was obtained by grafting poly(vinylamine) (PVA) with β-CD using *x* as the molar ratio (PVA to CDOTs).

[†] Degree of substitution obtained in the following way: SP, spectrophotometric method; EA, elemental analysis; and P, polarimetry.

coated and cross-linked onto the surface of the silica beads. In the second method, the polymer was coated, cross-linked, and functionalized with β-CD. We introduced this last method to obtain stationary phases with a high concentration of β-CD and maximum accessibility of the β-CD cavity. These two methods used the same intermediate, the monotosyl derivative of β-CD, 6-O-(*p*-tosyl)-β-cyclodextrin (β-CDOTS).

Synthesis of 6-O-(*p*-tosyl)-β-cyclodextrin

The monotosyl derivative of β-CD, β-CDOTS, was prepared by reaction between one primary hydroxyl group of β-CD in the six position and tosyl chloride

Table II. Characterization of Systems Obtained by Method I*

Name*	Polymer	Nitrogen (%)	Polymer deposit[†] (mg/g)	β-CD[‡] (μmol/g)	Surface area	Coating solvent	Crosslinking agent	Crosslinking solvent
SiPVAβCD7	PVAβCD7	3.42	109	13	300	H_2O		
SiPVAβCD7ret	PVAβCD7	2.32	99	7	305		Epichlorohydrin	MeOH
SiPVAβCD24	PVAβCD5	2.90	94	26	295	MeOH		
SiPVAβCD24ret	PVAβCD5	3.51	121	24	245		BUDGE	Ether
SiPVAβCD15	PVAβCD4	2.46	86	20	286	H_2O		
SiPVAβCD15ret	PVAβCD4	2.64	92	15	266		Epichlorohydrin	Dioxane
SiPVAβCD36	PVAβCD3	2.96	82	38	284	H_2O		
SiPVAβCD36ret	PVAβCD3	2.70	72	36	258		Epichlorohydrin	Dioxane
SiPVAβCD53	PVAβCD2	1.63	71	56	269	H_2O		
SiPVAβCD53ret	PVAβCD2	1.53	64	53	227		Epichlorohydrin	Dioxane

* SiPVAβCD36ret refers to a support obtained by coating silica with PVA and grafting with β-CD; the resulting system has 36 μmole of β-CD per gram of support. *ret* indicates that cross-linking (reticulation) was carried out as the last step.

[†] Amount of polymer (milligrams) per gram of support.

[‡] Amount of β-CD (micromoles) per gram of support.

Table III. Characterization of Systems Obtained by Method II

						Grafting conditions			
Name*	Nitrogen (%)	Polymer deposit[†] (mg/g)	β-CD[‡] (μmol/g)	Surface area	Cross-linking agent	Solvent	Temperature (°C)	Time (h)	CDOTS[§]
SiPVA	2.32	99		305		H_2O			
SiPVA	3.82	139		269		MeOH			
SiPVAret	4.36	147		245	Epichlorohydrin	Dioxane			
SiPVAret	4.02	132		270	BUDGE	Dioxane			
SiPVAretβCD12	4.82	158	12	242	Epichlorohydrin	H_2O	60	24	1
SiPVAretβCD23	6.22	204	23	231	Epichlorohydrin	MeOH	50	20	1.5
SiPVAretβCD36	4.26	142	36	260	BUDGE	MeOH	50	20	2
SiPVAretβCD46	5.34	175	46	247	Epichlorohydrin	MeOH/DMAC	60	24	1.5
SiPVAretβCD67	5.67	186	67	249	Epichlorohydrin	MeOH/DMAC	60	30	3
SiPVAretβCD95	6.54	242	95	227	Epichlorohydrin	MeOH/DMAC	65	48	5

* SiPVAretβCD36, for example, refers to a support obtained by first coating silica with PVA. *ret* indicates that cross-linking (reticulation) was carried out as the second step. In a third step, the support was functionalized with β-CD. The resulting system has 36 μmol of βCD per gram of support.

[†] Amount of polymer (milligrams) per gram of support.

[‡] Amount of β-CD (micromoles) per gram of support.

[§] In grams per gram of SiPVAret.

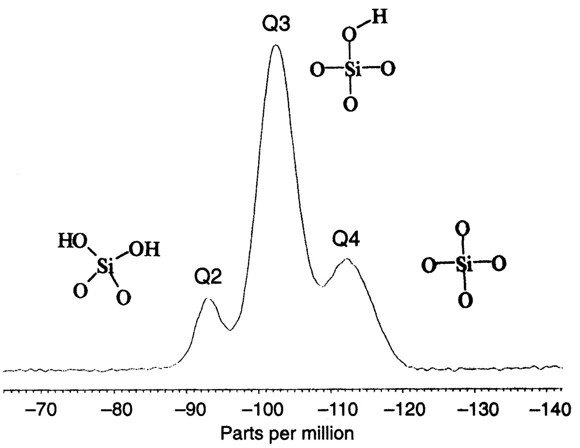

Figure 2. CP MAS ^{29}Si NMR spectrum of Lichrospher Si100 (30,000 scans).

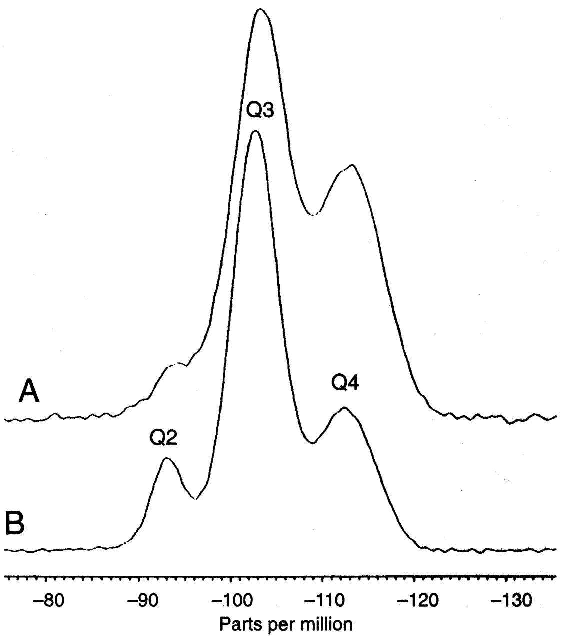

Figure 3. CP MAS ^{29}Si NMR spectra of SiPVA (A) and Si100 (B) (30,000 scans).

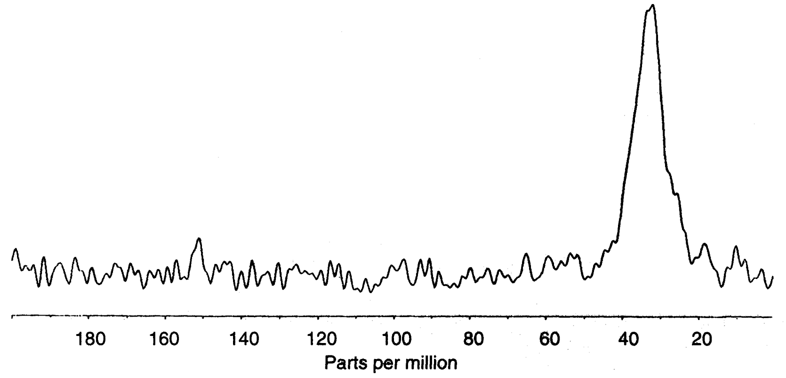

Figure 4. CP MAS ^{13}C NMR spectrum of SiPVA (4021 scans).

according to the method of Seo and co-workers (15). The crude product was purified by repeated recrystallization from water, and its purity was checked by proton nuclear magnetic resonance and mass spectrometry.

Preparation of a β-CD-containing poly(vinylamine)

A mixture of 4.6 g of β-CDOTS and 2 g of PVA, HCl, in 100 mL of water (adjusted to pH 11 with 1M potassium hydroxide solution) was heated for 24 h at 60°C. After cooling, the solution was dialyzed with water and the PVA–β-CD polymer was collected by freeze-drying (Table I).

The polymers are referred to as PVAβCDx, which means that the polymer was obtained by grafting poly(vinylamine) (PVA) with β-CD by using *x* as the molar ratio of PVA to CDOTs.

Coating of silica with PVA or PVA–β-CD polymers

A 10% solution of polymer (PVA or PVAβCD) in water (or methanol) was added to 1.5 g of Lichrospher Si-100. The solution was put under vacuum (10 mm Hg) for 30 s to remove air from the pores of silica. The solution was then stirred with a vortex mixer for 24 h at room temperature. The coated silica was filtered on a 0.45-µm pore diameter regenerated cellulose membrane. The SiPVA or SiPVAβCD support was extensively washed with water, methanol, and acetone, and then it was dried under vacuum at 60°C for 20 h. Characterization of the systems obtained by both methods is shown in Tables II and III.

β-CD coupling step

SiPVAretβCD46 (Table III) can be used to show the typical grafting reaction. Three grams of β-CDOTS was dissolved in 45 mL of methanol–dimethylacetamide (2:1, v/v). Two grams of SiPVAret support was added to this suspension (*ret* indicates cross-linking), and the mixture was kept at 60°C for 24 h. After cooling, the modified silica was filtered, washed with methanol, water, and acetone, and then dried under vacuum at 60°C for 20 h. Relative amounts of β-CDOTS, reaction time, temperature, and solvent mixture (Tables I and II) were varied to obtain different degrees of substitution of the amino groups by β-CDOTS.

Cross-linking

The SiPVA (or SiPVAβCD) support was transferred to a large test tube and covered with a 5% (w/v) solution of epichlorohydrin (or butan-1,4-diol diglycidyl ether) in dioxane. The mixture was degassed in vacuum (10 mm Hg for 10 min) and left overnight at room temperature. The solution was then heated with a steam bath (80°C) for 40 min with vortex mixing every 10 min. After successive washes with dioxane, acetone, water, and acetone again, the SiPVAret (or SiPVAβCDret) support was dried under vacuum at 60°C for 20 h.

Characterization of β-CD-bonded silica gel

First, the amount of polyamine deposited per gram of silica support was obtained from the results of elemental analysis of nitrogen. The best method for the determination of the amount of β-CD in the support was the determination of reducing sugars with tetrazolium blue by using glucose for the calibration curve (17,19,24). Fifty milligrams of the support was refluxed in 30 mL of 0.5M H_2SO_4 at 100°C for 12 h. The solution was neutralized with 3M NaOH up to pH 7, filtered, and diluted to 100 mL. The glucose calibration curve was constructed over the range 0–50 µg/mL. Measurements were made at 690 nm. Calculations took into account the fact that only six glucose molecules are obtained from one β-CD residue in the hydrolysis process since the N–C bond between the polymer and β-CD is not hydrolyzed.

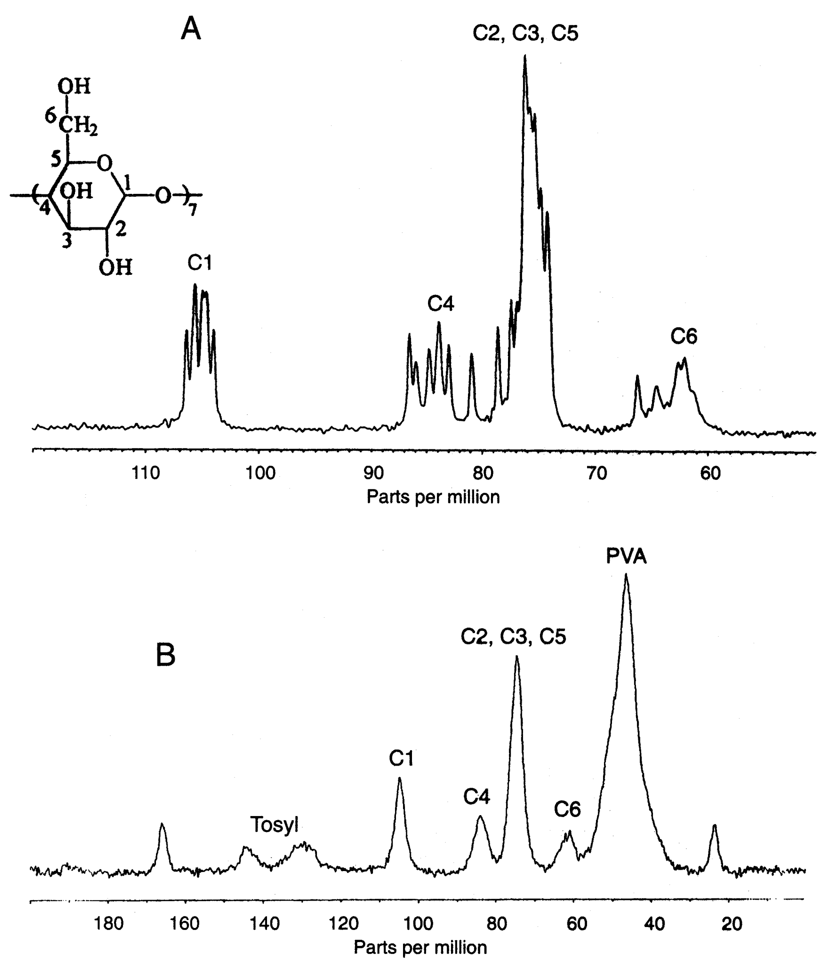

Figure 5. CP MAS ^{13}C NMR spectra of β-CD (128 scans) (A), SiPVAβCD7 (8758 scans) (B).

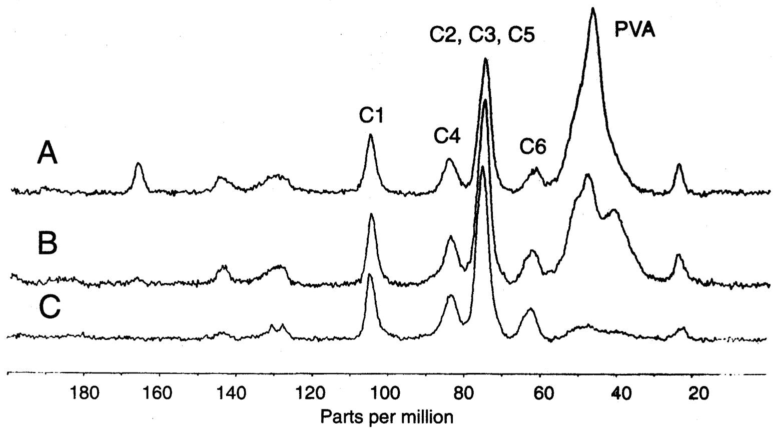

Figure 6. Influence of the amount of β-CD on the CP MAS ^{13}C NMR spectrum of SiPVAβCD supports: A, SiPVAβCD7; B, SiPVAβCD36; and C, SiPVAβCD53 (8758 scans).

We used surface areas and pore size distribution measurements for the characterization of silica beads; these data were obtained from the results of nitrogen desorption Brunauer-Emmett-Teller (21).

Cross polarization and magic-angle spinning solid-state nuclear magnetic resonance measurements were used for the characterization of the different supports. A Bruker CXP-300 spectrometer that was operated at 75.47 MHz for ^{13}C was used. The following conditions were applied: repetition time, 4 s; acquisition time, 30 ms; ^{1}H 90° pulse length, 4.5 µs; contact time, 1 ms; temperature, 303 K; and spin rate, 4 kHz. The compounds were placed in a zirconium rotor that was 7 mm in diameter and 18 mm high. The ^{13}C NMR signals were measured relative to benzene and those of ^{29}Si were measured relative to Q8M8 (trimethylsilyl ester of double four-ring silicate) as secondary standards. The chemical shifts were recorded with respect to tetramethylsilane.

Results and Discussion

Polymer content

The amount of PVA coated on silica gel (SiPVA) ranged from 100 to 140 mg/g depending on the experimental conditions (first two entries in Table III, method 2). This amount was less than that for PEI (170–180 mg/g) (17). This is related to differences in the extent of interaction between the primary or secondary amino groups of the polymers and the silanol groups at the surface of silica, as assessed by calorimetric experiments in progress in our laboratory.

The quantity of functionalized polymer adsorbed on silica (Table II, method 1) ranged from 70 to 110 mg/g and was less than in the previous case. In addition, when the amount of β-CD increased, the amount of polymer (A value) coated on silica decreased. This decrease, as well as the differences in the polymer deposit between the two methods, can be explained in terms of the conformation of PVA and

PVAβCD in the coating solvent (mainly water). A study conducted in our laboratory has shown that the grafting of β-CD on PVA causes the conformation of the chains to change. From the decrease of the viscosity and potentiometric measurements, it has been shown that the β-CD causes the polymer to fold back on itself by formation of hydrogen bonds between the OH groups of β-CD and the amino groups of PVA (25). Thus the amino groups are less available for an interaction with the surface silanol groups, they are responsible for the coating, and the amount of adsorbed polymer decreases. On the other hand, the interaction of silica surface and PVAβCD probably changes the conformation of the latter. As a matter of fact, the OH groups of β-CD and those of silanol compete for interaction with the amino group. The amount of adsorbed polymer results from the balance between these effects.

β-CD content

β-CD concentrations up to 4–5 mole % were obtained for the linear soluble PVA polymer before coating by changing the experimental conditions (Table I); 2–3 mole % was obtained for PEI (18). This is explained by the better accessibility of the primary amino group in PVA. The second method, which consisted of grafting β-CD after coating and cross-linking, allows stationary phases with higher capacity in β-CD to be obtained (from 10–100 μmol/g versus 10–50 μmol/g for the first method, Tables II and III). This is related to the differences in the strength of interactions of PVA and PVAβCD with silica as noted before. In addition, PVAβCD tended to desorb from silica during the cross-linking step, which is consistent with the above explanation and further reduces the β-CD concentration expressed in micromoles per gram of support.

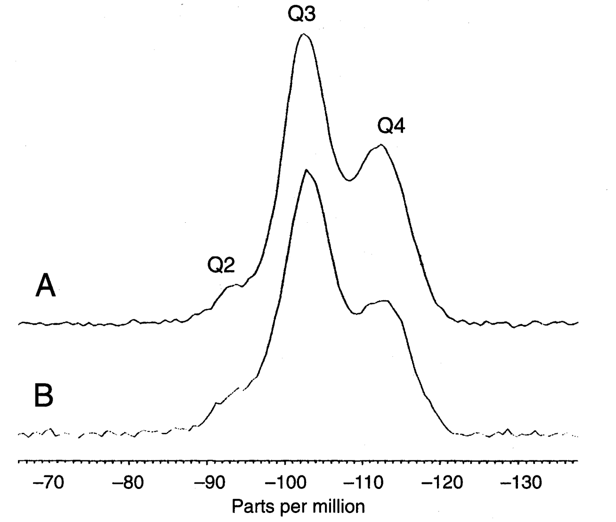

Figure 7. CP MAS ^{29}Si NMR spectra of SiPVA (A) and SiPVAret (B) (30,000 scans).

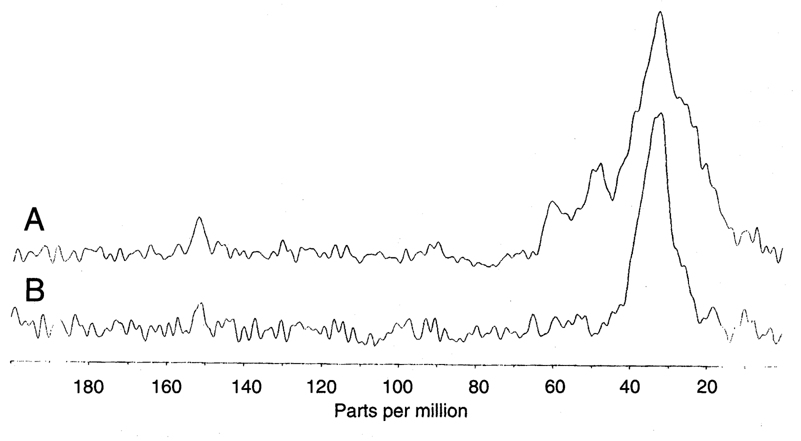

Figure 8. CP MAS ^{13}C NMR spectra of SiPVAret (6734 scans) (A) and SiPVA (4021 scans) (B).

Pore size distributions

Porous silica supports are usually characterized by their specific surface area and pore size distribution. The native Si100 silica exhibits a four peak distribution around 32, 47, 67 and 79 Å (Figure 1A). Figure 1A shows that the coating process affected mainly the small pores with a radius lower than 45 Å and, to a lesser extent, those higher than 70 Å. The cross-linking step has very little influence on the pore size distribution (Figure 1B). When the coatings by PVA and PVAβCD were compared, it appeared clearly that in the last case pores with radius lower than 40 Å have disappeared (Figure 1C). At the same time, the surface area is strongly decreased compared with native silica (Tables II and III). This is likely due to the sealing of the smallest pores mainly by parts of polymer chain bearing β-CD. After functionalization and cross-linking, only two peaks were found around 48 Å and 65 Å, regardless of the method of preparation (Figure 1D).

NMR analysis

Silicon-29 and Carbon-13 solid-state nuclear magnetic resonance techniques have already been used for the characterization of chemically modified silica gels (26–29), which are employed in HPLC. Different information can be obtained with the help of NMR spectroscopy. For example, this technique allows the differentiation between free silanol groups and geminal silanol groups (26,28), which is not possible by other techniques (e.g., infrared spectroscopy).

The silica surface chemical species can be separated into the following groups (28): $Si(O_{0.5})_4$, the siloxane groups Q^4 which form the bulk of the bead; $Si(O_{0.5})_3OH$, the single hydroxyl silanol groups Q^3; and $Si(O_{0.5})_2(OH)_2$, the geminal hydroxyl silanol groups Q^2. The CP MAS ^{29}Si NMR spectrum of the silica gel that was used shows three signals which reflect the presence of Q^2, Q^3 and Q^4 units (Figure 2). The chemical shifts of the three signals are respectively about −93, −103 and −113 ppm (Figure 2).

Coating step

Figure 3A shows the CP MAS ^{29}Si NMR spectrum of SiPVA support. This spectrum shows that the adsorbed polymer modifies the surface properties of the silica gel. By considering the signal-to-noise ratio of two spectra recorded strictly under the same conditions, it is clear that all three bands are weaker. Indeed Q^2 has practically disappeared. From this, we are led to the conclusion that surface silanols greatly participate in the coating process by interaction with the amino groups of the polymer chain. Maciel (26) and Legrand (28,29) have already shown that it is the surface silanols that take part in all modifications of silica gels.

Figure 4 shows the CP MAS ^{13}C NMR spectrum of a SiPVA support. We note the presence of a large band centered around 38 ppm and attribute it to both the CH and CH_2 carbons. The solid state ^{13}C NMR spectrum of PVA also gives only one large band centered about 48 ppm. Therefore we observe a shift toward higher fields (δ decreases) related to the increase of the rigidity of PVA when it is immobilized on the silica beads.

Figure 5 shows the CP MAS ^{13}C NMR spectra of β-CD and SiPVAβCD (5A and 5B respectively). The β-CD sample shows a high degree of crystallinity as revealed by the number of resonances due to C1, C4, and C6 atoms (Figure 5A). The assignment of the peaks for β-CD is in agreement with literature values (30,31). The spectrum of the SiPVAβCD (Figure 5B) shows the peaks of a disordered β-CD (broad signals) in the range 50–110 ppm and those, rather well defined, of the polymer chain between 20 and 50 ppm. As observed for PVA, all the peaks of SiPVAβCD are shifted toward high fields when compared to noncoated PVAβCD.

Figure 6 shows the solid state ^{13}C NMR spectra of the SiPVAβCD supports when varying the quantity of β-CD fixed on the PVA. The intensity of the alkyl chain band decreased as the amount of β-CD increased. This probably reflects a decrease of the mobility of the polymer chain at the surface. Additional hydrogen bonds with the OH groups of β-CD could be responsible for this effect.

Cross-linking step

The CP MAS ^{29}Si NMR spectrum of SiPVAret support shows the effect of the cross-linking step (Figure 7). The Q^2 band practically disappeared and the Q^4 also moved, probably due to the deposition of the polymer on the surface. The CP MAS ^{13}C NMR spectrum of SiPVAret support in Figure 8 exhibits two extra peaks (50 and 60 ppm) as compared with that of SiPVA. These have been attributed to the groups brought by the reticulating agent.

Figure 9 shows the spectrum of a stationary phase obtained by method 1 (SiPVAβCDret). We can clearly distinguish the peaks due to the alkyl group of the polymer from those due to the CD. The peak at 59 ppm is assigned to the carbons of epichlorohydrin. After the cross-linking, the main change is observed at the level of the intensity ratios of the polymer signal. This effect may reasonably be explained as being due to a change in the mobility of the chain and the insertion of the cross-linking agent. Relaxation experiments are in progress to confirm this hypothesis.

There is also an important difference between the spectra of the stationary phases prepared by method 1 and those prepared by method 2. Figure 10 shows the spectra obtained from a type 2 support: the peaks due to the β-CD appear much less clearly. It appears that the β-CD, and therefore the polymer, do not have the same mobility at the surface of the bead.

It can therefore be concluded that the two methods of preparation lead to different arrangements of the polymer layers and hence supports which are different with respect to mobility and rigidity.

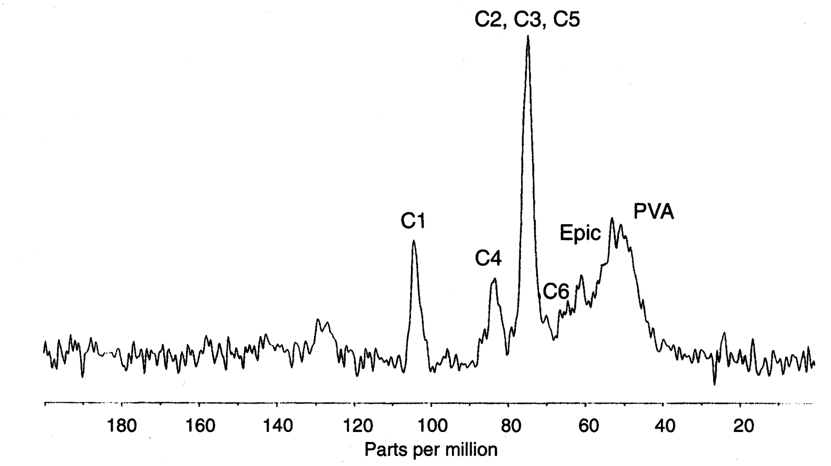

Figure 9. CP MAS ^{13}C NMR spectrum of SiPVAβCD53ret (8758 scans).

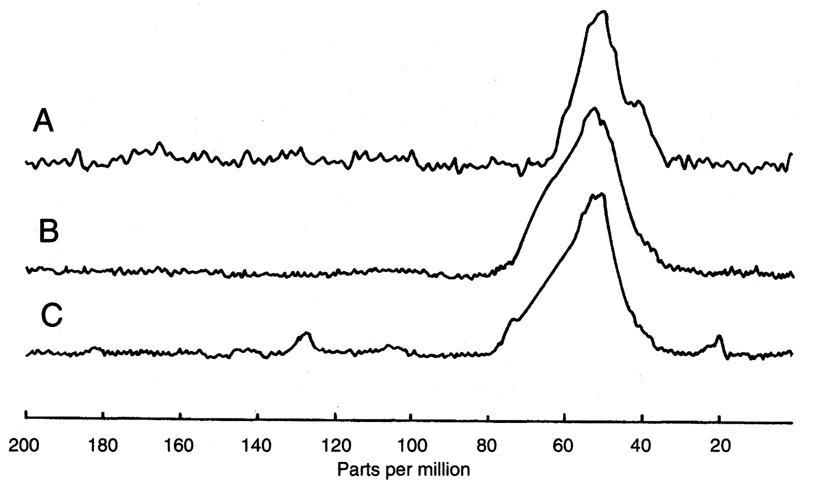

Figure 10. CP MAS ^{13}C NMR spectra of SiPVA (A), SiPVAret (B), and SiPVAretβCD67 (C) (9336 scans).

Conclusion

Two different methods have been used for the preparation of supports consisting of PVA coated on silica and functionalized by β-CD. These two methods lead to supports having close physical features (surface area, pore size distribution). Nevertheless, method 2 allows systems with larger amounts of coated polymer (which ensure a better masking of the silanol groups) and higher degrees of substitution by β-CD to be obtained. This should be better for chromatographic applications based on an inclusion process between substrate and β-CD. Nevertheless, NMR analyses show that the mobility and perhaps accessibility of β-CD are probably very different, with possible effects on chromatographic separations. It should also be pointed out that the cross-linking step in method 1 involves both the amino groups of PVA and all the hydroxyl groups of β-CD, whereas only amino groups participate in method 2. The chromatographic performances of the two types of supports will be compared in the second part of this work (32).

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