

Coating of Porous Silica Beads by *in Situ* Polymerization/Crosslinking of 2-Hydroxypropyl β -Cyclodextrin for Reversed-Phase High Performance Liquid Chromatography Applications

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ABSTRACT: Porous silica beads were coated with a crosslinked β -cyclodextrin polymer by *in situ* polymerization/crosslinking of 2-hydroxypropyl β -cyclodextrin with 1,6-hexamethylenediisocyanate in anhydrous dimethylsulfoxide. This method was developed for the preparation of reversed-phase high performance liquid chromatography stationary phases. The mass of polymer immobilized onto the silica surface was controlled by the amount of coupling agent, 1,6-hexamethylenediisocyanate, added during the coating process. The influence of the polymer coating on the physical features of the beads was investigated by means of nitrogen adsorption/desorption methods, scanning electron

microscopy and energy dispersion X-ray analysis. The column lifetime was found to be primarily dependent on the extent of crosslinking of the stationary phase. Moreover, it was demonstrated that the synthesis of highly crosslinked stationary phases with a reasonable column lifetime gave rise to a phase separation of the β -cyclodextrin polymer. To prove their usefulness as reversed-phase packing materials, they were used to separate mixtures of nitrophenol positional isomers, four pesticides, and drugs. © 2003 Wiley Periodicals, Inc. *J Appl Polym Sci* 91: 1419–1426, 2004

Key words: crosslinking; coatings; silicas; chromatography

INTRODUCTION

At present, the bonding of organic compounds onto inorganic support particles is the most common procedure for the preparation of reversed-phase high performance liquid chromatography (RP HPLC) packing materials. These hybrid organic-inorganic particles combine the advantageous properties of both inorganic support (excellent mechanical strength) and bonded organic compounds (high efficiency, reproducibility and selectivity). Chemically bonded stationary phases with silica,¹ titania² and zirconia³ supports have been widely developed to solve a broad range of different separation problems. Porous silica is certainly the most widely used because it is commercially available in a wide variety of shapes and sizes with different porosities and has well-defined surface silanol groups that can be easily modified by various organosilanes. However, investigation of these new packaging materials and optimization of their properties are of continuing interest to chromatographers. Thus, the immobilization of polymers onto oxide supports has been studied with the objective of improving

stability and minimizing undesirable interactions between the solute and the chromatographic support. There are two possibilities for the coating of silica with polymers. The first one, which is commonly used, consists of the physical adsorption of a pre-formed polymer. In this approach, the polymer is dissolved in a proper solvent and mixed with the support particles for awhile. Later, the support is recovered by filtration⁴ or evaporation⁵ of the solvent. The external synthesis of the polymer allows the use of operating conditions that may not be possible in the presence of support particles. Hence, polymers having well-defined structure, chemical composition and suitable molecular weight are expected to be prepared,⁶ since all of these parameters may influence coating efficiency.

In the second method, the formation of the macromolecular chains takes place directly on the surface of the chromatographic support via *in situ* polymerization of the monomer.^{7,8} To provide stable coatings, polymers should bear functional groups able to interact strongly with the silica surface by the formation of hydrogen bonds and van der Waals interactions with silica surface silanol and siloxane groups. Nevertheless, a radiation treatment⁹ or a crosslinking step¹⁰ generally further extends the coating stability. Over the past two decades, the progress in supramolecular chemistry has introduced a fascinating new field of

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liquid chromatography (LC) separation media. For example, stationary phases based on helical polymers,¹¹ cellulose derivatives,^{12,13} calixarene and resorcinarene,¹⁴ macrocyclic antibiotics¹⁵ and crown ether^{16,17} have been developed and commercialized for the direct separation of geometric and optical isomers. In these cases, the separation mechanism relies on the formation of inclusion complexes with the polymeric chains or the cyclic molecules. Among these original stationary phases, the ones which contain cyclodextrins (CDs) have proven to be a powerful tool for the separation of various compounds that cannot be resolved well on traditional reversed stationary phases.¹⁸

CDs are natural macrocyclic oligomers of glucose that contain six to twelve D-(+)-glucopyranose units (seven in the case of β -CD) which are bonded via α -(1,4) linkages.¹⁹ They have a toroidal or hollow truncated cone with all the glucose units in substantially undistorted C1 (D) (chair) conformations. The interior of the cavity, which contains two rings of C—H groups with a ring of glycosidic oxygen in between, is relatively hydrophobic, while the external faces with hydroxyl groups are hydrophilic. On account of this special feature, CDs can selectively allow some guest molecules into their hydrophobic cavity to form inclusion complexes with different stabilities.²⁰ With regard to the two coating procedures described previously, CD polymer coated stationary phases were prepared from either polymerizable CD derivatives²¹ and polymers containing CD units as a part of their macromolecular backbone^{22,23} or from those having pendant CD moieties.²⁴ A last, synthetic method of preparation may be distinguished, which depends upon the grafting of a monosubstituted CD derivative onto silica coated with functional polymers such as poly(vinylimidazole),²⁵ poly(allylamine)²⁶ or poly(ethyleneimine).²⁷ It is obvious that differences in the synthetic approach result in stationary phases that differ in their chromatographic behavior. In the present work, a method for the preparation of crosslinked 2-hydroxypropyl β -cyclodextrin (HP β -CD) polymer coated stationary phases is presented. The method is based on the *in situ* polymerization/crosslinking of HP β -CD with 1,6-hexamethylenediisocyanate (HMDI). The detailed characterization of the resulting supports highlights the effect of varying the crosslinking density on several parameters, such as the amount of adsorbed polymer, the silica porosity, and the column lifetime.

EXPERIMENTAL

Materials

The chromatographic support used to prepare the stationary phases was spherical Lichrospher Si 100 silica (Merck, Strasbourg, France) having a mean particle diameter of 10 μ m, a pore diameter of 100 nm, a specific pore volume of 1.25 mL/g and a specific sur-

face area of 350 m²/g. HP β -CD was provided by Roquette (Lestrem, France) and is described as native β -CD substituted on the 2 position by 2-hydroxypropyl groups. The degree of substitution was 0.5, leading to an estimated molecular weight of 1340 g/mol. The coupling agent, HMDI, (98%) and the anhydrous dimethylsulfoxide (DMSO, 99.9%), kept over molecular sieves, were obtained from Sigma-Aldrich (Saint Quentin Fallavier, France). HMDI was kept at 4°C to avoid moisture contamination. The chromatographic test substances, *ortho*- and *para*-nitrophenol, pentachlorophenol, 2,4-dichlorophenoxyacetic acid (Sigma-Aldrich), triadimenol, metribuzin (Riedel de Haën, Seelze, Germany) *meta*-nitrophenol (Janssen, Beerse, Belgium) were analytical reagent-grade and not further purified. Barbitol, phenobarbitol, hexobarbitol, luminol and rutonal were the generous gift of Prof. D. Blondeau (Laboratoire d'Ingénierie Moléculaire, Lille, France) and were used as received. Water and methanol of HPLC-grade, obtained from Sigma-Aldrich, were filtered through a membrane filter with a 0.45 μ m pore size (Type HVLP, Millipore) and degassed by ultrasonic vibration prior to use.

Coating of porous silica beads by *in situ* polymerization/crosslinking

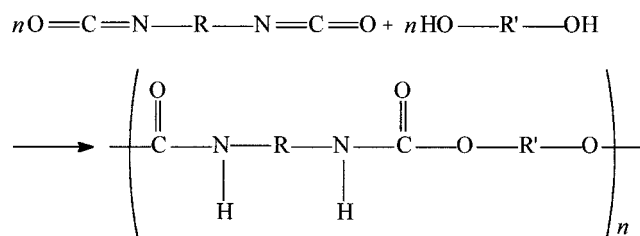
A series of coating experiments was carried out as follows. Into a 100 cm³ round-bottomed flask kept at 60°C with an oil bath, equipped with a condenser and a moisture-free nitrogen inlet, various amounts of HMDI were added dropwise within 15 min under stirring to 15 mL of anhydrous DMSO containing 3 g of HP β -CD and 1.5 g of Lichrospher Si 100 silica. Prior to use, HP β -CD and Lichrospher Si 100 were dried at 80°C and 110°C, respectively, under vacuum for 24 h. The mixture was then kept at 60°C and stirred for 16 h. The resulting modified silica was recovered by filtration, washed successively with DMSO (100 mL), water (200 mL) and methanol (200 mL) and then dried at 60°C under vacuum overnight.

Column packing

Columns (100 \times 4.6 mm I.D.) made from stainless steel tubing were downward packed using 10% (w/v) slurries of each stationary phase in carbon tetrachloride. A packing pressure of 200 bars (Touzard & Matignon packer model) was used, with dichloromethane as the propulsion solvent. Columns were conditioned for 1 h with methanol at 0.5 mL/min prior to use.

Analytical procedures

Thermogravimetric analysis (TGA) was carried out in air at temperatures ranging from 25°C to 800°C at a scan rate of 10°C/min, using a Shimadzu TGA-51



Scheme 1

analyzer to determine the amount of polymer adsorbed onto the silica surface. The latter value was calculated after correction for water from the difference between the TG loss of the coated silica and the initial weight determined. The specific surface area (S_{BET}) was obtained by nitrogen adsorption in the dry state following the Brunauer, Emmett, and Teller²⁸ (BET) method. The pore size distribution and the specific pore volume were obtained by nitrogen desorption measurements following the Barret, Joyner, and Halenda²⁹ (BJH) method. These analyses were carried out with a Sorptomatic 1990 apparatus. The surface properties of the coated materials were studied by energy dispersion X-ray analysis (EDXA) with a Cambridge Instrument model 600 as well as scanning electron microscopy (SEM) with a Hitachi S-4500 SEM apparatus, using an accelerating voltage ranging from 3.0 to 5.0 kV. The silica supports were directly shadowed with a thin layer of platinum (2 nm) without surface treatment.

Chromatographic evaluations

Chromatography was performed with a modular HPLC system equipped with a Merck model L6200A Intelligent pump, a Rheodyne model 7125 injector (20 μL), a Merck model L4250 ultraviolet-visible variable-wavelength spectrophotometric detector and a Merck model D2500 chromatogram integrator. All separations were carried out at room temperature under isocratic conditions with flow rates ranging from 1.0 to 2.0 mL/min. Test mixtures were prepared by dissolving the sample in water-methanol (v/v) mixtures at a concentration of 100 $\mu\text{g}/\text{mL}$. Injections of 20 μL of

these test mixtures provided satisfactory chromatographic peaks with detection at 254 nm. A saturated solution of potassium nitrite was used to determine the void volume of the column. The extent of separation of two peaks is usually represented by the factor of resolution (R_S). However, this R_S does not accurately represent the extent of the separation for the poorly resolved peaks, because their width cannot be precisely measured. Various factors were introduced to account for the separation of two peaks.³⁰ In this study, the extent of separation between the peaks of analytes was represented by R_H .³¹

$$R_H = \frac{H - H'}{H} \quad (1)$$

where H and H' are the height of the first eluted peak and that of the valley between the two peaks, respectively. The greater the value of R_H , the better the resolution, and a R_H value of 1 represents the complete separation of the two peaks.

RESULTS AND DISCUSSION

Coating of porous silica beads by *in situ* polymerization/crosslinking

It is well known that the reaction between diol and diisocyanate bi-functional monomers leads to the formation of linear polymers through the formation of urethane linkages, as described below (Scheme 1).

The polymer chains are produced during a polymerization process called step-growth polymerization, meaning that oligomers created in the first stage can further react with starting materials and/or products resulting from previous reactions. When a multi-functional molecule such as HP β -CD (21 hydroxyl groups) and a bi-functional molecule such as HMDI are reacted together, crosslinking occurs at the same time as polymerization. Hence, the polymerization gradually leads to a three-dimensional macromolecular network. Table I summarizes the results of *in situ* polymerization/crosslinking of HP β -CD on Lichrospher Si 100 silica using various amounts of HMDI crosslinker. Relatively "soft" conditions were chosen:

TABLE I
Characteristics of Silica Coated by *in Situ* Polymerization/Crosslinking of 2-Hydroxypropyl β -Cyclodextrin

Support	HMDI/HP β -CD (wt/wt)	Coated polymer on silica (mg/g)	%N	Monomer content ($\mu\text{mol}/\text{g}$)	
				HP β -CD	HMDI
Si HMDI 0	0	0	0	0	0
Si HMDI 1	0.01	55 \pm 3	< 0.05	nd ^a	nd ^a
Si HMDI 2	0.03	32 \pm 2	< 0.05	nd ^a	nd ^a
Si HMDI 3	0.08	52 \pm 3	0.15	33	54
Si HMDI 4	0.09	66 \pm 4	0.22	39	79

^a Not determined

TABLE II
Porosity of Silica Coated by *in Situ*
Polymerization/Crosslinking of 2-Hydroxypropyl
 β -Cyclodextrin Obtained from Nitrogen
Adsorption/Desorption Method

Support	Coated polymer on silica (mg/g)	S_{BET} (m ² /g)	V_{BJH} (mL/g)
Si HMDI 0	0	344	1.09
Si HMDI 1	55.4	324	1.08
Si HMDI 2	32.4	326	1.00
Si HMDI 3	52.3	313	1.04
Si HMDI 4	66.1	302	1.01

a molar ratio of HP β -CD to HMDI greater than 1 to ensure an excess of CD hydroxyl groups in comparison with isocyanate functional groups, and the drop-wise addition of HMDI solution in DMSO to the reaction mixture to avoid the phase separation of HP β -CD polymer. Indeed, if stoichiometric amounts of isocyanate and hydroxyl functional groups were used during the polymerization reaction, the polymer may have become highly crosslinked, and non-soluble polymer aggregates of infinite molecular weight may have formed.

TGA

The TGA curves (not presented) of each silica sample exhibited two steps in the temperature range studied. In the case of non-coated Lichrospher Si 100, these two sharp mass losses correspond to the dehydration of silica due to the elimination of water molecules physically adsorbed onto the surface (around 100°C) and, at higher temperature (above 500°C), the condensation of neighboring silanol groups with the formation of siloxane linkages.³² In the case of coated samples, the thermal degradation of the polymer occurs from 500°C due to the breakage of the covalent bonds along the polymer chains and the consequent formation of gaseous product. Thus, the mass of polymer adsorbed onto the silica surface was estimated from this second weight loss value with the assumption that the dehydration rate of bare silica was similar to that of coated silica. The amount of adsorbed polymer increased with the initial concentration of HMDI solution added to the reacting medium and reached a threshold value around 65 mg/g (Table I). It is interesting to note the abnormally high (but reproducible) amount of polymer adsorbed onto silica when a weight ratio of HMDI to HP β -CD of 0.01 was used. One limitation on the coating efficiency may be the smaller size pores, as it is difficult for branched polymer structures to arrange themselves within such a tight space. As attested by data for Si HMDI 0 support, HP β -CD monomer was not adsorbed onto the silica

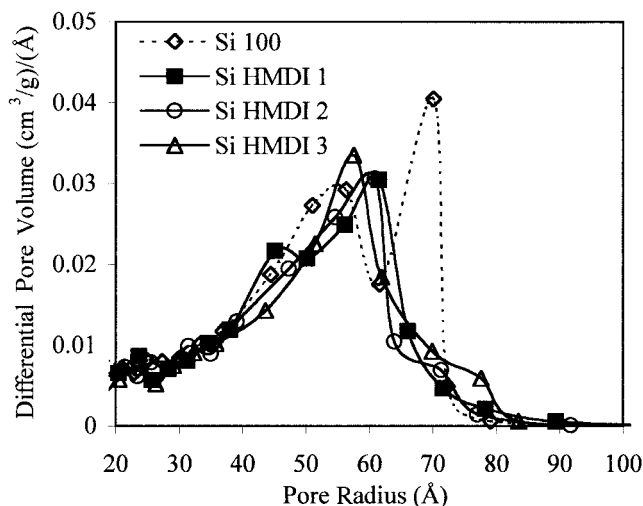


Figure 1 Pore size distribution curves of non-coated Li-chrospher Si 100 silica and Si HMDI coated silica obtained from the nitrogen desorption method.

surface. Therefore, a proposed mechanism for the *in situ* polymerization/crosslinking coating procedure involves the adsorption of oligomers previously formed in solution. The adsorption may occur as long as the polymer is soluble in the reacting solution. The adhesion of the polymer layer may be stabilized through the formation of multiple anchorages, including hydrogen bonds and van der Waals interactions between urethane linkages and/or free hydroxyl groups of the HP β -CD polymer with the surface silanol groups and siloxane linkages of silica. Table I also give the nitrogen content determined through elemental analysis and the monomer contents deduced in the same fashion.

Porosity analysis

BET measurements show a slight drop in the specific surface area from 344 m²/g for the non-coated Lichro-

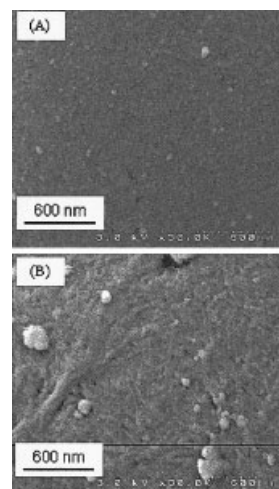


Figure 2 Micrographs of (a) Si HMDI 2 and (b) Si HMDI 3 coated silica at $\times 50,000$ magnification.

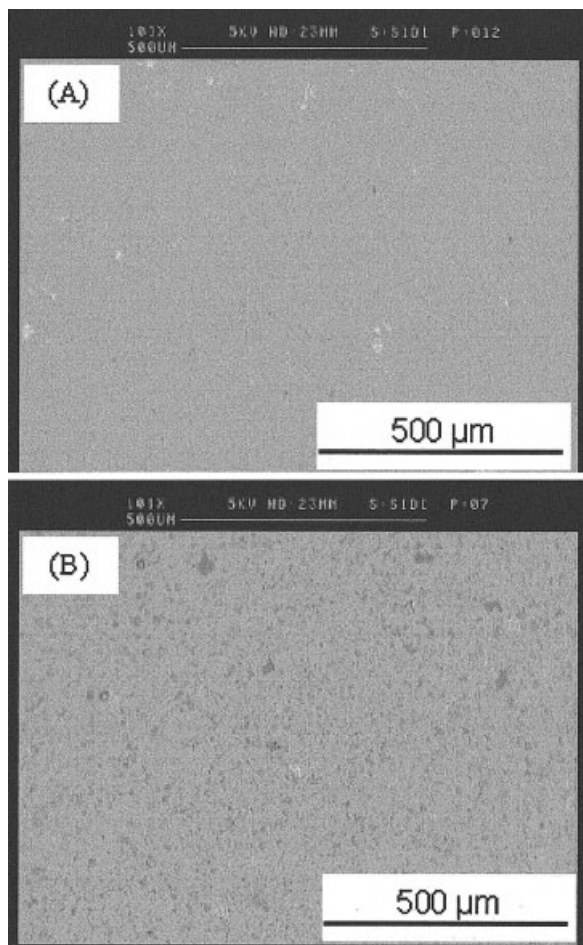


Figure 3 Micrographs of (a) Si HMDI 2 and (b) Si HMDI 3 coated silica.

spher Si 100 to 302 m²/g for the Si HMDI 4 coated silica with the highest degree of polymer coating (Table II). Similarly, the BJH specific pore volume was found to decrease slightly with coating, from 1.09 mL/g to 1.01 mL/g. Typical size distributions of non-

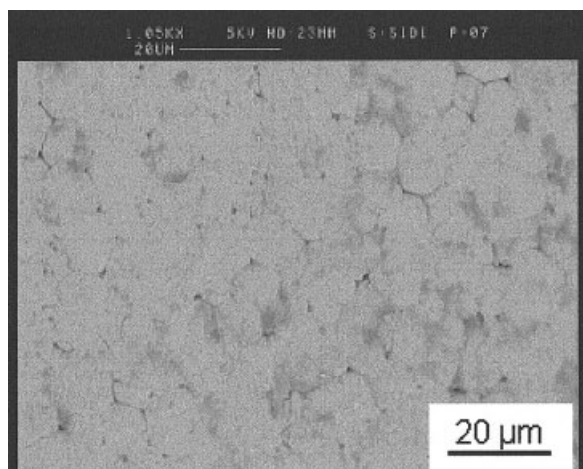


Figure 4 Micrograph of Si HMDI 3 coated silica.

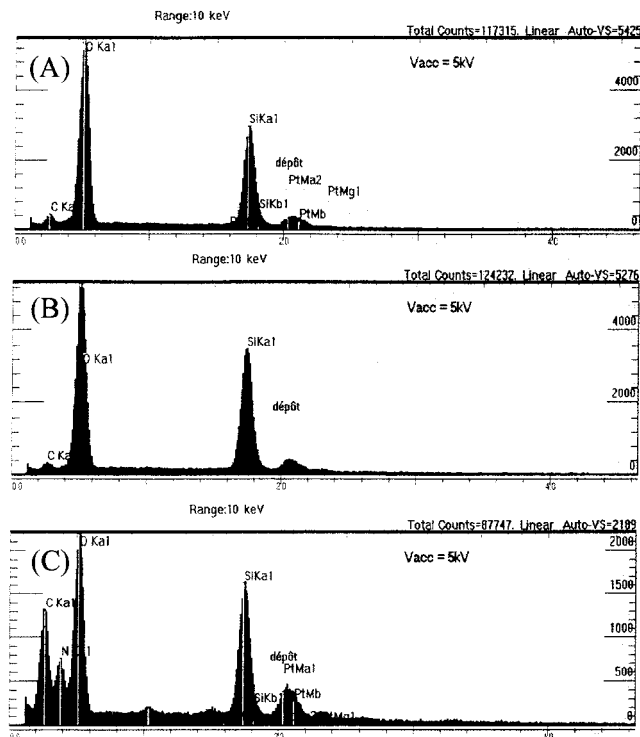


Figure 5 Energy dispersion X-ray spectra representative of (a) the sample as a whole, (b) a silica bead and (c) the polymeric aggregate shown in Figure 4.

coated and coated silica are shown in Figure 1. As a general rule, coated silica have a narrower size distribution in comparison with Lichrospher Si 100. The latter are characterized by a bimodal profile, with two distinct pore populations at about 55 and 70 nm. The distribution profiles obtained for Si HMDI 1 and Si HMDI 3 supports are rather dissimilar, despite their equivalent amounts of immobilized polymer. Si HMDI 1 exhibits the same bimodal distribution as Lichrospher Si 100, but the pore size is shifted to smaller radii (45 and 60 Å). On the other hand, in the case of Si HMDI 3 support, a monomodal distribution is observed, with a pore population centered on a 58 Å radius. This difference may be attributed to the macromolecular structure of the polymer, since the use of a small amount of coupling agent (HMDI) may lead to a branched structure of low molecular weight able to penetrate inside the two pore populations. The adsorption of the polymer chains onto the wall of the pores gives rise to a bimodal distribution with small pore radii (Si HMDI 1, Fig. 1). On the other hand, it is assumed that when using a higher proportion of HMDI, polymers have a globular structure and high molecular weight due to the high crosslink density. Consequently, only the largest pores are affected by the adsorption, resulting in a monomodal pore size distribution (Si HMDI 3, Fig. 1). Nevertheless, the ways in which the polymer molecules move and arrange themselves within the pores during the *in situ*

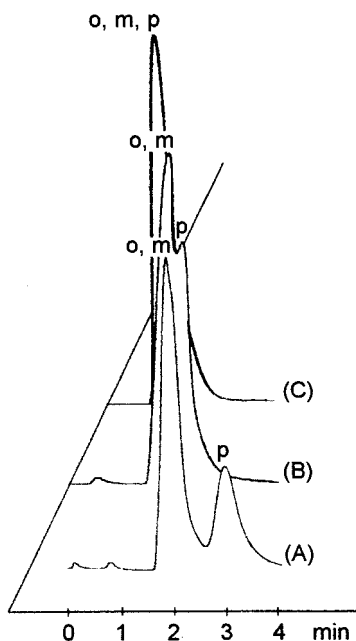


Figure 6 Chromatograms of (o) *ortho*, (m) *meta* and (p) *para* positional isomers of nitrophenol on the Si HMDI 1 packed column obtained within 2-3 h of operation obtained using a flow rate of 1 mL/min with water/methanol (90:10) as the mobile phase: (a) first injection, (b) after 2 hours and (c) after 3 hours.

polymerization/crosslinking coating process is not clearly established. Moreover, several parameters, such as the possible continuation of the polymerization of the polymer chains adsorbed onto silica, as well as the influence of this process occurring on the silica surface on the process in solution, are difficult to estimate.

Morphology of coated silica surfaces

The polymer coatings were characterized by SEM. Micrographs of Si HMDI 2 and Si HMDI 3 coated silica are shown in Figure 2(a,b), respectively. At the high magnification ($\times 50,000$), it is interesting to note that the morphologies observed are quite similar, since in both case the silica surface is homogeneously covered with a smooth layer of polymer that seems to be firmly adhered to the silica surface. Nevertheless, the polymer coating appears to be more condensed and thicker in the case of the Si HMDI 3 support. This is probably due to the higher crosslink density. The main requirement for the preparation of efficient stationary phases by the *in situ* polymerization/crosslinking method is to prevent, as much as possible, the formation of polymeric aggregates in solution. The images presented in Figure 3 indicate that, according to the weight ratio of HMDI to HP β -CD employed, inhomogeneous supports were produced. Indeed, in Figure 3(b), one can easily distinguish numerous black spots, corresponding to carbon-rich areas

that are insoluble polymeric aggregates. At higher magnification, it can be clearly seen that these polymeric particles of irregular shape and on the order of a few micrometers in size remain separated from the silica beads (Fig. 4). These findings were confirmed by EDXA by focusing the beam on different places of the sample shown in Figure 4. Thus, the spectra illustrated in Figure 5 are representative of the chemical composition of the surface of the sample as a whole [Fig. 5(a)], a silica bead [Fig. 5(b)] and a polymeric aggregate [Fig. 5(c)]. The presence of nitrogen, characteristic of HMDI, was established only in the latter case. This suggests that the polymer has a highly crosslinked structure.

Chromatographic evaluations

A preliminary evaluation of the chromatographic properties of the supports prepared above was made, using different sets of substrates. Despite the well-known favorable influence of a buffer added to the mobile phase, simple mixtures of water/methanol were chosen as the eluents in order to observe the undisturbed effects of solute/support interactions. The chromatograms depicted in Figure 6 were obtained from the Si HMDI 1 packed column with a water/methanol (90:10) mixture as the mobile phase. It seems clear that the mechanical stability of the polymeric coating of this support is rather low, as evidenced from a significant loss of resolving power of the stationary phase within 2-3 h [Fig. 6(a-c)]. This fast deterioration probably stems from the low amount of crosslinker used during the coating process. After use, the Si HMDI 1 coated phase was submitted again to TGA. The weight loss associated with the thermal degradation of the polymer layer was 23.1 mg/g, in contrast to the 55.4 mg/g prior to use, corresponding to the desorption of more than 40% of the initial mass of the adsorbed polymer during the chromatographic elution. This amount of

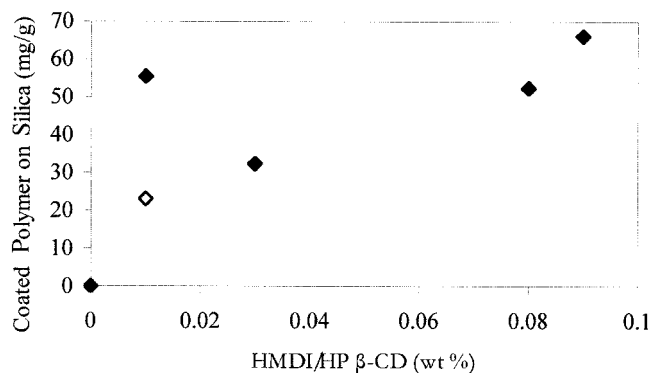


Figure 7 Influence of HMDI/HP β -CD weight ratio on the amount of polymer adsorbed onto silica obtained from TGA determined (filled diamond) prior to use and (open diamond) after use.

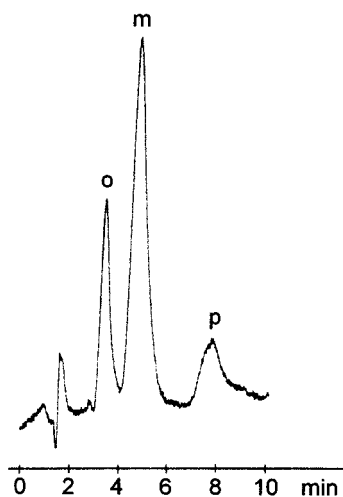


Figure 8 Separation of (o) *ortho*, (m) *meta* and (p) *para* positional isomers of nitrophenol using the Si HMDI 3 packed column obtained using a flow rate of 1 mL/min with water/methanol (85:15) as the mobile phase.

“firmly” adsorbed polymer is in line with the results obtained for the other coated phases (Fig. 7).

The Si HMDI 2 support demonstrated good stability but poor resolving power, since the three nitrophenol isomers were eluted within two peaks with water/methanol (98:2) as the eluent (chromatogram not presented). The *ortho* and *meta* isomers were eluted simultaneously, whereas the *para* isomer was retained longer. Figure 8 shows the separation of nitrophenol positional isomers obtained on the Si HMDI 3 packed column with a water/methanol (85:15) mixture as the elution solvent. The three isomers were completely resolved in the sequence *ortho*, *meta* and *para*. In this case, the higher extent of polymer crosslinking ensures the stability of the stationary phase by avoiding the long-time desorption of the coating. The experimental results clearly show that the retention and separation remained unaltered after more 30 h of operation for this column. In liquid chromatography, retention and resolution can be strongly influenced by the composition of the mobile phase. Water/methanol systems have been extensively studied with CD based stationary phases because inclusion complexes are less sensitive to the addition of methanol than to the addition of ethanol, acetonitrile or dimethylsulfoxide. The retention factors and the resolutions of the three nitrophenol isomers increased linearly with a decreasing amount of methanol in the mobile phase because of the enhancement of inclusion complex stability (Table III). The elution order of *ortho*, *meta* and *para* was independent of the organic modifier content. Based on values of the stability constants of the CD/nitrophenol inclusion complexes, the elution order should be *meta*, *ortho*, *para*. The strongest retention of the *para* isomer indicates that the formation of the inclusion complex plays the dominant role in the separation mecha-

TABLE III
Effect of Mobile Phase Water Content on Retention Factors and Resolutions of Nitrophenol Positional Isomers on Si HMDI 3 Packed Column

%H ₂ O	Retention Factor (<i>k</i>)			Resolution (<i>R_H</i>)	
	<i>ortho</i>	<i>meta</i>	<i>para</i>	<i>ortho/meta</i>	<i>meta/para</i>
65	0.33	0.56	1.07	0.57	0.93
75	0.66	1.12	2.28	0.812	1
85	1.08	1.8	3.79	0.93	1

nism.³³ On the other hand, the weakest retention of the *ortho* isomer leads us to assume that the crosslinking step introduces additional interactions between polymer and solute. The urethane groups generated by the reaction with 2,6-hexamethylenediisocyanate probably take part in the separation process by interacting with the phenol groups of the solute. Crosslinking reactions may also cause a significant change in the polarity (higher hydrophobicity due to the carbon 6 atom's alkyl chain) of the coated polymer external surface, providing additional adsorption sites. The last effect able to influence the elution order is the limitation of the mobility and accessibility of the CD units within crosslinked polymer. This may cause a decrease in the selectivity of the inclusion complex formation.

Figures 9 and 10 show other examples of separations completed on the Si HMDI 3 stationary phase. A mixture of four pesticides was effectively resolved by using water/methanol (70:30) as the mobile phase at a flow rate of 1.5 mL/min. The high retention of pentachlorophenol and some of the drugs was accompanied

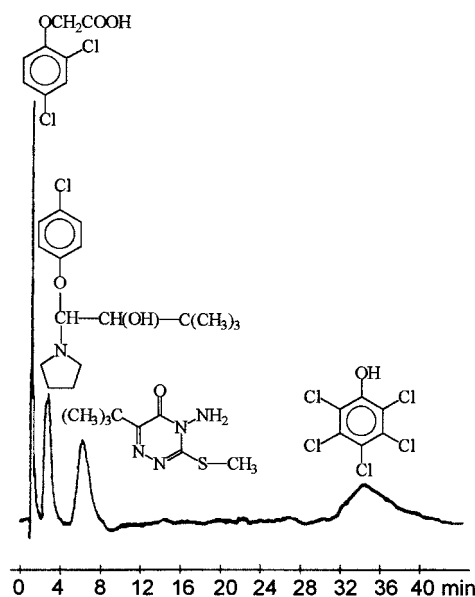


Figure 9 Separation of four pesticides using the Si HMDI 3 packed column obtained using a flow rate of 1.5 mL/min with water/methanol (70:30) as the mobile phase.

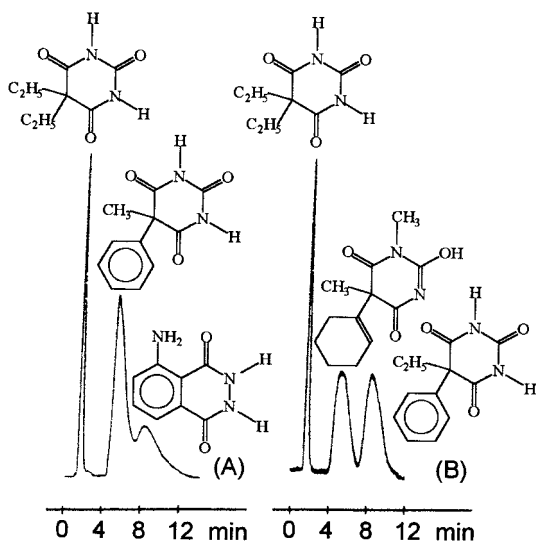


Figure 10 Separation of drugs on the Si HMDI 3 packed column obtained using a flow rate of 2.0 mL/min with water/methanol (85:15) as the mobile phase.

by a broadening of the peaks. As indicated above, the crosslinked phase has to withstand stress from the mobile phase and to allow a sufficiently fast diffusion of the analytes in the stationary phase polymer layer. Obviously, the presence of polymeric aggregates should be avoided because it seems to be the main reason for the poor efficiencies observed in some cases. Nevertheless, it is difficult to exclude the possible limiting effect of crosslinking on the rate of diffusion of the solutes.

CONCLUSIONS

In this study, a method for the fast and easy preparation of new stationary phases has been developed. It involves the coating of porous silica beads by the *in situ* polymerization/crosslinking of 2-hydroxypropyl β -cyclodextrin with 2,6-hexamethylenediisocyanate. The adsorption mechanism is a complex process, which seems to be governed primarily by the adsorption of oligomers. Nevertheless, many parameters must be considered, such as the polymerization/crosslinking of adsorbed polymer chains and the solubility of the polymer in the reacting solution. The potential use of the coated stationary phases as packing materials for RP HPLC separation has been illustrated. The influence of the extent of stationary phase crosslinking on column stability has been demonstrated. It was shown that the addition of an excessive amount of crosslinker during the polymerization/crosslinking process leads to the formation of poly-

meric aggregates that are certainly the cause of poor efficiency. Thus, the preparation of efficient phases requires careful adjustment of the molar ratios of the reactant in order to ensure both the long time stability of the phase and sufficiently fast diffusion of the analytes.

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