

# High-Performance Liquid Chromatographic Stationary Phases Based on Silica Coated by In Situ Polymerization of a Methacryloyl $\beta$ -Cyclodextrin Monomer: Synthesis, Characterization, and Chromatographic Evaluation

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## Abstract

Porous silica beads have been coated with a 2-hydroxy-3-methacryloyloxypropyl  $\beta$ -cyclodextrin polymer by in situ free-radical polymerization in water. This system has been developed for use as a stationary phase in high-performance liquid chromatography. In the conditions used, the coating efficiency is controlled by the initial concentration of the monomer. The polymer coating has been quantitated by thermogravimetric analysis. The stationary phases have also been characterized by means of the nitrogen adsorption/desorption method, energy dispersion X-ray analysis, and scanning electron microscopy to investigate the changes in the porosity, as well as in the surface properties generated by the coating process. Finally, the chromatographic evaluation has been made under normal- and reversed-phase elution conditions.

## Introduction

$\beta$ -Cyclodextrin ( $\beta$ -CD) is a torus-shaped cyclic oligosaccharide made up of seven  $\alpha$ -1,4-linked D-glucopyranose units, with a hydrophilic external surface and an internal apolar cavity (1). Porous silica beads modified with  $\beta$ -CD derivatives have been extensively developed for the reversed-phase (RP) (2), normal-phase (NP) (3), and polar/organic-phase (4) liquid chromatographic (LC) separation of enantiomers. Undoubtedly, the mechanism of resolution differs with the elution conditions. In RP applications, the separation is caused mainly by the formation of an inclusion complex between the solute and the CD cavity. Alternatively, it is assumed that under NP-LC the apolar eluent occupies the internal part of the cavity and that the separation

results mainly from interactions with the external hydroxyl groups of the CD molecule. Thus, the retention behavior is similar to that of a diol column. In the case of a polar-organic mobile phase, usually composed of acetonitrile mixed with a hydrogen-bonding modifier, the interaction process is not clearly defined. The separation may stem from hydrogen or dipolar interactions (or both) and a size-dependent interaction in which the solute acts like a "lid" over the CD cavity. Originally, CD supports for high-performance liquid chromatography (HPLC) were prepared by covalent linkage of the  $\beta$ -CD molecule to silica by the use of various spacers (5). Fujimura et al. showed that an increase in the length of the amino-type spacer of the bonded CD moieties on a silica surface led to an increase in both the retention and selectivity by inclusion complex formation (6). One of the problems often encountered is the presence of residual silanol groups that alter the retention mechanism. Another way consists of the adsorption on silica of a  $\beta$ -CD polymer having a well-defined chemical composition and macromolecular structure (7). Sébille et al. reported the chromatographic properties of methylated and acetylated polymers obtained from the condensation reaction of  $\beta$ -CD with epichlorohydrin used as chiral supports (8). Alternatively, a polymeric coating of the silica support can be accomplished in one step by means of in situ polymerization of the CD monomer on the surface of silica particles (9). The main advantages of these polymeric phases are the possibility of greater selectivity by the appropriate choice of the nature and amount of functional groups in the chains of the polymer, as well as the control of the polymer coating that permits modification of the porosity. The stability of the polymer coating can be improved by a crosslinking step (10). Nevertheless, the crosslinking must be controlled to avoid resistance to solute mass transfer. Moreover, Crini et al. demonstrated that the retention behavior of disubstituted benzene derivatives on  $\beta$ -CD grafted on poly(ethyleneimine)-coated silica beads was affected by the

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crosslinking procedure (11). The authors suggested that in this case, a size exclusion effect may be added to the other interactions. In this work, a method for the preparation of polymeric  $\beta$ -CD-coated silica stationary phases is presented. The method relies on the in situ free-radical polymerization of the 2-hydroxy-3-methacryloyloxypropyl  $\beta$ -CD ( $\beta$ W7 MAHP) monomer with simultaneous coating on the surface of silica beads. The effect of the concentration of the monomer on the coating efficiency has been evaluated. The coated phases were characterized by thermogravimetric analysis (TGA), nitrogen adsorption/desorption method, energy dispersion X-ray analysis (EDXA), and scanning electron microscopy (SEM). Finally, their potential utilization as a packing material in HPLC is described.

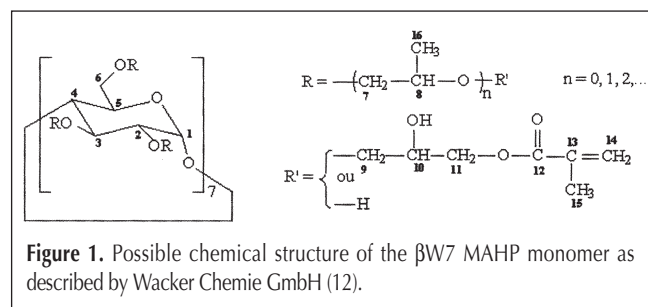
## Experimental

### Materials

The polymerizable  $\beta$ -CD derivative  $\beta$ W7 MAHP was a gift from Wacker Chemie GmbH (München, Germany). The manufacturer gives a molecular weight of 1400 g/mol and an average degree of hydroxypropyl (R) molar substitution of 2.8 per molecule of CD and of the methacryloyl group (R') of 1.05 per CD (12). Its chemical name is 2-hydroxy-3-methacryloyloxypropyl- $\beta$ -CD. However, it is not a homogeneous substance, but a mixture of numerous isomers. The possible chemical structure of  $\beta$ W7 MAHP is given in Figure 1. Water, methanol, 2-propanol, hexane (HPLC grade), and potassium peroxodisulfate were obtained from Sigma-Aldrich (L'Isle d'Abeau Chesnes, France). They were filtered through a membrane filter with a 0.45- $\mu$ m pore size (Type HVLP, Millipore, Bedford, MA) and degassed by ultrasonic vibration prior to use. All compounds used for chromatography were purchased from various suppliers. They were of analytical-reagent grade and not further purified. The silica support was Lichrospher Si 100 supplied from Merck (Nogent sur Marne, France), having regular spherical porous 10- $\mu$ m particles, with an average pore diameter of 100 nm, a specific surface area of 350 m<sup>2</sup>/g, and a porous volume of 1.25 mL/g.

### Coating procedure

A 1.5-g amount of porous silica Lichrospher Si 100 was suspended in 10 mL of distilled water under continuous stirring in the presence of a given amount of potassium peroxodisulfate as an initiator. The mixture was stirred for 15 min under a nitrogen atmosphere at 80°C. Afterwards, various amounts of  $\beta$ W7 MAHP monomer dissolved in 15 mL of distilled water were introduced dropwise. Stirring and heating were continued for 24 h. The



resulting coated supports were filtered off, washed successively with distilled water (200 mL) and distilled methanol (200 mL), and then dried at 60°C under vacuum for one night. The initiator- $\beta$ W7 MAHP ratio was kept constant to 1:100 (w%) for the whole series of coating experiments.

### Columns packing

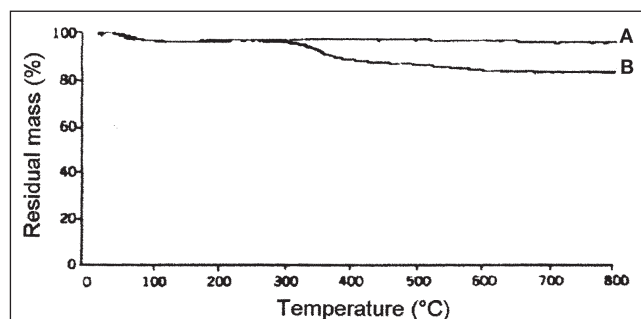
Columns (100- $\times$ 4.6-mm i.d.) made from stainless steel tubing were slurry packed with 10% slurries (w/v) of the stationary phase in carbon tetrachloride using a Touzard & Matignon (Les Ulis, France) packer model. A constant packing pressure of 200 bars was used, with dichloromethane as propulsion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

### Characterization of the coated silica phases

The TGA, allowing the determination of the mass of polymer adsorbed onto the silica surface, was performed under an air atmosphere using a Shimadzu (Duisburg, Germany) TGA-51 analyzer. The temperature range was from 25°C to 800°C at a heating rate of 10°C/min. The amount of  $\beta$ -CD was calculated from the difference between the weight loss of the coated silica and that of the reference bare silica. Porosity characterization was investigated in the dry state using the nitrogen adsorption/desorption isotherm with a Sorptomatic 1990 (Thermoquest, Paris, France) apparatus. The specific surface area ( $S_{\text{BET}}$ ) was calculated by the Brunauer-Emmett-Teller (BET) method (13), whereas the specific porous volume ( $V_{\text{BJH}}$ ) and pore distribution were determined using the Bourret-Joyner-Helenda (BJH) model (14). The surface properties of the coated materials were studied by energy dispersion X-ray analysis (EDXA) with a Cambridge Instruments (Witchford, U.K.) model 600, as well as SEM with a Hitachi (Ealing, London, U.K.) S-4500 SEM apparatus using an accelerating voltage ranging from 3.0 to 5.0 kV. The silica supports were suspended in ethanol, and several droplets of the solution were put down on the microscopy grids. After the ethanol was completely evaporated, they were directly shadowed with a thin layer of platinum (2 nm), without surface treatment.

### HPLC measurements

An L6200A Intelligent Pump (Merck) was used. Sample solution (20  $\mu$ L) was injected using a Rheodyne (model 7125, Merck) valve injector. The detection was performed with a UV-vis variable-wavelength detector (L4250, Merck) and a chromatogram-integrator (D2500, Merck). Chromatography was carried out at



**Figure 2.** Thermogram of (A) bare silica and (B) silica coated with Si  $\beta$ W7 MAHP 2.

room temperature under isocratic conditions at a flow rate of 1.0 mL/min. UV detection was performed at 280 nm. Sample solutions were prepared by dissolving the sample compounds using the respective mobile phase as a solvent at a concentration of 100 mg/mL.

## Results and Discussion

### Characterization of the coated silica phases

#### TGA

Owing to the way of synthesis, the  $\beta$ W7 MAHP polymer is physically entangled onto the silica surface and immobilized irreversibly by the formation of multiple hydrogen bonds and van der Waals interactions with the silica surface via the silanol groups and siloxane linkages. The thermograms of bare and coated silica (Figure 2) indicated two losses of weight in the temperature range studied. In both cases, the first loss of weight near 100°C was related to the dehydration of silica with elimination of water molecules physically adsorbed onto the surface. The second one was attributed in the case of the bare Lichrospher Si 100 to the dehydroxylation of silica, resulting from the condensation of neighboring silanol groups at a temperature above 500°C with formation of siloxane linkages. In the case of modified silica, this second loss of weight corresponded both to the deshydroxylation of silica, as previously described, and to the thermal degradation of the organic polymer layer. After correction for water loss, the mass of organic matter was determined from the difference between the mass loss of coated silica and that of native silica, with the assumption that the dehydroxylation was similar in both cases. The  $\beta$ W7 MAHP content was directly obtained from the mass of immobilized polymer. The data deduced from TGA are presented in Table I. The amount of adsorbed  $\beta$ W7 MAHP polymer presents a maximum with increasing the monomer con-

centration. This maximum may be explained by the protocol used for the coating process. The  $\beta$ W7 MAHP monomer was added dropwise to the aqueous medium containing the initiator and the silica beads. This approach was chosen to limit the formation of aggregates of polymeric  $\beta$ W7 MAHP nonadsorbed onto the silica because it could lead to a decrease of the chromatographic support efficiency. It is probable that the first  $\beta$ W7 MAHP molecules added to the reacting medium react instantaneously, leading to the polymerization of the monomer in solution. One can assume that in a second step, the polymerization of these growing  $\beta$ W7 MAHP polymer chains occurs simultaneously to the adsorption onto the silica surface. It was checked that only rather long polymer chains can adsorb onto the silica surface and that the  $\beta$ W7 MAHP monomer cannot. The radical polymerization rate of the chains adsorbed on the silica is determined first by the availability of the reactive sites and second by the diffusion of the monomer from the solution to the coated layer. Consequently, coated chains grow as long as a high activation barrier because one of these two effects does not restrict the attachment of an additional monomer unit. This explains the maximum obtained for the mass of adsorbed polymer. The decrease of the coating efficiency when a solution of higher monomer concentration was added may arise from the fact that a large amount of the  $\beta$ W7 MAHP polymer was not adsorbed onto the silica surface and remained free in solution. Thus, the higher viscosity because of the numerous macromolecular chains present in solution should be an obstacle for the diffusion of the monomer to the growing chains adsorbed on the silica.

#### Porosity analysis

The texture of the beads is an important feature that could explain the behavior observed in chromatography. The experimental value obtained for the  $S_{\text{BET}}$  (344 m<sup>2</sup>/g) of the bare Lichrospher Si 100 was found to be in perfect agreement with the value given by the supplier, whereas the  $V_{\text{BJH}}$  (1.09 mL/g) was found lower (Table II). As expected, the  $S_{\text{BET}}$  and  $V_{\text{BJH}}$  decrease when the amount of the adsorbed polymer increases, but this phenomenon is not very pronounced. In opposite, the pore size distribution curves are rather different (Figure 3). The Lichrospher Si 100 silica is characterized by a bimodal profile with two distinct maxima at approximately 55 and 70 nm. The pore size distribution profiles obtained with the  $\beta$ W7 MAHP

| Support              | $\beta$ W7 MAHP solution (w%) | Coated polymer on silica (mg/g) | $\beta$ W7 MAHP content ( $\mu$ mol/g) |
|----------------------|-------------------------------|---------------------------------|--|
| Si $\beta$ W7 MAHP 1 | 20                            | 84                              | 60                                     |
| Si $\beta$ W7 MAHP 2 | 9.1                           | 116.5                           | 83                                     |
| Si $\beta$ W7 MAHP 3 | 5.7                           | 33.9                            | 24                                     |

| Support              | Coated polymer on silica (mg/g) | $S_{\text{BET}}$ (m <sup>2</sup> /g) | $V_{\text{BJH}}$ (mL/g) |
|----------------------|---------------------------------|--------------------------------------|-------------------------|
| Lichrospher Si 100   | 0                               | 351                                  | 1.09                    |
| Si $\beta$ W7 MAHP 1 | 84                              | 302                                  | 0.92                    |
| Si $\beta$ W7 MAHP 2 | 116.5                           | 298                                  | 0.90                    |
| Si $\beta$ W7 MAHP 3 | 33.9                            | 336                                  | 1.06                    |

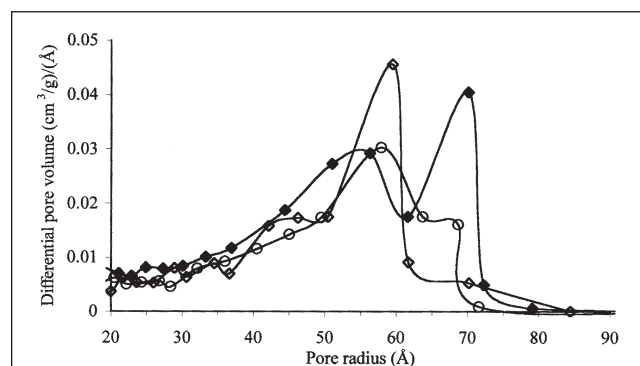


Figure 3. Pore size distribution curves of (♦) Lichrospher Si 100 silica, (○) Si  $\beta$ W7 MAHP 1, and (◇) Si  $\beta$ W7 MAHP 2 coated silica using the nitrogen adsorption/desorption method.

polymer coated silica show a decline of the largest pore, especially when the amount of immobilized polymer is important. For instance, the Si  $\beta$ W7 MAHP 2 support exhibits a much narrowed pore size distribution around 60 nm, with a shoulder near 45 nm. These findings demonstrate that the largest pores play a leading part in the coating efficiency.

#### EDXA

EDXA was used to confirm that the silica surface was polymer-rich. The extent of the phase coating was estimated from the content carbon through the following equation:

$$C\% = (I_C/I_{Si}) \times 100 \quad \text{Eq. 1}$$

where  $I_C$  and  $I_{Si}$  represent the intensity of the peaks of the carbon and silicium atoms, respectively. The carbon content was found to be proportional to the amount of polymer immobilized on the silica surface (Table III). The error was estimated from three EDXA spectra recorded at different places on the sample surface.

#### SEM

To investigate the origin of a possible difference in the performance of the Si  $\beta$ W7 MAHP HPLC columns, the coated silica surface was examined and compared using SEM. From the micrograph shown in part A of Figure 4, it can be deduced that the coating procedure of silica beads by in situ polymerization of the  $\beta$ W7 MAHP monomer led to an agglomeration of the particles, even after being extensively washed with water. The presence

of this polymer external to silica particles (also observed with the supports Si  $\beta$ W7 MAHP 1 and 2 prepared from a greater amount of monomer) suggests that these stationary phases will probably not present good results in chromatography, as polymeric aggregates may generate interstitial spaces within the column. At higher magnifications (Figure 4B and C) it can be seen that the surface is not homogeneously coated. Some  $\beta$ W7 MAHP polymer aggregates of irregular shape are dispersed onto the silica surface, a part of which is polymer free. In opposite, in the case of the Si  $\beta$ W7 MAHP 2 support, the fraction of the silica surface that is coated is much higher (Figure 4D).

#### HPLC results

The mixture of *o*-, *m*-, and *p*-positional isomers of nitrophenol was chosen as test sample for NP and RP experiments. Its aromatic ring allows hydrophobic interactions with the internal part of the  $\beta$ -CD ring, yet its phenol group is able to form hydrogen bonds with the external hydroxyl groups of the CD unit (15). Despite the well-known favorable influence of a buffer added to the mobile phase, simple mixtures of water–methanol were chosen as eluents in order to observe undisturbed effects of solute/support interactions. The RP properties of the Si  $\beta$ W7 MAHP 2 packing material are presented in the Figure 5. As can be seen, the *o*-isomer was eluted first, whereas the *m*- and *p*-isomers were poorly resolved. Injection of pure isomer solutions allowed attribute to the second peak shoulder to the *p*-isomer. An increase of the polarity of the mobile phase from a water–methanol ratio of 65:35 to 80:20 did not improve the resolution because the *m*- and *p*-isomers were then eluted within the same peak. Nevertheless, the assumed elution order *o*- < *p*- < *m*- does not follow the reten-

| Support              | Coated polymer on silica (mg/g) | %C              |
|----------------------|---------------------------------|-----------------|
| Si $\beta$ W7 MAHP 1 | 84                              | 3.02 $\pm$ 0.17 |
| Si $\beta$ W7 MAHP 2 | 116.5                           | 3.68 $\pm$ 0.12 |
| Si $\beta$ W7 MAHP 3 | 33.9                            | 2.33 $\pm$ 0.15 |

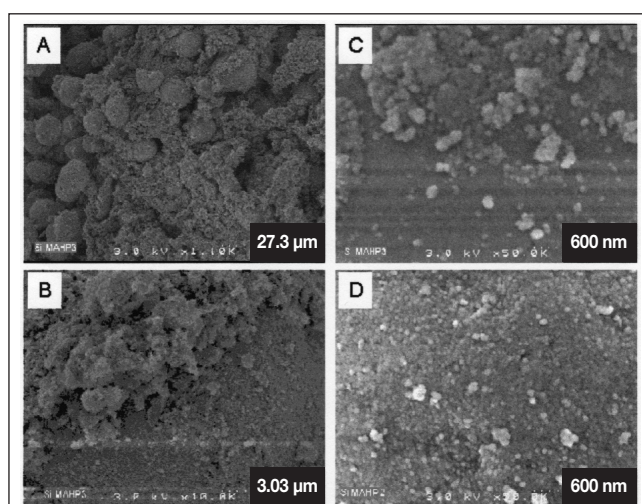


Figure 4. Scanning electron micrographs of the Si  $\beta$ W7 MAHP 3 support at (A)  $\times 1,100$ , (B)  $\times 10,000$ , and (C)  $\times 50,000$  magnifications and Si  $\beta$ W7 MAHP 2 support at (D)  $\times 50,000$  magnification.

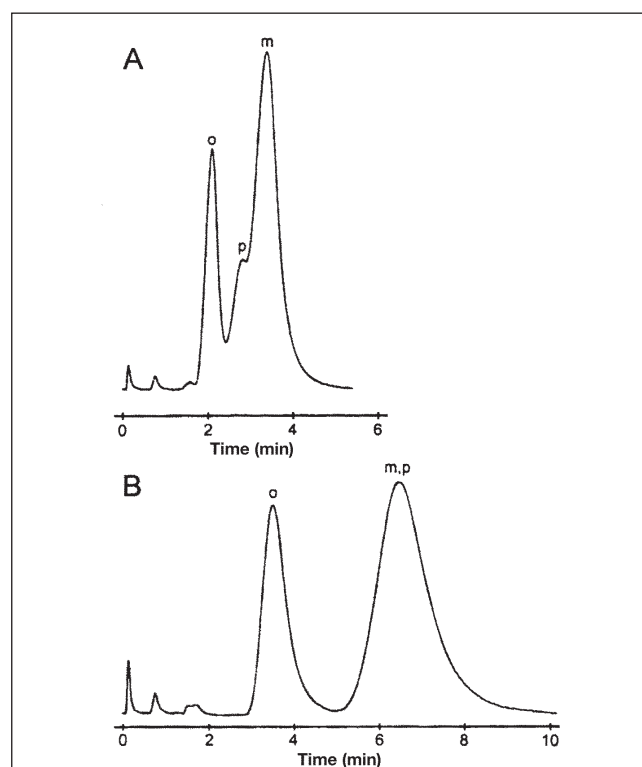
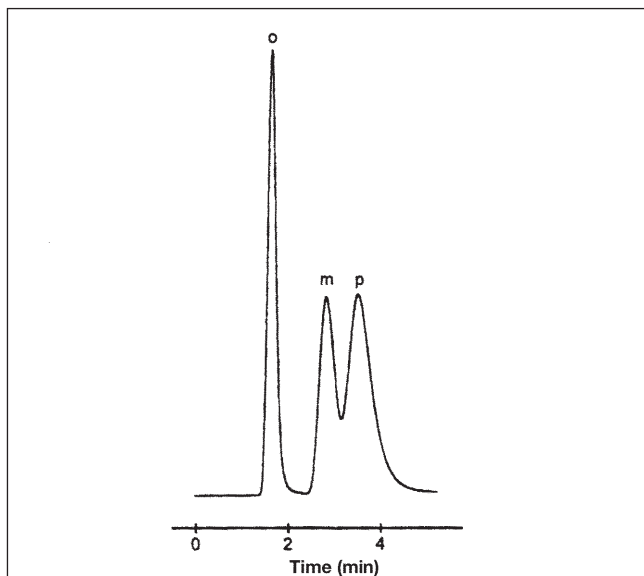


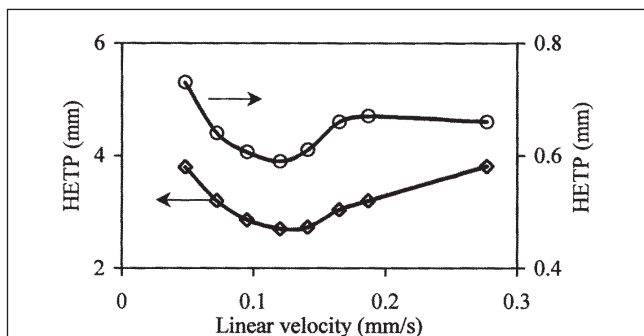
Figure 5. Separation of positional isomers of *o*-, *m*-, and *p*-nitrophenol on the Si  $\beta$ W7 MAHP 2 column. Conditions: (A) water–methanol (65:35) and (B) water–methanol (80:20) as mobile phase.

tion order expected with  $\beta$ -CD-based stationary phases when using aqueous eluents (16). For disubstituted benzene compounds, the separation mechanism is generally mainly governed by steric effects (17). The *p*-isomer, which exhibits the lowest steric hindrance, can penetrate deeply the cavity and is consequently eluted last. Whereas, the *m*-isomer that has the bulkiest structure interacts only partially with the interior of the cavity and is eluted first. These findings indicate that the selectivity of the  $\beta$ W7 MAHP polymer columns differs from the one of the common  $\beta$ -CD-bonded phases and, notably, that the retention and separation of nitrophenol isomers are also influenced by hydrogen bonding because of the phenol group. Evidences supporting this hypothesis came from unsuccessful attempts to separate the positional isomers of xylene and nitrotoluene. It must also be noted that the resolution was not enhanced with the Si  $\beta$ W7 MAHP 1 and 3 supports.

The chromatogram shown in Figure 6 demonstrates the NP separation of nitrophenol isomers on the Si  $\beta$ W7 MAHP 1 packed column with a hexane–2-propanol (90:10) eluent. The peak of the *o*-isomer eluted first was substantially separated from the *m*- and *p*-isomers that were solely partially resolved. In comparison with



**Figure 6.** Separation of positional isomers of *o*-, *m*-, and *p*-nitrophenol on the Si  $\beta$ W7 MAHP 1 column. Conditions: hexane–2-propanol (90:10) as mobile phase.



**Figure 7.** Dependence of HETP on linear-flow velocity for the ( $\diamond$ ) Si  $\beta$ W7 MAHP 2 and ( $\circ$ ) Si  $\beta$ W7 MAHP 3 columns determined for 2-naphtol. Conditions: water–methanol (80:20) as mobile phase.

the RP mode, the elution order of the *m*- and *p*-isomers was reversed, meaning that the interactions responsible for the separation were different. The use of stronger mobile phases (higher hexane content) led to a strong increase of retention with a weak separation improvement because of the peak broadening. The three Si  $\beta$ W7 MAHP packings did not exhibit large differences in effectiveness under the NP mode. Obviously, in the absence of water, hydrogen interactions involving the hydroxyl groups that line the mouth of the cavity and the phenol group may be significantly greater. Consequently, the enhanced resolution provided by using mobile phases consisting of hexane–2-propanol mixtures is assumed to reflect the greater amount and accessibility of the interaction sites. Nevertheless, it is difficult to compare the chromatographic results obtained with the two modes of elution. Indeed, the variation of the environment polarity of the polymer chains adsorbed onto the silica may lead to a change in their solvation and conformation. The classical van Deemter plot was investigated to point out the influence of the polymer coating on the solute diffusion and the mass transfer under RP mode (18). 2-Naphtol was chosen as probe molecule to determine the mass transfer kinetics because it was strongly retained. Resistance to mass transfer may be evaluated through quantitation of either the chromatographic band spreading or the solute resolution. Figure 7 shows the plots of the height equivalent to theoretical plate (HETP) versus the linear flow velocity for Si  $\beta$ W7 MAHP 2 and 3 supports determined with the water–methanol (85:15) mixture as mobile phase. These curves show a minimum at a linear velocity of 0.13 mm/s that corresponds to a flow rate of 1.25 mL/min. Nevertheless, despite similar shape in the profiles of the plots, the efficiencies achieved for 2-naphtol are rather different because a difference of one decade of magnitude is observed. The Si  $\beta$ W7 MAHP 2 support exhibited poor mass transfer (HETP values ten times higher), and the problem became worse when the mobile phase velocity through the column was increased, whereas the Si  $\beta$ W7 MAHP 3 had acceptable mass transfer. These findings are in good agreement with the SEM observations because it is assumed that the higher polymer loading in the Si  $\beta$ W7 MAHP 2 support may lead to a multilayer coating in which the mass transfer and solute diffusion are reduced.

## Conclusion

$\beta$ -CD polymer-coated stationary phases were produced during in situ polymerization of the  $\beta$ W7 MAHP monomer, a methacrylate derivative of hydroxypropyl  $\beta$ -CD. Taking into consideration the results of the different analysis, it appears that the coating is a complex process depending on different parameters such as: the initial concentration of the monomer, the type of interactions between the support and the polymer, the solubility of the polymer in the reacting medium, and the steric hindrance. This latter effect is presumed to limit the polymerization rate of the chains adsorbed onto the silica. However, the separation properties of the stationary phases towards the positional isomers of nitrophenol were demonstrated. Nevertheless, one must also keep in mind that the  $\beta$ W7 MAHP monomer is of industrial quality and, as indicated by Wacker Chemie GmbH, it was not at

all purified after synthesis. Thus, the  $\beta$ W7 MAHP is a mixture of various isomers including possible dimethacrylate  $\beta$ -CD derivatives, which may explain both the agglomeration of the silica beads during the in situ polymerization and the weak columns efficiency because of the formation of a crosslinked polymer.

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