New HPLC stationary phases based on (methacryloyloxypropyl-β-cyclodextrin-co-N-vinylpyrrolidone) copolymers coated on silica. Preparation and characterisation

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New silica based supports for use in HPLC separations have been prepared by the coating of a series of copolymers containing various molar fractions of vinylpyrrolidone (VP) and methacryloyloxypropyl beta cyclodextrin (βW7 MAHP) units on LiChrospher Si100. These chromatographic supports were fully characterised using the following techniques: thermogravimetric analysis, specific area and porous volume measurements, scanning electron micrography, NMR, EDAX and RAMAN spectroscopy. It was demonstrated that the interactions of the copolymer with the silica surface imply the VP units through H-bonding between the amide groups and the silanol functions explaining the increase of the polymer adsorption with the VP residue content. The analysis of the surface area, porous volume and pore size distribution showed that the coating process involved first the largest pores of silica and in a second step the smallest ones. From the ^{29}Si NMR spectroscopy and scanning electron microscopy analysis, it appears that only the supports with high amounts of polymer adsorbed ensured a complete passivation of the silanol groups.

Introduction

The discovery of the ability of β-cyclodextrin (β-CD) to form specific inclusion complexes with organic compounds has developed the production of numerous types of chromatographic supports able to separate compounds such as structural isomers (phenolic compounds, aromatic acids).

These supports can be produced by two main routes. The first route consists of chemically bonding β-CD moieties onto silica stationary phases.[1–3] The second approach is to use coating technology, which consists of covering, by physisorption, the silica bead surface with a polymer containing CD residues.[4–7]

In recent works[8,9] we have reported the synthesis of two series of β-CD containing polymers based on the copolymerisation of a new β-CD monomer (βW7 MAHP, produced by Wacker Chemie GmbH) with vinyl pyrrolidone (VP) and hydroxyethyl methacrylate (HEMA). The βW7 MAHP/VP copolymer has already been successfully used as a chiral selector in the mobile phase for capillary electrophoresis separation of enantiomers (especially D,L-epinephrine).[10] This paper now describes the preparation and characterisation of new stationary HPLC phases by the coating of βW7 MAHP/VP copolymers onto silica beads.

Experimental

Reagents and chemicals

βW7 MAHP/VP copolymers were prepared by radical copolymerisation according to a previously described procedure (Fig. 1).[9] Table 1 summarises the composition data of the βW7 MAHP/VP copolymers and poly(vinylpyrrolidone) (PVP) used to coat the silica beads.

The chromatographic support, LiChrospher Si-100 (particle diameter, 10 μm), is commercially available from Merck. The surface area determined by the BET method was 345 m^2 g^{-1} and the average pore diameter 100 Å.

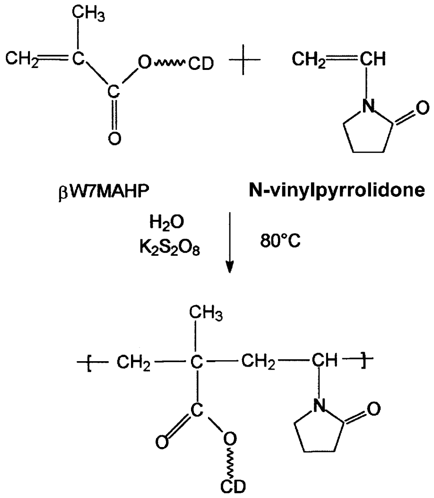

Fig. 1 Preparation of the βW7 MAHP/VP copolymers.

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Table 1 Composition of the βW7 MAHP/VP copolymers used as coating materials for the chromatographic supports preparation. M_w from light scattering measurements

Polymers	$10^{-3} \times M_w$	VP/ mol%	VP/ μmol g^{-1}	βW7 MAHP/ mol%	βW7 MAHP/ μmol g^{-1}
Copo 1		26	250	74	741
Copo 2	546.6	37.5	421	62.5	705
Copo 3	664.4	53	757	47	677
Copo 4	302.8	70.5	1471	29.5	619
Copo 5	67.9	88	3407	12	460
Copo 6	36.1	94	4964	6	332
Copo 7	24.6	96.5	6186	3.5	232
PVP		100		0	

Coating of silica with βW7 MAHP/VP copolymers

10 ml of a 5% (w/w) solution of polymer in distilled water was adjusted to pH 7 with 1 M HCl. 1 g of LiChrospher Si-100 was added to the solution and the mixture sonicated under vacuum (10 mm Hg) for 5 min 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 micrometer pore diameter regenerated cellulose membrane. The Si βW7 MAHP/VP support was extensively washed with water and ethanol and then dried under vacuum at room temperature.

Copo 1 was not suitable for the coating technique because of its insolubility in aqueous media, so it will not appear in the following results.

Characterisation of the supports

The mass of polymer coated on silica beads was determined by a thermogravimetric analysis in air with a TGA-51 Shimadzu thermogravimetric analyser. The specific surface area and the pore size distribution of the supports obtained were determined using a Sorbtomatic 1990 CE instrument. The porosity characterisation was carried out in the dry state using a nitrogen adsorption–desorption isotherm. Specific surface area S was calculated by the BET method.[11] The porous volume V_p and the pore size distribution were calculated using the BJH model.[12]

Raman microprobe analysis gave a local molecular analysis of the supports with a spatial resolution of about 1 μm. It was carried out using a Dilor XY Raman microspectrometer. Data were typically obtained with a laser excitation wavelength of 514.53 nm and 10–30 W power at sample.

Scanning electron micrographs (SEM) were taken on the surface of silica beads using a Hitachi S-4500 system at accelerated potentials of 3 and 5 kV. The sputtering thickness (Pt) was *ca.* 25 Å. The energy dispersion X-ray analysis (EDAX) was done with a Cambridge Instrument model 600.

Solid state NMR measurements (with magic angle spinning and cross polarization) were made with a Brucker CXP-300 spectrometer operating at 75.47 MHz.

Results and discussion

Specific surface area and porosity

The mass of the polymer deposit on silica beads (M_p) was obtained from a thermogravimetric analysis. This method is based on the measurement of the mass loss of a sample heated at high temperature (800 °C). The different organic components of the heated sample are taken off in the form of carbonisation gases. The remaining mass at the end of the heat treatment corresponds to silica. Typical TGA curves are given in Fig. 2. For pure silica, only a slight loss of water was observed from 100 °C. This is due to the take off of adsorbed water (about 2 wt%) and also to a condensation reaction involving the silanol groups at the surface of silica (about 1.5 wt%) (Scheme 1).

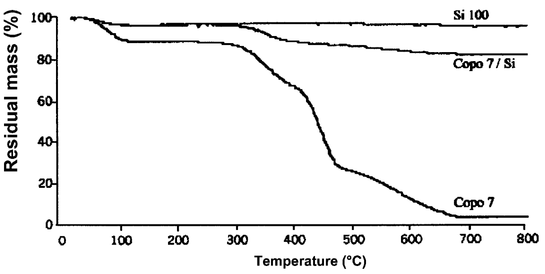
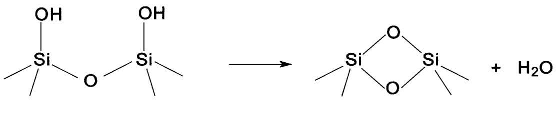


Fig. 2 TGA curves for silica, Copo 7 and Si/Copo 7.



Scheme 1 Condensation of the silanol groups.

The thermal decomposition of the polymers adsorbed was complete at *ca.* 700 °C (Fig. 2). The mass of the polymer deposit M_p was calculated by subtracting from the mass loss of a coated silica sample at 700 °C the mass of water evolved from the corresponding pure silica sample.

The data obtained for the different supports are given in Table 2. The βW7 MAHP content in the polymer layer coated on silica can be directly determined from M_p values using the copolymer composition data given in Table 1.

These results clearly show that the polymer deposit mass increased with the VP unit content in the copolymer as represented in Fig. 3.

A quasi plateau is observed when the quantity of polymer adsorbed is higher than 400 μmol g^{-1} suggesting that all the available surface of silica was covered by a layer of polymer.

At the same time, the specific surface area S decreased with the amount of polymer coated (Fig. 4) indicating an increasing fraction of pores blocked. This curve presents a distinct drop around 80 mg of polymer per gram of support. Thus, the coating process appeared to be divided into two steps. This phenomenon is confirmed by the two domains of variation of the porous volume V_p as a function of the VP unit content (Table 2).

It is well known that, in a porous material, the pores with the smallest diameters are responsible for the high values of the specific area. On the contrary, large pores make an important contribution to the overall porous volume. In our case, it

Table 2 Polymer deposit M_p, cyclodextrin content, surface area S and porous volume V_p of the different samples

Support	VP/ mol%	M_p/ mg g^{-1}	Cyclodextrin content/ μmol g^{-1}	S/ m^2 g^{-1}	V_p/ cm^3 g^{-1}
Silica	—	—	0	345	
Si/Copo 2	37.5	38	27	336	0.96
Si/Copo 3	53	85	58	294	0.94
Si/Copo 4	70.5	107	66	242	0.82
Si/Copo 5	88	170	78	229	0.71
Si/Copo 6	94	135	45	237	0.78
Si/Copo 7	96.5	134	31	246	0.83
Si/PVP	100	154	0		

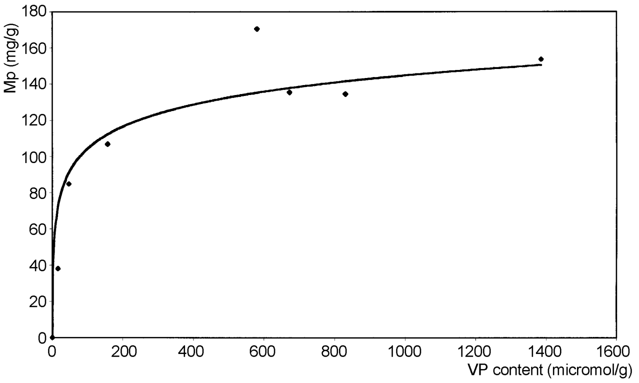


Fig. 3 Polymer deposit M_p *vs.* the adsorbed VP content in µmol g^{-1}.

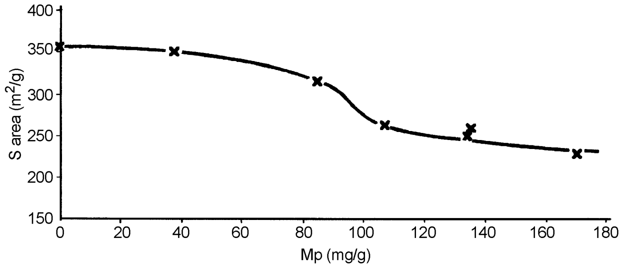


Fig. 4 Variation of S *vs.* M_p.

can be assumed that, for low M_p values, the polymer layer coated mainly the large pores so that the porous volume decreased whereas the surface area was not greatly affected. When reaching the saturation plateau (Fig. 3), *i.e.* when the coating was performed with VP rich copolymers, the smallest pores began to be affected reducing the surface area. For a better understanding of this process, it is useful to study the pore size distribution of the different samples.

Figs. 5a–d give the pore size distribution of the different supports prepared from Copo 2, 4, 5 and 6 compared to that of native silica (dashed line). The distribution of pores for pure silica presents two distinct regions. The bigger one, centred at about 65 Å, indicates that the size of a large fraction of pores ranged from 45 to 70 Å. A small peak can also be seen at about 40 Å showing the presence of a small number of pores with a size comprised between 25 and 45 Å. When silica was coated with Copo 2 (low M_p values) only the large pores were affected by the coating and the main peak was shifted to 55 Å (Fig. 5a). When the coating was performed using Copo 4 or Copo 5, the amount of polymer adsorbed increased. In these cases, both large and small pores were affected (Figs. 5b–c). The large pore peak was shifted in the same way as for Copo 2 and the fraction of small pores with diameter ranging from 10 to 40 Å decreased. For still higher polymer coverage ($M_p = 135$ mg g^{-1}, Fig. 5d), the same decrease was observed for the small pore region whereas the large peak shifts again to the upper values with, however, a disymmetrical shape. At this step it seems that the coating changes: the small ones are coated with a drastic effect on the specific surface area (Fig. 4). At the same time the large pores seem to be coated in a different way: instead of being simply obturated by the polymer layer, they could be really coated on all their inner surface leading to an increase of the porous volume. This better interaction between the polymer and the surface is related to the increase of the VP content (VP is responsible for the interactions through hydrogen bonds as shown by the Raman spectra described later) and to the simultaneous decrease of the steric hindrances due to the CD moieties. When using the PVP homopolymer for the coating, the amount of adsorbed polymer is still higher.

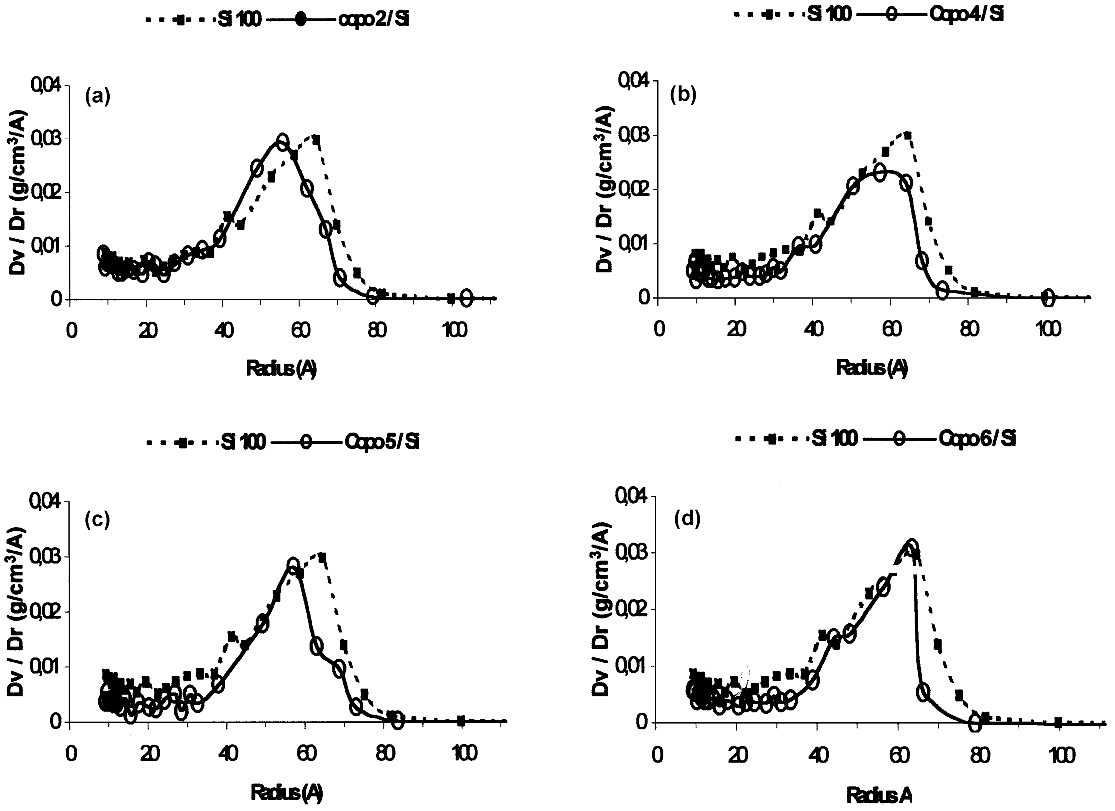


Fig. 5 Pore size distribution of (a) Si/Copo 2. (b) Si/Copo 4. (c) Si/Copo 5. (d) Si/Copo 6.

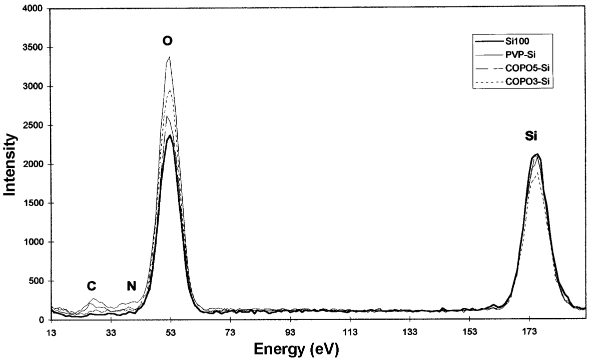


Fig. 6 EDAX spectra of silica (bold line), Si/PVP, Si/Copo 3 and 5.

Spectroscopic study

The presence of a polymer layer at the surface of silica was first checked by an energy dispersion X-ray analysis. Fig. 6 indicates an increase of the fraction of carbon, nitrogen and oxygen whereas a relative decrease of the Si peak is observed.

The above results (Fig. 4) suggest that the functional groups of the pyrrolidone units are responsible for the interaction with silica.

Raman spectroscopy helps to identify the chemical functions that act in the bonding between the polymer and the silica during the coating.

The spectra in Fig. 7 concern the silica/PVP system. The spectrum of PVP exhibits a band at 1650 cm^{-1} due to the valence vibration of the carbonyl group ($\nu_{C=O}$). In the spectrum of the Si/PVP support this band is split into two at 1650 and 1800 cm^{-1}. Thus two different populations of carbonyl groups are present: a population of carbonyl groups interacting with silica (1800 cm^{-1}) and a population of free carbonyl groups (1650 cm^{-1}). The splitting phenomenon is also observed in the spectra of supports coated with the copolymers containing the highest amounts of VP (Si/Copo 5, 6 and 7) (Fig. 8). This observation confirms that the carbonyl group of VP participates strongly in the coating mechanism and in the interaction with the silanol groups of the surface.

As a confirmation, a NMR study of the silanol groups was undertaken using ^{29}Si cross polarization magic angle spinning. It is well known that the ^{29}Si CPMAS NMR spectrum of pure silica (in this case LiChrospher Si 100) presents three distinctive peaks, named Q2, Q3 and Q4, at −92, −103 and −113 ppm. These peaks are attributed to the geminal hydroxy silanols, the single hydroxy silanols and the siloxane groups in the bulk, respectively.[13] Fig. 9 shows the NMR spectra of Si/PVP, Si/Copo 2 and Si/Copo 5. When the amount of VP increases, the Q2 and Q3 peaks decrease. For Si/PVP, the Q2 signal has completely disappeared and the Q3 signal is a slight shoulder to the main Q4 peak. This means that the mobility of the silanol groups especially the geminal ones (Q2) is strongly reduced by interaction with the amide groups of PVP.

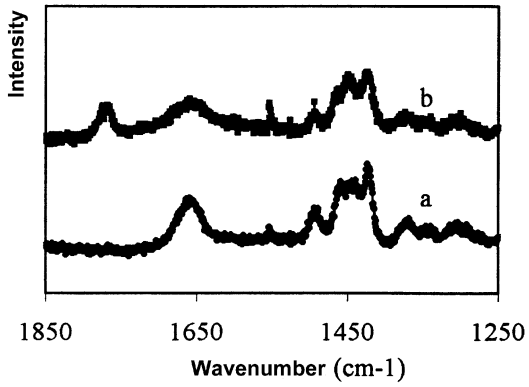


Fig. 7 Raman spectra of (a) PVP; (b) Si/PVP.

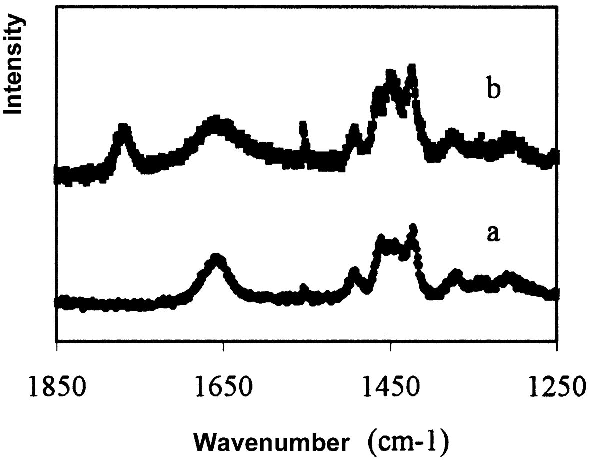


Fig. 8 Raman spectra of (a) Copo 7; (b) Si/Copo 7.

The micrographs in Fig. 10 show the surfaces of some supports with different M_p values and copolymer composition. There is a marked difference between samples coated with a thick layer of polymer (c and d) and samples with a thin or any polymer layer (b and a). Micrographs (a) and (b) present a quite smooth surface with some cracks peculiar to silica surface. The polymer deposit seems irregular at the surface of Si/Copo 3 (Fig. 10b). The cracks disappear in micrographs (c) and (d) and a homogeneous granular surface looking like cauliflower skin appears.

Conclusion

These new silica based supports have been prepared by the coating of different copolymers with various proportions of VP and βW7 MAHP. EDAX analysis and the amount of

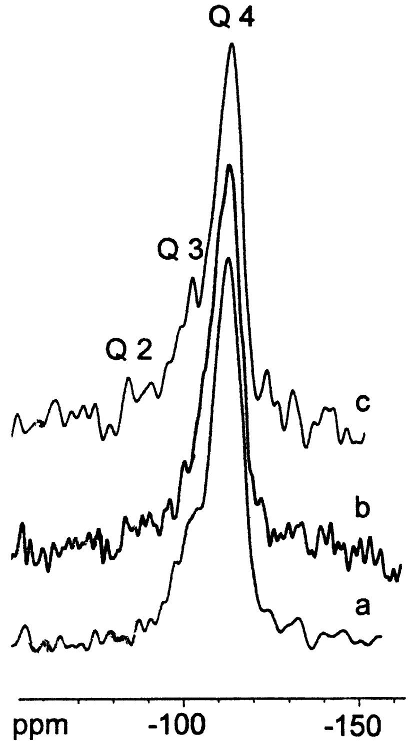


Fig. 9 ^{29}Si NMR spectra of (a) Si/PVP; (b) Si/Copo 5; (c) Si/Copo 2.

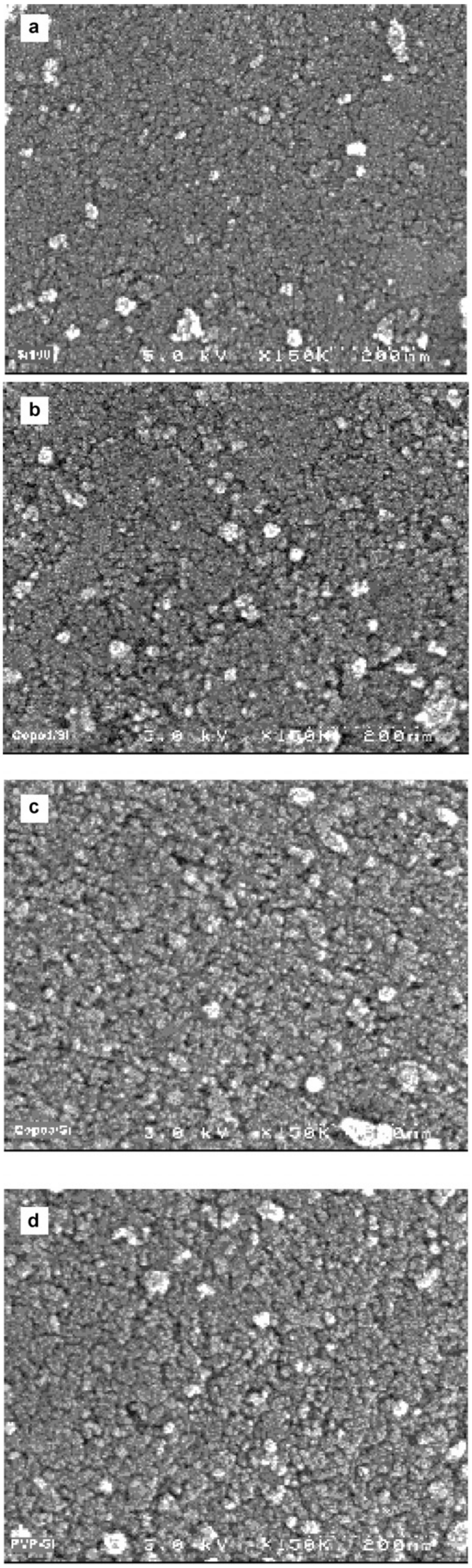


Fig. 10 Electron micrographs of: bare silica (a); Si/Copo 3 (b); Si/Copo 5 (c); Si/PVP (d).

deposit M_p evaluated by thermogravimetric analysis have shown the presence of the polymer at the surface. A Raman spectroscopic analysis has shown that the amide groups of VP are involved in the interaction with the silica surface. The silanol groups, especially the geminal ones, are also involved as shown by NMR. As a consequence the amount of deposit increases with the percentage of VP in the copolymer. A detailed analysis of the variations of the specific surface area, overall porous volume and pore size distribution was made. It has shown that, when increasing the amount of polymer, the coating affects first the large pores and that in a second step, the smallest pores are covered, inducing a sudden decrease of the surface area.

These supports are good candidates for use as stationary phases in HPLC. The polymer layer is firmly anchored to the surface by interaction of the VP constituent with the silanol groups. On the other hand, the βW7 MAHP unit, which is much less involved in the interaction process, remains available for interaction of the cyclodextrin cavity with the constituents of a solution. In order to avoid non-specific interactions of the solutes with the silica surface, it is important to ensure that the surface is completely covered by the polymer layer. From that point of view, electron micrographs and silicon NMR spectrometry have shown that samples Si/Copo 5–7 are satisfying. Among these three supports, Si/Copo 5 seems the best one since its cyclodextrin content is relatively high and therefore good separation properties may be expected. As an example, this system was tested as a stationary phase for the separation of the positional isomers of nitrophenol (Fig. 11). These isomers are eluted in the order *ortho/para/meta* with resolution parameters equal to 1 for *ortho/para* and 0.95 for *para/meta*. This order is different from the expected one, *meta/ortho/para*, using the values of the stability constants of the inclusion complexes. This indicates that other types of interactions, besides inclusion, participate in the separation process.

Results of various separations using these stationary phases will be published later.[14]

Acknowledgements

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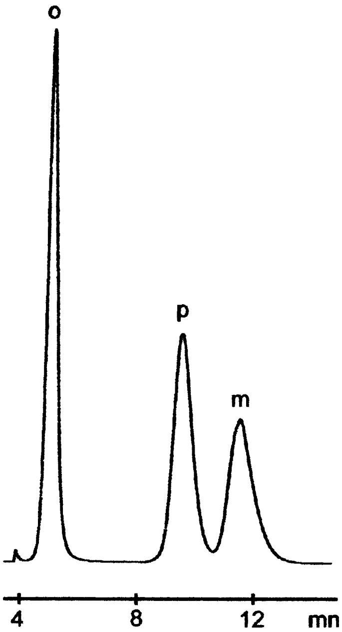

Fig. 11 Separation of the nitrophenol isomers on Si/Copo 5: mobile phase methanol–water 5/95.

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