

Methacryloylpropyl- β Cyclodextrin and Vinylpyrrolidone Copolymers: Synthesis and Characterization as Potential Chiral Selector

B. Carbonnier,¹ L. Janus,¹ A. Deratani,² M. Morcellet¹

¹Laboratoire de Chimie Macromoléculaire, UPRESA CNRS 8009, Université des Sciences et Technologies de Lille, F-59655 Cedex Villeneuve d'Ascq, France

²Institut Européen des Membranes, UMR 5635 CNRS-ENSCM-Université de Montpellier II, 2 Place Eugène Bataillon, F-34095 Montpellier, France

Received 7 April 2004; accepted 30 November 2004

DOI 10.1002/app.21829

Published online in Wiley InterScience (www.interscience.wiley.com).

ABSTRACT: The free radical copolymerization of vinylpyrrolidone (VP) with 2-hydroxy-3-methacryloyloxypropyl β -cyclodextrin (β W7 MAHP), a derivative of hydroxypropyl β -cyclodextrin (CD) substituted by polymerizable methacryloyl groups, is carried out in water by varying the molar ratio of the comonomers. It is found that the higher the molar ratio of β W7 MAHP to VP, the larger the molar mass of the water-soluble copolymer. A size exclusion chromatography analysis coupled with multiangle laser light scattering detection (SEC-MALLS) suggests that VP-rich copolymers with a mass-average molar mass (MM) of about 2.5×10^4 g mol⁻¹ may consist of β W7 MAHP dimers, trimers, and oligomers containing few CD units whereas β W7 MAHP rich copolymers with an MM of about 5.5×10^6 g mol⁻¹ are likely to be dominated by crosslinked polymer materials. β W7 MAHP-co-VP copolymers coated on porous silica are used as high-performance liquid chromatography chiral selectors. The effects of the structural features of the guest molecule and the characteristics of the chiral station-

ary phases on the retention and resolution are evaluated. SEC-MALLS detection shows that, by varying the comonomer feed, copolymers with different molar masses and macromolecular structures are formed. The chiral separation ability of Copo VP is evaluated toward enantiomers having one or more aromatic rings as a function of the amount of copolymer adsorbed onto the silica surface, the β W7 MAHP content, and the concentration of solute. It is clear that the column combining the greater amounts of adsorbed copolymer and β W7 MAHP exhibits better resolving power. Moreover, the size, geometry, and functionality of the guest molecule are important factors that strongly affect the enantioselectivity. © 2005 Wiley Periodicals, Inc. *J Appl Polym Sci* 97: 2364–2374, 2005

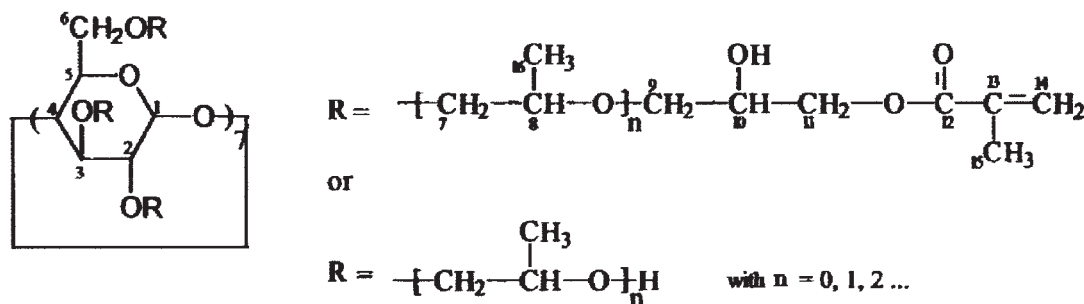
Key words: radical polymerization; high-performance liquid chromatography; gel permeation chromatography; light scattering; chiral; inclusion chemistry

INTRODUCTION

Cyclodextrins (CDs), which are cyclic glucose oligomers, are well known in supramolecular chemistry as receptors that are capable of including a range of organic, inorganic, and biological molecules into their hydrophobic cavity via noncovalent interactions.^{1,2} One especially important application of CD-based materials has been in the field of separation science.^{3–6} In terms of molecular recognition, these macrocycles consisting of six, seven, or eight α -D glucopyranose units that possess a truncated cone-shaped hydrophobic cavity that is able to coordinate guest molecules having different sizes and/or functional groups, depending on which native CD is used as the host molecule. To improve or enhance the original molecular

binding abilities of native CD, numerous studies have been directed toward the design and synthesis of chemically modified CD derivatives.^{7,8} Among these, CD-containing polymers have been extensively used as supramolecular selectors coated on a silica support in chiral chromatography.^{9,10} The preparation of chiral stationary phases (CSPs) with the desired enantioselective properties requires the prior synthesis of water-soluble CD polymers having well-defined structures and chemical compositions and suitable molar masses. Water-soluble CD polymers may be obtained from the limited polycondensation of CDs or CD derivatives with proper bifunctional agents such as epoxy-based reactive monomers.^{11–13} This method of synthesis presents two main problems. First, the polycondensation conditions must be drastically controlled because the formation of hyperbranched polymers would lead to poor chromatographic properties that are attributable to resistance to solute mass transfer. Moreover, when CD cavities are immobilized within a crosslinked microstructure, they may have a

Correspondence to: M. Morcellet (michel.morcellet@univ-lille1.fr).



Scheme 1 The chemical structure of β W7 MAHP.

limited mobility synonym of restricted inclusion ability and selectivity. Second, the chemical derivatization of the polymers, which is necessary to the introduction of functional groups that are able to interact with the chromatographic support, generally requires an additional step.⁹ In contrast, polymeric structures having side CD cavities allow the combination of the remarkable properties of CD to form inclusion complexes and properties of high molar mass materials. Such macromolecular architecture may be achieved through the covalent attachment of CD to linear functional polymers^{14–16} and by the polymerization of monofunctional acryloyl-CD derivatives.¹⁷ The latter preparation method allows easy incorporation of functional groups within the polymer chain by simple copolymerization.¹⁸

Our group has recently focused on the synthesis of water-soluble copolymers containing β -CD side chains and crosslinked β -CD materials through the free radical copolymerization of a new β -CD monomer, 2-hydroxy-3-methacryloyloxypropyl β -CD (β W7 MAHP), which was developed by Wacker Chemie GmbH and consists of methacryloyl moieties grafted to a 2-hydroxypropyl β -CD derivative.¹⁹ In a previous work, the characterization of this β W7 MAHP monomer by a matrix-assisted laser desorption/ionization time of flight mass spectrometry analysis demonstrated that β W7 MAHP is a complex mixture including at least 60 identified isomers.²⁰ Only a part of them possess a methacryloyl substituent, making it suitable for radical copolymerization. The presence of di- and tri-methacryloyl substituted CDs has also been clearly highlighted so that homopolymerization of β W7 MAHP leads to insoluble polymer materials.

The results of our study on the physicochemical characterization and high-performance liquid chromatography (HPLC) enantioselective properties of a series of 2-hydroxy-3-methacryloyloxypropyl β -CD-co-VP copolymers (Copo VP) are reported.

EXPERIMENTAL

Materials

β W7 MAHP (Scheme 1) was a gift from Wacker Chemie GmbH (batch 26 C 001). A careful analysis of this

compound was carried out in previous work.²⁰ It contains about 50 mol % hydroxypropyl CD substituted by methacryloyl groups and thus is able to undergo polymerization; the rest consists of nonpolymerizable molecules. Commercially available VP (Aldrich) was freshly distilled under a vacuum before use. The initiator $K_2S_2O_8$ (Janssen Chimica) was used without further purification.

Synthesis of Copo VP

Detailed information on the polymerization conditions can be found elsewhere.²⁰ Table I summarizes the monomer feed used for β W7 MAHP/VP copolymerization and the characteristics of the copolymers. Of course, the values given for β W7 MAHP take into account the exact content of polymerizable groups.²⁰

The chemical structure of Copo VP is presented in Figure 1.

Preparation and characterization of silica coated CSPs

Several CSPs based on Copo VP coated on silica (Si Copo VP) were obtained and characterized as reported previously.²¹ The main characteristics of the CSPs are recalled in Table II.

Analysis of chemical composition of Copo VP

The chemical composition of Copo VP was determined by titration of both β W7 MAHP and VP comonomers. The VP content was determined through elemental analysis of nitrogen. The content of the CD cavities was determined from titration of reducing sugars with tetrazolium blue²² and spectrophotometric measurement using phenolphthalein solutions at pH 10.5 as a probe for the inclusion complex formation.²³

Analysis by size exclusion chromatography with multiangle laser light scattering (SEC-MALLS)

Copo VPs were analyzed using SEC-MALLS detection following a procedure described elsewhere.¹⁰ The

TABLE I
 β W7 MAHP mol Fraction in Monomer Feed (f_θ), Yield of Copolymerization Reaction, and Copo VP Composition Expressed by Nitrogen Weight Fraction, β W7 MAHP mol Fraction (F_a), and Comonomer Concentration

Copo VP	f_a	Yield (%)	N (wt%)	F_a	Copo VP			
					β W7 MAHP ($\mu\text{mol/g}$)	VP ^a ($\mu\text{mol/g}$)		
2	0.44	36	0.59	0.625	706 ^a	705 ^b	151 ^c	421 ^a
3	0.14	33	1.06	0.47	678 ^a	678 ^b	221 ^c	757 ^a
4	0.04	17	2.06	0.295	619 ^a	619 ^b	246 ^c	1471 ^a
5	0.01	13	4.77	0.12	460 ^a	460 ^b	195 ^c	3407 ^a
6	0.0045	9	6.95	0.06	332 ^a	333 ^b	161 ^c	4964 ^a
7	0.002	6.5	8.66	0.0035	232 ^a	232 ^b	69 ^c	6186 ^a

^a Determined from elemental analysis of nitrogen.

^b Determined from titration of reducing sugars with tetrazolium blue.

^c Determined from titration using the spectrophotometric method.

analyses were carried out at a controlled temperature of 40°C (column, detectors) with a flow rate of 0.5 mL min⁻¹. The light scattering intensity was detected simultaneously at 13 angles ranging from 26.6° to 142°.

Data evaluation

The data were collected and analyzed using ASTRA SEC-software (Wyatt Technology Co.).²⁴ The calculation of the mass-average molar mass (MM) and radius of gyration ($\langle r_g^2 \rangle^{0.5}$) was carried out according to eqs. (1) and (2) for each sliced section of the SEC-MALLS profiles by plotting Kc/R_θ versus $\sin^2(\theta/2)$ (Zimm plot),²⁵

$$R_\theta = \frac{(I_{\theta(P)} - I_{\theta(S)})r_D^2}{I_0 V_0} \quad (1)$$

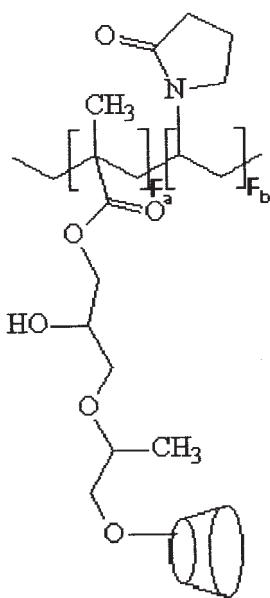


Figure 1 The chemical structure of Copo VP.

where R_θ is the Rayleigh ratio, I_θ is the scattering intensity of the polymer solution (P) and the solvent (S) at the θ angle, I_0 is the intensity of the incident radiation, r_D is the distance between the detector and the scattering volume V_0 , and

$$\frac{Kc}{R_\theta} = \frac{1}{MMP_{(\theta)}} + 2A_2c \quad (2)$$

where K is the optical constant, c is the concentration of polymer in solution, MM is the mass-average molar mass, and $P_{(\theta)}$ is the form factor. An extrapolation to $c \rightarrow 0$ was not necessary because the concentration in each slice was very low and the influence of the second virial coefficient could be neglected.²⁶ A first-order fitting of the values observed to be linear over a large range of molar mass was used to determine MM and $\langle r_g^2 \rangle^{0.5}$ values from extrapolation of the intercept and the initial slope. Well-suited experimental data representations such as a double logarithmic plot of $\langle r_g^2 \rangle^{0.5}$ versus MM allows the determination of the structure according to the scaling law presented in eq. (3).²⁷

$$\langle r_g^2 \rangle^{0.5} = KMM^\alpha \quad (3)$$

The α value gives information about the polymer conformation in solution as determined theoretically in the literature for different shapes of macromolecules.

Measurement of refractive index (RI) increment

The RI increment (dn/dc) was determined for both copolymers at 40°C using sample solutions with six different concentrations. The accurate knowledge of the (dn/dc) value is crucial for the molar mass calculations, because (dn/dc) appears in the term K of eq. (2), which is used to convert the detector signals into the molar mass through the following equation:

TABLE II
Refractive Index Increment (dn/dc), Mass-Average Molar Mass (MM) Root Mean Square Radius ($r_g^{2,0.5}$), and Recovery for Copo VP Samples Analyzed Using SEC MALLS

CSPs	M_p (mg g^{-1})	βW7 MAHP ($\mu\text{mol g}^{-1}$)	VP ($\mu\text{mol g}^{-1}$)	S ($\text{m}^2 \text{g}^{-1}$)	V_p ($\text{cm}^3 \text{g}^{-1}$)
Si Copo VP 2	38	27	16	336	0.96
Si Copo VP 3	85	58	47	294	0.94
Si Copo VP 4	107	66	157	242	0.82
Si Copo VP 5	170	78	581	229	0.71
Si Copo VP 6	135	45	673	237	0.78
Si Copo VP 7	134	31	831	246	0.83

$$K = \frac{4\pi^2 n_0^2}{N_A \lambda_0^4} \left(\frac{dn}{dc} \right)^2 \quad (4)$$

where n_0 is the RI of the solvent at the incident radiation wavelength (λ_0) and N_A is Avogadro's number. The (dn/dc) value is also important for the determination of the recovery. Dividing the change in the RI for each slice by the given (dn/dc) , one obtains the concentration of each slice, the sum of which gives the peak concentration. Finally, the recovery may be obtained comparing the mass obtained for the peak with the amount injected.

HPLC evaluations

Details about the HPLC apparatus and procedures as well as the column packing are found elsewhere.²⁸

RESULTS AND DISCUSSION

In the following text, the results are presented and discussed assuming a mass-average molar mass of 1350 g mol^{-1} for βW7 MAHP and taking into account that only 50 mol % βW7 MAHP is reactive. The polymerizability of βW7 MAHP was previously evaluated through the radical copolymerization with 2-hydroxyethyl methacrylate (HEMA), resulting in the formation of insoluble crosslinked materials.²⁹ As can be seen from Figure 2, the incorporation of βW7 MAHP in the VP copolymers is low compared to HEMA copolymers. This result may be interpreted in terms of different monomer reactivity ratios. It should be mentioned that, because of its polyfunctionality, the βW7 MAHP monomer is in fact a multicomponent system, so the determination of monomer reactivity ratios has a limited meaning. The monomer reactivity ratios are $r_a = 0.74$ and $r_b = 3.71$, where a and b represent βW7 MAHP and HEMA monomers, respectively. These data indicate a tendency of βW7 MAHP toward cross propagation and a clear trend toward HEMA unit block formation, suggesting that homopropagation of βW7 MAHP may be hindered because of steric considerations. Enhancement of the reactivity of such a

bulky monomer should be possible by copolymerization with monomers having poor reactivity with themselves. The combination of βW7 MAHP with VP yielded water-soluble copolymers for the monomer feed having a βW7 MAHP fraction of <60 mol %, whereas for higher βW7 MAHP mole percentages insoluble crosslinked gels were produced.²⁰ The compositions of the monomer feed and the resulting copolymers are compiled in Table I. The chemical composition of Copo VP was determined by both βW7 MAHP and VP comonomer titration as mentioned in the Experimental section. Results derived from the elemental analysis of nitrogen were used to plot the composition curve of Copo VP (Fig. 2). Table I indicates a discrepancy between the two methods used for the determination of the CD content. The tetrazolium blue method gave results similar to the ones obtained from the elemental analysis, indicating that it can be used as an absolute method. Data values obtained by the phenolphthalein complexation method were

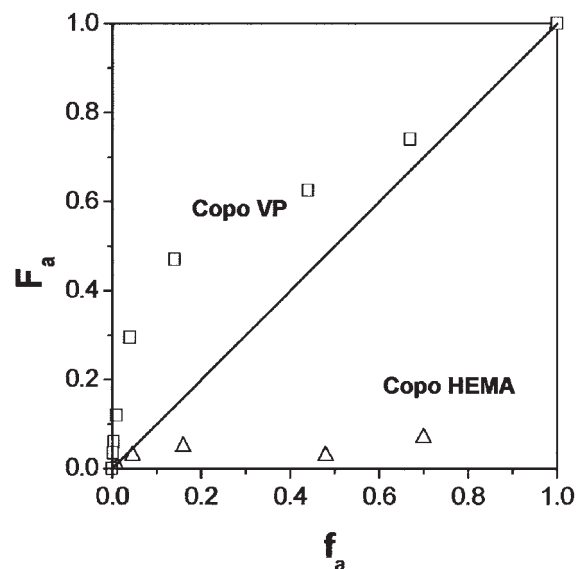


Figure 2 A plot of the dependence of the mol fraction (F_a) versus the monomer feed (f_a) for HEMA βW7 MAHP copolymers and VP βW7 MAHP copolymers.

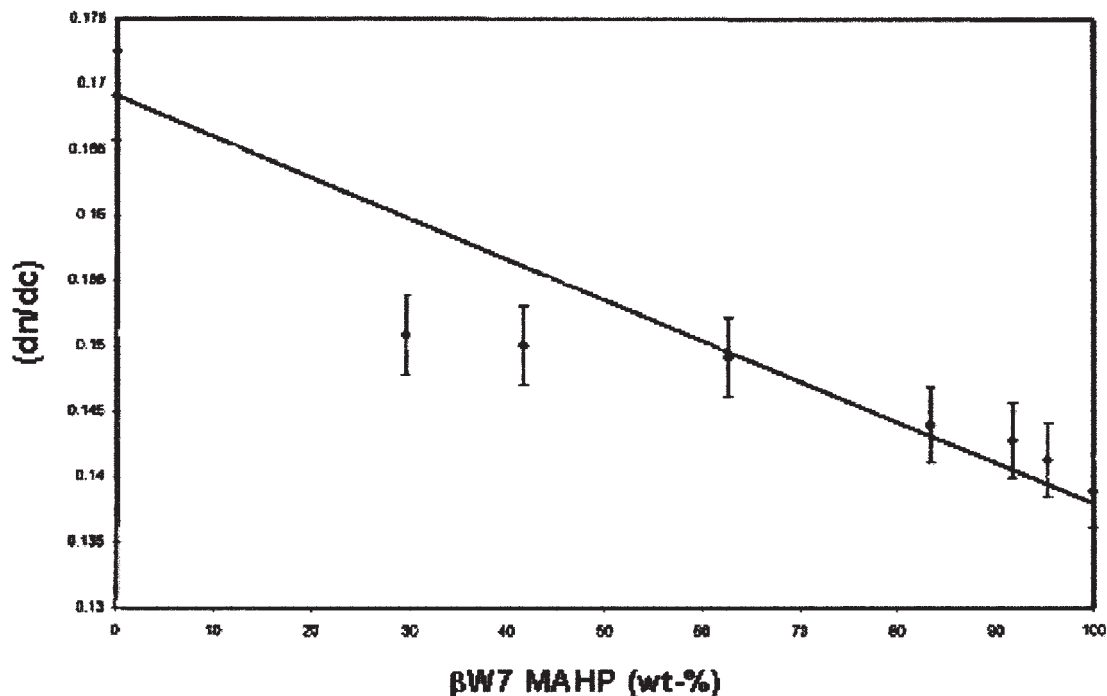


Figure 3 A plot of (dn/dc) as a function of the β W7 MAHP weight fraction of Copo VP.

found to be significantly lower. This may be ascribed to the fact that this method does not provide an absolute quantification of CD content but allows only the titration of CD units accessible to potential guest molecules. It is also of interest to note that the CD content determined by the phenolphthalein method is not correlated with the β W7 MAHP incorporation, revealing that β W7 MAHP rich copolymers do not necessarily possess a greater number of accessible complexing CD cavities. Monomer reactivity ratio values of 0.93 and 0.06 were obtained for β W7 MAHP and VP, respectively. It seems clear that the β W7 MAHP comonomer is more reactive, so its incorporation within the polymer chain is favored compared to VP. From these results, it appears that a key issue to obtain the desired product, crosslinked resins or soluble materials, relies on the reactivity of each monomer as well as on the monomer feed composition.

Measurement of (dn/dc)

The (dn/dc) values of a series of copolymers cannot be assumed constant as in the case of homopolymers. However, (dn/dc) is an additive function so that it varies with the relative weight fraction (wt %) of monomer units in the copolymers. Because homopolymerization of β W7 MAHP leads to insoluble polymers, the (dn/dc) was determined for the β W7 MAHP monomer and was equal to 0.139 mL g^{-1} . A value of 0.169 mL g^{-1} was obtained for poly(VP) (PVP). It follows from Figure 3 that (dn/dc) increases with a

decreasing β W7 MAHP weight percentage because of the lower specific refractivity of β W7 MAHP compared to PVP. In addition, it correlates well with the chemical composition of the copolymers except for Copo VP 6 and 7, for which a small negative deviation from the theoretical curve is observed.

SEC-MALLS analysis

Copo VPs were analyzed with SEC-MALLS on an agarose-based Superose[®] 12 HR 10/30 column (designed for high-performance gel filtration of biomacromolecules, Pharmacia Biotech) in 0.1M phosphate buffer (pH 7.0). The refractometric signals obtained for the six water-soluble copolymers are depicted in Figure 4(a,b). From Figure 4(a) it is obvious that Copo VP gives rise to bimodal SEC profiles with distinct peaks at around 8 and 14.5–16 mL. A slight shoulder, more clearly seen for Copo VP 7, is recognized at an elution volume close to the total volume of the column ($V_{\text{tot}} = 17.5 \text{ mL}$, arrow in Fig. 4). A progressive shift to lower elution volumes of the second eluted peak accompanied with an increase of the peak area is also seen together with a decrease of the area of the first eluted peak, which exhibits solely a slight shift along the elution volume axis. These findings, which are more clearly seen in Figure 4(b) where three selected SEC profiles (Copo VP 2, 4, and 7) are shown, reflect the increase of the VP content in the copolymer. Two extreme behaviors may be distinguished. Copo VP 2, which is representative of β W7 MAHP rich copoly-

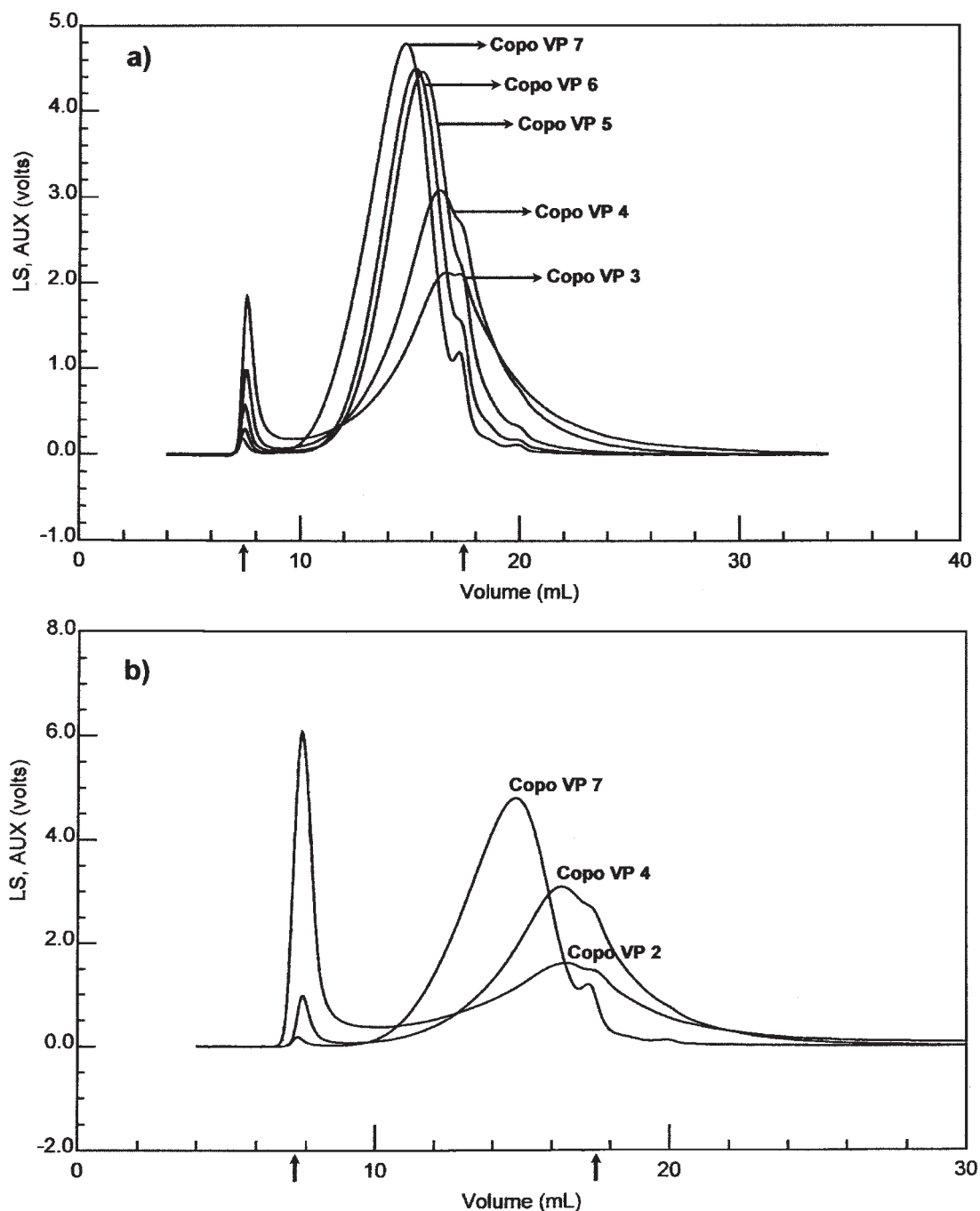


Figure 4 SEC RI elution profiles of Copo VP.

mers, presents a chromatogram typical of an exclusion mechanism. The main peak appears close to the exclusion limit because the void volume (V_{exc} , arrow in Fig. 4) of the column amounts to approximately 7.8 mL. The second peak extends over a broad range of elution volumes and even beyond V_{tot} , showing that the column that was used does not cover the entire range of molar masses. The sample amount that is eluted at V_{exc} corresponds qualitatively to very high molar mass macromolecules that are not separated by this

column. The part of the sample eluted after V_{tot} is consistent with macromolecules that enter within the pores of the column packing but drag along the column. The samples are not solely fractionated by molar mass but also according to other physicochemical parameters.

In contrast, the chromatogram of Copo VP 7, characteristic of low β W7 MAHP content copolymers, presents a weak exclusion peak at 7.8 mL and a second peak that is sharpened compared to Copo VP 2, the

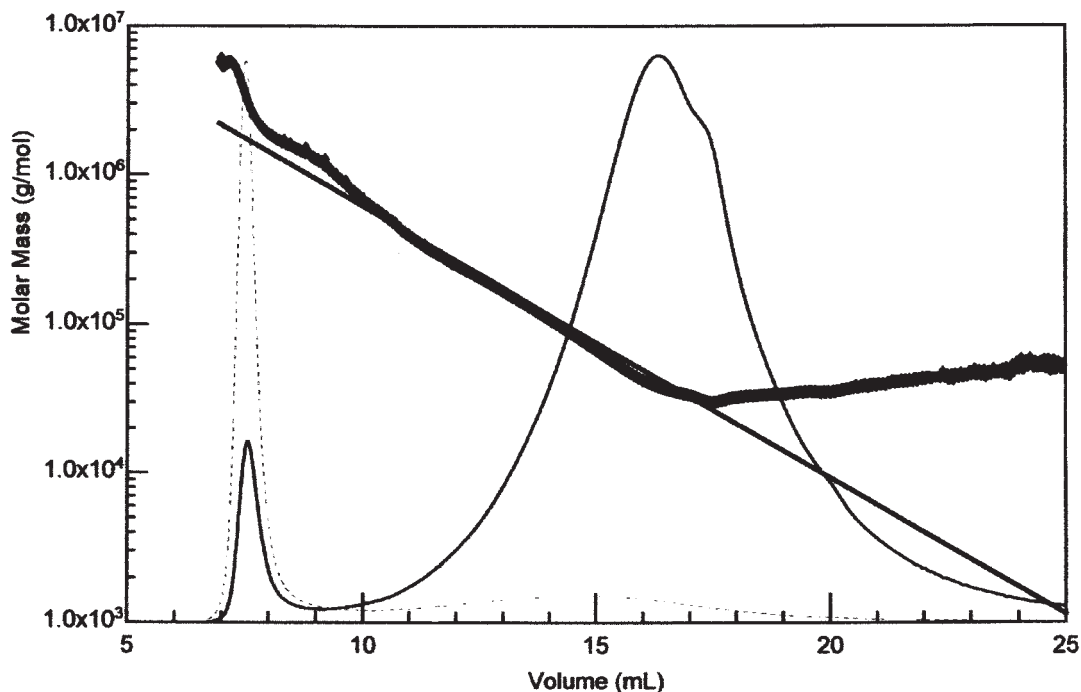


Figure 5 SEC RI elution profiles (—) of Copo VP 5 overlaid with (■) the calculated mass-average molar mass and (---) MALLS at a 90° angle.

main part of which is eluted well before V_{tot} . The fraction of the excluded particles is very low because only a slight RI signal can be detected at 7.8 mL. However, they show molar masses that are much higher than the major component of the copolymer. Figure 4(a) shows that Copo VP 3, 4, 5, and 6 have intermediary elution behaviors between the ones described for Copo VP 2 and 7 because, for instance, Copo VP 2 and 3, having mole fraction (F_a) values larger than 0.45, have elution profiles showing a tailing of the low molar mass peak and a rather intense exclusion peak. Thus, the change in the SEC profiles observed for Copo VP is closely related to the chemical composition of the macromolecules, suggesting that the MM of the copolymer is strongly correlated to the F_a . As mentioned above, β W7 MAHP consists of a complex mixture of at least 60 identified HP β -CD derivatives involving mono-, di-, and tri-methacryloyl substituted CDs, so that, depending on the monomer feed, various macromolecular architectures may be produced.

The calculated logarithmic plot of the MM versus the elution volume for Copo VP 5 is displayed with corresponding RI and MALLS (90°) signals in Figure 5 as an example. The two signals do not overlap because of the different type of response of both detectors. (An RI detector is only proportional to the concentration, whereas a MALLS detector is proportional to both the molar mass and concentration.) The calibration curve presents two distinct linear portions that are not parallel, revealing that the two polymer fractions belong

to different families of polymers that differ not only in molar mass but also in conformation.²⁷ Nevertheless, the overall evolution of log MM against the elution volume may be considered as linear in the elution volume range from 7.8 to 17.5 mL, indicating that any part of the sample eluted within the SEC window is separated according to size (hydrodynamic volume). A double logarithmic plot of $\langle r_g^2 \rangle^{0.5}$ versus MM allows the extraction of information on the macromolecular conformation. Spherical macromolecules should yield a linear dependence with a slope value of 0.33; if the macromolecules are rods, then it should be unity; whereas for random coils in a good solvent, a value between 0.5 and 0.6 should be calculated. A log-log plot of $\langle r_g^2 \rangle^{0.5}$ versus MM for the Copo VP 2 sample is shown in Figure 6, where the second eluted peak is not included. A straight line with a slope of 0.39 is observed. This slope is lower than that expected for a random coil, confirming that the high molar mass fraction has a compact structure corresponding to microgels. The slopes calculated from the excluded fractions of other Copo VPs were consistent with this 0.39 value, indicating that similar polymer microstructures are formed. Accurate estimates of the slope for the low molar mass fractions were rather difficult because of the weak MALLS signal and tailing at low molar mass beyond V_{tot} . Thus, the evaluated elution did not cover the entire range of eluted samples, so the data were not representative of the entire eluted sample. A general trend was that the treatment of this part of the chromatograms gave slope values higher than 0.39,

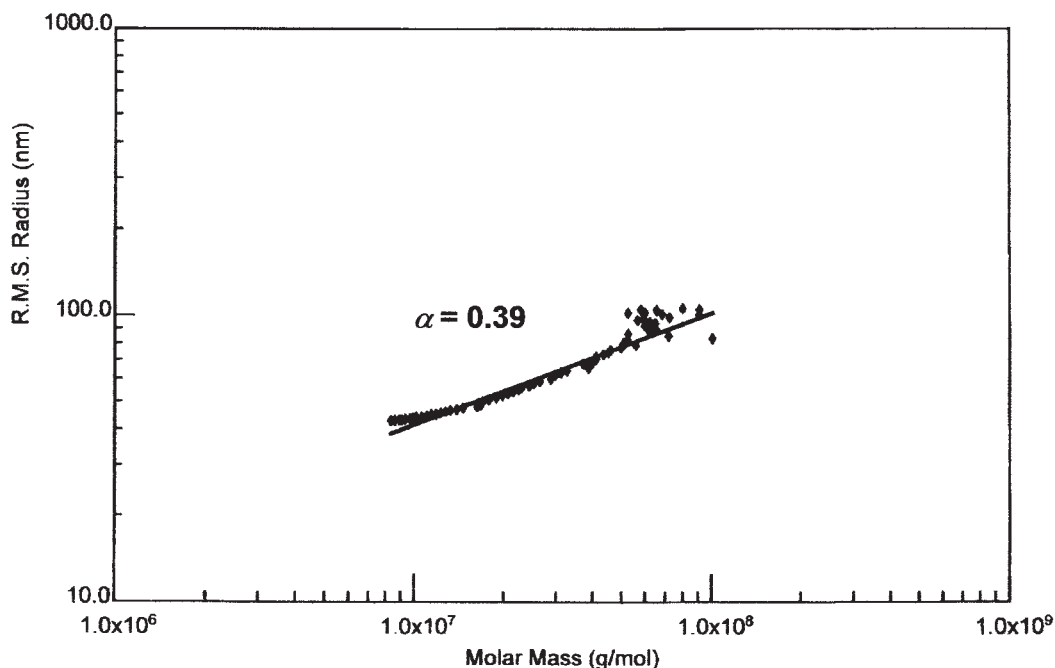


Figure 6 A double logarithmic plot of the root mean square radius ($\langle r_g^2 \rangle^{0.5}$) as a function of the mass-average molar mass (MM) obtained for the high molar mass eluted fraction of Copo VP 2. The slope of the regression line is 0.39.

and a slope value close to the theoretical predictions of 0.58 for a linear random coil polymer was obtained for Copo VP 7. These findings suggest that the low molar mass polymer fraction of Copo VP has a less compact microstructure compared to the excluded one and may comprise weakly branched macromolecules.

Additional information is provided by the cumulative weight fraction versus the mass-average molar mass determined for both copolymers of the series (Fig. 7). From the cumulative weight fraction of Copo VP 7 it is obvious that the sample has a molar mass distribution extending down to the oligomeric range

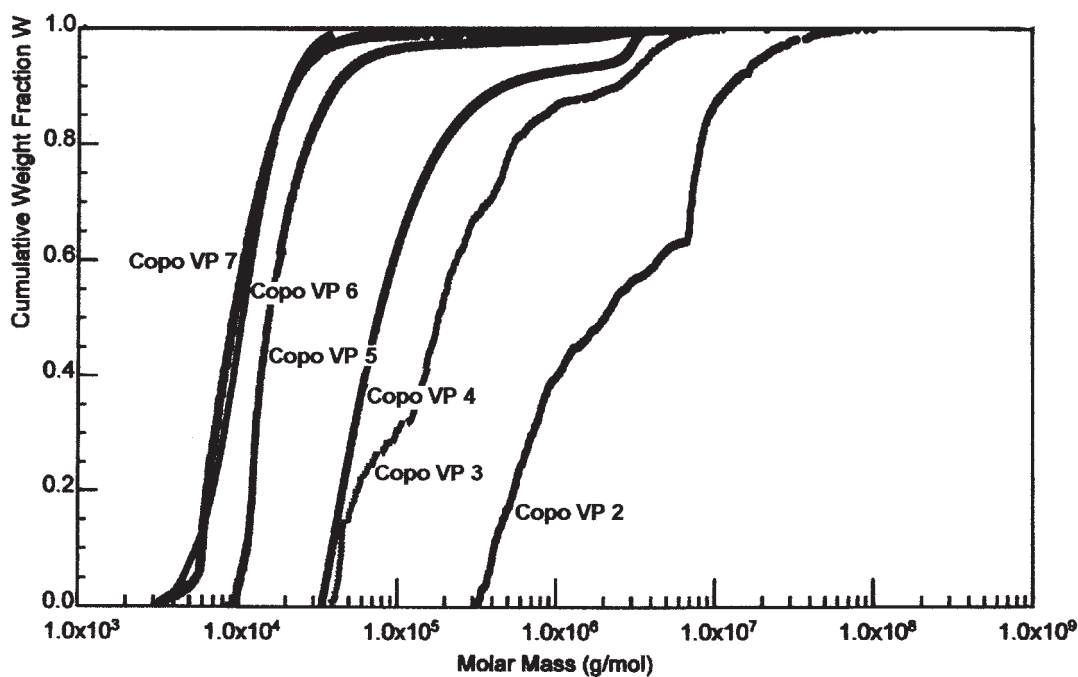


Figure 7 A plot of the cumulative weight fraction calculated for Copo VP as a function of the log molar mass.

TABLE III
Mass of Copo VP Adsorbed (M_p), Monomer Concentration, Surface Area (S), and Porous Volume (V_p) Determined for Silica Stationary Phases Coated with Copo VP

Copo VP	dn/dc	MM (g mol^{-1})	I_p	Recovery (wt %)
2	0.141	5.47×10^6	5	0.70
3	0.143	6.64×10^5	5.5	0.63
4	0.144	3.03×10^5	4.1	0.53
5	0.149	6.79×10^4	4.1	0.97
6	0.150	3.61×10^4	3.5	0.96
7	0.151	2.46×10^4	2.7	0.95

because compounds having MM values of about 3000, 4500, and 6000 g mol^{-1} corresponding to dimers, trimers, and tetramers, respectively, can be detected. Copo VP 7 revealed a rather sharp distribution ranging from approximately 3×10^3 to 3×10^4 g mol^{-1} with more than 60 wt % of the material having an MM of $<1 \times 10^4$ g mol^{-1} . By comparison, Copo VP 2–4 exhibited a wide distribution, as evidenced by a larger index of polymolecularity (Table III), ranging, for instance, from about 3×10^5 to 3×10^7 g mol^{-1} in the case of Copo VP 2. Furthermore, Figure 7 shows a fast change in the cumulative molar mass distribution profiles of β W7 MAHP rich copolymers, indicating the presence of two polymeric components identified as a high molar mass structure and a mixture of low molar mass materials. This finding is in agreement with the SEC profiles depicted in Figure 4. The weight percentage of microgels in each copolymer and the logarithmic plot of the MM are displayed in Figure 8(a,b) as a function of the mole fraction of β W7 MAHP in the copolymers. Quasiexponential growth was obtained for the weight percentage of microgels whereas a linear dependence with a positive slope was found for the log MM versus F_a . These data reveal the occurrence of an extensive crosslinking reaction as the β W7 MAHP content increases, leading to the formation of microgels that increase the mass-average molar mass to a great extent.

The areas under the RI signals were integrated, and the sample recoveries were calculated (Table III). Over 95% recovery was obtained for Copo VP 5–7 samples, indicating that little polymer material was adsorbed onto the column packing. Lower recovery (around 60%) was obtained for Copo VP 2–4. A relevant explanation might be that the copolymers that exhibit a major exclusion peak were not completely dissolved during chromatographic solutions preparation, so that larger aggregates were retained during filtration carried out before the solution was injected. Thus, macromolecular features determined for Copo VP 2–4 may be solely representative of the soluble part; hence, there may be an underestimation of the MM. By con-

trast, as attested by the low molar mass tailing observed for these polymers, residual adsorption on the stationary phase cannot be excluded.

HPLC evaluations

Direct correlation between the physicochemical characteristics of Copo VP and the chromatographic properties is rather difficult because the observed enantioselectivities are mainly governed by the physicochemical properties of the CSPs. The main properties of the CSPs are recalled in Table III. The chromatographic measurements were carried out at ambient temperature with a 1 mL min^{-1} flow rate and a water/methanol (85/15) mixture as the mobile phase. In many CD complex forming applications, the correlation between the structure of both host and guest molecules and the selectivity is a key parameter. Si

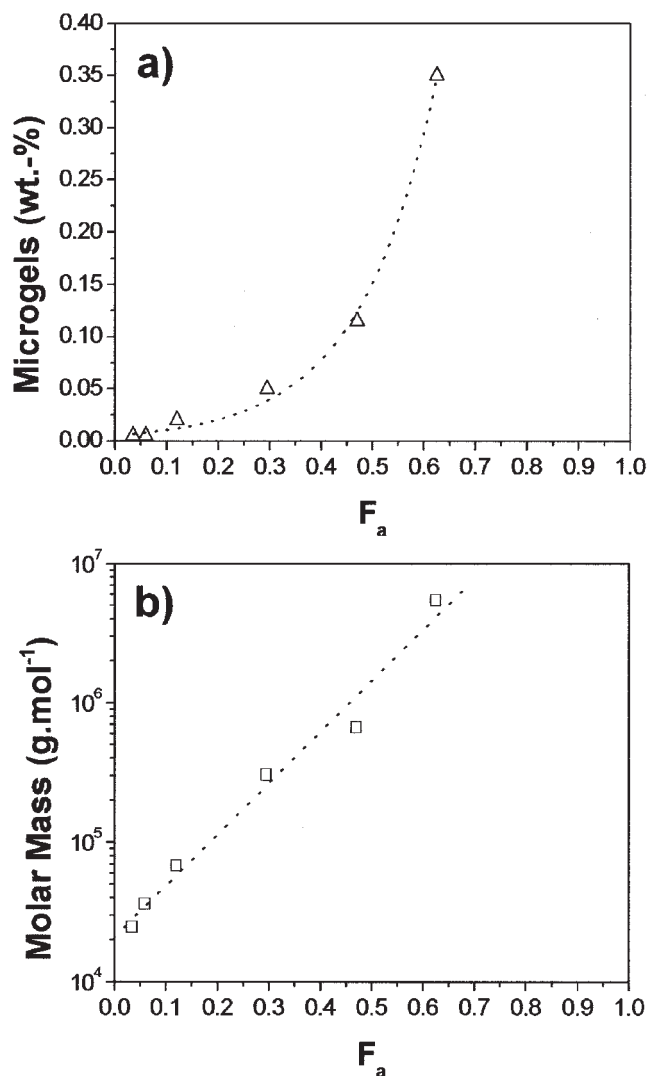


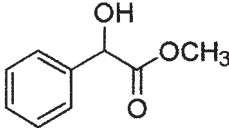
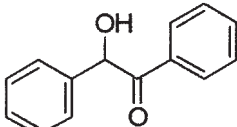
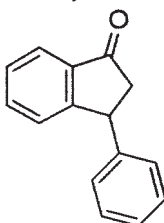
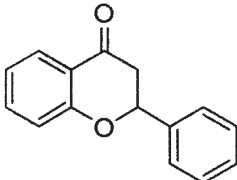
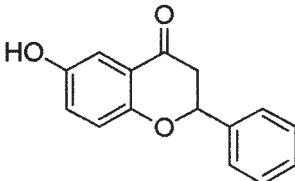
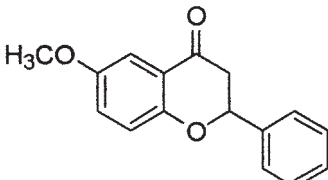
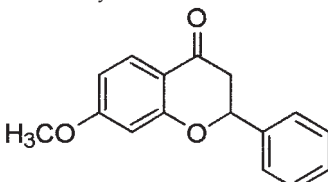
Figure 8 The plot of (a) the microgel weight fraction and (b) the log molar mass calculated for Copo VP as a function of the β W7 MAHP mole fraction (F_a) of Copo VP.

Copo VP 5, combining the greatest amounts of β W7 MAHP and mass of adsorbed copolymer, was used to estimate the chiral separation ability of Copo VP based CSPs toward varied guest molecules. The retention factors (k), separation factors (α), and resolutions (R_s) obtained for the test compounds are gathered in Table IV, where the k values given for 3-phenyl-1-indanone and the four flavanone derivatives correspond to the first eluted peak. Methylmandelate, which is the smallest of the evaluated compounds, was not resolved and exhibited low retention factors. Larger retention factors were obtained for benzoin and 3-phenyl-1-indanone and (S)-benzoin was eluted slightly before the (R)-enantiomer. The use of larger solute molecules, such as compounds belonging to the flavanones family, led to a substantial improvement of both chromatographic parameters. Flavanones are complex molecules possessing many moieties that are able to provide multiple sites of interaction with CD.³⁰ Baseline resolution ($R_s > 1.5$) and greater retention were achieved for the three monosubstituted flavanones, indicating that substituents in position 6 or 7 on the fused ring induce selective secondary interactions with the CD selector.³¹ It appears that Si Copo VP exhibits an extent of enantioselectivity toward the solute having between two and four rings with at least one being aromatic, as previously reported for other 2-hydroxypropyl β -CD based CSPs.^{10,32}

Influence of mass of adsorbed copolymer and β W7 MAHP content

Si Copo VP 7 (134 mg g⁻¹) exhibited greater chromatographic properties than Si Copo VP 2 (38 mg g⁻¹), which is expressed by greater resolutions and shorter retention factors. In addition, Si Copo VP 2 gave rise to serious peak broadening (Fig. 9). These findings indicate that larger amounts of copolymers adsorbed onto the silica surface provide CSPs with greater resolving power. Regardless of the mass of Copo VP adsorbed, chromatograms obtained with Si Copo VP 5 and Si Copo VP 7 enable a comparison of the effects of the β W7 MAHP concentration. As expected, the higher the CD content, the greater are the retention factors and resolutions. It was also found that when increasing the flavanone concentration of the injected solution up to 10⁻³ M, the enantioselectivity on Si Copo VP 7 was lost but $R_s = 1.08$ was obtained on Si Copo VP 5. Retention factors were found to decrease slightly (about 10%) for both CSPs. Note that both the conformation and microstructure of the adsorbed copolymer may influence the chromatographic behavior, because it is known that crosslinked structures may exhibit serious mass transfer limitations and erratic performance.^{28,33}

TABLE IV
Retention Factors (k) Separation Factors (α), and Resolutions (R_s) Obtained on Si Copo VP 5 Column for Test Compounds

Compounds	k	α	R_s
Methylmandelate (S) (R)	0.68 0.68	1	—
			
Benzoin (S) (R)	6.12 6.63	1	—
			
3-Phenyl-1-indanone	7.69	1	—
			
Flavanone	14.27	1.24	1.42
			
6-Hydroxyflavanone	21.73	1.33	1.71
			
6-Methoxyflavanone	18.96	1.33	1.61
			
7-Methoxyflavanone	20.32	1.31	1.57
			

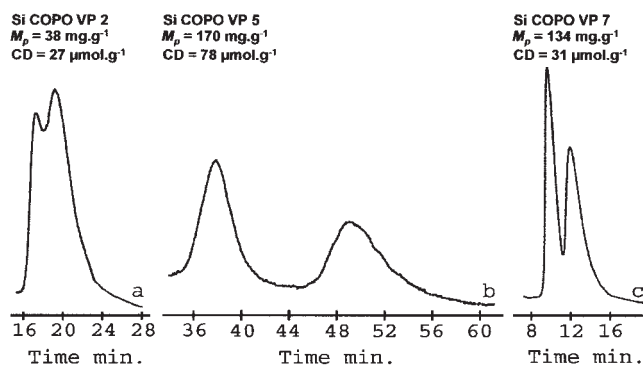


Figure 9 The enantiomeric separation of 6-hydroxyflavanone on (a) Si Copo VP 2, (b) Si Copo VP 5, and (c) Si Copo VP 7 columns.

CONCLUSION

A series of water-soluble hydroxypropyl- β -CD-containing copolymers was prepared by free radical copolymerization of a multifunctional methacryloyl- β -CD monomer with VP. SEC-MALLS revealed that, by varying the monomer feed, copolymers with varied molar masses and macromolecular architectures are produced. The trend toward the formation of larger molar mass polymer materials having a compact microstructure (microgels) with increasing β -CD monomer F_n was clearly evidenced. From the HPLC evaluation, it clearly appeared that structural features of the guest molecule play a key role in retention and chiral separation processes. Although it is not possible to draw conclusions on the chiral recognition mechanism, it seems that Copo VP based CSPs are well designed for the resolution of enantiomers that possess specific features such as a polycyclic structure, a chiral carbon atom included within the cyclic structure, a phenyl group attached to the asymmetric carbon, and a hydrogen bond forming substituent adjacent to the chiral center.

References

- Szejtli, J.; Osa, T. *Comprehensive Supramolecular Chemistry*; Pergamon: Oxford, UK, 1996; p 3.
- Szejtli, J. *Cyclodextrin Technology*; Kluwer Academic: Dordrecht, 1988.
- Lee, C. H. *J Appl Polym Sci* 1981, 26, 489.
- Armstrong, D. W.; Jin, H. L. *Anal Chem* 1987, 59, 2237.
- Miyata, T.; Iwamoto, T.; Uragami, T. *Macromol Chem Phys* 1996, 197, 2909.
- Yanagioka, M.; Kurita, H.; Yamaguchi, T.; Nakao, S.-I. *Ind Eng Chem Res* 2003, 42, 380.
- Croft, A. P.; Bartsch, R. A. *Tetrahedron* 1983, 39, 1417.
- Khan, A. R.; Forgo, P.; Stine, K. J.; D'Souza, V. T. *Chem Rev* 1998, 98, 1977.
- Thuaud, N.; Sébille, B.; Deratani, A.; Lelièvre, G. *J Chromatogr* 1991, 555, 53.
- Carbonnier, B.; Janus, L.; Deratani, A.; Morcellet, M. *e-Polymers* 2003, Article 4 [Internet only journal].
- Renard, E.; Deratani, A.; Volet, G.; Sebille, B. *Eur Polym J* 1997, 33, 49.
- Cserhati, T.; Fenyvesi, E.; Szejtli, J. *J Incl Phenom* 1992, 14, 181.
- Cserhati, T.; Forgacs, E. *J Chromatogr* 1994, 660, 313.
- Seo, T.; Kajihara, T.; Iijima, T. *Makromol Chem* 1987, 188, 2071.
- Weickenmeier, M.; Wenz, G. *Macromol Rapid Commun* 1996, 17, 731.
- Suh, J.; Lee, S. H.; Zoh, K. D. *J Am Chem Soc* 1992, 114, 7916.
- Harada, A.; Furue, M.; Nozakura, S. *Macromolecules* 1976, 9, 701.
- Liu, Y.-Y.; Fan, X.-D.; Gao, L. *Macromol Biosci* 2003, 3, 715.
- Reuscher, H. BETA W7 MAHP, Technical Data Sheet; Wacker Chemie GmbH: Munich, Germany, 1996.
- Janus, L.; Carbonnier, B.; Morcellet, M.; Ricard, G.; Crini, G.; Deratani, A. *Macromol Biosci* 2003, 3, 198.
- Janus, L.; Carbonnier, B.; Deratani, A.; Bacquet, M.; Crini, G.; Laureyns, J.; Morcellet, M. *New J Chem* 2003, 27, 307.
- Jue, C. K.; Lipke, P. N. *J Biochem Biophys Methods* 1985, 11, 109.
- Rohrbach, R. P.; Rodriguez, L. J.; Eyring, E. M.; Wojcik, J. F. *J Phys Chem* 1977, 81, 944.
- Podzimek, S. *J Appl Polym Sci* 1994, 54, 91.
- Zimm, B. *J Chem Phys* 1948, 16, 1093.
- Prochazka, O.; Kratochvil, P. *J Appl Polym Sci* 1986, 31, 919.
- De Gennes, P. G. *Scaling Concepts in Polymer Physics*; Cornell University Press: Ithaca, NY, 1979.
- Carbonnier, B.; Janus, L.; Lekchiri, Y.; Morcellet, M. *J Chromatogr Sci* 2004, 42, 37.
- Janus, L.; Crini, G.; El-Rezzi, V.; Morcellet, M.; Cambiaghi, A.; Torri, G.; Naggi, A.; Vecchi, C. *React Funct Polym* 1999, 42, 173.
- Krause, M.; Galensa, R. *J Chromatogr* 1990, 514, 147.
- Ng, S.-C.; Ong, T.-T.; Fu, P.; Ching, C.-B. *J Chromatogr A* 2002, 968, 31.
- Stalcup, A. M.; Chang, S.-C.; Armstrong, D. W.; Pitha, J. *J Chromatogr* 1990, 513, 181.
- Vanecek, G.; Regnier, F. E. *Anal Biochem* 1982, 121, 156.