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Note

Isolation by preparative chromatography and characterization of styrene oligomers from 1 to 18 and their application in pore size distribution mination

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The pore size distribution (PSD) determines the exclusion properties of a sorbent in liquid chromatography. Hence it is of importance to know the PSD when choosing a stationary phase to be used in an optimal chromatographic separation of large molecules, both when size exclusion is the chosen method and when, *e.g.*, reversed-phase chromatography is to be used. In the latter instance a substantial part of the active surface must be situated in pores of sufficiently large size to allow the penetration of the solutes.

When the sorbent geometry is changed considerably, *e.g.*, by chemically bonding a substituent or by hydrothermal treatment, it is of equal importance to know the PSD. Changes in the pore size range of 10 to 60 Å are especially interesting because modification by chemical bonding mostly influences this range. With hydrothermal treatment the aim is often to reduce the number of pores in this range.

It is therefore of great importance to have a detailed knowledge of the lower PSD of both native and substituted sorbents. A method based on size exclusion chromatography (SEC) developed by Halász and co-workers[1–4] offers this possibility, as it has been pointed out that PSD determinations of chemically modified silica sorbents made by capillary condensation of nitrogen at low temperatures can hardly offer valid results. However, the choice of commercial polystyrene standards with molecular weights to cover the range from 10 to 60 Å is restricted. These standards are products with a low degree of polymerization.

In order to obtain a sufficient number of styrene oligomers to allow a detailed PSD determination from 10 to 60 Å, three commercial standards were separated into single components by preparative non-aqueous reversed-phase chromatography. This method has been described previously[5–7], but only on an analytical scale. Di- and tristyrene have been prepared for NMR analysis of diastereoisomers by normal-phase chromatography[7].

In this investigation, styrene oligomers from $n = 1$ to 18 were isolated with a purity from 95 to 99.5% as determined by analytical high-performance liquid chromatography (HPLC). The identification was made by mass spectrometry and HPLC. In addition, the utility of single styrene oligomers in PSD determinations has been demonstrated by an example comparing a silica product before and after hydrothermal treatment[8–10].

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EXPERIMENTAL

Materials

Polystyrene standards A-300, A-500 and A-1000 were obtained from Toyo Soda (Tokyo, Japan). Polystyrene standard of M_w 2400 daltons was purchased from Waters Assoc. (Milford, MA, U.S.A.). Twenty-one polystyrene standards with M_w ranging from $3.6 \cdot 10^3$ to $4.48 \cdot 10^6$ daltons were obtained from Toyo Soda and Waters Assoc. Dichloromethane was of pro analysi grade from Merck (Darmstadt, G.F.R.). Methanol was of analytical-reagent grade from May & Baker (Dagenham, Great Britain) and was distilled before use. ODS(Me_2)-silica was prepared in the laboratory from LiChrosorb Si 100, 10 μm (Merck). ODS(Me_2)SiCl was prepared in the laboratory by Grignard reaction. Silica Si 60 was obtained from Merck.

Analytical HPLC

Polystyrene A-300, A-500, A-1000 and PST-2400 were separated on ODS(Me_2)-silica in a Spectra-Physics SP 8000 B liquid chromatograph, equipped with a Spectra-Physics SP 8400 variable-wavelength UV detector set for detection at 260 nm. The column (25 cm × 0.4 cm I.D.) was packed with ODS(Me_2)-silica. Injections of 10 μl containing approximately 100 μg of polystyrene mixture and 10–100 μg of single components were made. For the separation of A-300 and A-500, 3% of dichloromethane in methanol was used, and for A-1000 and PST-2400 a linear gradient from 3 to 10% and from 3 to 40%, respectively, of dichloromethane in methanol.

Preparative HPLC

The preparative chromatograph consisted of a Jobin Yvon column (4 cm I.D.), modified with a home-made injection system, consisting of a Whitey six-port valve with a 5-ml PTFE loop, a Lewa FD pump operated at 20 ml/min, a Uvicord S set for detection at 254 nm, a pressure transducer to record pressure during runs and a W + W recorder. The column was packed with 135 g of ODS(Me_2)-silica, the same material used for the analytical HPLC. Polystyrene A-300 portions of 1.4 g were dissolved in a small volume of dichloromethane, made up to 5 ml with methanol and injected on to the column. Elution was effected with 3% dichloromethane in methanol. Fractions were collected, cut in the valleys, the solvent was removed and the oligomers were further purified by rechromatography under the same conditions. Portions of 0.5–1 g of A-500 were processed in the same way.

It was found to be impossible to treat A-1000 in the same way. Instead, 3.5-g portions were eluted with a 2.5% step gradient from 0 to 40% of dichloromethane in methanol, simulating the linear gradient of the analytical work. The resulting fifteen fractions were concentrated and rechromatographed under the same conditions, and oligomers from $n = 3$ to 18 were isolated.

The purity of the oligomers was checked by analytical chromatography.

Determination of pore size distribution

The method of Halász and Martin[1] was used with minor modifications. The use of a greater number of standards did not allow the simultaneous injection of toluene, a high-molecular-weight standard and the standard to be tested. Instead, toluene was used as internal standard for polystyrenes of $M_w > 1.67 \cdot 10^4$ daltons and polystyrene of $M_w = 1.26 \cdot 10^6$ for polystyrenes of $M_w \leqslant 1.67 \cdot 10^4$ daltons.

Mass spectrometry

In order to identify the isolated products, mass spectrometry was performed on fractions 4 and 6.

Hydrothermal treatment

Silica Si 60 was refluxed for 24 h in 0.7 *M* sodium sulphate solution. The suspension was filtered and the silica washed with water, 6 *M* hydrochloric acid, water and acetone and dried under vacuum.

RESULTS AND DISCUSSION

Isolation and characterization of the oligomers

Analytical and preparative chromatograms of A-300 and A-500 are shown in Fig. 1a–d. Comparing Fig. 1a and 1c, it can be seen that a 200-fold increase in the load/column cross-sectional area did not severly impair resolution. Close to baseline separation of all components was achieved. Further, it can be seen that it is ad-

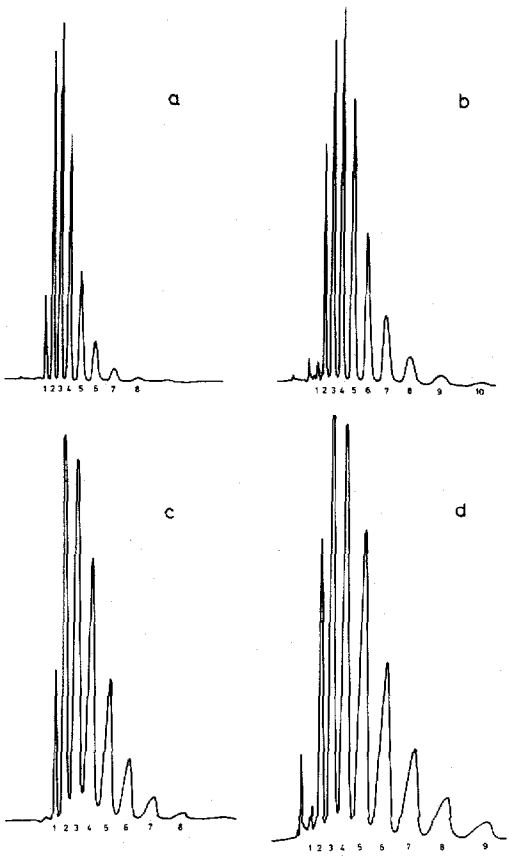

Fig. 1. Chromatograms of A-300 and A-500 polystyrene. Detection: UV at 260 nm. (a) A-300, analytical, *ca.* 100 μg; (b) A-500, analytical, *ca.* 100 μg; (c) A-300, preparative, 1.4 g; (d) A-500, preparative, 1 g. Mobile phase: methanol–dichloromethane (97:3). Numbers denote degree of polymerization.

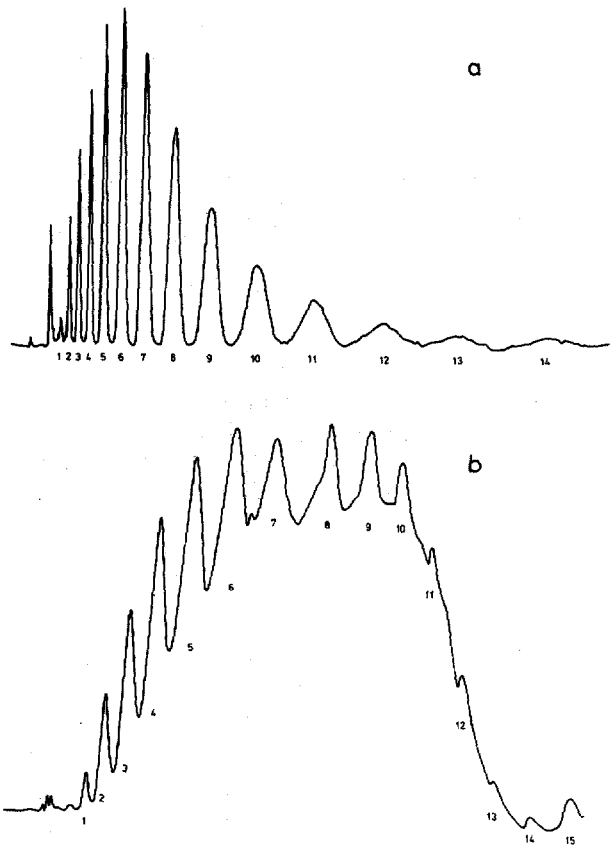

Fig. 2. Chromatograms of A-1000 polystyrene mixture. Detection: UV at 260 nm. (a) Analytical, *ca.* 100 μg, mobile phase methanol–dichloromethane gradient from 97:3 to 90:10; (b) preparative, 3.5 g, mobile phase methanol–dichloromethane gradient from 100:0 to 60:40. Numbers in (a) denote degree of polymerization, in (b) fractions.

vantageous to use the same stationary phase for both the analytical and the preparative column as results obtained analytically can be transferred directly to the preparative scale if the separation is simple, as in this instance. For A-300 a 15,000-fold increase in the amount injected was possible. The same observations are valid for the A-500 separations, although in this instance the increase in load/column cross-sectional area was 150-fold. However, the A-1000 separation was different. Owing to the increased amount of higher oligomers compared with A-300 and A-500, which differ only slightly in composition, it was not possible to use a simple isocratic solvent to achieve the separation.

Fig. 2a shows A-1000 eluted by a gradient from 3 to 10% of dichloromethane in methanol. When this system was tried on the preparative scale severe overlapping peaks was observed, and a very long run time was necessary to elute the mixture. Other systems were tried (not shown) with no more success. In the end we used a step gradient from 0 to 40% of dichloromethane in methanol, overloaded the column heavily (3.5 g per 125 g of stationary phase) and separated the product in fractions

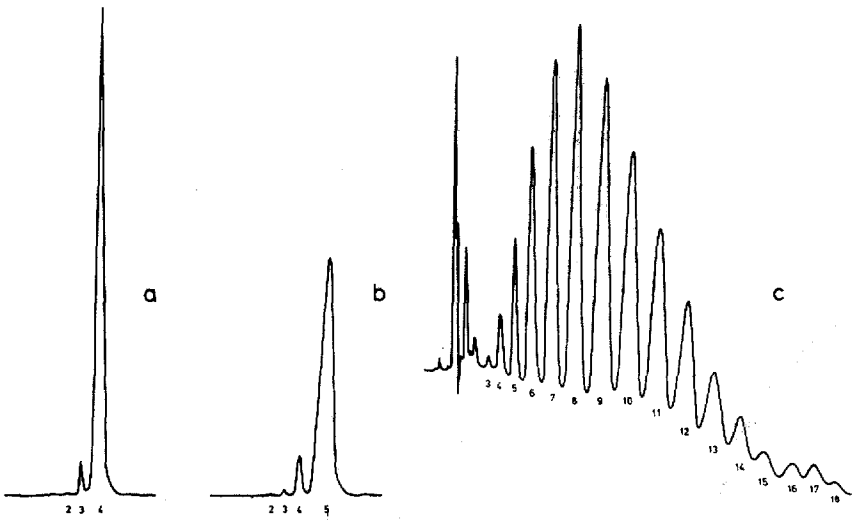

Fig. 3. Rechromatography of fractions isolated from A-1000 (Fig. 2b). Detection: UV at 260 nm. (a) Fraction 2; (b) fraction 3; (c) fraction 15. Mobile phase: methanol–dichloromethane gradient from 100:0 to 60:40. Numbers denote degree of polymerization.

determined by the valleys, as shown in Fig. 2b. In this way fifteen fractions were collected. These in turn were rechromatographed, using the same system, as shown by three examples in Fig. 3a–c.

In Fig. 3c the drift in the baseline is due to the gradient and is observed because of a higher detector sensitivity than in Fig. 3a and b. That all oligomers are present in fraction 15 is a result of the severe tailing observed in Fig. 2b.

By analytical chromatography the components from A-300, A-500 and A-1000 were classified and identical fractions were pooled. In this way eighteen fractions were obtained. The data provided by the manufacturer made a tentative identification possible. In A-300 the most abundant component should be the trimer, in A-500 the tetramer and in A-1000 the octamer.

From these data the peaks in the analytical and preparative chromatograms were identified. However, in order to allow identification of oligomers higher than $n = 14$, PST-2400 was chromatographed as shown in Fig. 4. More than 30 components could be distinguished. By comparison with A-300, chromatographed in the same system, the peaks in Fig. 4 were identified.

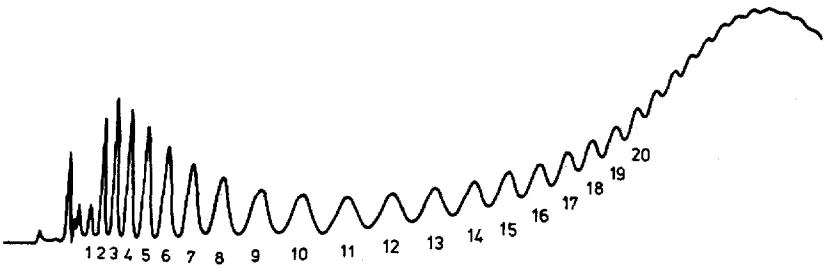

Fig. 4. Chromatogram of PST-2400 (100 μg). Detection: UV at 260 nm. Mobile phase: methanol–dichloromethane gradient from 97:3 to 60:40. Numbers denote degree of polymerization.

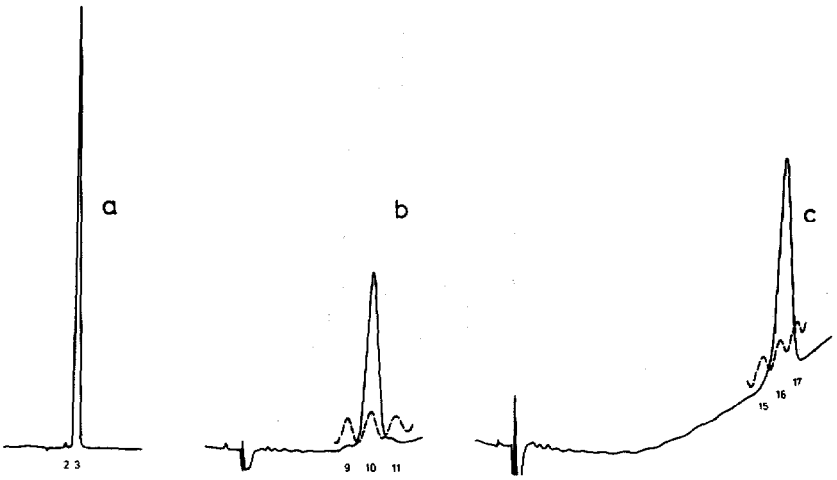

Fig. 5. Chromatograms of isolated styrene oligomers. Detection: UV at 260 nm. (a) Butyltristyrene, 10 μg; (b) decatetrastyrene, 50 μg; (c) butylhexadecastyrene, 100 μg. Mobile phase: methanol–dichloromethane gradient from 97:3 to 60:40. Dashed lines represent positions of relevant peaks from PST-2400. Numbers denote degree of polymerization.

In turn, all separated components were then injected with PST-2400 as the internal standard. In this way it was shown that the identification made on basis of the preparative separation agreed with the analytical method using PST-2400 as the internal standard. However, to obtain a more unequivocal proof of the identity, the components classified as the tetramer and the hexamer were analysed by mass spectrometry, and the identification was confirmed.

Thus it was possible to isolate all oligomers from $n = 1$ to 18.

The purity was tested by analytical HPLC and three examples are shown in Fig. 5. In this way it was shown that most of the oligomers were more than 98% and all more than 95% pure. It must be pointed out that the separation of diastereomers was not intended. The purity obtained was sufficient for our purpose, *viz.*, application as standards in the determination of pore size distribution by size exclusion chromatography.

Determination of pore size distribution

Together with the isolated oligomers, toluene and 21 polystyrene standards were used for PSD determinations. The advantage of using the single oligomers was evident on comparing the chromatograms of A-300, A-500 and A-1000 with those of the trimer, the tetramer and the octamer, respectively. The latter showed much narrower peaks, as peak broadening was due solely to the chromatographic system and not to dispersity of the standards[4,11].

PSD determinations were made on Si 60 before and after hydrothermal treatment. The pore volume is changed in the process, and thus plots of K_{av} *versus* pore size are not very informative. The results were consequently calculated, expressing the cumulative specific pore volume as a function of the pore size.

In order to obtain maximum information on the changes in the pore structure caused by the hydrothermal treatment, the difference in the cumulative specific pore volume between hydrothermally treated Si 60 and native Si 60 was plotted against the pore size (Fig. 6). In this plot a positive slope represents an increase in specific poor volume and a negative slope a decrease. This plot has the further advantage that the

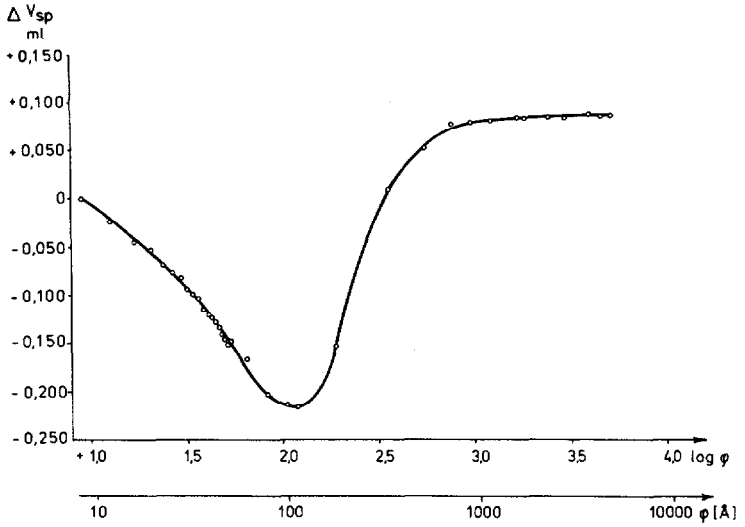

Fig. 6. Difference in cumulative specific pore volume between hydrothermally treated Si 60 and native Si 60 as a function of pore size.

error in the PSD effected by points of contact between particles[12] should be eliminated, as the particle size is 20 μm in both instances. It can be seen that pores between 100 and 1000 Å have become more abundant, whereas pores between 10 and 100 Å have become less abundant. From Fig. 6 it is evident that the use of the isolated oligomers was advantageous. The exclusive use of commercial PST standards would allow only an imprecise estimate of the first part of the curve.

The mean pore diameter determined by the usual K_{av} *versus* log ∅ plot showed that the mean pore diameter was changed from 60 Å for Si 60 to 145 Å for the hydrothermally treated material.

An investigation of hydrothermal treatment as a means of effecting controlled changes in the pore structure of silica is in progress.

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