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# Effects of Surface Area and Pore Size upon Liquid Chromatographic Fractionations of Polystyrene Oligomers Using Ethylphenyl-Derivatized Silicas

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# Effects of Surface Area and Pore Size upon Liquid Chromatographic Fractionations of Polystyrene Oligomers Using Ethylphenyl-Derivatized Silicas

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## Abstract

Oligomers of 800 and 2200 MW polystyrene have been separated on ethylphenyl-derivatized silicas. This study examined the effects of surface area, pore size, sample loading, and functionality of the silica surface upon oligomer separations. Particle size, endcapping, and polymerization of the silane were held constant. Silicas having small pores and high surface area yielded the best separation. Also, ethylphenyl was better than *n*-octyl for separating 800 MW polystyrene oligomers.

## INTRODUCTION

There is great interest in separating oligomers of polymers. Due to their unique properties, commercial preparations of polymers can provide demanding tests of chromatographic systems (*1*). An earlier study in our laboratory demonstrated that resolutions of oligomers of 800 MW polystyrene could be achieved to different extents using various chemically bonded phases (*2*). A phenyl pellicular packing (37–75 μm) gave the poorest resolution. Later, using a Macherey-Nagel 7.5-μm phenyl packing, the 800-MW polystyrene oligomers were resolved to nearly the extent as when an octadecyl stationary phase was used. For that reason, the present study was directed toward the effects of pore size and surface area on resolution.

Other studies have investigated the effects of surface area and pore size upon polystyrene oligomer separations. Hsu et al. (*3*) have investigated

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octadecyl-derivatized silicas of different average pore sizes using 800 MW polystyrene as the probe sample. That work showed how sample loading affected the resolution of the polystyrene oligomers and the baseline of the chromatogram. Recently, a somewhat more theoretical approach (*4*) than the present study was published. It also addressed the effects of pore size and surface area upon polystyrene separations, but our study examines the variables in a different way.

In the early stages of the present study, we employed a 7.5-μm isopropylphenyl-bonded phase. In contrast to the earlier results with a phenyl phase, this packing yielded an excellent separation very similar to that obtained using an octadecyl phase. Those results showed that factors other than organic functionality of the silica surface were important in the separation process. As a result, the present study attempted to control parameters such as particle diameter, endcapping, and polymerization of the silane on the silica surface while exploring effects of pore size, surface area, sample loading, and functionality of the silica surface. Most of the study was done using 800 MW polystyrene although a few fractionations of a 2200-MW polystyrene sample were also performed.

## EXPERIMENTAL

### Chemicals and Reagents

LiChrospher silicas for preparation of bonded phases were obtained from Alltech Associates (Norcross, Georgia). The properties of these silicas are shown in Table 1. Phenethyldimethylchlorosilane and *n*-octyldimethylchlorosilane were obtained from Petrarch Systems Inc. (Bristol, Pennsylvania). Trimethylchlorosilane was received from PCR Research Chemicals (Gainesville, Florida).

TABLE 1
Nominal Properties of the 10-μm Silica Starting Materials

| Packing | Specific surface area ($m^2/g$) | Pore volume (mL/g) |
|---|---|---|
| LiChrospher Si 100 | 250 | 1.2 |
| LiChrospher Si 300 | 250 | 2.0 |
| LiChrospher Si 500 | 50 | 0.8 |
| LiChrospher Si 1000 | 20 | 0.8 |

Monodisperse polystyrene standards (800, 2,200, and 1,800,000 MW) were obtained from Pressure Chemical Company (Pittsburgh, Pennsylvania). All standards were dissolved in pure tetrahydrofuran (THF). The sample concentration was always 2 mg/mL for size exclusion experiments, but other concentrations were used for adsorption studies as indicated in the figure captions.

Hexane and methanol, both J. T. Baker HPLC grade (Phillipsburg, New Jersey), were used as mobile phases. Tetrahydrofuran "Baker Grade" was distilled over potassium to remove the water and the butylated hydroxytoluene antioxidant prior to use. Water was deionized and doubly distilled prior to use as a mobile phase. All mobile phases were filtered through the appropriate Millipore filters (Bedford, Massachusetts), 0.45 $\mu$m cellulose acetate and nitrate (HAWP) for aqueous, 0.50 $\mu$m Teflon (FHUP) for organic, before the mobile phases were degassed with helium prior to use.

Methanol, toluene, diethyl ether, and acetone (all J. T. Baker reagent grade) were used to wash the bonded phases. The toluene for synthetic procedures was dried over anhydrous calcium chloride and stored over sodium. Pyridine (J. T. Baker reagent grade) was distilled over $CaH_2$ and stored over potassium hydroxide. Ethanol (U.S. Industrial Chemical Co., New York, New York), 1,2-dichloroethane (Eastman Kodak), and ethyl acetate (J. T. Baker HPLC grade) were used to activate the silica columns.

## Apparatus

Two Model 8500 pumps (Varian Instrument Group, Palo Alto, California) equipped with a solvent programmer were used to generate gradients. Samples were introduced onto the column by an injection valve Model ACV-6-UHPa-N60 (Valco Instruments Inc., Houston, Texas) with a 5-$\mu$L sample loop and detected using a LC-55 UV-vis detector (Perkin-Elmer Corp., Norwalk, Connecticut) set at 265 nm. Chromatograms were recorded using a Model 585 chart recorder (Linear Instruments Corp., Reno, Nevada).

Columns were packed using a Model 705 stirred slurry packer (Micromeritics Inc., Norcross, Georgia) that was pressurized by a Varian 8500 pump.

## Procedures

Bonded phases were prepared in a manner similar to that described by Berendsen and deGalan (5). Silica gel (4.0 g) was dried in a vacuum oven at

175°C (5 × $10^{-3}$ torr) for 24 h. Then the silica was added to a flame-dried, nitrogen-purged reflux apparatus followed by dry toluene (100 mL), a twofold excess of the silane reagent, and dry pyridine (5 mL). The amount of the silane reagent added was calculated by assuming 4.0 $\mu$mol/$m^2$ of accessible hydroxyl groups. The pyridine served as a catalyst by complexing the liberated HCl to form the pyridinium hydrochloride salt. The silica was refluxed for 96 h at a temperature of 10°C below the boiling point of the most volatile component. The silica was collected and washed successively with 50 mL of each of the following solvents: toluene, methanol, methanol:$H_2O$ (50:50), water, methanol, diethyl ether, and acetone. The silica was then dried in a vacuum oven at 110°C (5 × $10^{-3}$ torr) for 12 h. The product was then endcapped using a fourfold excess of the trimethylchlorosilane reagent (TMCS) in the same manner as that used for the initial silane reagent. The endcapped product was washed successively with 50 mL of each of the following solvents: toluene, methanol, methanol:water (50:50), water, methanol, diethyl ether, and acetone before being dried at 110°C (5 × $10^{-3}$ torr) for 12 h. In cases where the silica was to be hydrolyzed before derivatization, hydrolysis was performed in 150 mL of 0.1 *M* HCl at 90°C for 24 h. However, silica was usually not hydrolyzed since the pore structure may be altered (6).

The derivatized silica was packed into 316 stainless steel tubing, 6.35 mm o.d. × 4.60 mm i.d. (Alltech Associates, Norcross, Georgia). The columns were terminated with 6.35 mm (1/4") to 1.59 mm (1/16") zero dead volume Swagelok reducing unions (Georgia Valve and Fitting, Atlanta, Georgia) having 6.35 mm o.d., 2 $\mu$m stainless steel frits (Alltech). All tubing and reducing unions were washed successively with THF, methanol, water, 3 *N* nitric acid, water, methanol, and acetone before being dried with nitrogen.

In order to pack the bonded-phase columns, the appropriate packing was slurried in 1:1 chloroform:cyclohexane. This slurry was then placed in the bomb and displaced into the column using MeOH at 200–400 bar and the Varian pump. The bonded-phase columns were equilibrated with pure THF and the appropriate mobile phase prior to use. The underivatized silica columns were packed by slurrying the silica in 1 × $10^{-3}$ *M* $NH_4OH$ and packing at 200–400 bar. These silica columns were activated by passing successively through the column prior to use 15–20 column dead-volumes of each of the following solvents: ethanol, acetone, ethyl acetate, 1,2-dichloroethane, and hexane. The specifications of the ethylphenyl columns used in this study are shown in Table 2. The *n*-octyl column was 25 cm long.

For adsorption studies the column was equilibrated with 60:40 THF:$H_2O$ for 30 min prior to an injection. The THF concentration was increased at

TABLE 2
Ethylphenyl Columns

| Column | Silica[a] | Surface area[b] ($m^2/g$) | Length (cm) | Total area ($m^2$) |
|---|---|---|---|---|
| A | LiChrospher Si 100 | 250 | 25 | 620 |
| B | LiChrospher Si 100 | 250 | 5 | 124 |
| C | LiChrospher Si 300 | 250 | 25 | 620 |
| D | LiChrospher Si 300 | 250 | 5 | 124 |
| E | LiChrospher Si 500 | 50 | 25 | 124 |
| F | LiChrospher Si 500 | 50 | 10 | 50 |
| G | LiChrospher Si 1000 | 20 | 25 | 50 |
| H | LiChrospher Si 100 hydrolyzed | 250 | 25 | 620 |

[a] Assume packed density is 0.60 $g/cm^3$.
[b] Manufacturer's specifications.

0.2%/min after sample injection. The flow rate in all adsorption experiments was 1.0 mL/min.

For size-exclusion studies the column was equilibrated with 40 mL of THF before injection. The eluent was 100% THF (isocratic) and the flow rate was 0.25 mL/min.

## RESULTS

### Elemental Analyses

Elemental analyses of the bonded phases are shown in Table 3. Berendsen and deGalan's (5) expressions were employed to calculate the surface coverage in terms of the number of silane molecules bonded per unit surface area. Our phenyl results agreed with theirs, 3.49 $\mu mol/m^2$, and with those of Hemetsberger et al. (7), 3.70 $\mu mol/m^2$. An increase in surface coverage was obtained with wider pore silicas. This increase in coverage could be due either to the ability of the silane to diffuse into the larger pores, to improved accuracy of the elemental analyses for higher carbon values, or to the use of the manufacturer's surface area, which may be inaccurate. The ethylphenyl silane gave a higher surface coverage compared to the *n*-octyl silane under identical experimental conditions.

TABLE 3
Surface Coverages of Bonded Phases

| LiChrospher Silica, 10 | Surface Area[a] ($m^2$/g) | Wt% carbon | $\mu$mol/$m^2$ | mmol/g |
|---|---|---|---|---|
| Si 100 ethylphenyl | 250 | 9.37 | 3.57 | 0.89 |
| Si 100 ethylphenyl hydrolyzed silica | 250 | 8.59 | 3.23 | 0.81 |
| Si 300 ethylphenyl | 250 | 8.45 | 3.18 | 0.79 |
| Si 300 octyl | 250 | 6.91 | 2.54 | 0.63 |
| Si 500 ethylphenyl | 50 | 2.69 | 4.65 | 0.23 |
| Si 1000 ethylphenyl | 20 | 1.36 | 5.77 | 0.12 |

[a] Underivatized silica, surface area values are those of the manufacturer.

## Effect of Hydrolysis

The effect of hydrolyzing the silica prior to bonding was examined. The separations of 800 MW polystyrene oligomers on the hydrolyzed and nonhydrolyzed ethylphenyl silicas are shown in Fig. 1. Both columns gave near-baseline separation of all of the oligomers present in the sample. Retention was somewhat greater on the hydrolyzed silica, but the oligomers were not as well separated due to tailing.

## Comparison of Pore Sizes at Constant Column Length

***Constant Sample Size.*** Figure 2 shows that Columns A and C almost gave baseline separation of the polystyrene oligomers. The greater retention on Column A apparently resulted from the higher carbon loading. Column E provided adequate resolution of the oligomeric mixture for qualitative purposes, but it gave poor baseline resolution. Column G gave no resolution due to the low surface area. A large surface area, high carbon-loading column clearly gave better resolution of the polystyrene oligomers than a low surface area, low carbon-loading column.

***Constant Ratio of Sample Size to Surface Area.*** The poor resolution of the polystyrene oligomers may have been due to overloading the low surface area (low capacity) columns. Therefore, we changed the sample size to match the surface area for the data in Fig. 3. Baseline resolution of 12 oligomers was obtained using Columns A and C whereas Column E resolved

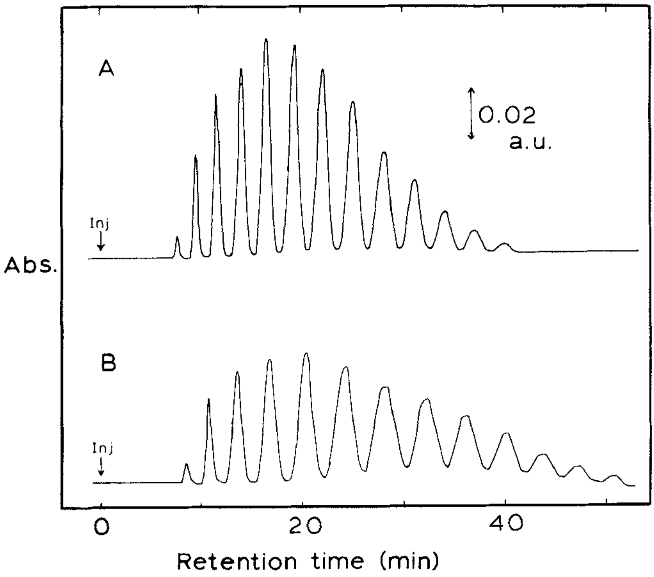

FIG. 1. Effect of hydrolysis of silica using a 100 mg/mL sample of 800 MW polystyrene: (A) Column A and (B) Column H.

only 10 oligomers. A large envelope of poorly resolved species was observed for Column E. Again, Column G provided little or no resolution. Thus, constant sample size per unit surface area did not compensate for the loss of surface area by the wider pore silicas.

## Effect of Pore Size at Constant Surface Area

Bonded-phase columns of different average pore sizes and constant surface area were compared by using columns of different lengths and by assuming uniform packing densities for each of the silicas. Some packings were not studied due to the difficulty of packing columns greater than 30 cm. Columns of about 620 $m^2$/column are compared in Fig. 4. Columns A and C gave baseline separation of the oligomers with retention being greater on Column A. Pore size showed no effect upon this separation, as expected.

The effects of a fourfold decrease in surface area per column compared to Column A are shown in Fig. 5. The oligomers were poorly resolved on Columns B and D. However, 12 oligomers were resolved on Column E. This could be due to the ability of the solute to better interact with the available

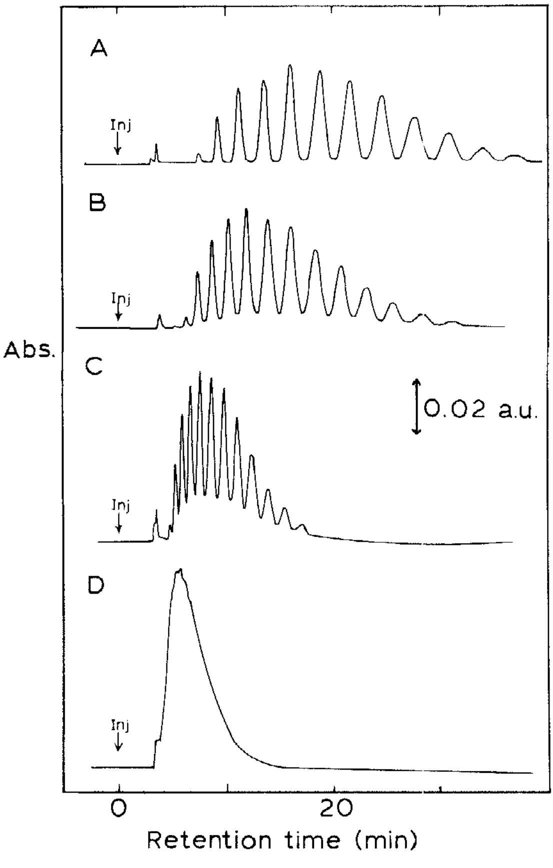

FIG. 2. Effect of pore size using a constant length column and a 25-mg/mL sample of 800 MW polystyrene: (A) Column A, (B) Column C, (C) Column E, and (D) Column G.

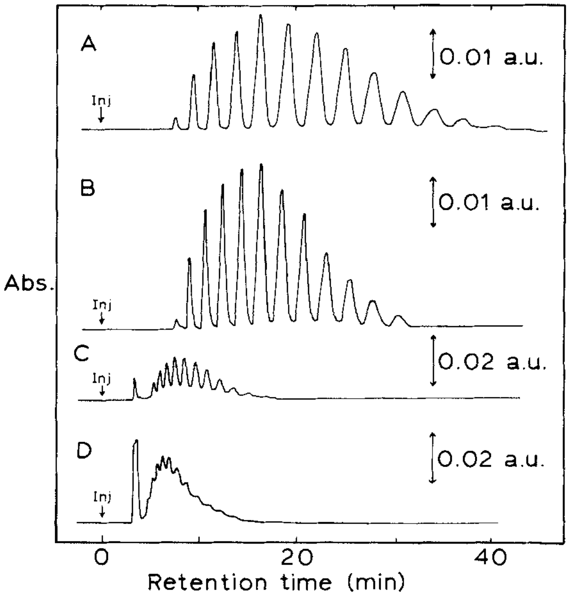

FIG. 3. Effect of pore size at constant column length and constant ratio of sample size using 800 MW polystyrene: (A) Column A, 50 mg/mL; (B) Column C, 50 mg/mL; (C) Column E, 10 mg/mL, and (D) Column G, 4 mg/mL.

surface area since the sample concentration per unit column length is lower on Column E. The column length may have been less important because area was not the limiting factor, as indicated by the retention volume of the $n = 10$ oligomer.

Finally, columns having a 12-fold decrease in surface area compared to Column A were compared in Fig. 6. The oligomers were partially resolved on Column F but were less resolved on Column G. These columns had similar retention times and about the same sample capacity. The longer column gave wider but less resolved peaks.

## Functionality of the Silica Surface

The effect on oligomer separations of the functionality of the silica surface was examined. Ethylphenyl silane and *n*-octyl silane, both having 10 carbon atoms, are compared in Fig. 7. The ethylphenyl column gave baseline resolution whereas the octyl column did not, and the latter resolved fewer

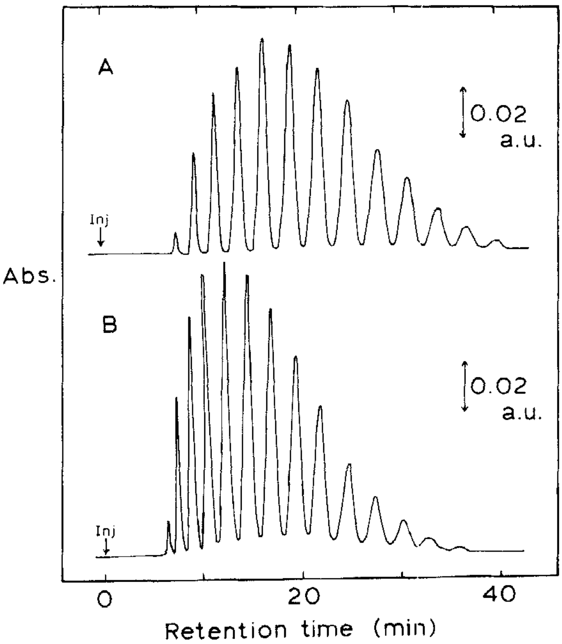

FIG. 4. Effect of pore size at a constant surface area of 620 $m^2$/column and constant sample size of 100 mg/mL sample of 800 MW polystyrene: (A) Column A and (B) Column C.

oligomers. The loss in resolution might have been due to the lower surface coverage of the *n*-octyl surface, but a 20% decrease in coverage seems too small to account for the loss in resolution. Furthermore, one should note that the retention volumes of the individual oligomers were larger on the *n*-octyl column.

## 2200 MW Polystyrene

Figure 8 shows that Column A gave larger retention volumes and better resolution of the oligomeric mixture than Column C. These columns gave near-baseline resolution of the 800-MW polystyrene oligomers; therefore, the loss of resolution for 2200 MW polystyrene oligomers must not have been due to exclusion but, instead, to the inability of the bonded phase to separate the oligomers.

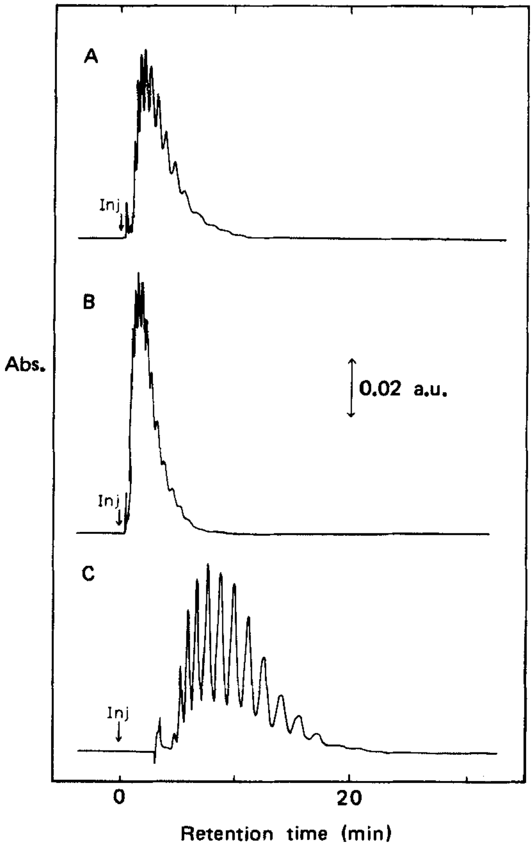

FIG. 5. Effect of pore size at a constant surface area of 125 $m^2$/column and a constant sample size of 25 mg/mL sample of 800 MW polystyrene: (A) Column B, (B) Column D, and (C) Column E.

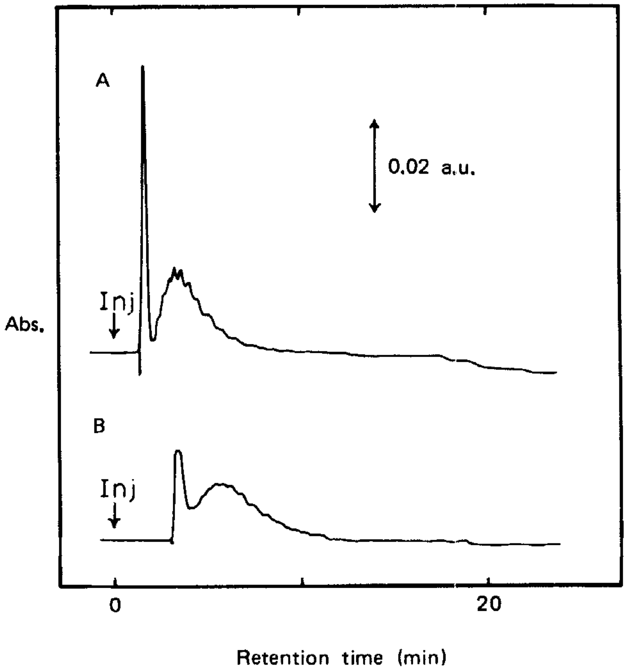

FIG. 6. Effect of pore size at a constant surface area of 50 $m^2$/column using a constant sample size of 2.5 mg/mL sample of 800 MW polystyrene: (A) Column F and (B) Column G.

## Size-Exclusion Studies

Size-exclusion results for the bonded phase and silica columns are shown in Table 4. The size-exclusion terms are defined as follows (*8*). The mobile phase volume, $V_m$, is the retention volume $V_R$ of toluene in pure THF. The void volume, $V_o$, is the retention volume of 1,800,000 MW polystyrene in pure THF. The internal volume, $V_i$, is defined as $V_i = V_m - V_o$. The mobile phase volume in the column, $V_m$, follows the expected trend of increasing $V_m$ for the larger pore underivatized silicas. One should note that Si 300 has a larger pore volume which, in turn, yields a mobile phase volume that is significantly different from those of the other silicas. The larger void volumes, $V_o$, for the underivatized silica packings could result from experimental error, adsorption, or differences in packing between the underivatized and the bonded phases. Throughout the experiments, the internal volume, $V_i$, was almost constant, within experimental error.

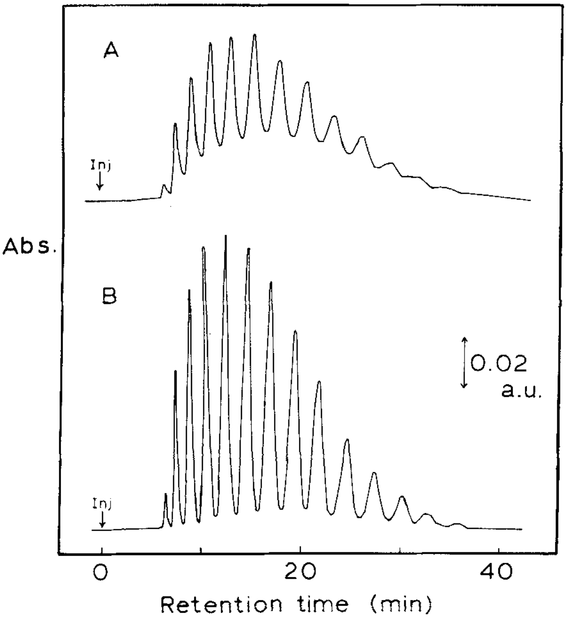

FIG. 7. Comparison of ethylphenyl and octyl packings using a 100-mg/mL sample of 800 MW polystyrene: (A) Column C and (B) *n*-octyl column.'

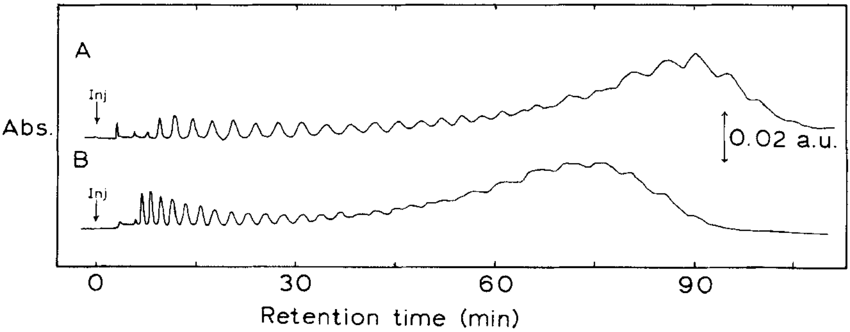

FIG. 8. Separations of 2200 MW polystyrene sample using a 100-mg/mL sample: (A) Column A and (B) Column C.

TABLE 4
Size-Exclusion Results

| Column | $V_m$[a] (+ 0.08 mL) | $V_o$[b] | $V_i$[c] |
|---|---|---|---|
| Si 100 phenyl | 3.17 | 1.38 | 1.79 |
| Si 100 phenyl[d] | 3.01 | 1.38 | 1.63 |
| Si 100 silica | 3.22 | 1.60 | 1.62 |
| Si 300 phenyl | 3.44 | 1.40 | 2.04 |
| Si 300 silica | 3.49 | 1.48 | 2.01 |
| Si 500 phenyl | 3.22 | 1.67 | 1.55 |
| Si 500 silica | 3.40 | 1.82 | 1.73 |
| Si 1000 phenyl | 3.28 | 1.84 | 1.54 |
| Si 1000 silica | 3.38 | 2.00 | 1.38 |

[a] $V_m$ based on $V_R$ for toluene in pure THF.
[b] $V_o$ based on $V_R$ for 1,800,000 MW polystyrene in THF.
[c] $V_i = V_m - V_o$
[d] Hydrolyzed silica.

The bonded phases gave smaller $V_o$ values than the underivatized silica, but the internal volumes were larger. Thus, one can see small changes due to derivatization. It is interesting to note that the nonhydrolyzed silica may have had a larger internal volume than the hydrolyzed silica.

## DISCUSSION

The use of wider pore silicas resulted in poorer resolution of the polystyrene oligomers than did the smaller pore silicas. The higher molecular weight oligomers gave evidence of either exclusion or of poorer retention. However, the decrease in surface area for the wider pore silicas appears to be the principle reason for the degradation of the resolution.

For columns of nominally equivalent surface area but different lengths, the wider pore columns gave better separations. This may have been due to better utilization of the column by the sample, i.e., as the sample concentration decreased on going through the column, the sample interacted better with the available surface area. In an earlier study (*3*) a given column gave better baseline resolution of the oligomeric mixture with decreasing sample load.

When comparing the 100 and 300 Å ethylphenyl-derivatized silicas, the latter gave smaller retention volumes and poorer baseline resolutions for the 800 and 2200 MW polystyrene oligomers. Therefore, on the ethylphenyl column the loss of resolution was not due to an exclusion effect but to the inability of the ethylphenyl column to adsorb the oligomeric mixture to a sufficient extent. Unfortunately, for low specific area silicas ($<50$ m$^2$/g), if the sample were decreased to a level that should permit maximum resolution to be achieved, the oligomers may be too dilute to be detectable in the eluate.

In the present study we have also attempted to control experimental variables such as endcapping, pore size, surface area, and polymerization of the silane bonded to the silica. Polymerization was avoided by using a monofunctional silane. In that way ethylphenyl-derivatized silicas were shown to give separations of 800 MW polystyrene oligomers equivalent to those achieved using octadecyl-derivatized silicas. However, the ethylphenyl yielded separations better than those achieved using *n*-octyl-derivatized silica. Although this may have been the result of lower surface coverage of the *n*-octyl-derivatized silica, a 20% decrease in surface coverage seems too small to account for the difference in resolution. Likewise, wide pore silicas having low surface area provided little resolution of the polystyrene oligomers. Pore size was not as important as the surface area for small oligomers.

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