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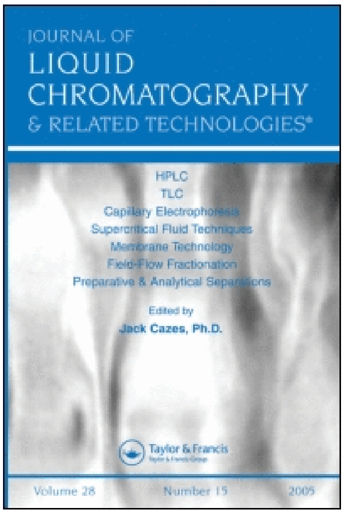

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A Polymer Coated Silica Gel for High-Performance Liquid Chromatography

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A POLYMER COATED SILICA GEL FOR HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

The treatment of microparticulate silica gel with Carbowax 20M results in a stable and reproducible support which greatly reduces the irreversible adsorption effects observed for polyoxyethylene compounds on untreated silica gel. The selectivity of this coated support, likely a result of selective deactivation, permits the rapid separation of oligomers differing by <3% in molecular weight. The advantages and disadvantages of this support and a C_{18} reversed-phase support for the separation and analysis of polyoxyethylene oligomers and their adducts are illustrated and discussed.

INTRODUCTION

Silica gel is a widely used adsorbent in high-performance liquid chromatography (HPLC) in spite of disadvantages such as batch to batch irreproducibility, irreversible adsorption, and variations in the energy of adsorption for different adsorption sites (1). These variations make it difficult to control adsorbent activity and to reproduce retention times. These disadvantages have contributed to the popularity of

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reversed-phase supports and to the development of sophisticated methods to control support deactivation. Recently a series of reports (2-6) have described a unique polymer-coated silica gel for gas chromatography (GC). We have found this irreversibly adsorbed (possibly bonded (2)) Carbowax 20M to be quite useful for GC and have been interested in its application to HPLC. Carbowax 20M has been used to deactivate controlled-pore glasses for steric-exclusion separations (7,8) but its behaviour in liquid-solid adsorption chromatography has not been investigated in any detail (2). It was felt that this type of packing might have several attractive features; the preparation of these supports should be simpler relative to bonded-phase packings and the disadvantages of silica gel described above should be eliminated or greatly reduced.

Part of the impetus behind this study was an interest in the separation and analysis of Carbowaxes and other polyoxyethylene (POE) compounds. POE compounds are widely used and their separation and analysis is still a problem (9,10) in spite of the fact that a number of GC (11-14), thin-layer chromatographic (15-17), LC (18-24) and colorimetric (25) methods have been described. Separations on silica gel are particularly difficult due to irreversible adsorption (19,26) which also leads to pronounced tailing and irreproducible chromatograms.

MATERIALS

The mobile phases were high purity solvents (Burdick and Jackson, Muskegon, Michigan, U.S.A.). The POE compounds (Chromatographic Specialties, Brockville, Ontario, Canada), 5-dimethylamino-1-

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napthalenesulfonyl chloride (DANSYL Cl; Eastman Organic Chemicals, Rochester, N.Y., U.S.A.), and 3,5-dinitrobenzoylchloride (DNB Cl; Eastman) were used as received. The liquid chromatographs were custom-built from commercial components and have been described elsewhere (27). Eluted samples were detected with one or more of the following detectors: UV (Model 430001-00, Varian, Palo Alto, Calif., U.S.A.; Model 970 variable wavelength, Tracor Instruments, Austin, Texas, U.S.A.) and fluorescence (Fluro-Monitor, American Instrument Co., Silver Spring, Md., U.S.A.). A C_{18} μ-Bondapak (Waters Associates, Milford, Mass., U.S.A.) was used for reversed-phase studies.

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METHODS

Preparation of POE Coated Support. The procedure described by Aue et al. (5) was used to coat the microparticulate silica gel (LiChrosorb SI-60 and SI-100, 10 μm; Brinkmann, Rexdale, Ontario, Canada) with Carbowax 20M. The silica gel was washed with several volumes of concentrated hydrochloric acid until the washings were colourless, washed to neutrality with water and then dried for several hours at 200°C. The hot, dry silica was then coated (6% w/w) with the Carbowax 20M by rotary evaporation (the solvent was chloroform) and placed in a glass tube which was heated to 270°C for 16-18 hours while a slow flow of nitrogen was passed through it.

Column Packing. Approximately 8 ml of the packing (coated or uncoated silica gel) were added to 50 ml CCl_4 and placed in an ultrasonic bath for 1-2 minutes. This slurry was then transferred to a reservoir and packed into a 4 mm x 30 cm 316 stainless steel (polished with Brasso^R) or glass-lined steel column

(Alltech Associates, Arlington Heights, Ill., U.S.A.) at 48 MPa (7000 psi) with a constant pressure pump (Model PIN 26890-4, Haskel Engineering, Burbank, Calif., U.S.A.). The Carbowax coated column was then conditioned with 20-50 column volumes of CH_2Cl_2, CH_3CN, CH_3OH and H_2O in that order. The silica-gel columns were washed with CH_3OH, CH_3CN, CH_2Cl_2 and n-C_6H_{14}in that order.

Linear Column Capacity. Linear column capacities were determined with ethylene glvcol monoethyl ether diphenylethanol and o-cresol. The linear capacity was taken as the amount of solute required to reduce the column capacity factor, k', by 10%.

DNB Cl Derivatives of POE. Approximately 1 mg of a POE dissolved in methylene chloride and a 5:1 molar excess of DNB Cl (freshly prepared 2.5% (w/v) solution in methylene chloride) were sealed in a 5 ml reaction vial and 30 µl of dry pyridine (distilled over BaO) were then added. This mixture was then heated to 55^{o}C for one hour. The pyridine and methylene chloride were evaporated with nitrogen and excess DNB Cl was hydrolyzed with one or two drops of 2N HCl. If desired the dinitrobenzoic acid can be removed by extraction with 1% Na_2CO_3.

DANSYL Cl Derivatives of POE. The DANSYL Cl derivatives were prepared as described for DNB Cl derivatives.

RESULTS AND DISCUSSION

Derivatization. For POE and many POE adducts derivatization is required for sensitive LC detection. DANSYL Cl is widely used (28) for the preparation of fluorescent derivatives of amines (29) and phenols (30).

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The derivatives formed with POE exhibited strong fluorescence and offered sensitive detection but the reaction was very slow and three series of products were obtained; monosubstituted, disubstituted and an unknown third product produced at long reaction times. Consequently this reagent was not studied further.

DNB Cl has been used previously (31,32) for the derivatization (UV chromophore) of large quantities (several mg) of POE compounds. When smaller quantities are derivatized it is necessary to use pure solvents which have been carefully dried and then stored in sealed vials. Solvents such as pyridine (31) and dimethylformamide (32) were tried but with unsatisfactory results. Methylene chloride, which is a good solvent for POE and other POE compounds, gave good results when a small amount of pyridine was added as a catalyst. For 1 mg samples the yield of the derivatives for eight POE oligomers was the same for reagent to sample mole ratios from 32:1 to 5:1. Yields were constant after a one hour reaction at 55^{o}C and the relative standard deviation (determined by integration of LC peaks as discussed below) for eight Carbowaxes was 2-3%. Both free OH groups of the Carbowaxes react to give disubstituted DNB Cl derivatives and the detection limit (twice baseline noise) for ethylene glycol was ∿0.5 ng.

<u>Carbowax Coated Silica Gel</u>. Figure 1 shows a representative chromatogram for the separation of the DNB Cl derivatives of a mixture of Carbowax 200 and 750. The excellent selectivity of this packing for the separation of the individual POE oligomers is evident. The separation of these oligomers on untreated silica gel was not possible due to badly skewed peaks and irreproducible retention times. The selectivity of the

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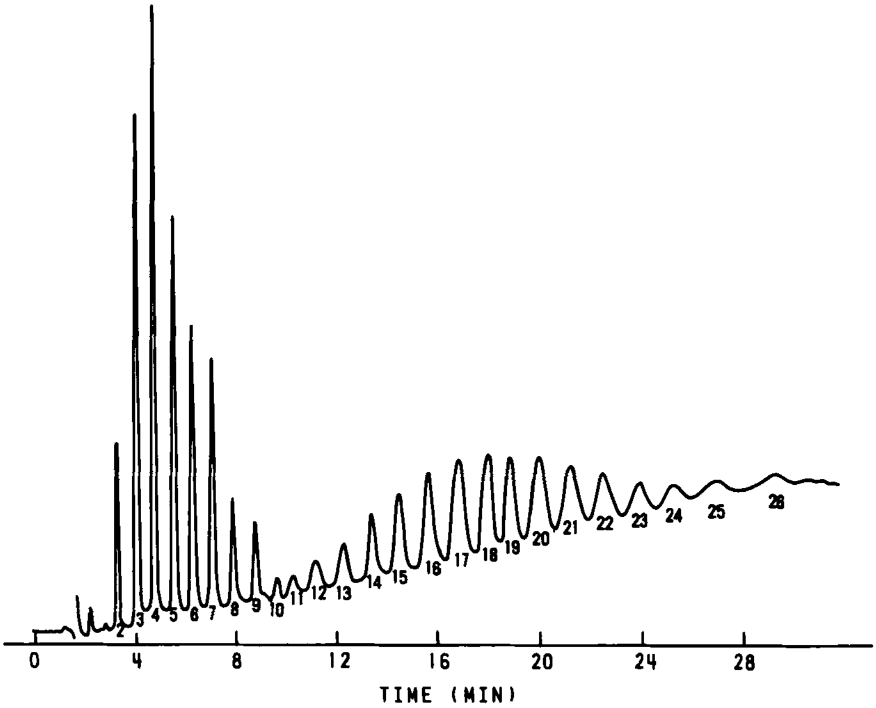

FIGURE 1

Separation of DNB Cl Derivatives of Carbowax 200 and 750 on Carbowax 20M Silica Gel. Column, glass lined 4 mm x 25 cm packed with coated 10 µm silica gel (10 nm pore); Flow, 2 ml min^{-1}; Solvent, 3% (v/v) acetonitrile in 1:1 methylene chloride-hexane to 100% acetonitrile; Gradient, linear, 30 min.

coated columns is such that POE oligomers with molecular weights of 1300 can be separated with baseline resolution on a single 25 cm column. This corresponds to the separation of molecules with a molecular weight difference of only 3%. Such a separation would be very difficult if not impossible by steric-exclusion chromatography.

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Retention time reproducibility was excellent and the relative standard deviation for the separation and analysis (peak area) of DNB Cl derivatives was 2-3%. The differences observed from one column to another were small as long as the columns were conditioned with the same solvents. No deterioration was observed in any of the columns either after long continuous use (1-3 months) or after sitting idle for several months.

The effect of the polarity of the conditioning solvent on the behaviour of new columns was determined with DNB Cl Carbowax derivatives used as test solutes. Increases in the polarity of the conditioning solvent caused a corresponding increase in the retention of the Carbowax derivatives. This change was quite noticeable from CH_2Cl_2 to CH_3CN to CH_3OH and then there was very little change as the conditioning solvent was gradually changed to water. Injection of Carbowax 20M solutions onto the column decreased retention times with liquid-liquid behaviour eventually being observed. Column performance could be restored with a methanol-water wash.

The performance of columns prepared from coated silica gel with and without heat treatment at 270^{0}C was essentially identical. Consequently the heat treatment is not required for LC applications. This result suggests that the function of the Carbowax may not be the same for LC as it is for GC (2). Recently (26) it has been shown that a combination of hydrophobic and hydrophilic sites on the silica gel will result in stronger adsorption of POE than on hydrophilic silica gel alone. Consequently it would be of interest to examine the GC and LC behaviour of these partially hydrophobic silica gels prepared either by heat treatment at 600°C (26) or by partial silanization.

The linear column capacities found for the coated silica were 2-5 times those found for untreated silica gel. The largest linear capacity was found for ethylene glycol monoethyl ether which contains two POE units. Relative k' values for a number of small molecules (molecular weights <200) were the same on both coated and uncoated silica gel and this suggests that the adsorbent-adsorbate interaction is very similar for the two packings. It would appear that the strongly adsorbed Carbowax acts mainly as a stable deactivator and since Carbowax is a POE, it is particularly selective for the separation of POE molecules.

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Comparison of Coated Silica Gel and C_{18} Reversed-Phase System. The excellent selectivity of the coated columns for the separation of POE oligomers is illustrated in the following examples. These examples, which are representative of three of the most common classes of POE compounds, illustrate the advantages and disadvantages of Carbowax coated silica versus a C_{18} reversed-phase system. Figure 2 shows a representative chromatogram for the reversed-phase separation of DNB Cl derivatives of POE. This poor selectivity was found for all POE compounds. Changes in the solvent systems gave no significant improvement. This result can be compared to the excellent selectivity of the coated silica gel illustrated in Figure 1. Figure 3 shows the reversed-phase separation of a fatty acid diester (mixture of oleic and linoleic) of POE with an average of 9-10 POE units (determined by steric exclusion chromatography). Since the reversed-phase

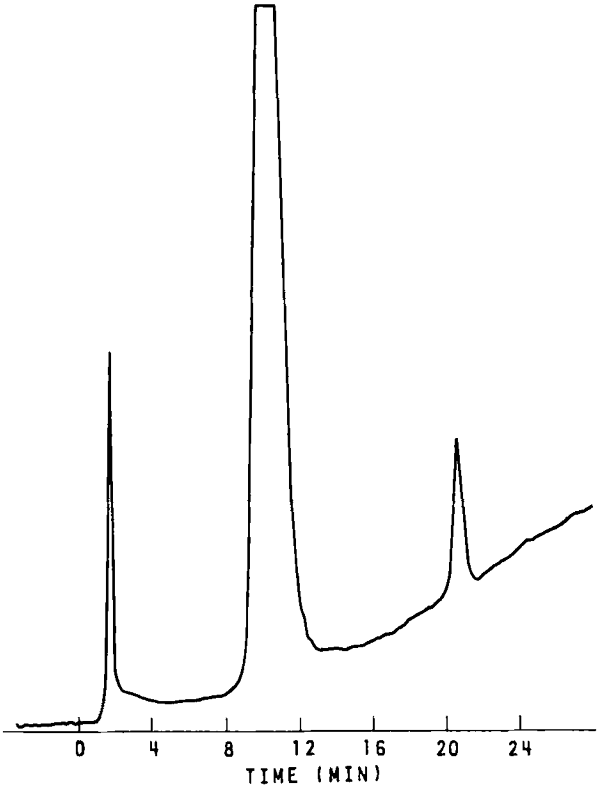

FIGURE 2

Separation of DNB Cl Derivatives of Carbowax 750 on C_{18} μ-Bondapak Column. Column, 4 mm x 30 cm; Flow, 2 ml min^{-1}; Solvent, 20% (v/v) to 70% tetrahydrofuran in H_2O; Gradient, linear, 40 min.

system is sensitive to changes in the hydrophobic content of a solute there is noticeable broadening of the peaks (relative to Figure 2) due to the presence of two fatty acids in the POE formulation and to impurities such as monosubstituted POE esters. The advantage of coated silica gel for the separation of this sample is obvious from the results shown in Figure 4. Since the separation between the peaks eluting from 16 to

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FIGURE 3

Separation of Fatty Acid Diesters of POE on C_{18} μ-Bondapak. Column, 4 mm x 30 cm; Flow, 2 ml min^{-1}; Solvent, 60% (v/v) tetrahydrofuran in H_2O to 100% tetrahydrofuran; Gradient, linear, 30 min.

25 min is characteristic of monosubstituted POE these peaks are likely due to small amounts of monosubstituted fatty acid esters. The hydrophobic portion of POE compounds has very little influence on their retention. For example, a comparison of the average retention time for the oligomers shown in Figure 4 with retention times in Figure 1 suggests

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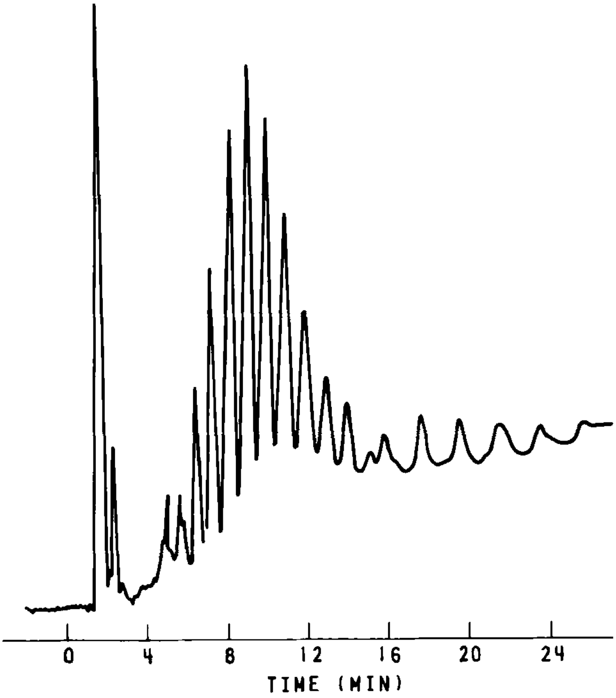

FIGURE 4

Separation of Fatty Acid Diesters of POE on Carbowax 20M Silica Gel. Experimental conditions as for Figure 1.

that there are an average of 9-10 POE units in this diester and analysis of the free POE obtained on hydrolysis confirmed this result. Figure 5 shows the partial reversed-phase separation of the POE oligomers of Triton X-100 (monoalkylsubstituted phenol-POE adduct) followed by a small peak due to the dialkyl substituted impurity. Figure 6 shows that the POE oligomers are well separated with the coated silica gel but, due to poor response to hydrophobic sub-

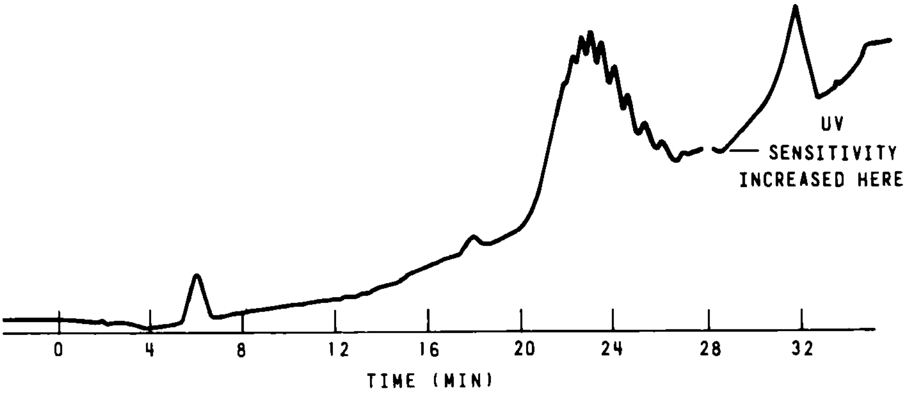

FIGURE 5

Separation of Triton X-100 on C_{18} μ-Bondapak. Column, 4 mm x 30 cm; Flow, 2 ml min^{-1}; Solvent, 20% (v/v) to 50% tetrahydrofuran in H_2O; Gradient, linear, 30 min.

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stituents, the presence of dialkyl impurity is not evident.

Although the C_{18} reversed-phase system is of limited use for the separation of POE oligomers it could be quite useful for the preconcentration of free POE and POE adducts from aqueous systems. Figure 7 shows that preconcentration is feasible for all free POE except n = 1 or 2, and the preconcentration of these two oligomers could likely be achieved with an increase in ionic strength. The retention of adducts with hydrophobic groups is stronger than that for the corresponding free POE. For example, Triton X-100 contains an average of 9-10 units of POE but is eluted at the same position as a free POE with 22-23 units. Such a preconcentration scheme should prove to be faster and more efficient than extraction procedures (33), especially if it is possible to combine pre-

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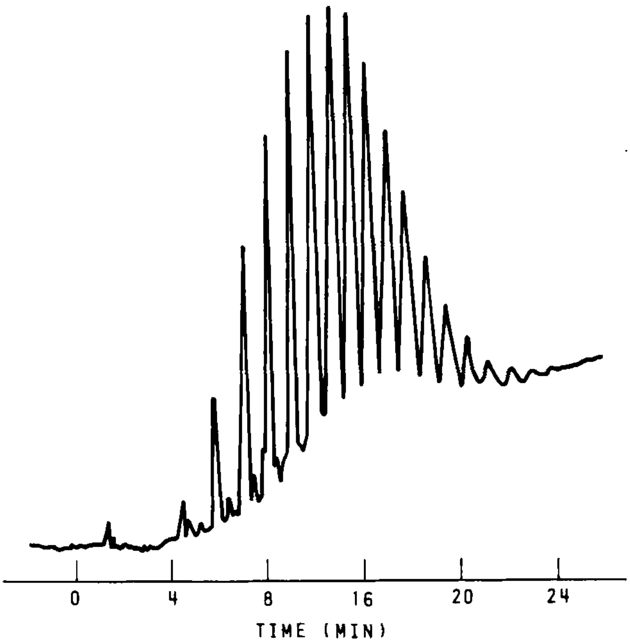

FIGURE 6

Separation of Triton X-100 on Carbowax 20M Silica Gel. Column, glass lined 4 mm x 25 cm packed with coated 10 µm silica gel (10 nm pore); Flow, 2 ml min^{-1}; Solvent, 1.5% (v/v) and 3% acetonitrile in 1:1 hexane-methylene chloride to 10% isopropanol in acetonitrile; Gradient, linear, 30 min.

concentration and analysis in one operation on the same analytical column (34).

CONCLUSIONS

In summary, the above results have shown that Carbowax treatment of silica gel eliminates irreversible adsorption effects and results in an adsorbent with excellent selectivity for the separation of POE oligomers. In addition to quality control and

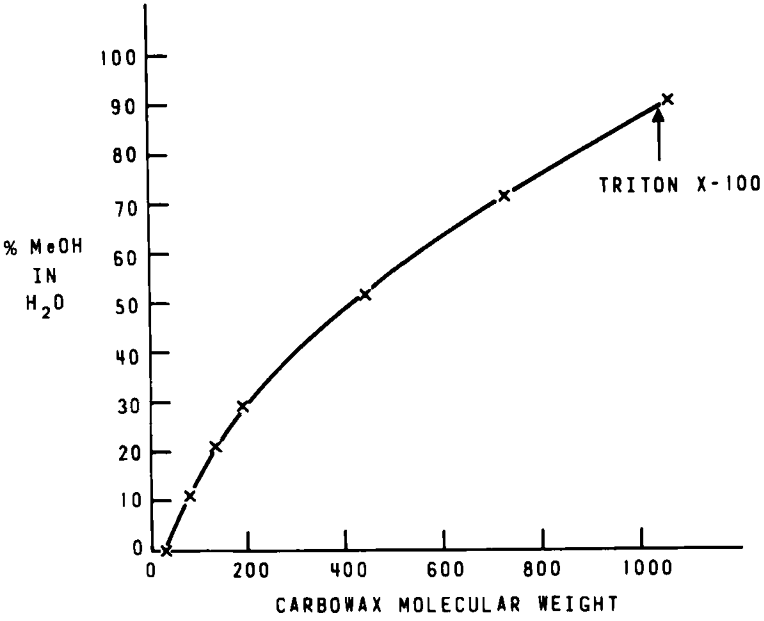

FIGURE 7

Percentage Methanol Required to Elute Carbowaxes with k' = 1.

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effluent analysis, this adsorbent should prove valuable for biodegradation studies (9) and for the preparation of pure POE oligomers. This technique could also offer a simple and reproducible method for the permanent deactivation of silica gel for other kinds of analysis. It is also possible that other polymers could be used to give adsorbents with different selectivities. Reversed-phase systems are of limited use for the separation of POE oligomers but should prove useful for the preconcentration of POE compounds directly from aqueous systems. If the molecular size of the POE compound is large enough

(>2000-3000) then the utility of both the coated silica gel and the reversed-phase systems is limited due to steric exclusion, and slow desorption and/or irreversible adsorption. However most POE compounds of interest do not fall in this molecular-weight range.

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