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CHROMATOGRAPHIC PROPERTIES OF A VINYL ALCOHOL COPOLYMER GEL COLUMN FOR THE ANALYSIS OF NON-IONIC SURFACTANTS

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SUMMARY

A high-performance size-exclusion chromatographic column packed with Asahipak GS-310 vinyl alcohol copolymer gel was investigated for its applicability to the analysis of non-ionic surfactants with mobile phases containing water and acetonitrile or other organic solvents in various ratios. The surfactant series consisted of $R(OCH_2CH_2)_nOH$, where R is an alkylaryl group and n = 1–100.

In the water-rich region, with acetonitrile concentrations of 0–30%, surfactants either were not eluted or their elution was extremely delayed, indicating a hydrophobic interaction between the gel and the alkylaryl group in the surfactant. In the acetonitrile-rich region, with acetonitrile concentrations of 60–100%, the surfactants were eluted relatively rapidly in order of decreasing molecular weight, without a fine separation between compounds with different n values. These results indicate that the organic solvent effectively inhibited the hydrophobic interaction and that size exclusion was the predominant separation mechanism. In the intermediate region, with 30–60% acetonitrile concentrations, the surfactants were eluted in the same order as for the acetonitrile-rich region, but less rapidly and with a fine separation between n values. The results indicate that the separation mechanism is mainly hydrophobic interaction between the alkylaryl groups and the gel, the hydrophobicity of the gel being too weak for effective interaction with the oxyethylene groups.

The results indicate that the Asahipak GS-310 hydrophilic polymer gel column can be effectively employed for the practical, efficient high-performance liquid chromatographic analysis of non-ionic surfactants.

INTRODUCTION

The Asahipak GS series consists of high-performance liquid chromatographic (HPLC) columns packed with vinyl alcohol copolymer gel, which were developed and are now widely utilized for aqueous size-exclusion chromatography (SEC). In a previous study[1], we found that two columns in the series, GS-310 and GS-510, could be characterized as "amphipathic SEC columns", capable of SEC of both pullulan with an aqueous mobile phase and polystyrene with chloroform as the mobile phase. Both columns were found to be characterized, in particular, by their stability and effective

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performance using mobile phases with a broad range of freely varied organic solvents and aqueous organic solvents of various concentration, because their gel volume is not significantly affected by changes in mobile-phase polarity. This characteristic led to the present investigation of the application of amphipathic Asahipak GS-310 to the analysis of non-ionic surfactants with mobile phases containing water and acetonitrile or other organic solvents in various ratios.

EXPERIMENTAL

Chromatography was performed with a Tri-Rotar HPLC apparatus (Jasco, Tokyo, Japan), equipped with a Shodex SE-51 refractometer (Showa Denko, Tokyo, Japan).

Asahipak GS-310 (500 mm × 7.6 mm I.D.) HPLC columns (Asahi Chemical Industries, Tokyo, Japan), packed with vinyl alcohol copolymer gel having a mean particle diameter of 9.0 μm and an exclusion limit of 40 000 Da, were employed.

Surfactants were obtained from Nippon Nyukazai (Tokyo, Japan), in the series described by the formula $R(OCH_2CH_2)_nOH$, where R = C_8H_{17}-*p*-C_6H_4– and C_9H_{19}-*p*-C_6H_4– and n = 1–100. Organic solvents were obtained from Wako (Osaka, Japan).

RESULTS AND DISCUSSION

The chromatographic behaviour of the non-ionic surfactants on isocratic elution with water–organic solvent mixtures using the vinyl alcohol copolymer gel column (GS-310) is shown in Fig. 1 in terms of the relationship between the retention volume of nonylphenoxyoligo(ethylene glycol)s and acetonitrile concentration in a water–acetonitrile mobile phase.

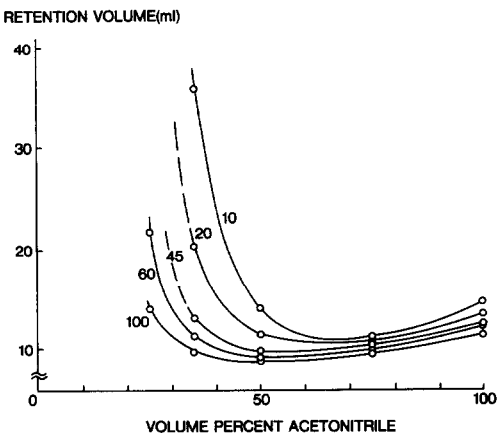


Fig. 1. Graphs illustrating plots of the retention volume (ml) against the composition of the acetonitrile–water mixture used as the eluent. The number on each curve indicates the number of EO units contained in the solute molecule. Column, Asahipak GS-310 (500 × 7.6 mm I.D.); temperature, 30°C; flow-rate, 1.0 ml/min; detection, 280 nm; samples, nonylphenoxy oligo(ethylene glycol)s.

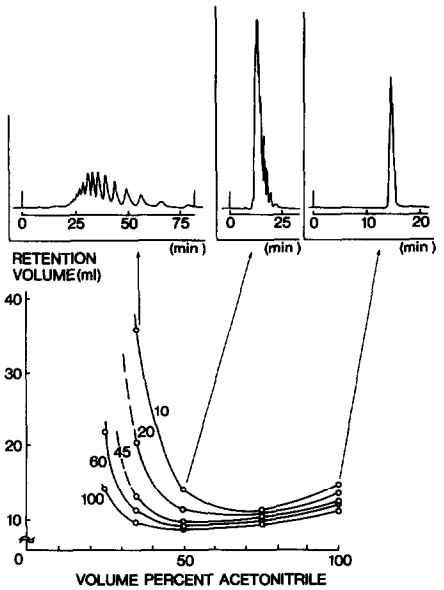


Fig. 2. Chromatograms of nonylphenoxy oligo(ethylene glycol)s with an average of 10 EO units at various acetonitrile concentrations, and graphs illustrating plots of the retention volume against the composition of the acetonitrile–water mixture used as the eluent. Column, Asahipak GS-310 (500 × 7.6 mm I.D.); temperature, 30°C; flow-rate, 1.0 ml/min; eluent, water–acetonitrile; detection, 280 nm.

The chromatograms in Fig. 2 are typical of those obtained for these surfactants on the GS-310 column. They were obtained with eluents containing acetonitrile at three concentrations for a homologous mixture containing an average of ten ethylene oxide (EO) units per solute molecule. These homologues were not eluted when the mobile phase was water alone or water containing up to 30% acetonitrile. In a series of analyses with successively higher acetonitrile concentrations, elution resulting in late, poorly defined peaks was first observed with about 30% acetonitrile, and further increases in the acetonitrile concentration resulted in increasingly rapid elution. It was also observed that, at a given acetonitrile concentration, smaller n values in C_9H_{19}-p-$C_6H_4(OCH_2CH_2)_n$–tended to result in longer elution times. Conversely, the acetonitrile concentration necessary to elute the non-ionic surfactant in a given time tended to increase with decreasing n.

In numerous similar analyses of these surfactants with n values ranging from 1 to 100, they were eluted in the intermediate region of about 30–60% acetonitrile with a fine separation between homologues with different n values. No reversal in the order of elution of these homologues occurred with changes in organic solvent concentration, in contrast to the results reported by Melander *et al.*[2] for similar analyses of phenyloligo(ethylene glycol)s on ODS columns.

It was therefore possible to analyse the non-ionic surfactants isocratically, despite the large variation in hydrophobicity known to occur with different n values. Under the given conditions that yielded this fine separation, moreover, the same homologue was eluted at the same elution volume, with excellent reproducibility, even when in different homologue mixtures, as shown in Fig. 3 and Table I.

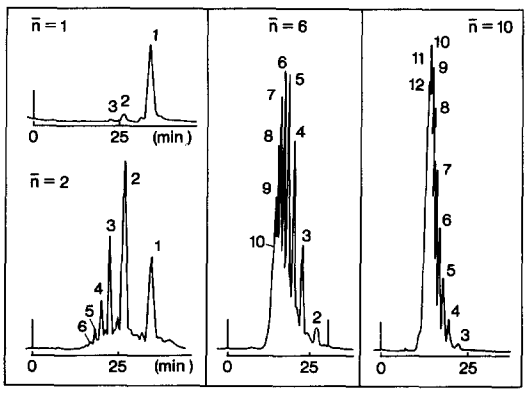


Fig. 3. Chromatograms for several nonylphenoxy oligo(ethylene glycol)s differing in the average number of EO units. Column, Asahipak GS-310 (500 × 7.6 mm I.D.); temperature, 30°C; flow-rate, 1.0 ml/min; eluent, water–acetonitrile (50:50); detection, 280 nm.

From these analyses, the number of theoretical plates (N) for these surfactants was determined to be about 5000–8000, with the value tending to increase with increasing k' value, as shown in Fig. 4. This indicates a capability for excellent separations of surfactants with small n values.

Fig. 5 shows the dependence of the logarithm of the retention factor for octylphenyloligo(ethylene glycol)s on the number of EO units with eluents of various acetonitrile concentrations. No inversion in the order of elution occurred with changes in the acetonitrile concentration, again in contrast to the behaviour of phenyloligo (ethylene glycol)s on ODS columns reported by Melander *et al.*[2].

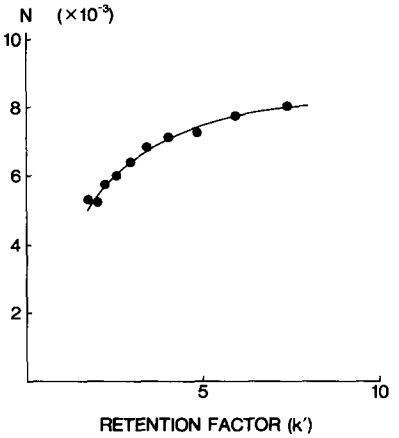
As shown by the Van 't Hoff plots in Fig. 6, the retention factors decreased with increasing temperature for homologues with n values smaller than 10, as is usual in reversed-phase chromatography, but increased for those with larger n values. Although the elution order with respect to the n values in the homologous series is the reverse of that reported by Melander *et al.*[2] for phenyloligo(ethylene glycol)s on ODS columns, the tendency for the retention factors to decrease with increase in temperature for homologues with small n values and to increase for those with larger n values is similar.

TABLE I

REPRODUCIBILITY OF RETENTION VOLUMES

Average number of ethylene oxide units	*Retention volume (ml)*					
	*10**	*6**	*4**	*3**	*2**	*1**
2	–	16.91	20.14	22.76	27.01	35.10
6	13.99	16.96	20.09	22.70	27.01	–
10	14.15	16.91	19.99	22.70	–	–

* Number of ethylene oxide units in nonylphenoxyoligo(ethylene glycol)s.



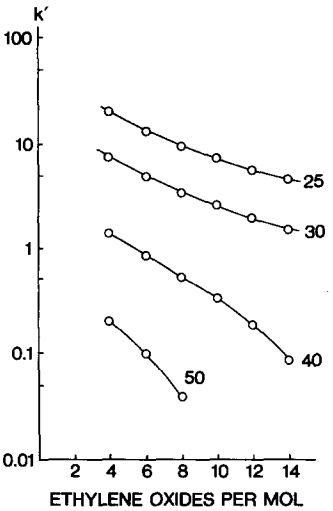


Fig. 4. Graph of the number of theoretical plates against retention factors (k'). Column, Asahipak GS-310 (500 × 7.6 mm I.D.); temperature, 30°C; flow-rate, 1.0 ml/min; eluent, water–acetonitrile (70:30); detection, 280 nm; sample, octylphenoxyoligo(ethylene glycol)s with an average of 10 EO units.

Fig. 5. Graphs of retention factors (k') on a logarithmic scale against number of EO units in octylphenoxy oligo(ethylene glycol)s with an average of 10 EO units. Column, Asahipak GS-310 (500 × 7.6 mm I.D.; temperature, 30°C; flow-rate, 1.0 ml/min; eluent, water–acetonitrile mixtures with the acetonitrile concentrations (%, v/v) indicated on the curves; detection, 280 nm.

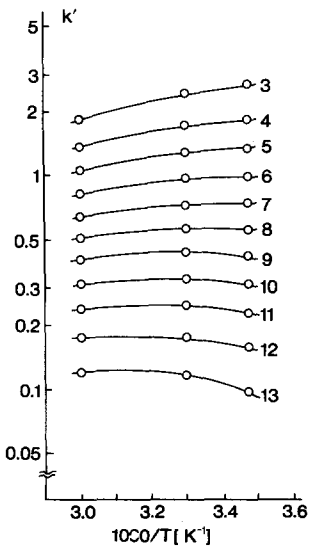


Fig. 6. Van 't Hoff plots of retention factors (k') for octylphenoxy oligo(ethylene glycol)s containing the number of EO units indicated on the curves. The retention data were measured with an Asahipak GS-310 column (500 × 7.6 mm I.D.) at an eluent flow-rate of 1.0 ml/min and with detection at 280 nm; eluent, water–acetonitrile (60:40).

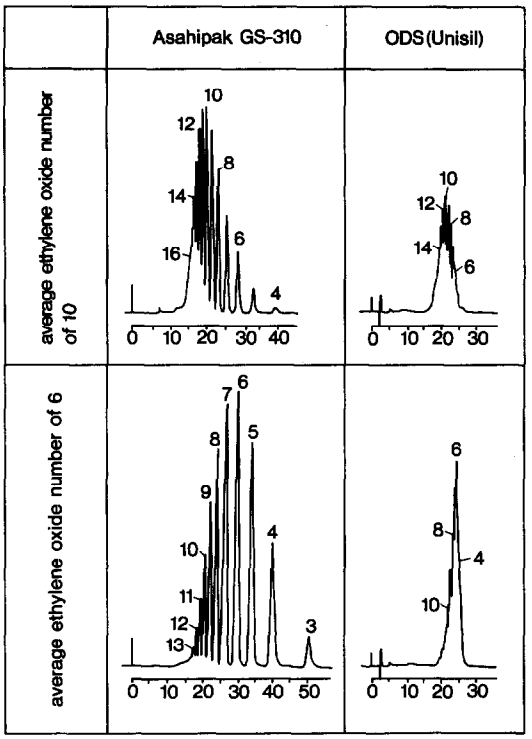


Fig. 7. Chromatograms of octylphenoxy oligo(ethylene glycol)s containing the number of EO units indicated on the peaks on an Asahipak GS-310 column (500 × 7.6 mm I.D.) at an eluent flow-rate of 1.0 ml/min and on a 5-μm Unisil Pack ODS column. Detection at 280 nm. Eluents: water–acetonitrile (60:40) on the Asahipak column and water–acetonitrile (45:55) on the ODS column. Temperature, 30°C.

For comparison, similar analyses were performed on a widely used ODS column. Fig. 7 shows typical examples of attempts to achieve a fine separation between homologous with different n values. The surfactants were eluted from the ODS column in order of decreasing n, as on Asahipak GS-310. However, the concentration of acetonitrile required to give similar retention factors was about 15–20% higher than on GS-310, indicating a higher hydrophobicity of the ODS column. The inferior separation between homologues with smaller n values on the ODS column corresponds to a far smaller difference between retention factors on the ODS column, especially for small n.

Elution mechanism

Three broad regions of non-ionic surfactant elution behaviour were observed, corresponding to different ranges of acetonitrile concentration: the water-rich region with acetonitrile concentrations of 0–30%, the intermediate region (30–60%) and the acetonitrile-rich region (60–100%).

In the water-rich region, elution of the non-ionic surfactants either did not occur or required very large eluent volumes, probably as a result of strong hydrophobic interactions between the alkylaryl groups in the surfactant and the gel skele-

ton. As an SEC column, on the other hand, GS-310 generally exhibits only weak hydrophobic interactions with poly(ethylene glycol)s and other hydrophilic solutes in water. It may therefore be expected to provide a means of effective removal of surfactants from protein–surfactant mixtures, with a capability for repeated use after washing with organic or aqueous–organic solvents to remove adsorbed surfactants.

In the acetonitrile-rich region, the non-ionic surfactants exhibited elution volumes of 9–14 ml, in order of decreasing molecular weight, with no fine separation between homologues with different n values. Plots of this relationship between molecular weight and elution volume are shown in Fig. 8, together with calibration graphs for standard polystyrenes in chloroform and for standard pullulans and poly(ethylene glycol)s in water. The plots for the non-ionic surfactants lie between the two calibration graphs; the K_d values, as calculated from the retention volume for each surfactant, ranged from 0.2 to 0.8. These results indicate that the organic solvent effectively inhibited hydrophobic interactions with the GS-310 gel, and that size exclusion was the predominant separation mechanism.

Essentially the same results, indicating a size-exclusion mechanism, were observed with methanol or n-propanol in place of acetonitrile. The organic solvent concentrations required for elution volumes similar to those obtained with acetonitrile were higher with methanol and lower with n-propanol, as shown in Fig. 9. The comparatively high efficiency of SEC with n-propanol indicates that organic solvents with longer alkyl groups inhibit more effectively the hydrophobic interactions between the nonylphenyl groups in the surfactant and the gel.

In the intermediate region, the surfactants were eluted in the same order as for the acetonitrile-rich region, but less rapidly and with a fine separation between n values. The K_d values for all of the homologues with smaller n values were greater than 1, as shown in Fig. 10, indicating that the separation mechanism is not predominantly one of size exclusion.

Fig. 11, obtained with three columns having different OH densities, shows that

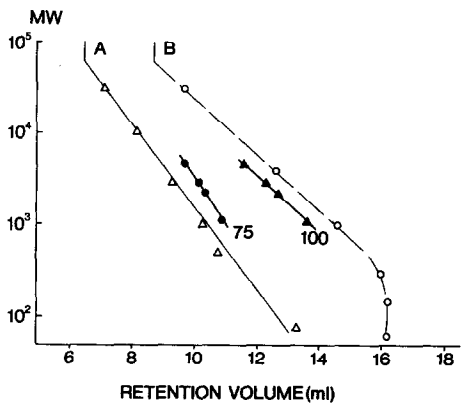


Fig. 8. Graphs of molecular weight on a logarithmic scale against retention volume. The number on each curve indicates the acetonitrile concentration in water–acetonitrile mixtures. Column, Asahipak GS-310 (500 × 7.6 mm I.D.); temperature, 30°C; flow-rate, 1.0 ml/min; detection at 280 nm; sample, nonylphenoxy oligo(ethylene glycol)s. (A) Calibration graph with standard polystyrene in chloroform; (B) calibration graph with standard poly(ethylene glycol)s in water.

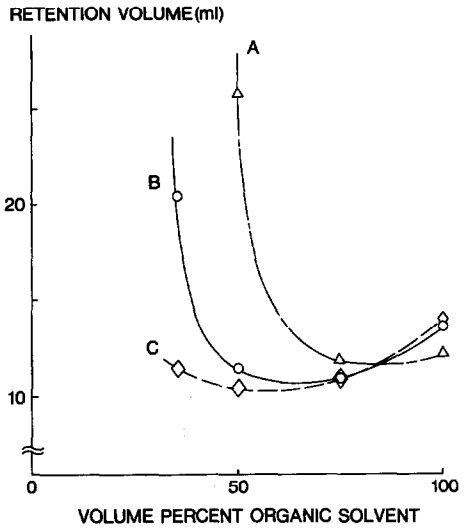


Fig. 9. Graphs of retention volume (ml) against composition of water–organic solvent mixtures used as eluent. Column, Asahipak GS-310 (500 × 7.6 mm I.D.); temperature, 30°C; flow-rate, 1.0 ml/min; detection at 280 nm; sample, nonylphenoxy oligo(ethylene glycol)s, average 20 EO units. Organic solvent: (A) methanol; (B) acetonitrile; (C) *n*-propanol.

the retention volume for surfactants with a given n value increases with decreasing OH density in the column gel. Hence with a gel of low OH density, as in GS-310, hydrophobic interactions predominate over hydrogen bonding.

Fig. 12 shows plots of retention volumes against n for GS-310 and ODS columns. With the ODS column, the slope is nearly constant throughout the investigated range of n values. With the GS-310 column, the slope increases rapidly with

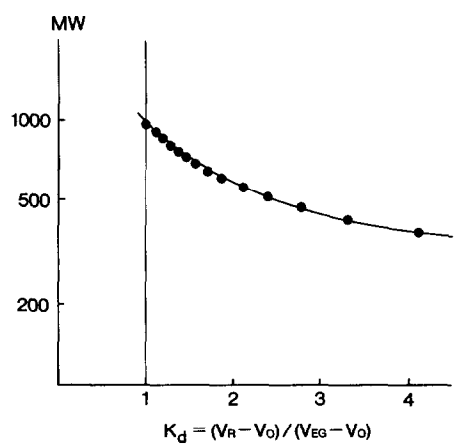


Fig. 10. Graphs of the molecular weight on a logarithmic scale against K_d value. Column, Asahipak GS-310 (500 × 7.6 mm I.D.); temperature, 30°C; flow-rate, 1.0 ml/min; detection at 280 nm; sample, octylphenoxy oligo(ethylene glycol)s, average 10 EO units; eluent, water–acetonitrile (60:40).

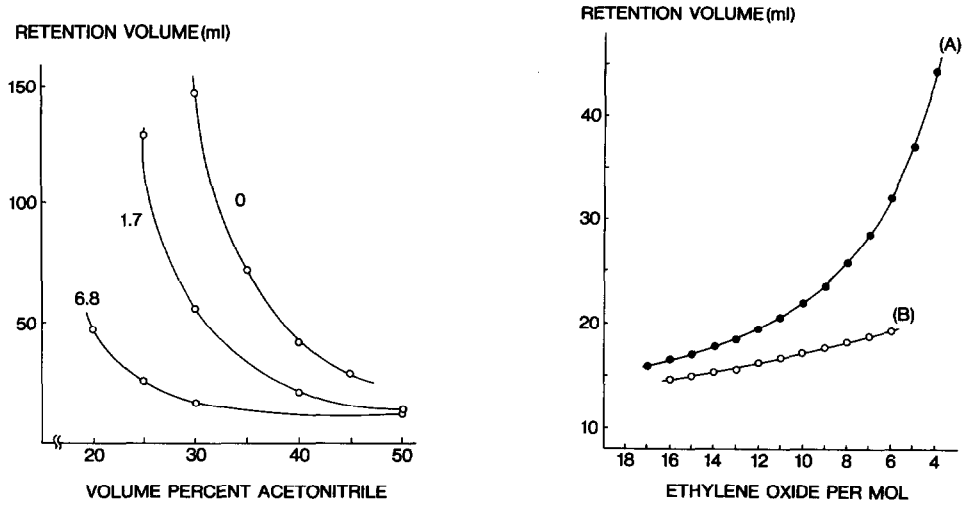


Fig. 11. Graphs of retention volume (ml) against composition of water–acetonitrile mixtures. The numbers on the curves indicate the density of OH groups in the gel (mequiv. per gram of dry resin). Columns, Asahipak GS-310 (500 × 7.6 mm I.D., OH group density 1.7), Asahipak GS-320 (500 × 7.6 mm I.D., OH group density 6.8) and a column packed with unhydrolysed gel (500 × 7.6 mm I.D., no OH groups); temperature, 30°C; flow-rate, 1.0 ml/min; detection at 280 nm; sample, octylphenoxy oligo(ethylene glycol)s, average 10 EO units.

Fig. 12. Graphs of retention volume (ml) against the number of EO units in the solute molecule. The retention data were measured (A) on an Asahipak GS-310 column (500 × 7.6 mm I.D.) at a flow-rate of 1.0 ml/min and (B) on a 5-μm Unisil Pack ODS column (150 × 4.6 mm I.D.) at a flow-rate of 0.8 ml/min; detection 280 nm; temperature, 30°C; flow-rate, 1.0 ml/min; sample, octylphenoxy oligo(ethylene glycol)s; eluent, (A) water–acetonitrile (60:40) and (B) water–acetonitrile (45:55) solution.

diminishing n values, indicating a capability for far better separations of surfactants with small n values.

As shown in Fig. 13, poly(ethylene glycol)s were eluted by what is apparently a size exclusion mechanism from the GS-310 column, even with water alone as eluent, and hence they were also eluted in order of decreasing n with eluents containing organic solvents. For the ODS column, as shown here and also reported by Melander *et al.*[2], their elution occurs in order of increasing molecular weight. The reversal of the elution order between these two columns may be attributed to a difference in gel hydrophobicity. It appears that the strongly hydrophobic ODS, with C_{18} groups, effects a slower elution at higher moleculer weights because of its hydrophobic interaction with the dimethylene groups of the poly(ethylene glycol)s, whereas the lack of significant hydrophobic interactions between the weakly hydrophobic vinyl alcohol copolymer gel and the poly(ethylene glycol)s results in separation predominantly by size exclusion.

The difference between the capabilities of the two columns for fine separation of the non-ionic surfactants may also be explained on the basis of the reversed order of poly(ethylene glycol) elution, as related to the difference in hydrophobicities. It appears that significant hydrophobic interactions between the GS-310 gel and the surfactants involves only their alkylaryl groups, and that longer poly(ethylene glycol)

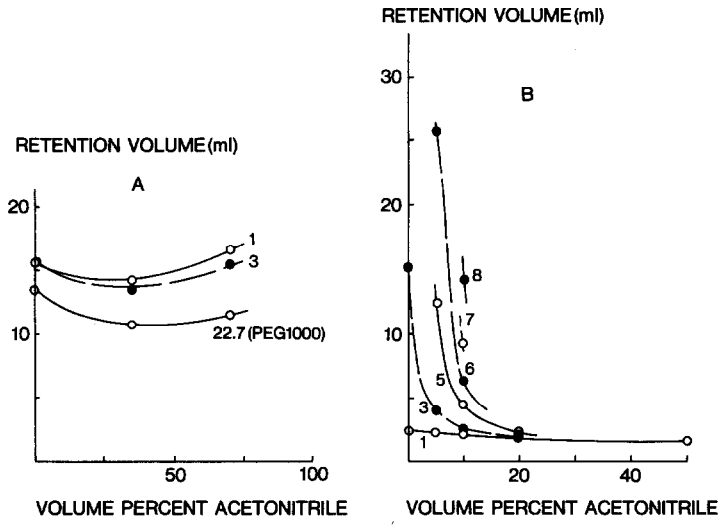


Fig. 13. Graphs of retention volume (ml) against composition of water–acetonitrile mixtures used as the eluent. The numbers on the curves indicate the number of EO units in the solute molecule. Columns (A) Asahipak GS-310 (500 × 7.6 mm I.D.) and (B) 5-μm Unisil Pack ODS (150 × 4.6 mm I.D.); sample, oligo(ethylene glycol)s; temperature, 30°C; flow-rate, (A) 1.0 ml/min and (B) 0.8 ml/min; detection, refractive index.

chains simply serve to interfere sterically with this interaction, these two effects in combination resulting in later elution of surfactants with smaller *n* values. On the other hand, the hydrophobic interaction between the ODS gel and the surfactants seems to involve both the alkylaryl and the poly(ethylene glycol) groups. Further, the interaction with the former groups is apparently stronger for the surfactants with smaller *n* values, whereas that with the latter groups appears stronger for those with larger *n* values. A cancelling effect therefore seems to occur, resulting particularly in a resolution between smaller *n* values that is poorer than that of the hydrophilic polymer gel column.

Any influence by micelle formation by the surfactants was considered to be negligible, as measurements were generally made with mobile phases having organic solvent concentrations known to prevent micelle formation, and also because essentially identical chromatograms were obtained at sample concentrations differing by as much as 10 000-fold.

CONCLUSION

The results of the analyses of alkylphenoxyoligo(ethylene glycol) non-ionic surfactants on GS-310 indicated that this column will be useful for various purposes, such as the selective removal of surfactants from protein–surfactant mixtures, the SEC of surfactants and the fine separation of surfactant series differing in the number of EO units. The surfactants were eluted in order of decreasing molecular weight, with no reversal in elution order for mobile phases with all water–organic solvent ratios.

Surfactants generally involve the presence of both a hydrophobic and a hydrophilic group in the same molecule. In the surfactants used in this study, these were alkylaryls and oligo(ethylene glycol)s, respectively. The ODS column apparently interacted hydrophobically with both of these groups, presumably more strongly with the former. It may further be assumed that the longer oligo(ethylene glycol) groups not only interacted hydrophobically with the ODS gel to effect a stronger retention, but also tended to interrupt sterically the interaction between the alkylaryl groups and the gel. Although the interactions appear to have a mutually cancelling effect, tending to impede fine separations, the stronger interaction between the ODS and the alkylaryl groups appears to predominate, thus determining the elution order.

GS-310, which is a hydrophilic polymer gel column with no C_{18} groups, apparently interacted hydrophobically only with the alkylaryl groups, at least at temperatures of 30°C and less, where longer oligo(ethylene glycol) chains simply tended to interrupt the interaction sterically. It may be presumed, furthermore, that this type of separation can be effected only on hydrophilic polymer gel columns which are amenable to various aqueous–organic solvent mobile phases and display weak hydrophobicity.

The results indicate that the GS-310 column can be effectively employed for practical, efficient HPLC analysis of various samples involving hydrophobic groups and hydrophilic groups in the same molecules, including surfactants.

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