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AMPHIPATHIC CHARACTER OF HYDROPHILIC POLYMER GEL COLUMNS

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SUMMARY

An investigation of the Asahipak GS series of aqueous high-performance liquid chromatographic columns, particularly of the characteristics of the gels in organic solvents, showed that two of the columns, GS-310 and GS-510, are amenable to chromatographic analysis with both hydrophilic and non-polar organic solvents, such as methanol and chloroform, as the gels swell more in organic solvents than in distilled water. Experiments confirmed that it is possible to perform gel permeation chromatographic analysis of polystyrene in the organic mobile phase on these two columns, as well as of water-soluble polymers in the aqueous mobile phase.

INTRODUCTION

Asahipak GS columns are packed with hydrophilic gels composed of a vinyl alcohol copolymer. Aqueous solutions are generally used as the mobile phase[1]. As noted previously[2], however, we also found it possible to use organic solvents such as methanol in the mobile phase on the GS-320 column.

Columns for gel permeation chromatography (GPC) have generally been limited in respect of the solvent or solvent type which may be used as the eluent on a particular column. Aqueous high-performance liquid chromatographic (HPLC) columns which can be used with organic as well as aqueous solvents would allow a considerable expansion in their range of application. For this reason, we investigated the relationship between the decrease in column efficiency caused by various organic solvents and the gel characteristics, for GS-320 and other columns in the Asahipak GS series.

EXPERIMENTAL

Chromatography was performed with a Hitachi 638-30 high-speed liquid chromatograph, equipped with a Jasco Uvidec 100-IV UV detector or Shodex SE-51 refractometer. Asahipak GS-310, GS-320, GS-510 and GS-520 columns (500 mm × 7.6 mm I.D.) were employed.

Organic solvents were obtained from Wako, standard samples of pullulan from Showa Denko, poly(ethylene glycol) from Kishida Kagaku and polystyrene from Toyo Soda.

The resistance of each column to lowering in efficiency by the organic solvent was determined by first measuring the number of theoretical plates, N_b, of the ethylene glycol peak using distilled water as the mobile phase. Fresh organic solvent was then passed through the column at room temperature and 1 ml/min for 2 h followed by recirculating the solvent under the same conditions for 2 days. Finally, the solvent was displaced with distilled water at 1 ml/min and room temperature. The number of theoretical plates, N_a, of the ethylene glycol peak was then remeasured. The passage of chloroform was preceded by methanol for 2 h because of its immiscibility with water.

The parameters $N(4\sigma)$ and $N(5\sigma)$ were calculated by the equations $N(4\sigma) = 5.54\,(V_R/W_{0.5})^2$ and $N(5\sigma) = 25\,(V_R/W_{0.044})^2$, where $W_{0.5}$ and $W_{0.044}$ are the widths of the peak at half-height and 4.4% height, respectively.

The determination of the influence of various solvents on the gel volume was based on measurement of the solvent regain, S_R. The dry gel was first dispersed in the solvent and left at room temperature for 16 h or more. The gel dispersion was then centrifuged at 1318 *g* and 0°C for 1 h, or in the case of water at 20°C for 90 min, to remove interstitial solvent. The weight, W_1, of the resulting wet gel was measured. The gel was then dried in a vacuum at 60°C for 16 h or more, and again weighted, W_2. The solvent regain, S_R, of the gel was calculated by the equation S_R (ml/g gel) $= [(W_1 - W_2)/\mathrm{SG} - 0.036\,W_2/d]/W_2$, in which SG and d are the specific gravities of the solvent and the gel, respectively.

The quantity of alcoholic hydroxyl groups in each gel was determined from the difference between the gel weights before and after acetylation. The dry gel was treated with acetic anhydride in pyridine at 90°C for 16 h or more, with continuous purging of the evolved nitrogen gas. The acetylated gel was filtered off, washed in methanol, dried in a vacuum and weighed.

RESULTS

Resistance to organic solvents

The resistance of GS-310, GS-320, GS-510 and GS-520 to lowering in efficiency by organic solvents is shown in Table I. GS-310, GS-510 and GS-520 generally showed no significant change in $N(4\sigma)$ or $N(5\sigma)$ with the passage of any of the organic solvents. The column efficiency of GS-320 was stable when methanol, ethanol and N,N-dimethylformamide (DMF) were used as eluents, but decreased substantially with acetonitrile, tetrahydrofuran (THF) and chloroform, the $N(5\sigma)$ values decreasing by 61, 44 and 27% respectively. The lowering of the efficiency of the GS-320 column by acetonitrile was particularly marked. For each column grade, the column pressure varied during the passage of the organic solvent, largely as a result of differing solvent viscosities.

Change of gel volume in organic solvents

For each of the gels GS-310, GS-510, GS-320 and GS-520, the ratio of the gel volume in an organic solvent to that in distilled water, V_S, was calculated by the

TABLE I

INFLUENCE OF ORGANIC SOLVENTS ON COLUMN EFFICIENCY

Solvent passed at 1.0 ml/min and room temperature. Efficiency determined with 50 μl of 1% ethylene glycol; distilled water as eluent; 30°C, RI detector. Values are: (1) $N(4\sigma) - N(5\sigma)$ before solvent passage; (2) $N(4\sigma) - N(5\sigma)$ after solvent passage; (3) pressure drop (kg/cm^2) during solvent passage.

Solvent	*GS-310*	*GS-510*	*GS-320*	*GS-520*
Methanol	(1) 18 700–13 900	19 700–17 100	18 900–14 100	18 700–12 400
	(2) 19 600–14 600	19 000–16 400	19 900–14 400	18 000–12 700
	(3) 24	17	26	20
Ethanol	(1) 26 100–23 100		23 300–17 100	20 400–16 800
	(2) 27 900–23 600		23 200–16 200	20 400–16 700
	(3) 56	53	42	30
Acetonitrile	(1) 24 700–16 700	21 600–17 000	24 100–17 100	20 100–14 600
	(2) 26 800–17 200	23 200–16 500	24 400– 6700	20 700–15 200
	(3) 14	18	15	10
Tetrahydrofuran (THF)	(1) 22 700–15 800	20 200–15 000	25 300–19 800	20 900–15 800
	(2) 24 400–15 400	20 300–15 200	21 600–11 000	19 700–15 300
	(3) 22	25	22	15
N,N-Dimethylformamide (DMF)	(1) 21 400–17 100	20 100–15 400	21 800–20 200	19 100–15 600
	(2) 18 600–15 900	19 400–14 800	22 500–20 900	19 500–16 000
	(3) 33	44	50	40
Chloroform	(1) 24 300–15 900	20 800–15 100	25 900–20 700	20 700–14 000
	(2) 23 900–18 500	21 000–14 900	18 500–15 100	21 600–14 800
	(3) 35	38	27	20
Distilled water	(3) 21 ± 7	30 ± 7	35 ± 5	30 ± 5

equation $V_S = (S_R + 1/d)/(S_{R,\text{ water}} + 1/d)$. The results are shown in Tables II and III.

All of the V_S values of GS-310 and GS-510 are larger than 1.00, indicating that these gels swell in all of the organic solvents tested. In contrast, the V_S values of GS-520 are less than 1.00 for several of the solvents, and those of GS-320 are lower than 1.00 for all solvents except DMF. This indicates that GS-520 and GS-320 tend to decrease in volume, or shrink, in organic solvents. It may also be noted that the gel pore size is larger in the GS-500 series than in the GS-300 series, and the above

TABLE II

SOLVENT REGAIN VALUES

Solvent	*Specific gravity*	*GS-310*	*GS-510*	*GS-320*	*GS-520*
Methanol	0.79	0.93	1.31	1.20	1.59
Ethanol	0.79	0.92	1.33	1.18	1.59
Acetonitrile	0.79	0.95	1.35	0.87	1.45
THF	0.89	0.92	1.29	0.98	1.47
DMF	0.95	0.95	1.38	1.25	1.49
Chloroform	1.49	0.98	1.34	1.02	1.67
Distilled water	1.00	0.81	1.25	1.22	1.59

TABLE III

V_S VALUES

Solvent	*GS-310*	*GS-510*	*GS-320*	*GS-520*
Methanol	1.08	1.03	0.99	1.00
Ethanol	1.07	1.04	0.98	1.00
Acetonitrile	1.09	1.05	0.82	0.94
THF	1.07	1.02	0.88	0.95
DMF	1.09	1.06	1.02	1.03
Chloroform	1.11	1.04	0.90	0.96

results thus indicate a tendency for the degree of swelling or shrinkage to increase with decrease in pore size.

Quantity of alcoholic hydroxyl groups

The quantity of alcoholic hydroxyl groups in GS-310, GS-510, GS-320 and GS-520 gels was found to be 1.8, 1.7, 6.9 and 5.7 mequiv./g gel, respectively. These values are in accord with the tendency for the GS-310 and GS-510 gels to swell more in organic solvents than in water and the opposite tendency of the GS-320 and GS-520 gels, if it is assumed that small quantities of hydroxyl groups tend to result in gel swelling in the presence of organic solvents while large quantities tend to cause gel shrinkage.

Relationship between gel swelling or shrinkage and resistance of column to organic solvents

Fig. 1 shows the relationship between gel swelling or shrinkage and the resistance of the column to lowering in efficiency by organic solvents. The parameter N_a/N_b is the ratio of the $N(5\sigma)$ value after solvent passage to that before solvent passage. In terms of this ratio, the original column efficiency is maintained if $V_S \geqslant 0.95$, but is decreased when $V_S \leqslant 0.9$. The original packing medium of the GS column

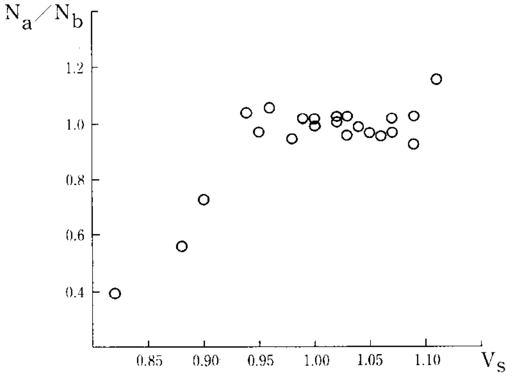
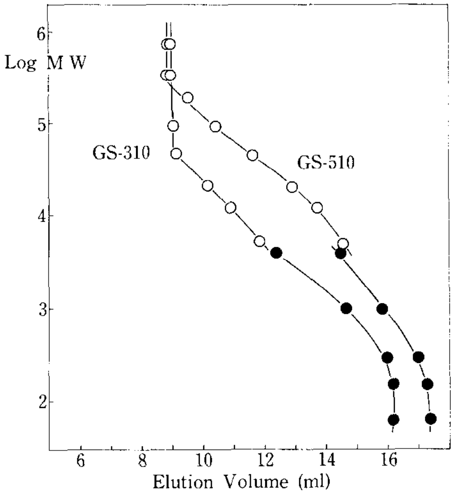


Fig. 1. Relationship between the V_S value and the column efficiency ratio before and after passage of organic solvents.



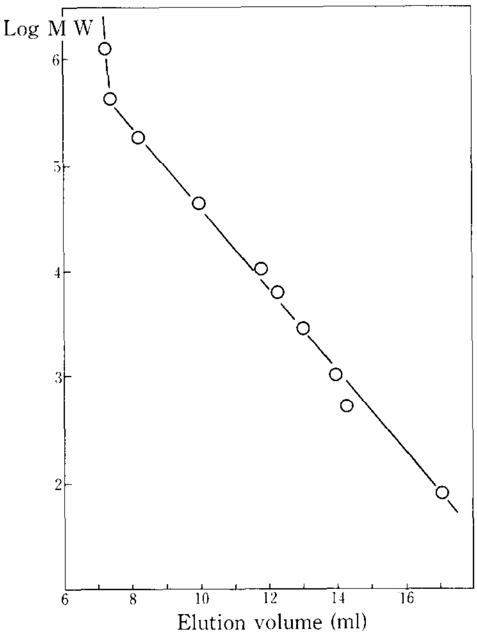


Fig. 2. Calibration curves obtained for pullulan (○) and poly(ethylene glycol) (●). Conditions: eluent, distilled water; flow-rate, 1 ml/min; sample concentration, 1%; charge, 50 μl; detection, refractive index; temperature, 30°C. MW = Molecular weight.

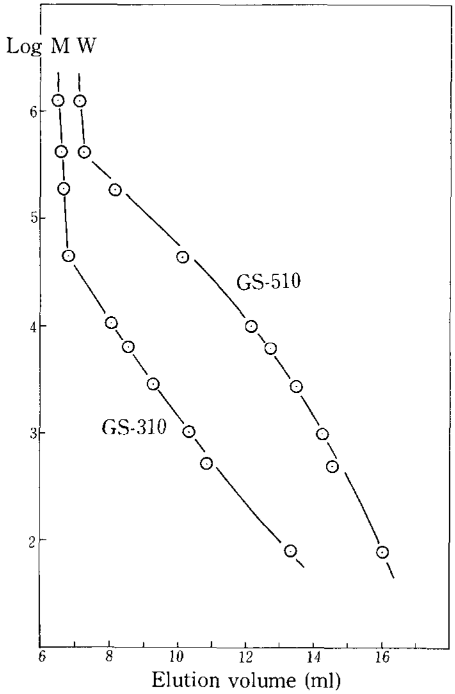
Fig. 3. Calibration curve obtained for standard polystyrene on GS-510 with DMF as the eluent. Conditions: flow-rate, 1 ml/min; sample concentration, 0.1%; charge, 50 μl; detection, UV 270 nm; temperature, 30°C.

series is an aqueous suspension and the column efficiency is thus stable with organic solvents causing at least the same degree of gel swelling as the original medium. It may reasonably by assumed that the configuration of the gel packing becomes non-uniform with solvents which cause more than 10% gel shrinkage, and that this results in decreased column efficiency.

Chromatographic analysis of hydrophobic substances

In view of the affinity of GS-310 and GS-510 gels for the hydrophobic solvents DMF and chloroform, as shown by their tendency to swell in these solvents more than in water, we evaluated the performance of these two columns in GPC analysis of hydrophilic substances.

Fig. 2 shows the calibration graphs for pullulan and poly(ethylene glycol) on GS-310 and GS-510 with distilled water as the eluent. The corresponding graph for polystyrene of the same GS-510 column with DMF is shown in Fig. 3, and those on both columns with chloroform are shown in Fig. 4. Although not shown here due to insufficient data, the calibration graph on the GS-310 column with DMF also appears to be linear and appropriate for chromatographic analysis. Sharp polystyrene peaks were obtained with the same columns, as shown in Fig. 5 for GS-510, and it



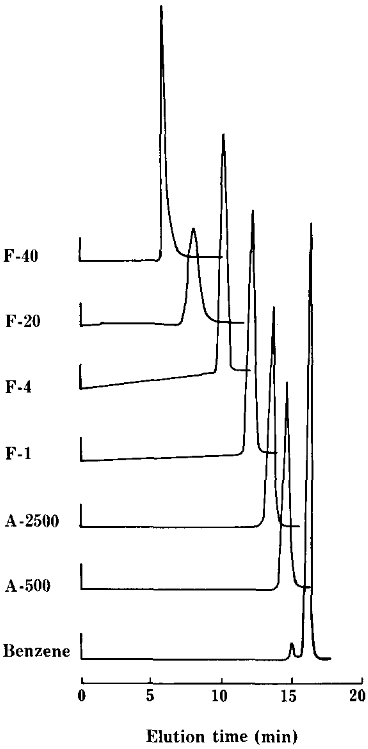


Fig. 4. Calibration curves obtained for standard polystyrene on GS-310 and GS-510 with chloroform as the eluent. Conditions as in Fig. 3 except detection, UV 250 nm.

Fig. 5. Analysis of standard polystyrene. The molecular weights of F-40, F-20, F-4, F-1, A-2500 and A-500 are 422 000, 186 000, 43 900, 10 300, 2800 and 500, respectively. Column: GS-510. Conditions as in Fig. 4.

thus appears that GS-310 and GS-510 will be useful for GPC analysis of hydrophobic as well as hydrophilic substances.

DISCUSSION

The results of this study show that the gels GS-310 and GS-510 both tend to swell in organic solvents, and indicate that each is amenable to the use of eluents consisting of special solvents such as DMF or chloroform as well as aqueous solutions. The two columns were also evaluated and found effective for GPC analysis of hydrophilic and hydrophobic substances. We are now planning to investigate the application of the amphipathic character of these columns with a broad range of solvents.

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