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Effect of polystyrene–divinylbenzene resin sulfonation on solute retention in high-performance liquid chromatography

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Abstract

Polystyrene–divinylbenzene (PS–DVB) resins were sulfonated under different conditions to give a series of resins with sulfonic acid capacities ranging from 0.27 to 2.63 mmol/g. Each of the resins was packed into a conventional column and used for HPLC separation of various substituted benzenes using acetonitrile–water solutions as the mobile phase. In a mobile phase of acetonitrile–water (30:70), a nearly linear decrease in retention factor with increasing sulfonic acid capacity was noted for a set of substituted benzenes (polar and non-polar). The retention factors at the highest sulfonic acid capacity studied (2.63 mmol/g) were only 0.10 to 0.16 that of the unsulfonated resin. The sulfonated resins were also used for quasi-normal-phase separations of polar analytes such as glycols and sugars. In binary acetonitrile–water mobile phases, retention factors increased in a regular fashion as the concentration of acetonitrile was increased from 70 to 95%. Retention factors of the same analytes also increased substantially with increases in sulfonic acid content of the resin. © 1998 Elsevier Science B.V.

Keywords: Stationary phases, LC; Polystyrene–divinylbenzene resins; Glycols; Sugars; Benzenes

1. Introduction

Sulfonated polystyrene–divinylbenzene (PS–DVB) polymers have been used for many years in ion-exchange chromatography of cations, as well as other analytes [1–8]. For modern ion chromatography the preferred resins are sulfonated under mild conditions and have low exchange capacities. For example, Sevenich and Fritz [9,10] presented extensive data on metal separations on resins containing only 6.1 μmol/g sulfonic acid exchange capacity. Linear plots were obtained for log adjusted retention time (or log k') versus log eluent ion activity. A paper by the same authors reported conditions for the preparation of sulfonated PS–DVB beads for ion chromatography [11]. Sulfonation was shown to occur on the surface of the 12–17 μm, 12% cross-linked resin beads.

Schmidt and Fritz [12] used low exchange capacity sulfonated PS–DVB material to pre concentrate organic solutes from aqueous matrices, followed by group separation on the same material. Morris and Fritz [13,14] compared carboxylated polacrylate and sulfonated PS–DVB packings for the separation of organic acids and small polar compounds by ion-exclusion. They concluded that the mechanism of retention was a partitioning of solutes between the mobile and stationary phases.

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Sun [15] used sulfonated PS–DVB stationary phased in high-performance liquid chromatography (HPLC). These investigations showed that sulfonated PS–DVB packing could be used for separating basic compounds from neutral compounds and weakly basic amines using a pre-column. A range of sulfonation capacities was used in an on-line mode to group-separate basic compounds before further separation on a porous PS–DVB HPLC column. Sulfonated polystyrenes were also used to separate basic and neutral compounds. Plots of log k' versus sulfonation capacity showed linear correlations for several compounds on a 10 cm×4.6 mm column packed with 10 μm sulfonated PS–DVB.

Dumont and coworkers [16–18] demonstrated the use of sulfonated macroporous PS–DVB packings for ion-exchange chromatography in non-aqueous solvents. The results suggested that solvation of the stationary phase contributes significantly to analyte selectivity. The use of non-aqueous eluents allowed separations that are often difficult to achieve in aqueous mobile phases. Addition of 18-crown-6 to the eluent improved peak shape and resolution for many ions [16]. For cation-exchange of several amines, plots of log k' versus log H^+ had a slope of nearly -1, indicating a purely cation-exchange mechanism instead of a mixed-mode mechanism involving hydrophobic interactions [18].

Dumont and Fritz [16] also used sulfonated PS–DVB material (8 μm) in solid-phase extraction (SPE) studies. It was shown that surface sulfonation of PS–DVB SPE material facilitated contact between solutes dissolved in water and the surface of the SPE packing. The best recoveries for many polar compounds were found at a PS–DVB sulfonation capacity of 0.6 mmol/g.

Recently, Otteneder and Marx [7] reported the use of sulfonated PS–DVB resins for the analysis of sugars and alcohols in wine. The stationary phase was a gel-type sulfonated polystyrene in the calcium form eluted with water.

The purpose of the present investigation was to determine how the degree to which polymer beads have been sulfonated will affect their properties when used in HPLC of neutral analytes. Resins with a broad range of sulfonic acid capacities (0 to 2.6 mmol/g) have been prepared and tested both for use in reversed-phase HPLC and in quasi-normal-phase chromatography of glycols and sugars.

2. Experimental

2.1. Apparatus

The chromatograph consisted of an Alltech Model 425 HPLC pump (Deerfield, IL, USA), a Model 783A UV absorbance detector (Applied Biosystems, Stone Mountain, GA, USA), Model 7000 switcher (Rheodyne, Cotati, CA, USA, used as injector), Hitachi Model D-2000 Chromato-Integrator (EM Science, Cherry Hill, NJ, USA), and a Model LP-21 Lo-Pulse pulse dampener (Scientific Systems, State College, PA, USA). The columns were 10 cm×4.6 mm I.D. stainless steel slurry-packed in our laboratory with bare or derivatized PS–DVB beads of 10 μm average particle diameter and 80–100 Å average pore diameter (Sarasep, Santa Clara, CA, USA). An Eldex Labs. column heater was used to maintain column temperature at 26°C.

2.2. Chromatographic procedure

Sample compounds were dissolved in acetonitrile and water. Chromatographic eluents were sparged with helium (Air Products, Des Moines, IA, USA) for 5 min before transferring to the eluent reservoir. Binary aqueous–organic solvent mixtures were used as mobile phases at a flow-rate of 1.2 ml/min. Samples were injected using a 5-μl injection loop (Rheodyne). Chromatographic peaks were detected using UV absorbance at 254 nm or 195 nm. Retention times were recorded with an integrator and the chromatographic retention factor, k', was calculated for each analyte using the relation: $k'=(t_R-t_0)/t_0$. Column hold-up time was determined by measuring the retention time of bromide ion or nitrate ion and ranged from 0.78 for unsulfonated PS–DVB to 0.55 for resin sulfonated at 2.63 mmol/g. All chromatograms were generated isocratically and retention data were reported as the average of at least three injections.

2.3. Column sulfonation

The method of PS–DVB resin sulfonation described by Dumont and Fritz was followed [16]. Briefly, 2 g of underivatized bulk PS–DVB particles (55% crosslinked) were slurried in 5 ml acetic acid in an ice bath followed by treatment with concen-

Table 1
Reaction conditions for PS–DVB sulfonation

Capacity (mmol/g)	H_2SO_4 (ml)	Reaction time	Temperature
0.00	0		
0.27	5	25 s	Ice
0.78	50	4 min	Ice
0.93	50	90 min	Room temperature
1.30	50	40 min	50°C
2.03	50	90 min	50°C
2.34	50	90 min	70°C
2.63	50	120 min	90°C

trated sulfuric acid for a designated reaction time and subsequently poured over ice to quench the reaction. Reaction times depended on the surface capacity of sulfonic acid groups desired. Capacities were determined by titration according to the method described in Ref. [6]. The sulfonated PS–DVB material was slurry-packed into stainless steel hardware at 3000 p.s.i. (1 p.s.i.=6894.76 Pa). Eight columns were used having capacities and reaction conditions as listed in Table 1.

3. Results and discussion

3.1. Reversed-phase HPLC

The chromatographic behavior of several organic analytes was compared on eight columns, each packed with one of the resins described in Table 1. The mobile phase consisted of either 30% or 50% acetonitrile in water. These concentrations were chosen with the expectation that retention times and retention factors would become progressively lower as resins with higher sulfonation capacities were used. Both 30% and 50% acetonitrile mixtures kept retention times within a reasonable range. A plot of retention factor versus sulfonation capacity is shown in Fig. 1 for benzene using 30% acetonitrile in water as the mobile phase. A progressive and large decrease in k' was observed as the sulfonation capacity was increased from 0.0 to 2.63 mmol/g. The graph is approximately linear. Deviations from linearity were probably due to errors in determining sulfonic acid capacity by titration or by differences in location of some of the sulfonic acid groups within the resin.

Similar trends were observed for four other aromatic compounds chromatographed under the same conditions. Table 2 lists retention data as a function of sulfonation capacity for each of the five solutes tested (standard deviations ranged from 0.01 to 0.9 for averages of at least three injections). The reduction in retention factor with increasing sulfonation is quite large. For example, the ratio of retention factor at the highest degree of sulfonation to that of the unsulfonated resin ranges from 0.10 for toluene to 0.16 for *p*-cresol. Thus, the retention factor can be adjusted to any desired value within the range simply by controlling the degree of resin sulfonation. The advantage here is that, where a polymeric column is beneficial, such as for the removal of octadecylsilane leachates in preparing a sample for nuclear magnetic resonance (NMR) analysis, the retention of large, late-eluting compounds can be abbreviated using a sulfonated resin. PS–DVB packings can also be used when pH constraints prohibit the use of silica-based

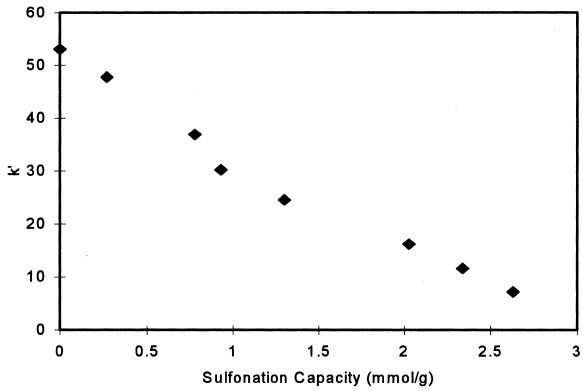

Fig. 1. k' versus column sulfonation capacity (mmol/g) for benzene in acetonitrile–water (30:70). UV absorbance at 254 nm. Flow-rate 1.2 ml/min. 100×4.6 mm stainless steel columns, 10 μm d_p sulfonated PS–DVB packing. Column temperature held at 26°C.

Table 2
Retention versus PS–DVB sulfonation capacity (mmol/g) for various compounds in acetonitrile–water (30:70) (retention times taken from the average of at least three injections)

Solute	Retention factor (k')	Sulfonation capacity (mmol/g)
p-Cresol	11.5	0.00
	11.4	0.27
	8.72	0.78
	6.88	0.93
	5.54	1.30
	3.77	2.03
	2.74	2.34
	1.83	2.63
Nitrobenzene	45.7	0.00
	41.5	0.27
	31.6	0.78
	25.9	0.93
	21.3	1.30
	14.1	2.03
	10.2	2.34
	6.40	2.63
Benzene	53.0	0.00
	47.8	0.27
	36.9	0.78
	30.2	0.93
	24.6	1.30
	16.2	2.03
	11.6	2.34
	7.18	2.63
Toluene	114.1	0.00
	101.9	0.27
	76.5	0.78
	61.1	0.93
	48.3	1.30
	29.7	2.03
	19.9	2.34
	11.1	2.63
Bromobenzene	–[a]	0.00
	184.1	0.27
	147.2	0.78
	120.6	0.93
	97.8	1.30
	60.9	2.03
	40.9	2.34
	23.6	2.63

[a] Prohibitively high retention.

materials. In this case, a sulfonated PS–DVB resin of the appropriate capacity can be chosen to keep analysis times within a reasonable range. Examples of actual chromatograms for three different sulfonation capacities are given in Fig. 2.

These results differ from those of Dumont and

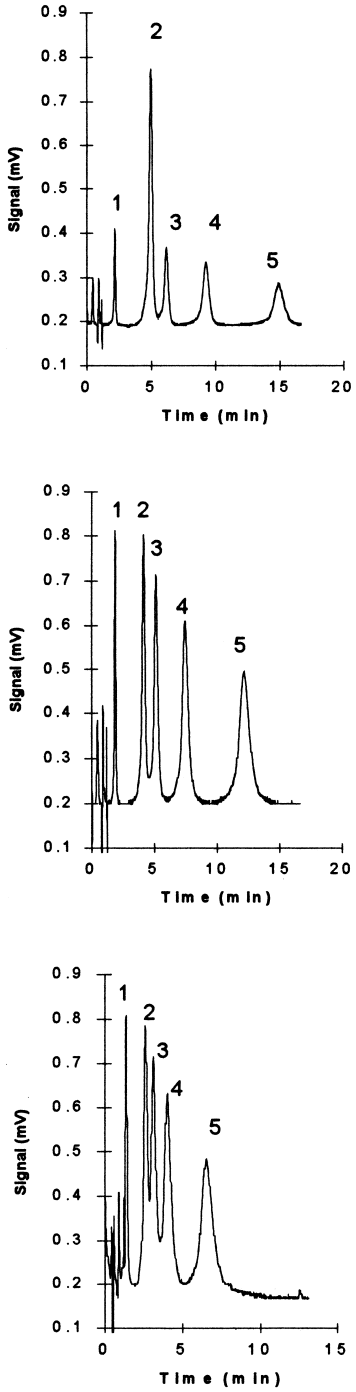

Fig. 2. Chromatograms of (1) *p*-cresol, (2) nitrobenzene, (3) benzene, (4) toluene and (5) bromobenzene in acetonitrile–water (50:50) on a PS–DVB column sulfonated at (a) 0.78 mmol/g, (b) 0.92 mmol/g and (c) 2.03 mmol/g. UV absorbance at 254 nm. Flow-rate 1.2 ml/min. Column temperature held at 26°C.

Fritz [16] where similar experiments were carried out in pure water. Their work showed a retention maximum at 0.6 mmol/g for plots of retention factor versus sulfonation capacity for phenol and catechol. At zero sulfonation capacity, the surface of the particle is hydrophobic and the polar solutes are poorly retained in pure water. At 0.6 mmol/g there are enough polar sulfonic acid groups on the surface to allow sufficient wetting of the surface by water, thus bringing the analyte into close contact with the stationary phase. At higher sulfonation capacities, the lower retention was attributed to decreasing overall hydrophobicity of the stationary phase. In the present work, the mobile phase contains a significant amount of organic solvent. This provides an excellent interface between the mobile phase and the stationary phase surface, thereby eliminating the difficulties encountered where water alone was in contact with unsulfonated or very slightly sulfonated resin.

3.2. Hydrophilic interaction

Normal-phase liquid chromatography is performed using a hydrophilic stationary phase such as silica gel or a RP silica with a very polar substituent. The mobile phase is generally a mixture of two organic solvents, a carrier solvent such as hexane and a more polar modifier solvent. The use of sulfonated PS–DVB columns in normal-phase applications has received comparatively little attention. Recently, separations have been shown for polar compounds such as sugars and alcohols in acetonitrile–water (80:20) and under gradient conditions using 0 to 45% water in acetonitrile [19]. These separations were performed on 25 cm Hamilton PRP-X400 columns (sulfonated PS–DVB). Several sugars were separated to near baseline resolution in roughly 15 min.

In the present work, the same set of sulfonated columns that was used for the reversed-phase separations was used to investigate the retention of glycols and sugars in a predominantly organic mobile phase (acetonitrile–water, 95:5). With highly polar groups on the surface of the stationary phase particles and an eluent of high organic content form a hydrophilic interaction system [20–28] which mimics conventional normal-phase chromatography. The advantage in this case, however, is that the

Table 3
Comparison of retention factors for a column at 2.34 mmol/g sulfonation capacity in the hydrogen and calcium forms in acetonitrile–water (95:5)

Compound	Hydrogen form	Calcium form
Ethylene glycol	3.13	12.67
Propylene glycol	2.49	10.83
Glycerol	5.40	35.57

number of polar groups on the surface can be controlled.

Preliminary results on the most highly sulfonated resin gave well shaped peaks for ethylene glycol, propylene glycol, and glycerol with retention factors ranging from 2.5 to 5.4. These values were obtained with the sulfonated resin the H^+ form. Conversion of the chromatographic column to the Ca^{2+} form (by passing an excess of calcium nitrate solution through the column) gave much higher retention factors, as shown in Table 3. The longer retention was probably due to the formation of weak complexes between the glycols and Ca^{2+} on the column. Since longer retention provided better resolution of glycols and sugars, all experiments in the hydrophilic interaction mode were performed with columns in the calcium form.

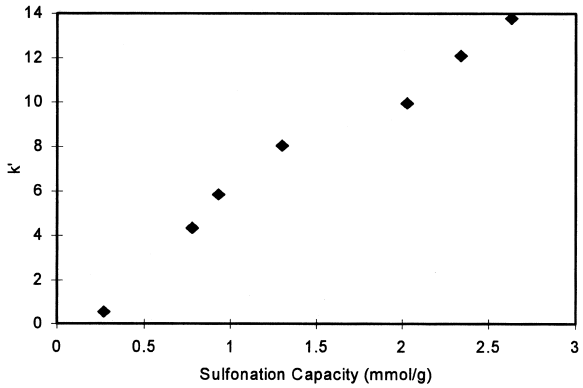

Fig. 3. k' versus column sulfonation capacity (mmol/g) for ethylene glycol in acetonitrile–water (95:5). UV absorbance at 254 nm. Flow-rate 1.2 ml/min. Column temperature held at 26°C. Column flushed with 10 m*M* $Ca(NO_3)_2$ before separations.

A plot of k' versus sulfonation capacity is given in Fig. 3 for ethylene glycol. Again, the change in retention as a function of sulfonation capacity is approximately linear. As expected, the graph has the opposite slope compared to benzene in acetonitrile–water (30:70) (Fig. 1), denoting *increased* retention

Table 4
Retention versus PS–DVB sulfonation capacity (mmol/g) for various compounds in acetonitrile–water (95:5)

Solute	Retention factor (k')	Sulfonation capacity (mmol/g)
Ethylene glycol	0.56	0.27
	4.32	0.78
	5.85	0.93
	8.05	1.30
	9.95	2.03
	12.11	2.34
	13.79	2.63
Propylene glycol	0.65	0.27
	4.69	0.78
	6.15	0.93
	7.99	1.30
	9.21	2.03
	10.42	2.34
Glycerol	0.96	0.27
	7.41	0.93
	22.92	1.30
	29.27	2.03
	37.77	2.34

Retention times from average of at least three injections. Column flushed with 10 m*M* $Ca(NO_3)_2$ before separations.

as sulfonation capacity is increased. It was noted in related experiments that polar compounds such as ethylene glycol and propylene glycol were hardly retained at all when the mobile phase contained a high percentage of water, indicating that hydrophobic interactions between the carbon atoms of the glycols and the PS–DVB surface are not strong enough to significantly influence retention under these conditions. However, the presence of the sulfonic acid group provides a site for possible hydrogen-bonding with the hydroxyls of the diols, thus increasing retention for these solutes in a mobile phase of high organic content. Values for retention factor as a function of sulfonation capacity for a set of polar compounds are listed in Table 4 (average of at least three injections with standard deviations ranging from 0.0 to 0.5).

This system can be very useful for the separation of glycols and sugars. An example is shown in the chromatogram in Fig. 4 for the separation of three of these compounds in acetonitrile–water (95:5) on a column of 0.78 mmol/g sulfonation capacity. A sulfonation capacity of 0.78 mmol/g or another intermediate sulfonation capacity is a good choice for this separation. High sulfonation capacities in this case would cause analysis times to be too high (refer to Table 4). However, a resin of higher sulfonation capacity might be used to resolve small polar solutes.

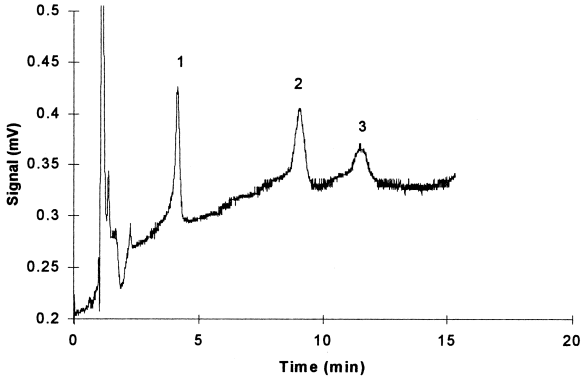

Fig. 4. Separation of (1) propylene glycol, (2) glycerol, (3) dextrose in acetonitrile–water (95:5). PS–DVB resin sulfonated at 0.78 mmol/g. UV absorbance at 195 nm. Flow-rate 1.2 ml/min. Column temperature held at 26°C. Column flushed with 10 m*M* $Ca(NO_3)_2$ before separations.

3.3. Retention as a function of mobile phase composition

In reversed-phase HPLC with an organic–water mixture as the mobile phase, a plot of log k' versus φ_{ACN}, where φ_{ACN} is the volume fraction of acetonitrile in the mobile phase, shows a smooth decrease in log k' with increasing values of φ_{ACN}. With normal-phase chromatography of glycols and sugars with acetonitrile–water mobile phases, the retention factors would be expected to *increase* as φ_{ACN} becomes larger. Table 5 gives data for log k' as a function of

Table 5
Log k' versus φ where φ=volume fraction of acetonitrile

Compound	Log k'	φ
Ethylene glycol	0.11	0.70
	0.17	0.75
	0.23	0.80
	0.31	0.85
	0.44	0.90
	0.69	0.95
Propylene glycol	0.05	0.70
	0.09	0.75
	0.14	0.80
	0.21	0.85
	0.32	0.90
	0.57	0.95
Glycerol	0.15	0.70
	0.24	0.75
	0.33	0.80
	0.46	0.85
	0.64	0.90
	0.98	0.95
D-Fructose	0.23	0.70
	0.36	0.75
	0.52	0.80
	0.72	0.85
	1.02	0.90
	1.48	0.95
Dextrose	0.24	0.70
	0.38	0.75
	0.56	0.80
	0.78	0.85
	1.11	0.90
	–[a]	0.95

Column sulfonation capacity 2.63 mmol/g, flushed with 10 m*M* $Ca(NO_3)_2$ before separations.
[a] Not measured.

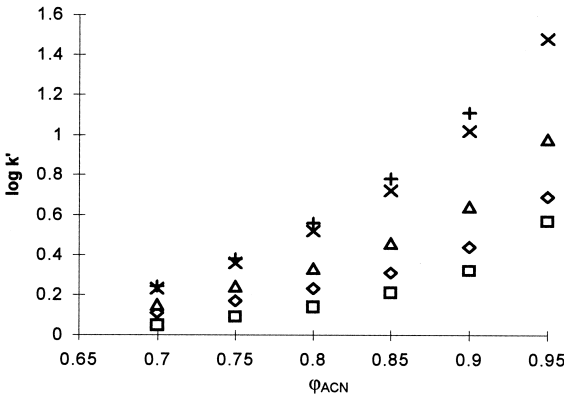

Fig. 5. Graph of log k' versus φ_{ACN} (volume fraction of acetonitrile) for ethylene glycol, propylene glycol, D-fructose and dextrose on a PS–DVB column sulfonated at 2.63 mmol/g. UV absorbance at 195 nm. Flow-rate 1.2 ml/min. Column temperature held at 26°C. Column flushed with 10 m*M* $Ca(NO_3)_2$ before separations.

φ_{ACN}, the volume fraction of acetonitrile. Fig. 5 shows smooth curves for log k' versus φ_{ACN} for the five polar compounds on a column packed with resin of 2.63 mmol/g sulfonation capacity in the Ca^{2+} form.

In these quasi-normal-phase chromatograms, water is acting as the stronger solvent in the mobile phase. By denoting φ_{H2O} as the volume fraction of water, plots such as that in Fig. 6 were obtained. Plotted in this manner, the curve is similar in appearance to a typical curve for reversed-phase chromatography. In fact, the curve in Fig. 6 gives a reasonable fit to the quadratic equation that best describes the effect of solvent composition on retention factor, in this case: $\log k' = A\varphi^2_{H2O} + B\varphi_{H2O} + C$.

For the plot in Fig. 6, $A=12.1$, $B=-9.1$ and $C=1.9$ with a quadratic fit coefficient of 0.999.

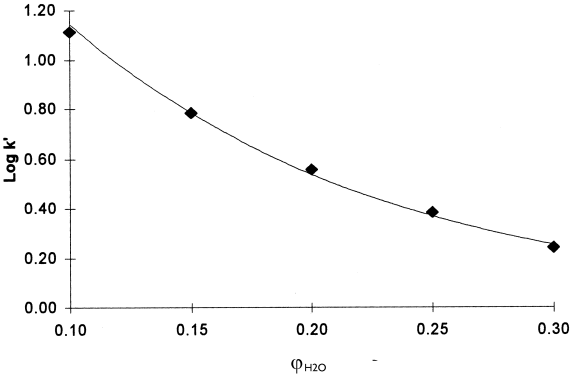

Fig. 6. Graph of log k' versus φ_{H2O} (volume fraction of water) for dextrose on a PS–DVB column sulfonated at 2.63 mmol/g. UV absorbance at 195 nm. Flow-rate 1.2 ml/min. Column temperature held at 26°C. Quadratic fit correlation coefficient=0.999; $A=12.1$, $B=-9.1$, $C=1.9$ for the quadratic equation log $K' = A\varphi^2 + B\varphi + C$.

4. Conclusions

For any given reversed-phase system the retention factors of organic analytes can be varied over a broad range simply by changing the sulfonic acid content of PS–DVB resins. The same resins may be used in hydrophilic interaction separations of glycols and sugars by using a high concentration of acetonitrile in a binary eluent with water. Although not yet as efficient as conventional column packings, sulfonated polymeric resins are easy to prepare and are very stable.

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References

[1] D.H. Freemen, S. Goldstein, G. Schmuckler, Isr. J. Chem. 7 (1969) 741.
[2] A. Klingenberg, A. Seubert, J. Chromatogr. 640 (1993) 167.
[3] L.C. Hansen, T.W. Gilbert, J. Chromatogr. Sci. 12 (1974) 458.
[4] K.H. Lieser, Radiochem. Radioanal. Lett. 18 (1974) 323.
[5] K.W. Pepper, J. Appl. Chem. 3 (1951) 124.
[6] F. Schoebrechts, E. Mercing, G. Daylkaerts, J. Chromatogr. 174 (1979) 351.
[7] H. Otteneder, R. Marx, Wein-Wiss. 50(No. 2) (1995) 67.
[8] T. Hargitai, P. Reinholdsson, B. Toernell, R. Isaksson, J. Chromatogr. 630 (1992) 79.

[9] G.J. Sevenich, J.S. Fritz, J. Chromatogr. 347 (1985) 147.
[10] G.J. Sevenich, J.S. Fritz, J. Chromatogr. 371 (1986) 361.
[11] G.J. Sevenich, J.S. Fritz, Reactive Polymers 4 (1986) 195.
[12] L. Schmidt, J.S. Fritz, J. Chromatogr. 640 (1993) 145.
[13] J. Morris, J.S. Fritz, Anal. Chem. 66 (1994) 2390.
[14] J. Morris, J.S. Fritz, LC·GC 11 (1993) 513.
[15] J.J. Sun, Thesis, Iowa State University, Ames, IA, 1991.
[16] P.J. Dumont, J.S. Fritz, J. Chromatogr. A 705 (1995) 149.
[17] P.J. Dumont, Thesis, Iowa State University, Ames, IA, 1995.
[18] P.J. Dumont, J.S. Fritz, L.W. Schmidt, J. Chromatogr. A 706 (1995) 109.
[19] Hamilton HPLC Application Handbook, Hamilton Company, Reno, NV, 1993, pp. 85–86.
[20] B.-Y. Zhu, C.T. Mant, R.S. Hodges, J. Chromatogr. 594 (1992) 75.
[21] A.J. Alpert, J. Chromatogr. 499 (1990) 177.
[22] H. Lindner, B. Sarg, C. Meraner, W. Helliger, J. Chromatogr. A 743 (1996) 137.
[23] M. Pauly, W.S. York, R. Guillen, P. Albersheim, A.G. Darvill, Carbohydr. Res. 282 (1996) 1.
[24] N. Takahashi, J. Chromatogr. A 720 (1996) 217.
[25] S.C. Churms, J. Chromatogr. A 720 (1996) 75.
[26] B. Sarg, W. Helliger, C. Meraner, H. Lindner, GIT Spez. Chromatogr. 15 (1995) 50.
[27] S.C. Churms, in: Z. El Rassi (Ed.), Carbohydrate Analysis – High-Performance Liquid Chromatography and Capillary Electrophoresis (Journal of Chromatography Library, Vol. 58), Elsevier, Amsterdam, 1995, p. 103.
[28] J. Yu, Z. El Rassi, J. High Resolut. Chromatogr. 17 (1994) 773.