

# Comparison of isocratic and gradient elution reversed-phase behaviour of high-molecular-mass polystyrenes in dichloromethane and acetonitrile

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## Abstract

Isocratic and gradient elution of high-molecular-mass polystyrenes were compared in a dichloromethane–acetonitrile mobile phase and C<sub>18</sub> bonded phase on a variety of different pore size silicas. Plots of the capacity factor versus the solvent composition were drawn from which solvent strength parameters, *S*, were determined for different molecular masses. Linear gradient elution data were also used to estimate *S* values using a graphical procedure. It was found that *S* depended on gradient rate and that only gradient rates of less than 2%/min gave constant values of *S*. Only molecular masses less than 50 000 gave good agreement with isocratic values. Plots of log *S* against log molecular mass were different for isocratic and gradient determined values of *S*.

## 1. Introduction

Several studies are reported [1] to have shown that the log of the isocratic capacity factor, *k'* varies linearly with  $\varphi$ , the volume fraction of good solvent, for low-molecular-mass compounds in water–methanol and water–acetonitrile reversed-phase solvent systems. The empirical relationship initially discussed by Snyder and co-workers [2,3] is

$$\log k' = \log k_0 - S\varphi \quad (1)$$

where *k*<sub>0</sub> is capacity factor in the poor solvent and *S* is a constant. Aguilar and Hearn [4] have proposed that the constant *S* is related to the

contact area of the molecule with the stationary phase surface and that log *k*<sub>0</sub> values reflect the affinity of the solute for the poorer solvent. Schoenmakers et al. [5,6] have argued on theoretical grounds that a quadratic relationship is more precise but that for normal operating conditions in an analytical separation, any differences would be negligible for low-molecular-mass compounds. With high-molecular-mass compounds the situation is less clear. Hearn [7] has clearly shown that plots of log *k'* against  $\varphi$  are curved due to two different modes of adsorption for peptides and proteins over large ranges of  $\varphi$ . Nevertheless, they have continued to use Eq. 1 over narrow ranges of  $\varphi$ . Polystyrenes are non-polar and would not be expected to show silanophilic interactions as do

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peptides and proteins. However, as the proportion of good solvent in the mobile phase increases, the capacity factors are expected to go to zero as size-exclusion chromatography becomes dominant.

Using gradient elution data to determine  $S$  and  $k_0$  is of special importance in the reversed-phase separation of high-molecular-mass synthetic polymers because, as the molecular mass increases, isocratic elution becomes more and more difficult to observe [8–11] and the direct application of Eq. 1 is difficult. Nevertheless, Lochmuller and McGranaghan [9] have been able to compare isocratically obtained values of  $S$  with gradient estimated values  $S$  for polystyrenes up to  $M_r$  300 000 in a dichloromethane–acetonitrile solvent system. They found linear relationships between  $\log k'$  and solvent composition for both tetrahydrofuran–water and dichloromethane–acetonitrile solvent systems using a 10 nm pore size,  $C_{18}$  column. A 30 nm pore size  $C_8$  bonded phase column also gave a linear plot. Using this column, agreement between isocratic and gradient  $S$  values was good, for both the dichloromethane–acetonitrile and the tetrahydrofuran–water systems. Gradient rates were between 1.6 and 5.0%/min. Alhedai et al. [10] investigated polystyrenes up to  $M_r$  390 000 in dichloromethane–methanol with 10 nm and 30 nm pore size  $C_{18}$  columns. They found a non-linear relationship between  $\log$  capacity factor and mobile phase composition. They also found poor agreement between isocratic and gradient estimated values of  $S$ . The gradient estimated values of  $S$  were considerably higher than the isocratic values but agreement improved as molecular mass increased. The gradient rates used were 2 to 4% dichloromethane per minute. Larman et al. [8] investigated the gradient elution of polystyrenes on four different pore size  $C_{18}$  columns in a tetrahydrofuran–methanol mobile phase but only up to a molecular mass of 50 000. They found reasonable agreement between isocratic and gradient derived  $S$  values. Gradient rates up to 15%/min were used. Later work by Quarry et al. [12] reexamined this work and concluded that better results were obtained when two well separated

gradient rates were used to estimate  $S$  and  $k_0$ . However, the highest gradient rate used in this later study was lowered to 2%/min.

Hearn [7] has found little correlation of  $S$  with molecular mass for peptides and proteins. However, this is to be expected in adsorption type chromatography of polymers with different chemical structures. All workers have found that  $S$  generally increases with molecular mass for synthetic polymers with the same chemical structure. Boehm et al. [13,14] found this increase to be monotonic for polystyrenes in both the dichloromethane–methanol and tetrahydrofuran–acetonitrile solvent systems. However, Lochmuller and McGranaghan [9] found a monotonic increase in  $S$  with molecular mass for a 30 nm pore size  $C_8$  column but not with a 10 nm pore size  $C_{18}$  column. They attributed this to available surface area effects. The BMAB theory [13–15] predicts that the slope of a  $\log S$  versus  $\log M$  plot should be one. However, all results reported so far, are lower than one.

When linear solvent strength (LSS) gradients [3] are used, Eq. 1 can be used to predict isocratic retention data from two or more gradient elution experiments [12]. The gradient steepness parameter,  $b$ , is proportional to  $S$  and given by

$$b = \Delta\phi St_0/t_G \quad (2)$$

where  $\Delta\phi$  is the change in the gradient in time  $t_G$  and  $t_0$  is the column void volume. To determine  $S$  and  $k_0$  for a solute, this relationship can be combined with Eq. 1 to give

$$t_g = (t_0/b) \log [2.3bk_0(t_s/t_0) + 1] + t_s + t_d \quad (3)$$

where  $t_g$  is the solute gradient elution time,  $t_s$  is the retention time of the solute under non-retained conditions and  $t_d$  the delay time of the gradient to column inlet [8]. Larman et al. [8] used an iterative method to estimate average values for  $S$  and  $k_0$ . Lochmuller and McGranaghan [9] used two gradient runs to solve for the two unknowns. Alhedai et al. [10] used a graphical method which allowed them to estimate  $S$  and the critical solvent composition from the gradient data. The critical solvent

composition was defined as the composition when  $k' = 1$ .

The aim of this paper is to report a simple graphical procedure for estimating  $S$  and  $k_0$  from gradient elution data and to compare  $S$  values for polystyrenes determined by both isocratic and gradient elution. The results were obtained using  $C_{18}$  bonded phases in a dichloromethane–acetonitrile solvent system, and were conducted over a wider range of molecular masses and column pore sizes than previous work. A column packed with pellicular material is included.

## 2. Experimental

All chromatographic experiments were performed using two M6000A pumps, a 660 solvent programmer and a U6K injector (Waters, Milford, MA, USA). The detector was a variable wavelength UVIS 200 set at 262 nm (Linear Instruments, NV, USA). Column temperature was maintained at 25°C in a thermostatted water jacket. In this work, flow-rates were 1.0 ml/min unless otherwise stated. All gradients were linear and started at 0.4 volume fraction of dichloromethane. Injection mass was 10  $\mu$ g. Injection volume was 10  $\mu$ l. Data acquisition was done with a laboratory-built system. Table 1 lists the columns that were used throughout this study. All columns were packed at  $400 \times 10^6$  Pa into 150 mm  $\times$  4.6 mm diameter column blanks. These columns are described in more detail elsewhere [16].

Acetonitrile and dichloromethane (HPLC

grade) were obtained from Mallinckrodt Australia. The monodisperse polystyrene standards used were molecular masses  $2.35 \cdot 10^3$ ,  $1.1 \cdot 10^5$ ,  $2.0 \cdot 10^5$  (Waters) and  $9.00 \cdot 10^3$ ,  $1.75 \cdot 10^4$ ,  $5.0 \cdot 10^4$ ,  $4.10 \cdot 10^5$  and  $9.29 \cdot 10^5$  (Polysciences, Warrington, PA, USA).

In the present study, the parameters  $S$  and  $k_0$  were determined by the following graphical method. Rearranging Eq. 2 gives

$$t'_G = \left( \frac{t_G}{\Delta\varphi} \right) = S \left( \frac{t_0}{b} \right) \quad (4)$$

where  $t'_G$  is the gradient time from 0 to 100%.

If  $t'_g = t_g - t_s - t_d$ , the gradient elution time corrected for size exclusion and gradient delay, then by substitution of Eq. 4 and  $t'_g$  into Eq. 3 we obtain,

$$t'_g = \left( \frac{t'_G}{S} \right) \log \left[ \left( \frac{2.3Sk_0t_s}{t'_G} \right) + 1 \right] \quad (5)$$

If  $k_0$  is very large as would be the case for high-molecular-mass polymers in most circumstances, (typically  $10^4$ – $10^5$  [9–11]) then the 1 could be neglected in Eq. 5, assuming realistic values of  $b$ ,  $\Delta\varphi$  and  $t_G$  are used. Rearrangement of Eq. 5 gives

$$\left( \frac{t'_g}{t'_G} \right) = \frac{1}{S} \log(2.3Sk_0t_s) - \frac{1}{S} \log t'_G \quad (6)$$

By plotting  $t'_g/t'_G$  against  $\log t'_G$ , a straight line with a slope of  $-1/S$  should be obtained with an intercept of  $1/S \log(2.3Sk_0t_s)$ . This allows  $S$  and  $k_0$  to be determined.

Table 1  
Column data

Column	Silica	Surface area (m <sup>2</sup> /g)	Carbon (%)	Reduced plate height <sup>a</sup>	
Pore size (nm)	Particle size ( $\mu$ m)				
7	6	Zorbax	350	14.5	2.08
50	10	LiChrospher	60	4.6	3.39
100	10	LiChrospher	30	1.8	10.0
Pellicular	30–40	Perisorb	14	0.9	20.4

<sup>a</sup> Measured from phentole using conditions of Bristow and Knox [20].

### 3. Results and discussion

The isocratic elution of high-molecular-mass polystyrenes was observed in agreement with previous workers [8–11]. The isocratic elution of the higher-molecular-mass polymers became increasingly more difficult to observe as the molecular mass increased and this was also found by these previous workers. Typically, the solvent range would vary by as little as 1% between complete retention and elution at the solvent front for the  $M_r$   $4.10 \cdot 10^5$  and  $9.29 \cdot 10^5$  polystyrenes. Without making solvent mixtures, which introduces composition stability problems with such volatile solvents as dichloromethane, we observed the isocratic elution of these polystyrenes but only by chance. Increasing the column pore size allowed observance of isocratic elution of higher molecular masses, as Fig. 1 illustrates for the  $M_r$  929 000 polystyrene on the 100 nm pore size column.

To determine the capacity factor, the unretained elution volume of the solute is required. For small molecules this is closely approximated by the solvent void volume. This approximation worsens on porous columns as the size of the solute increases relative to the solvent. Previous workers [8–12] have used the size-exclusion volume of the solute in pure good solvent as a measure of the unretained retention volume.

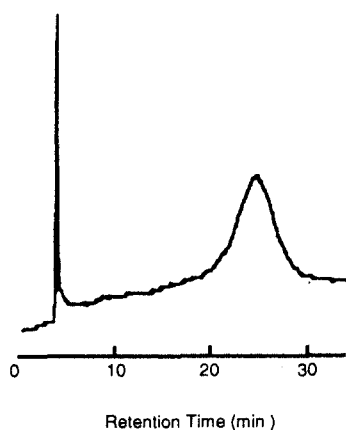


Fig. 1. Isocratic elution of  $M_r$  929 000 polystyrene. Mobile phase dichloromethane–acetonitrile (58:42), flow-rate 1.0 ml/min, sample  $10 \mu\text{g}$  in  $10 \mu\text{l}$  dichloromethane, 100 nm pore size,  $10 \mu\text{m}$  particle size  $C_{18}$  column.

However it is known [17] that the hydrodynamic volume of a polymer molecule varies with the solvent composition. Viscosity measurements [18] indicate that the radius of gyration of an  $M_r$  113 000 polystyrene molecule in 0.6 volume fraction dichloromethane–acetonitrile solvent is about 15% smaller than in pure dichloromethane. For  $M_r$  470 000 polystyrene the percentage decrease is 25. Hence the size-exclusion elution volume will only be an approximation to the unretained retention volume.

Size-exclusion experiments in dichloromethane–acetonitrile solvent mixtures with decreasing dichloromethane composition show that the elution volume increases exponentially with acetonitrile volume fraction. Fig. 2 shows these results for  $M_r$  110 000 polystyrene on the 50 nm pore size column. Benzene showed an elution volume of 2.0 ml on this column which does not change with mobile phase composition. The mobile phase composition at which the  $M_r$  110 000 polystyrene just dissolves (solubility composition) is 0.465 volume fraction dichloromethane [19]. Yet at a volume fraction of 0.6 dichloromethane, considerably higher than the solubility composition where size exclusion might

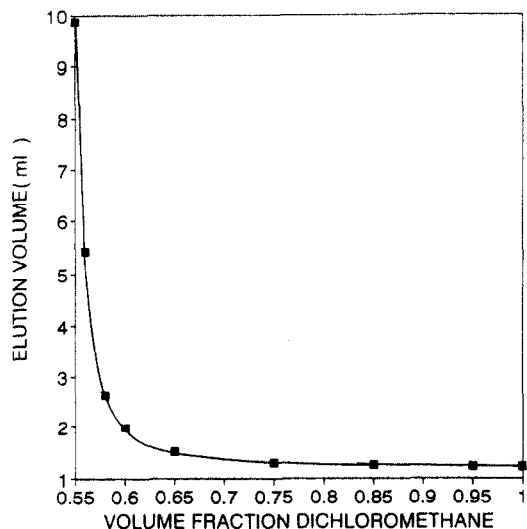


Fig. 2. Retention volume as a function of dichloromethane composition;  $10 \mu\text{g}$   $M_r$  110 000 polystyrene in  $10 \mu\text{l}$  dichloromethane, flow-rate 1.0 ml/min, 50 nm pore size,  $10 \mu\text{m}$  particle size  $C_{18}$  column.

be expected to be dominant, the elution volume was 2.0 ml. Obviously  $M_r$  110 000 polystyrene cannot have the same size as benzene and there must be retention to explain the same elution volume. There was no sharp change from size-exclusion to reversed-phase chromatography. Adsorption effects must be present at much higher concentrations of dichloromethane than the solubility composition. In these circumstances an accurate estimate of the unretained elution volume is not possible from chromatography and so we also used the elution volume in 100% dichloromethane as a measure of the unretained volume of a solute for capacity factor calculations. These elution volumes, for each column and each molecular mass, are shown in Table 2.

Fig. 3 illustrates the relationship between  $\log k'$  and the solvent composition for various molecular mass polystyrenes up to  $M_r$  200 000 eluted isocratically on the 50 nm pore size column. This graph used isocratic retention times reported in Table 3 and the size-exclusion volumes reported in Table 2. Similar graphs were drawn for the other columns using data reported in Tables 3 and 4. Isocratic  $S$  values for each column were determined from these graphs. There is evidence of very slight curvature in

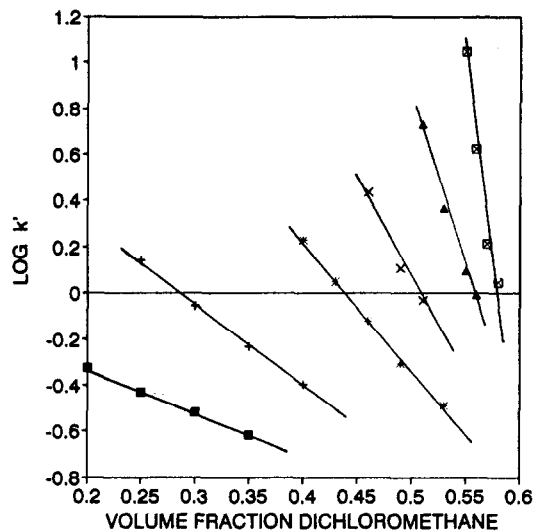


Fig. 3. Isocratic  $\log k'$  against volume fraction dichloromethane. Sample 10  $\mu\text{g}$  polystyrene in 10  $\mu\text{l}$  dichloromethane, flow-rate 1.0 ml/min, 50 nm pore size, 10  $\mu\text{m}$  particle size  $C_{18}$  column. Polystyrene  $M_r$ :  $\blacksquare$  = 800; + = 2350; \* = 9000;  $\times$  = 12 500;  $\blacktriangle$  = 50 000;  $\square$  = 110 000.

some of these graphs at the highest molecular masses. Curvature in these graphs has also been reported by Alhedai et al. [10]. The  $S$  values reported are obtained from the slopes of straight lines of best fit in all cases.

Gradient elution of the polystyrenes became more difficult to observe as the molecular mass decreased and as the gradient rate decreased. As the molecular mass and gradient rate decreased, peaks became broader and eventually merged into the baseline. Table 5 lists the retention data for the polystyrenes on the various  $C_{18}$  columns for various gradient rates. Fig. 4 illustrates a graph of  $t'_g/t'_G$  versus  $\log t'_G$  for molecular masses 17 500 to 929 000 on the 50 nm pore size column, using data from Table 5 and values of the size-exclusion elution volume, given in Table 2. Graphs for the other columns were similar. When steep gradients were used, the graphs were initially curved and became linear as the gradient steepness decreased. At gradient rates greater than 2%/min, curvature was evident even for the lowest molecular mass studied (17 600) but the curvature increased as the molecular mass increased. This implies that the

Table 2

Size-exclusion volumes on the various columns for the polystyrenes

Molecular mass	Size-exclusion elution volume (ml) on column of pore size			
	7 nm	50 nm	100 nm	Pellicular
78	1.600	2.000	2.150	1.030
800	1.310	1.935	2.150	0.980
$2.35 \cdot 10^3$	1.225	1.885	2.080	0.980
$9.00 \cdot 10^3$	1.115	1.783	2.050	0.970
$1.75 \cdot 10^4$	1.090	1.666	2.030	0.950
$5.00 \cdot 10^4$	1.075	1.475	1.970	0.950
$1.10 \cdot 10^5$	1.060	1.240	1.850	0.950
$2.00 \cdot 10^5$	1.048	1.075	1.580	0.950
$4.10 \cdot 10^5$	1.035	1.036	1.570	0.950
$9.29 \cdot 10^5$	1.032	0.975	1.570	0.950

Flow-rate = 0.5 ml/min, 1.0 dichloromethane.

Table 3  
Isocratic retention times for 7 nm and 50 nm pore size columns

Dichloromethane (volume fraction)	Retention time (min) for molecular mass						
	800	2350	9000	17 500	50 000	110 000	200 000
<i>7 nm pore size column</i>							
0.32	3.50						
0.38	2.92						
0.42	2.63	4.87					
0.46	2.48	3.58					
0.50		2.78					
0.52			4.52				
0.54		2.23	3.30	7.10			
0.56			2.50	3.78	15.00		
0.57				2.89	6.45		
0.58		1.58	2.03	2.42	3.52	13.05	
0.59				2.07	2.34	3.25	
0.60					2.05	2.45	
<i>50 nm pore size column</i>							
0.20	2.35						
0.25	2.65	4.50					
0.30	2.52	3.55					
0.35	2.40	2.99					
0.40		2.65	4.80				
0.43			3.78				
0.46			3.13	6.20			
0.49				3.81			
0.50			2.03	3.22			
0.51					9.40		
0.53					4.90		
0.55					3.73	15.00	
0.56					2.93	6.44	
0.57						3.26	7.26
0.58						2.62	3.44

rate of diffusion of mobile phase into and from these large solvated molecules may be a limiting factor in this chromatography. Similar suggestions have been made by Lochmuller and McGranaghan [9] and Shalliker [18] to explain anomalous elution behaviour of polystyrenes.  $S$  values were estimated from the slopes of these curves using only gradient rates of less than 2% dichloromethane per minute.  $S$  values were also estimated from these same data using the method described by Larman et al. [8]. No significant differences were observed.

The large variations in  $S$  values observed by other workers for high-molecular-mass polymers

may have resulted because of this curvature when steep gradients were used. The result of this curvature is that values of  $S$ , estimated at high gradient rates, are lower than values estimated at gradient rates less than 2%/min as the slope equals  $-1/S$ . In the study by Larman et al. [8],  $S$  values were estimated from gradient rates between 0.75 and 16%/min. Data from Larman et al.'s study for two different columns were plotted to produce graphs of  $t'_g/t'_G$  versus  $\log t'_G$  that were non-linear (Fig. 5). Later work by this group [12] used gradients of lower steepness and ascribed the differences in  $S$  obtained at different gradient rates to errors introduced by large

Table 4  
Isocratic retention times for 100 nm pore size and pellicular columns

Dichloromethane (volume fraction)	Retention time (min) for molecular mass						
	800	2350	9000	17 500	50 000	110 000	200 000
<i>100 nm pore size column</i>							
0.20	2.55						
0.25	2.41	3.21					
0.30	2.30	2.76					
0.35	2.20	2.45					
0.40		2.29	3.16				
0.43			2.67	4.46			
0.46			2.40				
0.49				2.91			
0.50			2.39	2.71	5.23		
0.51			2.26	2.48	3.42		
0.53				2.34	2.79	5.40	
0.55						3.90	
0.56					2.24	2.88	6.41
0.57						2.60	3.46
0.58							2.30
<i>Pellicular column</i>							
0.25		1.62					
0.30		1.42					
0.35		1.29					
0.42			1.90	6.00			
0.46			1.37	3.00			
0.50				1.54	18.80		
0.52				1.29	4.12		
0.54					1.75		
0.55					1.43		

values of  $k_0$ . It seems likely that at least part of this difference can be assigned to obtaining low values of  $S$  for high gradient rates, especially as a similar trend has now been obtained by different workers, in two different solvent systems and on columns of different pore sizes.

$S$  values estimated from isocratic and gradient data are shown in Table 6. Where possible a range, estimated from all possible slopes, is included. The range of molecular masses for which a comparison of the  $S$  values can be made is not large. The range is smaller when gradient rates of less than 2% dichloromethane per minute are used and larger when larger pore size columns are used. Reasonable agreement was obtained for molecular masses up to 50 000.

Above this molecular mass, isocratic  $S$  values tend to be higher than gradient  $S$  values. The differences are of the order of 100% for molecular masses 110 000 and 200 000. This is too large to be experimental error. The error of neglecting the size decrease in poorer solvents and hence underestimating the unretained retention volume can be estimated for the  $M_r$  110 000 polystyrene. By using size estimates from viscosity data of  $M_r$  110 000 polystyrene, it was estimated that  $S$  values would increase by only about 5% for both isocratic and gradient determined values. Thus the reasons for the difference remain unclear. Lochmuller and McGranaghan [9] obtained good agreement between isocratic and gradient  $S$  values, with a 30 nm pore size  $C_8$  column up to a

Table 5  
Retention times at various gradient rates for the polystyrenes

Molecular mass	Retention time (min) at gradient rate					
	10%/min	5%/min	2%/min	1%/min	0.5%/min	0.25%/min
<i>7 nm C<sub>18</sub> column</i>						
1.75 · 10 <sup>4</sup>	9.55	11.25	15.80	22.67	34.58	
5.00 · 10 <sup>4</sup>	9.50	11.37	16.51	24.83	39.92	
1.10 · 10 <sup>5</sup>	9.50	11.35	16.71	26.05	43.33	
2.00 · 10 <sup>5</sup>			16.80	26.47	44.20	
4.10 · 10 <sup>5</sup>	9.50	11.37	16.85	26.50	44.40	
9.29 · 10 <sup>5</sup>			16.85	26.54	44.71	
<i>50 nm C<sub>18</sub> column</i>						
1.75 · 10 <sup>4</sup>	9.38	10.30	12.38	14.77	18.15	21.28
5.00 · 10 <sup>4</sup>	9.77	11.25	14.86	20.58	29.70	45.82
1.10 · 10 <sup>5</sup>	9.88	11.70	16.30	23.97	37.75	63.08
2.00 · 10 <sup>5</sup>			16.83	25.43	41.43	71.22
4.10 · 10 <sup>5</sup>	9.87	11.88	16.88	25.78	42.48	72.19
9.29 · 10 <sup>5</sup>			16.90	26.22	43.28	76.36
<i>100 nm C<sub>18</sub> column</i>						
1.75 · 10 <sup>4</sup>			12.07	11.55	12.35	
5.00 · 10 <sup>4</sup>			14.15	18.78	27.40	
1.10 · 10 <sup>5</sup>			16.06	23.43	37.73	
2.00 · 10 <sup>5</sup>			16.58	24.77	39.93	
4.10 · 10 <sup>5</sup>			17.00	25.65	41.00	
9.29 · 10 <sup>5</sup>			17.23	26.03	42.81	
<i>Pellicular column</i>						
1.75 · 10 <sup>4</sup>			10.90	13.57	17.95	
5.00 · 10 <sup>4</sup>			13.40	19.35	29.98	
1.10 · 10 <sup>5</sup>			15.10	22.91	37.36	
4.10 · 10 <sup>5</sup>			15.55	24.20	40.58	
9.29 · 10 <sup>5</sup>			15.68	24.62	41.55	
7.00 · 10 <sup>6</sup>			15.85	24.98	42.48	
1.50 · 10 <sup>7</sup>			15.85	25.00	42.65	

Delay time,  $t_d = 5.4$  min. Flow-rate = 1.0 ml/min. Initial  $\phi = 0.40$  dichloromethane, final  $\phi = 1.0$  dichloromethane.

molecular mass of 300 000 in a dichloromethane–acetonitrile mobile phase. Gradient rates were 1.7 and 2.5% dichloromethane per minute. Alhedai et al. [10] found differences of greater than 100% between gradient and isocratic  $S$  values on 10 nm and 30 nm pore size  $C_{18}$  columns using dichloromethane–methanol solvent system. Gradient rates were 2–4% dichloromethane per minute. These workers found gradient  $S$  values to be significantly higher than

isocratic values with agreement improving as the molecular mass increased to 100 000. This is directly opposite to the trend reported here. The three sets of results are not directly comparable because they were conducted with different columns and mobile phases. However it is clear that more effort is required to reconcile these disparate trends.

Fig. 6 shows plots of  $\log S$  versus  $\log$  molecular mass for the isocratically determined values



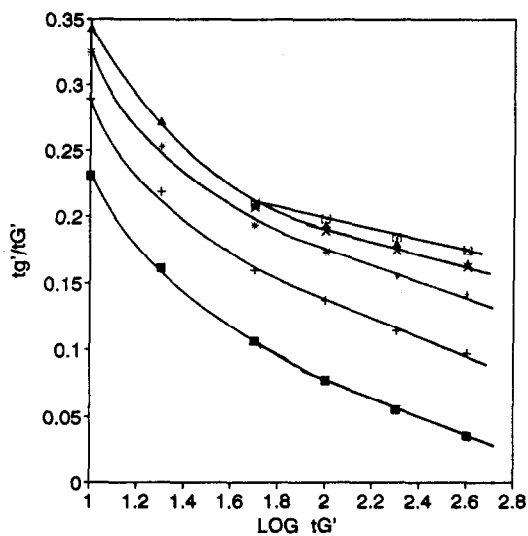


Fig. 4. Gradient  $S$  determination. Initial composition 0.4 dichloromethane, final composition 1.0 dichloromethane, flow-rate 1.0 ml/min, delay time 5.4 min, 50 nm pore size, 10  $\mu\text{m}$  particle size column. Polystyrene  $M_r$ : ■ = 17 500; + = 50 000; \* = 110 000; × = 200 500; ▲ = 410 000; □ = 929 000.

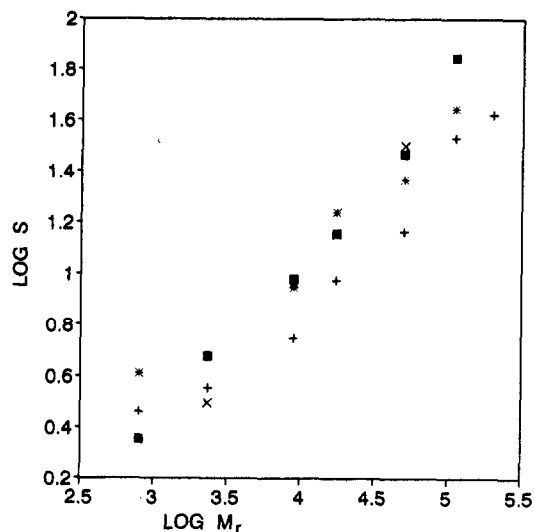


Fig. 6. Isocratic  $\log S$  against  $\log$  molecular mass. ■ = 7 nm pore size  $C_{18}$  column; + = 50 nm pore size  $C_{18}$  column; \* = 100 nm pore size  $C_{18}$  column; × = pellicular  $C_{18}$  column.

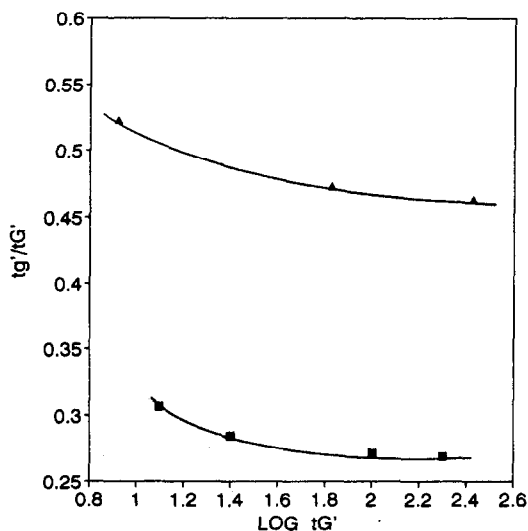


Fig. 5. Gradient  $t'_g/t'_G$  against  $\log t'_G$  using data of Larman et al. [8]. Mobile phase tetrahydrofuran–water, flow-rate 2.0 ml/min, delay time 2.2 min, sample  $M_r$  50 000 polystyrene. ■ = 15 nm pore size  $C_{18}$  column, gradient 0.6 to 1.0 tetrahydrofuran; ▲ = 30 nm pore size  $C_{18}$  column, gradient 0.4 to 1.0 tetrahydrofuran.

on the four different columns. There is not much difference. The slopes of all plots are similar and equal to 0.6. There is no evidence of a lower slope for the higher molecular masses and the virtually non-porous and low surface area pellicular column falls into the same set as the porous supports. Fig. 7 shows the same plots with  $S$  values obtained from gradient elution data and the plots differ appreciably. Closest agreement with isocratic data is shown by the 7 nm pore size column and the pellicular column. Both these columns do not allow significant pore access to the polystyrenes for which gradient elution data could be obtained. However, both plots tail off at higher molecular masses and only have slopes of 0.4 on the linear portion at low molecular masses. The graphs for the two columns with larger pores have much smaller slopes than the equivalent isocratic ones. Possible reasons for the poor agreement between isocratic and gradient results are that either LSS theory does not hold for large molecules, or that different mechanisms, or different available surface areas operate in the retention of large molecules for gradient elution versus isocratic elution.

Table 6  
S values for isocratic and gradient elution

Molecular mass	S							
	Isocratic				Gradient			
	Column pore size				Column pore size			
	7 nm	50 nm	100 nm	Pellicular	7 nm	50 nm	100 nm	Pellicular
800	2.3 ± 0.1	3.1 ± 1.3	4.1 ± 0.6					
2350	4.7 ± 0.2	3.6 ± 0.2	4.9 ± 0.3	3.2 ± 0.2				
9000	9.6 ± 0.3	5.6 ± 0.3	5.6 ± 1.3	9.2				
17 500	15.9 ± 1.5	9.4 ± 2.5	8.8 ± 1.5	14.4 ± 1.4	13.2 ± 0.9	10.1 ± 3.1	18.0	18.2 ± 3.0
50 000	29.4 ± 3.0	14.7 ± 2.3	17.5 ± 5.2	31.5 ± 3.8	18.0 ± 0.5	15.9 ± 3.7	17.0 ± 2.9	26.3 ± 1.1
110 000	69.9	34.1 ± 6.6	22.3 ± 7.6		29.4 ± 4.0	18.6 ± 2.5	25.3 ± 6.7	30.2 ± 1.1
200 000		41.7	44.1 ± 2.8		33.0 ± 7.0	21.1 ± 2.3	22.1 ± 1.5	46.9 ± 7.9
410 000					33.3 ± 7.3	22.4 ± 1.8	20.0 ± 1.0	56.8 ± 20
929 000					36.4 ± 7.3	26.0 ± 3.8	23.2 ± 3.3	64.4 ± 17

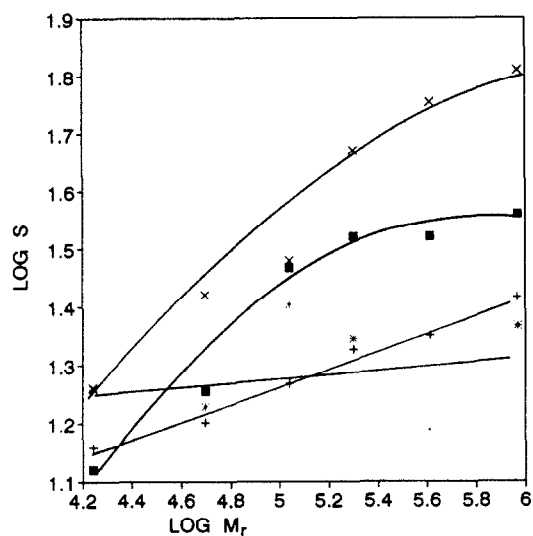


Fig. 7. Gradient log S against log molecular mass. ■ = 7 nm pore size C<sub>18</sub> column; + = 50 nm pore size C<sub>18</sub> column; \* = 100 nm pore size C<sub>18</sub> column; × = pellicular C<sub>18</sub> column.

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