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ISOCRATIC AND GRADIENT-ELUTION LIQUID CHROMATOGRAPHY OF STYRENE OLIGOMERS ON SILICA GEL

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SUMMARY

The separation of styrene oligomers on silica gel was compared using 1,4-dioxane–*n*-heptane and tetrahydrofuran–*n*-heptane mobile phases. A simple equation can be used to describe the dependence of the capacity factors for oligomers in normal-phase chromatography both on the degree of polymerization and on the mobile phase composition. It can also be used as the basis of calculations of retention volumes under isocratic conditions and in gradient-elution chromatography. The application of a convex gradient of dioxane in *n*-heptane enables at least the partial separation of individual oligostyrenes having up to 25–30 oligomer units and the concentration ratios of the individual oligomers in polystyrene samples can be estimated.

INTRODUCTION

Gel permeation chromatography (GPC) is frequently used for separations of styrene oligomers. More recently, supercritical fluid chromatography has been introduced for this purpose[1,2]. Reversed-phase liquid chromatography yielded separations of lower oligomers far superior to those achieved by GPC[3]. Styrene oligomers having polymerization degrees, N, up to 11 were separated on octadecylsilica columns eluted with pure acetonitrile[4]. Better separations can be achieved using gradient elution with an increasing concentration of tetrahydrofuran in water[5–8] or of tetrahydrofuran in methanol[6,9]. The influence of various factors on the separation of styrene oligomers has been investigated, such as the nature, pore size and surface area of chemically bonded non-polar phases[7,10–13] and the mobile phase[12,13]. Some combinations of bonded phases and solvents used as the mobile phases allow both the separation of styrene oligomers according to their degree of polymerization and the resolution of some stereoisomers of polystyrene oligomers[11–13]. Non-aqueous reversed-phase chromatography on octadecylsilica columns eluted with a gradient of dichloromethane in methanol separated styrene oligomers having values of N of up to 20 and of some higher polymers of low polydispersity[14,15]. The separation is believed to occur by a combination of hydrophobic and precipitation mechanisms[14–18] and a theory describ-

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ing this process was developed[16–18]. Aqueous and non-aqueous reversed-phase chromatography on phenyl-bonded phases were also used for isocratic separations of styrene oligomers with tetrahydrofuran–water, acetonitrile–water or tetrahydrofuran–*n*-hexane as mobile phases, and an equation was derived to describe the relationships between the logarithms of capacity factors, k', of the oligomers and both the degree of polymerization and the concentration of the organic solvent in water as the eluent[19,20].

Normal-phase chromatography on nitrile-bonded phases eluted with a gradient of dichloromethane in isooctane separated the first twelve styrene oligomers, and a linear relationship between log k' of the oligomers and the degree of polymerization, N, was reported[21].

Similar results were achieved on silica gel with a convex gradient of dichloromethane in *n*-hexane, which allowed the partial separation of 8–10 low-molecular-weight styrene oligomers[22]. Better resolution was obtained by using isocratic elution with tetrahydrofuran in a non-polar aliphatic hydrocarbon[23]. When the mobile phase was comprised of tetrahydrofuran and *n*-hexane or of ethyl acetate and *n*-hexane, the styrene oligomers were separated only according to the number of oligomer units, whereas with dichloromethane–*n*-hexane, separation of the stereoisomers of the styrene oligomers also occurs[24]. The retention behaviour was explained on the basis of additive contributions to the adsorption from identical oligomer units and from end groups[24,25].

The aim of the present work was to derive and to verify relationships between the retention of oligomers and both the degree of polymerization and the mobile phase composition in normal-phase chromatography and to compare the separation of oligostyrenes in mobile phases containing 1,4-dioxane and tetrahydrofuran under isocratic conditions and in gradient-elution chromatography.

THEORETICAL

Retention relationships

The basic equation describing the retention of solutes in normal-phase chromatography on polar adsorbents was derived by Snyder[26]:

$$\log K_D = \log V_a + \alpha\,(Q^0 - A_s\varepsilon^0) \tag{1}$$

where K_D is the distribution coefficient, V_a is the volume of the adsorbed solvent monolayer per unit weight of adsorbent, α characterizes the activity of the adsorbent, Q^0 is the dimensionless free energy of adsorption of the sample compound from *n*-pentane as the solvent, A_s is the area occupied by an adsorbed sample molecule on the adsorbent surface and ε^0 is the solvent strength of the mobile phase used. The distribution coefficient is related to the capacity factor, k'

$$k' = \frac{V_R}{V_M} - 1 = K_D \cdot \frac{V_s}{V_M} = K_D\varphi \tag{2}$$

where V_R is the retention volume of the solute, φ is the phase ratio in the column and V_M, V_s are the volumes of the mobile and stationary phases, respectively. Using eqns. 1 and 2 and a simplified relationship between the solvent strength and the mole fraction, x, of a polar solvent (b) in a solvent (a) of low polarity (such as an aliphatic hydrocarbon) in a binary mobile phase, the following equation was derived for the dependence of the retention on the mobile phase composition

$$\log k' = \log \varphi + \log V_a + \alpha Q^0 - \alpha A_s \varepsilon_b^0 - \frac{A_s}{n_b} \cdot \log x = \log k'_0 - m \cdot \log x \quad (3)$$

which is valid over a limited range of mobile phase compositions[26,27]. Here, n_b is the area occupied by an adsorbed molecule of the stronger (more polar) solvent b on the adsorbent surface, ε_b^0 is the ε^0 value of the pure solvent b and k'_0 is the k' value in pure solvent b.

The adsorption energy and the adsorbed area of a solute molecule are comprised of additive contributions from the individual structural elements in the molecule (the Martin rule)[26]. If each oligomer unit has an equal contribution, A_i to A_s and Q_i to Q^0, and Q_0 and A_0 represent the sums of the contributions from the end groups:

$$Q^0 = NQ_i + Q_0 \quad (4a)$$

$$A_s = NA_i + A_0 \quad (4b)$$

Eqn. 1 may be rewritten in the following way to describe the distribution coefficient of an oligomer with N repeat units[25]:

$$\log K_D = \log V_a + \alpha(Q_0 - A_0\varepsilon^0) + N\alpha(Q_i - A_i\varepsilon^0) \quad (5)$$

Combining eqns. 4a and b with eqn. 3, the following equation can be derived for the dependence of the capacity factor on both the mobile phase composition, x, and the degree of polymerization, N:

$$\log k' = \log \varphi + \log V_a + \alpha(Q_0 - A_0\varepsilon_b^0) + N\alpha(Q_i - A_i\varepsilon_b^0) - \frac{A_0 + NA_i}{n_b} \cdot \log x = a_0 - m_0 \cdot \log x + N(a_i - m_i \cdot \log x) \quad (6)$$

Here, a_0, a_i and m_i are auxiliary constants, for which:

$$\log k'_0 = a_0 + Na_i \quad (7a)$$

$$m = m_0 + Nm_i \quad (7b)$$

Eqn. 6 is similar to the empirical equation introduced earlier by Lai *et al.*[19] for reversed-phase chromatography of styrene oligomers on phenyl-bonded phases, where, however, the semilogarithmic relationship of log k' *vs.* x is used instead of log k' *vs.* log x in eqn. 6.

As both $\log k'_0$ and m increase with N, these constants should be correlated with one another:

$$m = m_0 + m_i \cdot \frac{\log k'_0 - a_0}{a_i} = q + p \cdot \log k'_0 \tag{8}$$

A linear correlation in the form of eqn. 8 between the intercept and slope of semi-logarithmic $\log k'$ *vs.* x relationships in reversed-phase chromatography has been reported by Schoenmakers *et al.*[28] for various solutes in aqueous methanol and it was subsequently shown that such an equation applies exactly only for members of a given homologous[29] or oligomeric[30] series.

It follows from eqn. 6 that the logarithm of the capacity factor increases linearly with increasing number of repeat units in individual oligomers. The slopes of such dependences decrease with decreasing concentration of the more polar solvent in the mobile phase.

Eqns. 6 and 8 are valid only in systems where two basic assumptions apply: (1) $\log k'$ is linearly dependent on $\log x$ and (2) $\log k'$ increases linearly with increasing degree of polymerization (the Martin rule).

Gradient elution

Gradient-elution chromatography on polar adsorbents offers the possibility of resolution of more individual oligomers in a shorter time than under isocratic conditions. If an appropriate gradient profile is chosen, the peaks of the individual oligomers are spaced more regularly and their individual widths differ far less than with a constant mobile phase composition, which is useful for quantitation of chromatograms.

The following equation for the net retention volume, V'_g, in gradient-elution liquid chromatography on polar adsorbents can be derived

$$V'_g = \frac{1}{B}\left[(\kappa m + 1)Bk'_0V_m + A^{\frac{\kappa m + 1}{\kappa}}\right]^{\frac{1}{\kappa m + 1}} - \frac{A^{1/k}}{B} \tag{9}$$

Here, A, B and κ are parameters characterizing the gradient profile, *i.e.*, the dependence of the volume concentration, c, of the stronger solvent component in a binary mobile phase on the volume of eluate, V, according to the following gradient function:

$$c = (A^{1/k} + BV)^{\kappa} \tag{10}$$

B is the slope of the gradient, A is the initial concentration of the stronger solvent component in the mobile phase and κ characterizes the shape (curvature) of the gradient.

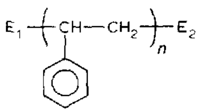
When eqns. 7a and b are introduced into eqn. 9 the following relationship is obtained between the net retention volume in gradient-elution chromatography and the degree of polymerization:

$$V'_g = \frac{1}{B}\left[(\kappa m_0 + \kappa N m_i + 1) B V_m \cdot 10^{(a_0 + N a_i)} + A^{\frac{\kappa m_0 + \kappa N m_i + 1}{\kappa}}\right]^{\frac{1}{\kappa m_0 + \kappa N m_i + 1}} - \frac{A^{1/k}}{B} \qquad (11)$$

According to Snyder and co-workers[7,32], approximately equal bandwidths of the solutes are generated in chromatography with so-called linear solvent strength gradients, where log k' of the solutes change linearly during the elution. In chromatography on polar adsorbents, linear solvent strength gradients require an exponential (concave) gradient profile, *i.e.*, $\kappa > 1$, assuming approximately constant m values for various solutes. However, for the members of an oligomeric series, where m increases regularly with the degree of polymerization, this increase ought to be compensated by a regular decrease in the slope of the gradient. This means that convex ($\kappa < 1$) concentration gradients would best approximate linear solvent strength gradients.

The response of a fixed-wavelength UV detector is proportional to the absorbance in the detector cell, *i.e.*, to the product of the molar concentration, c_m, and of the molar absorption coefficient, ε, at the wavelength used for detection. For quantitation, the response can be evaluated either from the peak areas or from peak heights, h. Assuming approximately constant bandwidths for different sample solutes in a single gradient-elution experiment, the peak heights are proportional to the peak areas.

The molar absorption coefficient, ε, is a characteristic property of a part of a molecule, called a chromophore, which contains a system of π- and n-electrons. If a molecule contains two or more chromophores the electron systems of which are not conjugated with each other, ε of the solute is comprised of additive contributions from each chromophore, ε_i. Some UV-absorbing oligomers have an identical chromophore in each repeat unit, non-conjugated with chromophores in other oligomer units, such as the benzene ring in oligostyrenes:



If the end groups E_1, E_2 in the oligomer do not absorb UV radiation at the wavelength used for detection, the molar absorption coefficient of the oligomer is approximately equal to that of a single repeat unit, ε_i, multiplied by the number, N, of these units in the oligomer, $\varepsilon \approx N\varepsilon_i$. Then, the peak area or the peak height, h, is as follows (assuming approximately constant bandwidths):

$$h = \text{const}_1 \cdot \varepsilon c_M = \text{const}_1 \cdot N \varepsilon_i c_M \qquad (12)$$

Consequently, the molar concentration, c_M, is proportional to the peak height, h, of the oligomer divided by its degree of polymerization, N

$$c_M = \text{const}_2 \cdot \frac{h}{N} \tag{13a}$$

and the weight concentration, c_w, is proportional to the peak height:

$$c_w = c_M \cdot M = \text{const}_2 \cdot \frac{h}{N} \cdot (M_0 + M_iN) = \text{const}_2 \cdot h'M_i = \text{const}_3 \cdot h' \tag{13b}$$

Here, M is the molecular weight of the oligomer, $M = M_0 + NM_i$ and $h' = h[1 + (M_0/M_iN)]$ is the corrected peak height; h is the measured peak height, M_i is the mass of the monomer (or of the repeat oligomer unit, *i.e.*, $M_i = 104$ for styrene, and M_0 is the combined mass of both end groups of the polymer, *i.e.*, of the butyl group and of the hydrogen atom, $M_0 = 58$, for an anionically polymerized polystyrene sample[8]). Hence, it is possible to estimate the molar or mass ratios of the individual oligomers in a polymer sample, without an impractical calibration for each oligomer.

EXPERIMENTAL

Instrumentation

The equipment used consisted of a M 6000A pump or of two M 6000A pumps and a M 660 gradient controller, an U 6K injector and a M 440 UV detector operated at 254 nm (all from Waters Assoc., Milford, MA, U.S.A), a TZ 4221 line recorder and two high-pressure CGC glass cartridge columns, 150 mm × 3.2 mm I.D., connected in series, packed with silica gel Separon SIX, 5 μm (Laboratory Instruments Works, Prague, Czechoslovakia). The efficiency of the two connected columns was 34 000 theoretical plates for benzene as the solute and tetrahydrofuran–*n*-heptane (6:94) as the eluent at a flow-rate of 0.5 ml/min.

Mobile phases

The mobile phases were prepared from spectroscopic grade *n*-heptane and 1,4-dioxane (both from Lachema, Brno, Czechoslovakia) and reagent grade tetrahydrofuran (Laborchemie Apolda, G.D.R.). Tetrahydrofuran was purified from UV-absorbing stabilizers and impurities by repeated shaking with solid sodium hydroxide followed by adsorption on a preparative glass column, packed with silica gel L 40/100 (Lachema). *n*-Heptane was dried over molecular sieves 5A and all the solvents were filtered using a 0.5-μm Millipore filter.

Sample

A polystyrene standard of a nominal molecular weight 2350 (Waters Assoc.) was dissolved in the mobile phases used for isocratic experiments, or in 1,4-dioxane–*n*-heptane (50:50) for gradient elution. Sample volumes of 40–100 μl were injected into the chromatograph.

Procedure

The column dead volume, V_M, was measured as the elution volume of benzene in 1,4-dioxane–*n*-heptane (20:80) and was used for all the mobile phases tested, as it was found not to change significantly with changing mobile phase composition (V_M = 2.35 ml for the two connected columns used). Capacity factors were calculated in the usual way, but a correction for the extra-column contribution to the elution volumes, V_z = 0.15 ml, was adopted where V_z was measured in an independent experiment using direct injector–detector connection; $k' = (V_R - V_M)/(V_M - V_z)$, where V_R is the experimental retention volume. The flow-rate of the mobile phase was measured during each experiment using a stop-watch and a graduated cylinder. Linear and non-linear regressions were used to fit log k' *vs.* log x, log k' *vs.* log c and log k' *vs.* N plots to the experimental data.

RESULTS AND DISCUSSION

Verification of the retention equations under isocratic conditions

Chromatograms of the polystyrene standard (mol.wt. 2350) were recorded using various mobile phases containing 2–10% tetrahydrofuran in *n*-heptane and 3–15% 1,4-dioxane in *n*-heptane. An example of a separation achieved under isocratic conditions is shown in Fig. 1. The individual oligomers yielded clearly separated peaks up to N = 10–27, depending on the mobile phase composition. To aid in identifying the least strongly retained oligostyrenes, styrene was also injected in a separate experiment in each mobile phase. The experimental k' values in Tables I and II are the means from two or three repeated experiments.

Plots of log k' of the individual styrene oligomers against log x of tetrahydrofuran or of 1,4-dioxane in *n*-heptane as the mobile phase are fairly linear over the mobile phase composition range studied in accordance with eqn. 3. Moreover, good linearity is observed also for the plots of log k' against the logarithm of the volume

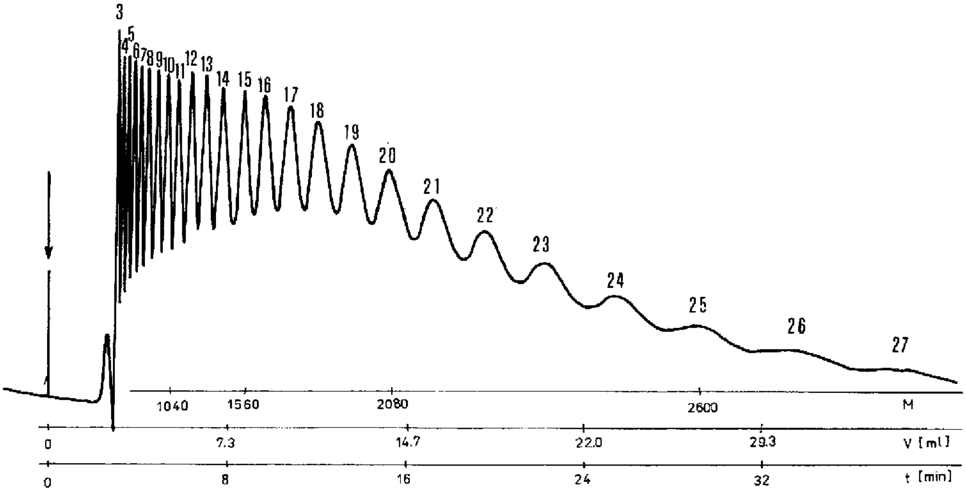


Fig. 1. Chromatographic separation of styrene oligomers in the polystyrene standard (mol.wt. 2350) on two Separon SIX columns connected in series using isocratic elution with 15% (v/v) 1,4-dioxane in *n*-heptane. Flow-rate: 1 ml/min. Detection: UV, 254 nm, 0.5 a.u.f.s. Sample volume: 100 μl. The numbers above the peaks give the degrees of polymerization of the individual oligomers. M = Molecular weight; V = volume (ml) of eluate; t = time (min).

TABLE I

EXPERIMENTAL (k'_{ex}) AND CALCULATED (k'_c) CAPACITY FACTORS OF SOME STYRENE OLIGOMERS (N = DEGREE OF POLYMERIZATION) ON TWO SEPARON SIX COLUMNS IN SERIES USING TETRAHYDROFURAN-*n*-HEPTANE ELUENTS

k' was calculated: I, using eqn. 15; II, using eqn. 6; III, as in II, but concentrations c in % · 10^{-2} were used instead of mole fractions, x. Constants in eqns. 6 and 15 are given in Table III.

N	k'	*Mobile phase*				
		$c = 0.02$, $x = 0.036$	$c = 0.04$, $x = 0.07$	$c = 0.06$, $x = 0.103$	$c = 0.08$, $x = 0.136$	$c = 0.10$, $x = 0.167$
4	k'_{ex}	0.811	0.537	0.424	0.325	0.293
	k'_c I	0.809	0.528	0.412	0.344	0.301
	k'_c II	0.766	0.621	0.550	0.506	0.474
	k'_c III	0.765	0.622	0.551	0.506	0.473
8	k'_{ex}	2.470	1.453	1.090	0.868	0.760
	k'_c I	2.491	1.459	1.070	0.855	0.725
	k'_c II	2.296	1.508	1.184	0.999	0.878
	k'_c III	2.289	1.513	1.187	1.000	0.875
12	k'_{ex}	7.559	3.708	2.587	1.880	1.576
	k'_c I	7.323	3.754	2.547	1.926	1.567
	k'_c II	6.880	3.662	2.547	1.976	1.628
	k'_c III	6.848	3.679	2.558	1.977	1.619
16	k'_{ex}	–	8.508	5.620	3.795	3.025
	k'_c I	20.556	8.987	5.558	3.933	3.046
	k'_c II	20.618	8.894	5.479	3.905	3.016
	k'_c III	20.490	8.949	5.513	3.909	2.994
20	k'_{ex}	–	–	10.966	7.462	5.602
	k'_c I	55.111	20.015	11.113	7.278	5.323
	k'_c II	61.781	21.599	11.787	7.721	5.590
	k'_c III	61.303	21.767	11.878	7.729	5.538

concentrations, c (Figs. 2 and 3). Of course, the values of the regression constants for the two types of dependences differ from one another (Tables III and IV). The log k' *vs.* log c plots offer better possibilities than the log k' *vs.* log x plots for correlation of the retention volumes in gradient-elution chromatography with the retention data obtained under isocratic conditions. In agreement with eqns. 7a and b, the parameter m of eqn. 3 increases with the degree of polymerization, N, in both types of eluents tested (Tables III and IV). The parameter log k'_0 increases with N in tetrahydrofuran–*n*-heptane eluents, but decreases with N in dioxane–*n*-heptane mobile phases. This can be explained by the higher polarity (solvent strength, ε_b^0) of dioxane in comparison with tetrahydrofuran. The slope of the log k'_0 *vs.* N plots, $a_i = \alpha(Q_i - A_i\varepsilon_b^0)$ according to eqn. 6 and $a_i > 0$ if $Q_i > A_i\varepsilon_b^0$; whereas $a_i < 0$ if $Q_i < A_i\,\varepsilon_b^0$. Consequently, solvents of higher polarity in the mobile phase may cause negative a_i values.

Correlations between the parameters m and log k'_0 according to eqn. 8 are observed with both mobile phases tested for the styrene oligomers the capacity factors

TABLE II

EXPERIMENTAL (k'_{ex}) AND CALCULATED (k'_c) CAPACITY FACTORS OF SOME STYRENE OLIGOMERS ON TWO SEPARON SIX COLUMNS IN SERIES USING 1,4-DIOXANE–*n*-HEPTANE ELUENTS

Details as in Table I, except constants in eqns. 6 and 15 are given in Table IV.

N	*k'*	*Mobile phase*			
		c = *0.03*, *x* = *0.0505*	*c* = *0.05*, *x* = *0.0830*	*c* = *0.10*, *x* = *0.1604*	*c* = *0.15*, *x* = *0.2328*
4	k'_{ex}	1.308	0.918	0.571	0.385
	k'_c I	1.324	0.908	0.550	0.415
	k'_c II	1.329	0.996	0.679	0.547
	k'_c III	1.325	0.999	0.682	0.545
8	k'_{ex}	5.629	3.532	1.549	0.861
	k'_c I	5.720	4.191	1.373	0.868
	k'_c II	5.845	3.284	1.528	0.992
	k'_c III	5.809	3.306	1.539	0.984
12	k'_{ex}	–	12.249	3.615	1.664
	k'_c I		13.755	3.291	1.731
	k'_c II	–	10.826	3.439	1.798
	k'_c III		10.939	3.474	1.776
16	k'_{ex}	–	–	7.035	3.017
	k'_c I			7.550	3.289
	k'_c II			7.736	3.259
	k'_c III			7.843	3.206
20	k'_{ex}	–	–	17.690	5.344
	k'_c I			16.605	5.950
	k'_c II			17.403	5.906
	k'_c III			17.704	5.787

of which fit to linear log *k'* *vs*. *N* plots (see below). The constants (*p*,*q*) of eqn. 8 are given in Tables III and IV; their negative values in dioxane–*n*-heptane eluents are due to the higher polarity of dioxane in comparison to tetrahydrofuran, as discussed above.

The constant m_i in dioxane–*n*-heptane eluents is approximately twice the corresponding value for tetrahydrofuran–*n*-heptane mobile phases. This means that the change in concentration of tetrahydrofuran required to cause an equivalent change in retention of the individual oligostyrenes is twice that of dioxane (Figs. 2 and 3). This can again be explained by the higher polarity of dioxane with two oxygen atoms than that of tetrahydrofuran with only one oxygen atom; approximately twice as many molecules of tetrahydrofuran than of dioxane are necessary to displace an oligostyrene molecule.

Figs. 4 and 5 show plots of experimental log *k'* against the degree of polymerization, *N*, using mobile phases with various concentrations of tetrahydrofuran or of 1,4-dioxane in *n*-heptane. These plots are approximately linear for oligostyrenes

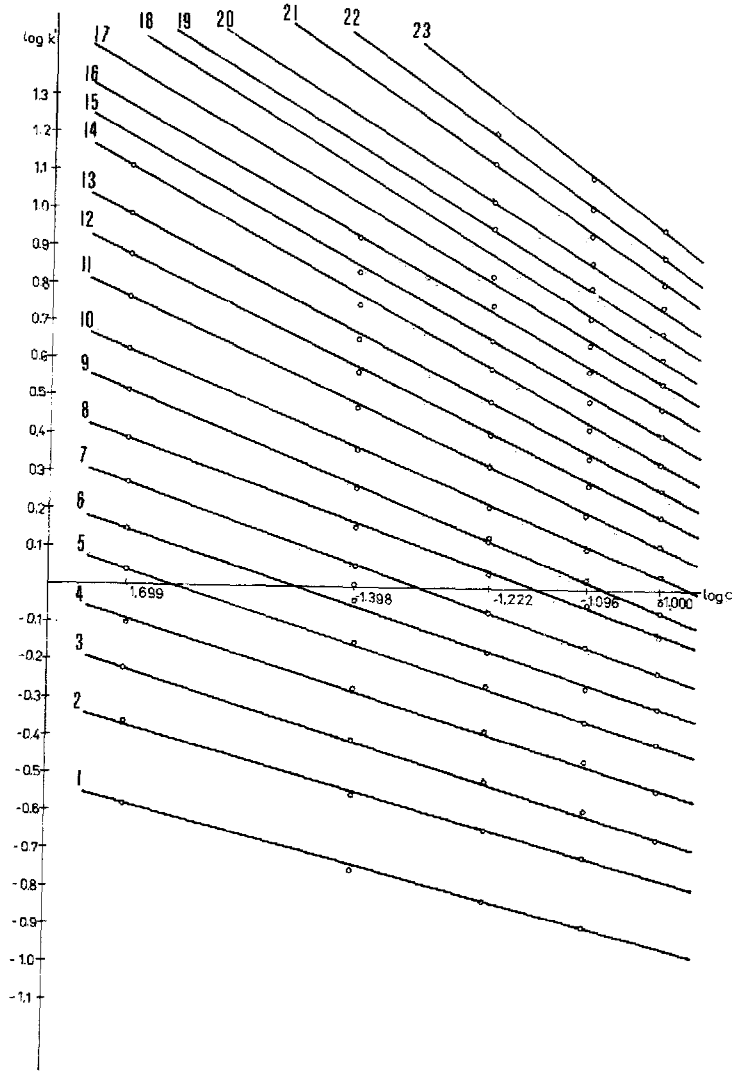


Fig. 2. Logarithmic plots of the capacity factors, k', for styrene oligomers against the concentration, c (% · 10^{-2}), of tetrahydrofuran in *n*-heptane as the eluent. Two Separon SIX columns were used in series. The numbers on the curves give the degrees of polymerization of the oligomers.

with $N > 6$–10 in tetrahydrofuran–*n*-heptane mobile phases and for oligostyrenes with $N > 5$–8 in 1,4-dioxane–*n*-heptane mobile phases. In agreement with eqn. 6, the slopes of these lines decrease with increasing concentration of the more polar solvent in the mobile phase, whereas the intercepts are practically independent of the type or the concentration of the more polar solvent. This means that $m_0 = 0$ in eqn. 6 and the end groups do not contribute significantly to the adsorption of higher oligomers. Consequently, log k' *vs.* N plots using mobile phases of various compositions have a common intersection point.

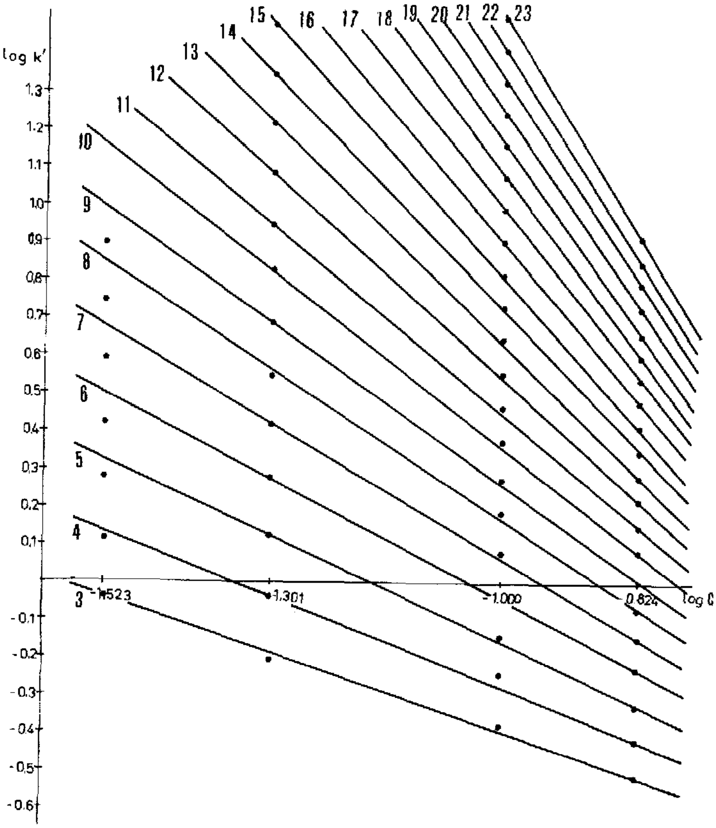


Fig. 3. Logarithmic plots of the capacity factors, k', for styrene oligomers against the concentration, c (% · 10^{-2}), of 1,4-dioxane in n-heptane as the eluent. Other details as in Fig. 2.

Deviations from the Martin rule have previously been observed in reversed-phase chromatography for styrene oligomers with $N > 20$ and these deviations have been attributed to a collapse of the extended configuration of larger oligostyrenes due to hydrophobic effects or to entropic exclusion of larger oligostyrenes from the surface layer[7]. Also log k' *vs.* N plots for styrene oligomers in non-aqueous reversed-phase chromatography show a distinct deviation from linearity (Fig. 7 in ref. 19).

The deviations from linearity of log k' *vs.* N plots in normal-phase chromatography observed for lower oligostyrenes may be attributed to non-equal contributions to the energy of adsorption, Q_i, and (or) to the area occupied by an adsorbed oligomer unit, A_i. Hence, eqns. 4a and b do not apply exactly. Better to describe the dependence of the retention on N, we adopted quadratic relationships:

$$Q^0 = Q_0 + NQ_1 + N^2Q_2 \tag{14a}$$

$$A_s = A_0 + A_1N + A_2N^2 \tag{14b}$$

TABLE III

REGRESSION CONSTANTS OF THE EQUATIONS: $\log k' = \log k'_0 - m \cdot \log x$ (I); $\log k' = \log k'_0 - m \cdot \log c$ (II); $\log k' = \alpha' + \beta' N$ (III); $\log k' = \alpha' + \beta' N + \gamma' N^2$ (IV); CONSTANTS p, q IN EQN. 8; a_0, a_i, m_0, m_i IN EQN. 6 (A, FOR CONCENTRATIONS IN MOLE FRACTIONS, x; B, FOR CONCENTRATIONS, c, IN % · 10^{-2}) AND C, CONSTANTS a_0, a_1, a_2, m_0, m_1, m_2 IN EQN. 15

Mobile phases comprised of tetrahydrofuran and *n*-heptane; r_k = correlation coefficient.

N	*I*			*II*		
	log k'_0	*m*	r_k	*log* k'_0	*m*	r_k
3	−1.150	0.644	0.9991	−1.271	0.620	0.9994
5	−0.930	0.675	0.9994	−1.058	0.650	0.9996
7	−0.779	0.725	0.9999	−0.916	0.698	0.9999
9	−0.710	0.846	0.9999	−0.869	0.814	0.9999
11	−0.622	0.955	0.9999	−0.802	0.919	0.9999
13	−0.564	1.068	0.9995	−0.766	1.028	0.9994
15	−0.516	1.186	0.9994	−0.740	1.141	0.9992
17	−0.442	1.276	0.9982	−0.666	1.214	0.9984
19*	−0.303	1.274	—	−0.513	1.199	—

A**: $q = 2.027$, $p = 1.697$, $r_k = 0.983$. B**: $q = 2.641$, $p = 2.125$, $r_k = 0.950$.

		c(x)				
		0.02 (0.036)	*0.04 (0.070)*	*0.06 (0.103)*	*0.08 (0.136)*	*0.1 (0.167)*
III	α'	−0.5655	−0.6028	−0.5755	−0.6050	−0.6124
	β'	0.1196	0.0965	0.0816	0.0737	0.0683
	r_k	0.9998	0.9992	0.9986	0.9999	0.9999
IV	α'	−0.597	−0.754	−0.836	−0.898	−0.952
	β'	0.128	0.125	0.119	0.113	0.113
	γ'	−0.00047	−0.00125	−0.00122	−0.00123	−0.00139
	r_k	0.99987	0.99982	0.99981	0.99966	0.99950

A: $a_0 = -0.592$, $a_i = 0.00669$, $m_0 = 0$, $m_i = 0.0776$. B : $a_0 = -0.592$, $a_i = -0.00791$, $m_0 = 0$, $m_i = 0.0747$. C: $a_0 = -1.354$, $a_1 = 0.0928$, $a_2 = -0.0024$, $m_0 = 0.522$, $m_1 = 0.0255$, $m_2 = 0.0012$.

* Two points only.
** N = 7–17.

Using these relationships, the following equation results:

$$\log k' = a_0 - m_0 \cdot \log x + N(a_1 - m_1 \cdot \log x) + N^2(a_2 - m_2 \cdot \log x) \qquad (15)$$

The constants of this equation determined for the systems studied are given in Tables III and IV.

Mourey[25] also reported a decrease in Q_i and A_i for the repeat units in styrene oligomers with $N \leqslant 6$–8 in adsorption chromatography on silica gel with *n*-hexane–dichloromethane mobile phases.

To test the fit of eqns. 6 and 15 to the experimental data, the capacity factors

TABLE IV

REGRESSION CONSTANTS OF THE SAME EQUATIONS AS IN TABLE III AND VALUES OF THE CONSTANTS THEREIN FOR MOBILE PHASES COMPRISED OF 1,4-DIOXANE–*n*-HEPTANE

N	*I*			*II*		
	log k'_0	*m*	r_k	*log* k'_0	*m*	r_k
3	−0.774	0.566	0.8482	−1.043	0.652	0.9982
5	−0.883	0.913	0.9969	−1.023	0.868	0.9972
7	−0.829	1.121	0.9954	−1.001	1.066	0.9963
9	−0.826	1.362	0.9956	−1.036	1.295	0.9964
11	−0.953	1.764	0.9993	−1.206	1.659	0.9996
13	−1.018	2.074	0.9995	−1.316	1.951	0.9998
15	−1.078	2.371	0.9999	−1.418	2.230	0.9999
17*	−1.216	2.779	—	−1.561	2.554	—

A**: $q = -2.633, p = -4.622, r_k = 0.978$. B**: $q = -1.513, p = -2.620, r_k = 0.998$.

		c(x)			
		0.03 (0.050)	*0.05 (0.083)*	*0.10 (0.160)*	*0.15 (0.233)*
III	α'	−0.516	−0.518	−0.508	−0.538
	β'	0.158	0.136	0.088	0.063
	r_k	0.9999	0.9999	0.9998	0.9999
IV	α'	−0.525	−0.672	−0.669	−0.729
	β'	0.162	0.164	0.110	0.085
	γ'	−0.00029	−0.0014	−0.00070	−0.00058
	r_k	0.9999	0.9998	0.9997	0.9996

A: $a_0 = -0.520$, $a_i = -0.0272$, $m_0 = 0$, $m_i = 0.145$. B: $a_0 = -0.520$, $a_i = -0.0495$, $m_0 = 0$, $m_i = 0.138$. C***: $a_0 = -0.916$, $a_1 = 0.0171$, $a_2 = -0.00099$, $m_0 = 0.302$, $m_1 = 0.1122$, $m_2 = 0.0005$.

* Two points only.

** $N = 7$–17.

*** Data for $c = 0.05$ are not included.

calculated in different ways are compared with experimental values in Tables I and II. For simplicity, only the values for a part of the oligomers tested are listed. In tetrahydrofuran–*n*-heptane mobile phases, quadratic equation 15 fits better the experimental k' than does linear equation 6, but in 1,4-dioxane–*n*-heptane mobile phases the fit of the linear equation to the experimental data is comparable to or better than the fit of the quadratic equation for styrene oligomers with $N > 5$–6, except for the mobile phase containing 15% dioxane, where the quadratic equation fits better the experimental data for oligostyrenes with *N* up to 14.

Gradient elution

Only concentration gradients of 1,4-dioxane in *n*-heptane were tested for separation of styrene oligomers, for the following reasons: (1) 1,4-dioxane was available

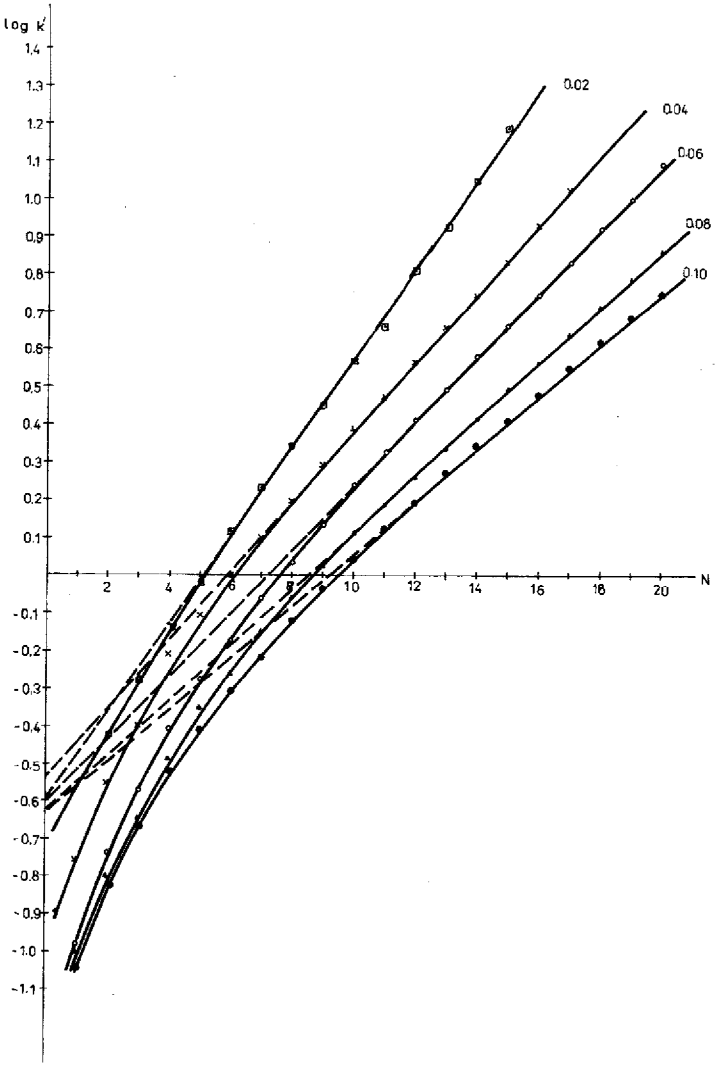


Fig. 4. Plots of log k' for styrene oligomers against the degree of polymerization, N, on two Separon SIX columns connected in series. The concentrations of tetrahydrofuran in n-heptane (in % · 10^{-2}) are given on the individual plots.

in higher purity than tetrahydrofuran; (2) the validity of the linear equation 6 is better for dioxane–n-heptane mobile phases than for tetrahydrofuran–n-heptane eluents; this should allow easier predictions of retention volumes in gradient-elution chromatography; (3) other possible polar mobile phase components, such as n-propanol, are poor solvents for polystyrene samples.

Unfortunately, the present theory of gradient elution does not allow prediction of the optimum gradient profile necessary to achieve equal bandwidths for com-

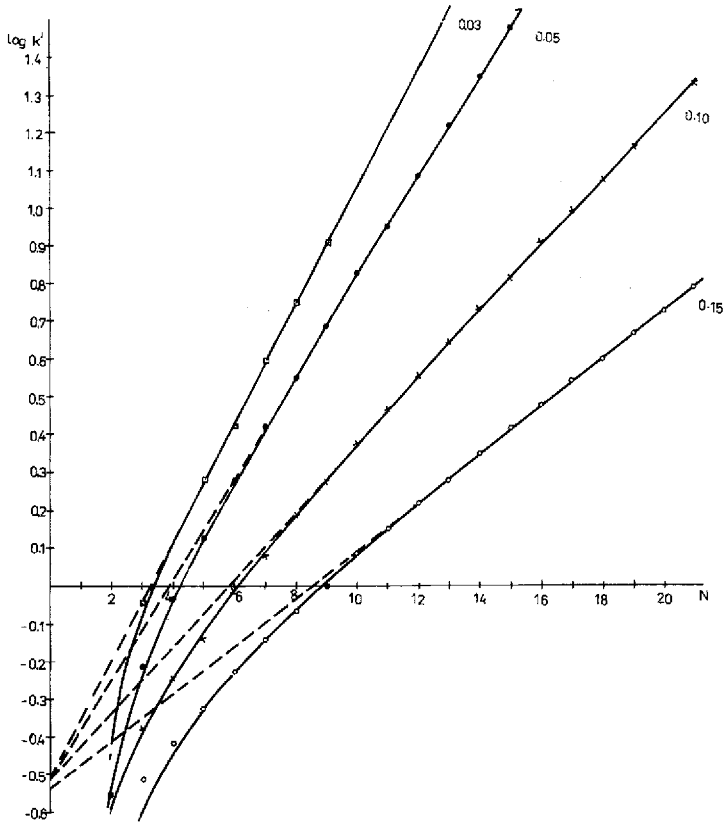


Fig. 5. Plots of log k' for styrene oligomers against the degree of polymerization, N, on two Separon SIX columns connected in series. The concentrations of 1,4-dioxane in n-heptane (in % · 10^{-2}) are given on the individual plots.

pounds with changing m in eqn. 3. It was therefore necessary to test experimentally various gradient profiles. The best results (most uniform bandwidths for the individual oligomers) were achieved for the gradient with a convex profile (curve 5 selected on Waters M660 gradient controller) from 3 to 30% of 1,4-dioxane in n-heptane in 2 h at a flow-rate of 1 ml/min. This gradient profile can be described by:

$$c = 0.03 + 0.02465\sqrt{V} \tag{16}$$

where V is the volume of the eluate from the start of the gradient in ml; c is in % · 10^{-2}. Fig. 6 shows the separation of the polystyrene standard with nominal molecular weight 2350 under these conditions. The baseline obtained with a "blank" gradient is shown as a broken line.

Net retention volumes, V'_g, of the individual styrene oligomers are listed in Table V, together with the values calculated using eqn. 11. It is not possible to calculate retention volumes in normal-phase chromatography using concentration gra-

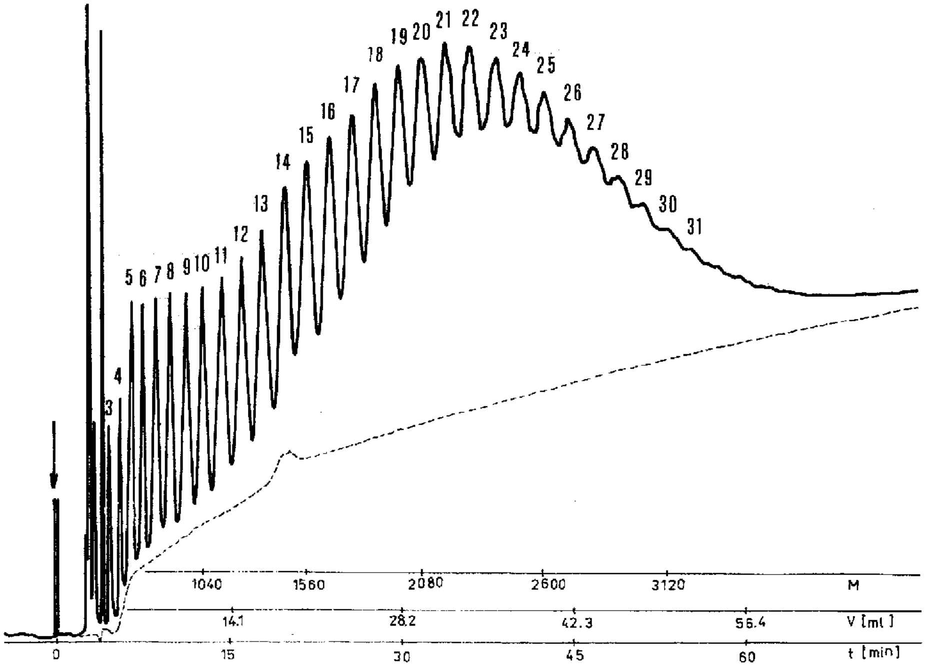


Fig. 6. Chromatographic separation of styrene oligomers in the polystyrene standard (mol.wt. 2350) on two Separon SIX columns connected in series using gradient elution with 1,4-dioxane in *n*-heptane at a flow-rate of 1 ml/min. Other conditions as in Fig. 1. The broken line shows the "blank" gradient.

dients directly according to the eqn. 16. Hence, the gradient function given by eqn. 16 was approximated by another, similar function in the form of eqn. 10, the profile of which was calculated so as to yield the values of c most close to those calculated from eqn. 16 for $V \leqslant 60$ ml:

$$c = (0.000237 + 0.000619V)^{0.46} \qquad (17)$$

The differences between the experimental net retention volumes and the values calculated using eqns. 11 and 17 are less than 1 ml, which is acceptable bearing in mind the simplifying assumptions adopted.

Unfortunately, it was not possible to achieve exactly equal distances between the neighbouring peaks for oligostyrenes with $N < 14$; the bandwidths also changed slightly from one oligomer peak to another. One complicating factor, which is difficult to account for, is a gradual loss of efficiency (increase in plate height) with increasing degree of polymerization because of the decrease in diffusion coefficients. Approximately constant bandwidths were assumed for styrene oligomers with $N \geqslant 14$ in calculations of the concentration ratios of the individual oligomers in the polystyrene sample using eqn. 13, but the experimental peak heights of lower oligomers were multiplied by a correction factor taking into account the lower bandwidths for these peaks. The results are given in Table V.

TABLE V

EVALUATION OF GRADIENT-ELUTION CHROMATOGRAM FOR THE POLYSTYRENE STANDARD, MOL.WT. 2350 (FIG. 6)

N = Degree of polymerization of individual oligomers; V'_g = net retention volume in ml, exp = experimental, cal = calculated using eqn. 11; h = experimental peak height from baseline in mm; $Y_{1/2}$ = bandwidth at $h/2$ in mm; h' = corrected peak height, see eqn. 13; f = correction factor for non-equal bandwidths = $Y_{1/2}/Y_{1/2(N=15)}$; c_{rel} is expressed in terms of $c_w/c_{w(N=21)} \cdot 10^2$.

N	V'_g *exp*	V'_g *cal*	h	$Y_{1/2}$	$h'f$	c_{rel}
3	2.04	1.87	55.0	0.9	13.98	13.6
4	2.98	2.48	59.0	0.9	14.41	14.1
5	3.92	3.22	73.4	0.9	17.48	17.1
6	4.79	4.10	69.9	1.1	20.01	19.5
7	5.80	5.11	69.8	1.3	23.32	22.8
8	7.05	6.25	67.3	1.6	27.43	26.8
9	8.40	7.52	63.0	2.0	31.86	31.4
10	9.81	8.90	63.1	2.4	38.07	30.7
11	11.28	10.39	63.5	2.8	44.48	43.4
12	13.00	11.98	64.6	3.2	51.51	50.3
13	14.63	13.67	68.0	3.6	60.79	59.3
14	16.29	15.43	71.0	4.0	70.31	68.6
15	18.17	17.28	79.1	4.2	82.04	80.1
16	20.05	19.18	83.7	4.2	86.62	84.5
17	21.90	21.15	88.1	–	90.99	88.8
18	23.84	23.17	94.1	–	97.02	94.7
19	25.63	25.23	97.2	–	100.05	97.7
20	27.64	27.33	98.0	–	100.73	98.3
21	29.55	29.46	99.8	–	102.45	100
22	31.55	31.62	96.7	–	99.15	96.8
23	33.62	33.80	91.8	–	94.03	91.8
24	35.63	36.00	86.0	–	88.00	85.9
25	37.60	38.21	79.1	–	80.87	78.9
26	39.51	40.42	70.8	–	72.32	70.6
27	41.58	42.64	60.9	–	62.16	60.7
28	43.62	44.86	51.8	–	52.83	51.6
29	45.59	47.08	43.0	–	43.83	42.8
30	47.63	49.30	34.5	–	35.14	34.3
31	49.51	51.51	27.5	–	28.00	27.3

The polystyrene sample (standard with nominal molecular weight 2350) shows the concentration maximum for the oligomer with $N = 21$ and mol.wt. = 2180. The calculated concentration ratios of the oligostyrenes may be subject to some errors due to the simplifying assumptions adopted, but it is believed that the calculation method described may be useful for a rough estimation of the concentration distribution of individual oligomers in a polymer sample.

CONCLUSIONS

The retention of styrene oligomers on silica gel in mobile phases comprised of n-heptane and tetrahydrofuran or 1,4-dioxane may be described adequately by eqn.

15, which takes into account deviations from Martin's rule, *i.e.*, a non-linear increase of log k' with increasing degree of polymerization, N, for the lower oligomers. In dioxane–*n*-heptane mobile phases, the simple linear eqn. 6 may be used for calculations of the retention of styrene oligomers with $N \geqslant 5$, without significant errors. This equation may also be used as the basis for calculating the retention volumes in gradient-elution chromatography from eqn. 11.

Styrene oligomers may be separated as well in 1,4-dioxane–*n*-heptane mobile phases as in tetrahydrofuran–*n*-heptane eluents. The change in tetrahydrofuran concentration required for an equivalent change in retention under isocratic conditions is twice that of 1,4-dioxane.

Gradient-elution chromatography on silica gel with 1,4-dioxane–*n*-heptane as the eluent enables a separation of styrene oligomers at least comparable to that in aqueous or non-aqueous reversed-phase gradient-elution liquid chromatography. The resolution obtained yields not only a "fingerprint" chromatogram, but also allows an estimate of the relative concentrations of the individual oligomers in a polystyrene sample, up to polymerization degrees, N, of 25–30.

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