EFFECT OF ULTRASONIC AGITATION IN HIGH-PERFORMANCE SIZE-EXCLUSION CHROMATOGRAPHY

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SUMMARY

A new effect on separation by liquid chromatography is reported. When the chromatographic column is used under ultrasonic agitation, peak widths typically increased while retention is unchanged. This is found to be general for a variety of compounds and chromatographic columns, and is reversible. Correlation with molecular diffusion coefficients is presented.

INTRODUCTION

High-performance size-exclusion chromatography (HPSEC) is a very popular branch of separation science. This popularity is due to the simplicity in which correlations are made between molecular weight and chromatographic retention time[1-3]. Yet, correlations between molecular weight and retention time may be subject to error unless a study is limited to a homologous series of molecular species, or specific corrections are made[4]. Since the original work of Giddings and Mallik[5], continued effort has been made to further understand the separation mechanism in HPSEC, in terms of the various contributions to chromatographic band broadening[2,6-8]. This includes studies pertaining to size distributions of the column packing material, namely, pore size and shape[9], and particle diameter and uniformity[10]. The importance of specific molecular properties, beyond considering just the molecular weight, are numerous. The relationship of diffusion coefficient[5,8], and molecular size and shape[1,7], to retention behavior and band broadening in HPSEC has been studied in great detail. Yet, fundamental relationships between molecular properties and chromatographic band broadening have been difficult to derive, and it is noted that empirical relationships are commonly applied[1,3]. The interest in finding exact solutions in relating HPSEC data to fundamental expressions is dependent upon designing experiments that can provide novel types of information[5]. This information should allow for the deconvolution of the various contributions to chromatographic band broadening, so each contributing term can be more precisely understood. We have designed an experiment in which an additional band broadening effect is observed when a HPSEC system is operated while the column is agitated in an ultrasonic bath. For a series of standard compounds, the height equivalent to a theoretical plate

(HETP) is compared for the HPSEC system with, and without, agitation. The magnitude of the effect observed is correlated with the diffusion coefficients of the series of standard compounds.

EXPERIMENTAL

Chromatography system

The HPSEC system used was conventional and consisted of a Beckman (Berkeley, CA, U.S.A.) Model 110A pump connected through conventional tubing to a Beckman, Model 7010 10-μl injection valve which was connected to a 30 cm $\times$ 7.7 mm I.D. HPSEC column having 10-μm μSpherogel packing with 10^4-Å pores (Anspec; Ann Arbor, MI, U.S.A.), with the effluent sent through a Rheodyne (Woburn, MA, U.S.A.) Model 15 commercial absorbance detector operating at 254 nm. The output was sent to a chart recorder for the measurement of the chromatographic data. The eluent was highly purified tetrahydrofuran (THF) (Burdick and Jackson; Muskegon, MI, U.S.A.) stored under nitrogen before use. Flow-rates ranging from 0.44 to 2.48 ml/min were used.

Samples studied

Polystyrene samples were obtained commercially (Altex; Berkeley, CA, U.S.A.), as was the toluene (Burdick and Jackson). Molecular information for the samples are given in Table I.

HPSEC system calibration

The retention volumes, V_e, of the samples were independent of flow-rate in the ranges studied and are listed in Table I. These data are used later to relate ln M to V_e, where M is the solute molecular weight. Temperature was controlled to $\pm 1°C$. The effect of temperature on the HETP was found to be negligible compared to the agitation effect when the temperature was controlled. Samples injected were at a concentration well within the linear range of the detector and well below the column saturation level. Extra-column broadening was minimized by use of proper tubing lengths and inner diameters.

TABLE I

PHYSICAL CONSTANTS AND HPSEC CALIBRATION DATA FOR SAMPLES STUDIED

Sample	M^*	$\bar{M}_w/\bar{M}_n^{**}$	R_s^{***}	ln M	V_e $(\sigma)^\S$
Polystyrene	115 000	1.04	85	11.65	6.25 (0.07)
Polystyrene	10 200	1.06	22	9.23	8.37 (0.11)
Polystyrene	950	1.20	6	6.86	9.41 (0.13)
Toluene	92	1.00	1.5	4.52	10.47 (0.16)

  * Molecular weight in g/mol.
 ** Polydispersity, weight-average M to number-average M ratio.
*** Stokes radius in Å.
  $\S$ Retention volume in ml; standard deviation of V_e is σ.

Production of agitation effect

The agitation effect was produced by submerging about two-thirds of the column in a Sonicor ultrasonic bath (Ace Scientific; East Brunswick, NJ, U.S.A.) filled with water at 40 ± 1°C. Agitation produced some heating that leveled off at that temperature. The ultrasonic bath agitates at 60 kHz. The peak width at half-height, $W_{\frac{1}{2}}$, and V_e data were measured manually. This may be the reason for the low precision in the results presented. The accuracy is however sufficient to demonstrate the effect. Three or more trials are used for all data points.

RESULTS AND DISCUSSION

HPSEC system performance

It is essential to test the HPSEC system with established treatments of chromatographic data, in order to be confident that any effect observed during the agitation experiment can be properly discussed. The HPSEC system was tested without agitation at a variety of flow-rates with the polystyrene standards and toluene as samples. The HETP values were calculated for each species at each flow-rate according to the known expression

$$H = \frac{L}{5.545}\left(\frac{W_{\frac{1}{2}}}{V_e}\right)^2 \tag{1}$$

where H is the HETP in mm, for a given sample species of width at half-height, $W_{\frac{1}{2}}$, and retention volume, V_e, for a column of length, L. The H vs. u plots, where u is the linear flow velocity of the eluent in mm s^{-1}, are shown in Fig. 1 for the HPSEC system. According to the literature[2,8], the relationship between H and experimental
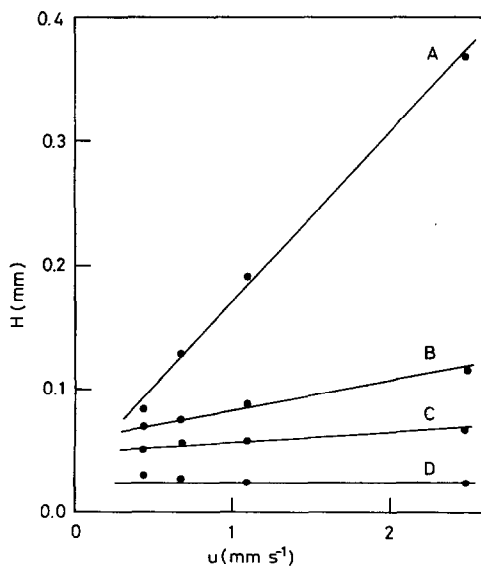

Fig. 1. H (mm) *vs.* u (mm s^{-1}) for the polystyrene standards (PS) and toluene eluted from the 10^4-Å, 10-μm, μSpherogel HPSEC column. A = PS 115000; B = PS 10200; C = PS 950, D = toluene.

parameters is given by

$$H = \sum_{i=1}^{N} [1/A_i + 1/C_{m,i}u]^{-1} + \frac{B}{u} + C_su + P \tag{2}$$

where A_i is the eddy diffusion term given by

$$A_i = 2\lambda_i d_p \tag{3}$$

and $C_{m,i}u$ is from solute non-equilibrium effects in the mobile phase, with $C_{m,i}$ given by

$$C_{m,i} = \frac{\omega_i d_p^2}{D_m} \quad \cdot \tag{4}$$

where the summation, i, is taken over N different effects[5]. In eqn. 2, B is related to longitudinal diffusion, with

$$B = 2\gamma D_m \tag{5}$$

and C_s is a coefficient resulting from solute dispersion due to mass transfer effects in the stationary phase, or pores, given by

$$C_s = \frac{qR(1-R)d_p^2}{D_s} \tag{6}$$

and, finally, the P term is due to the polydispersity of a polymer, given by

$$P = \frac{L[\ln (\bar{M}_w/\bar{M}_n)]}{D_2^2 V_e^2} \tag{7}$$

where $\bar{M}_w$ is the weight-average molecular weight and $\bar{M}_n$ the number-average molecular weight.

The remainder of the parameters in eqns. 2–7 not yet discussed are defined as follows. The parameters λ, ω, γ, and q are geometrical structure factors depending upon the packing material. The values of λ, ω, and γ are near unity, while q has an accepted value[5] of 1/30. The particle diameter is given by d_p. D_m and D_s are the diffusion coefficient for a solute in the mobile phase and stagnant mobile phase, respectively. D_2 is the slope of the HPSEC calibration curve, obtained by plotting ln M vs. V_e. Finally, R is the retention ratio, V_0/V_e, with V_0 equal to the interstitial volume of the HPSEC column.

For polymers, $1/A_i \gg 1/C_{m,i}u$, and B/u is negligible[8]. Thus eqn. 2 reduces to

$$H = A + C_su + P \tag{8}$$

where A is the sum of all A_i terms. It is apparent from Fig. 1 that H for toluene is

not affected significantly by u. Since toluene is monodisperse, there is no P term. Thus, H_T (average H for toluene) should be only due to A in eqn. 2. This is a good approximation, since substituting known parameters into eqn. 2 predicts A to be nearly the same as H_T. Thus, eqn. 8 for polymers can be simplified to include only the mass transfer and polydispersity terms,

$$H^* = H - A \approx H - H_T = C_s u + P \tag{9}$$

where H^* is the adjusted H values after subtracting the multipath contribution.

From Fig. 1, the slope of each H vs. u line provided a value for C_s in eqn. 9. Using eqn. 6, a value for D_s was calculated. Further, from the literature[11], an expression for D_m for polystyrene samples in THF provided

$$D_m = 3.45 \cdot 10^{-4} \, M^{-0.564} \tag{10}$$

Using eqn. 10, D_m values were calculated for the polystyrene standards. From the D_s and D_m values, the restriction ratio[8,12], D_s/D_m, was calculated for each standard. These data are listed in Table II. The values listed are consistent with literature values.

From eqn. 9, and using Fig. 1, extrapolating to $u = 0$ provided the value of P for each polymer standard. From the data in Table I, the slope of the $\ln M$ vs. V_e plot is equal to D_2 in eqn. 7. Using eqn. 7, the value of $\bar{M}_w/\bar{M}_n$, or the polydispersity, for each polystyrene standard was calculated. Data and results of these calculations are reported in Table III. The experimental $\bar{M}_w/\bar{M}_n$ values are better than the manufacturer's values. Yet, the difference is not unreasonable.

The result of the preceding calculations is to substantiate that the HPSEC system used in this study was functioning properly and to properly characterize the samples tested. Now it is possible to present the data for the effect of ultrasonic agitation on chromatographic band broadening, with a discussion in the context of eqn. 2.

Ultrasonic agitation effect

Ultrasonic agitation did not influence solute retention volume or column pres-

TABLE II

CALCULATED D_m AND D_s VALUES FROM EXPERIMENTAL HPSEC DATA

Sample	M^*	D_m^{**}	D_s^{***}	$D_s/D_m^{\S}$
Polystyrene	115000	$4.8 \cdot 10^{-7}$	$3.8 \cdot 10^{-8}$	0.080
Polystyrene	10200	$1.9 \cdot 10^{-6}$	$3.4 \cdot 10^{-7}$	0.18
Polystyrene	950	$7.2 \cdot 10^{-6}$	$2.0 \cdot 10^{-6}$	0.14
Toluene	92	$2.7 \cdot 10^{-5\S\S}$	$1.2 \cdot 10^{-5\S\S}$	0.44

   * Molecular weight in g/mol.
  ** Diffusion coefficient in moving mobile phase, eqn. 10 in cm^2 s^{-1}.
 *** Diffusion coefficient in stagnant mobile phase, as explained in text and eqn. 6 in cm^2 s^{-1}.
   $\S$ Restriction ratio[8,12].
  $\S\S$ Approximate values for toluene.

TABLE III

CALCULATED POLYDISPERSITY ($\bar{M}_w/\bar{M}_n$) VALUES FOR POLYSTYRENE STANDARDS

Sample	M^*	H_{INT}^{**}	$\bar{M}_w/\bar{M}_n$ (calculated)	$\bar{M}_w/\bar{M}_n$ (manufacturer)
Polystyrene	115 000	33	1.004	1.04
Polystyrene	10 200	60	1.03	1.06
Polystyrene	950	49	1.02	1.20
Toluene	92	22	1.00	1.00

* Molecular weight in g/mol.

** HETP at $u = 0$ (y-intercept), H_{INT} for toluene is assumed equal to A in eqn. 9.

*** Calculated polydispersity from eqn. 7, with $D_2 = -1.65$ ml^{-2} from slope of the linear regression of Table I data for ln M vs. V_e plot.

§ Manufacturer's value for polydispersity.

sure. The effect did not appear to be correlated with linear flow velocity, either. Yet, for flow-rates ranging from 0.67 ml/min to 2.36 ml/min, agitation produced the same general trend. Essentially, the $W_{\frac{1}{2}}$ values increased due to agitation, but the magnitude of the increase was directly related to the diffusion coefficient of the solute in the mobile phase, D_m. The effect is completely reversible by turning the agitation on and off. The results of this experiment are shown in Fig. 2. The data fit the empirical expression

$$\Delta H = a \log D_m + b \tag{11}$$

where a is the slope, b the y-intercept, and ΔH is given by
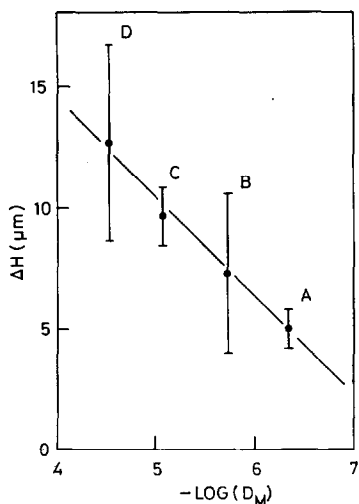
$$\Delta H = H_A - H_0 \tag{12}$$

Fig. 2. Effect of agitation on H as a function of D_m. ΔH (μm) vs. $-\log (D_m)$ for same system as in Fig. 1, with ΔH defined as in eqn. 12, averaged for three flow velocities: 0.67, 2.0, and 2.4 mm s^{-1}. A through D as in Fig. 1.

where H_A is the HETP with agitation, and H_0 is the HETP without agitation, given by eqn. 2. Since eqn. 12 is a difference in H values, the ΔH values provided should be due entirely to the agitation effect.

The large uncertainty (error bars) on the data points in Fig. 2 may be of some concern. Since the retention volumes did not change due to agitation, substitution of eqn. 1 into eqn. 12 provides

$$\Delta H = \frac{L}{5.545V_e^2}(W_{1/2,\,A}^2 - W_{1/2,\,0}^2) \tag{13}$$

The experimental uncertainty in ΔH, given by $\sigma_{\Delta H}$, can then be approximated by

$$\sigma_{\Delta H} = 2\sqrt{2}(H\sigma_{W_{1/2}}) \tag{14}$$

where H is the average of H_A and H_0, and $\sigma_{W_{1/2}}$ is the average of the relative standard deviation of $W_{1/2,\,A}$ and $W_{1/2,\,0}$. Thus, it is reasonable to expect uncertainties ($\sigma_{\Delta H}$) in ΔH on the order of 5% of H if the individual $W_{1/2}$ data are precise to roughly 1.5%. This was the case with the data presented here.

The plot given by eqn. 11 provides molecular information that did not have a retention volume dependence such as other HPSEC methods[1–3,8]. Agitation appears to affect the solutes in the moving mobile phase, and not the stagnant mobile phase, since retention times did not change. This is why a dependency on D_m is suggested. For a more limited set of data (all collected on one day), a good correlation was obtained by plotting ΔH vs. $D_m^{1/2}$. Yet, it is difficult to ascribe an unambiguous exponential behavior with confidence. Essentially, it is suggested that agitation produces an additional term in eqn. 2, that correlates ΔH with D_m, independent of the other H terms.

The agitation effect was also observed with three other chromatography systems. Columns having 5-μm polystyrene–divinylbenzene particles with 10^2-Å pores, 5-μm silica particles with 60-Å pores, and 5-μm silica-based C_{18} particles all exhibited the agitation effect with THF as eluent. The magnitude of the effect varied with these columns as compared to the study reported here. More work is needed to determine any trends between molecular properties and the agitation effect for these other three systems.

CONCLUSIONS

The performance of a HPSEC system using a 10^4-Å, 10-μm, μSpherogel column with THF as eluent was substantiated using established theory. An experiment using ultrasonic agitation provided an additional reversible contribution to the HETP that correlated well with the diffusion coefficients of a series of standard compounds. The correlation appeared to be independent of the ln M vs. V_e correlation already provided by HPSEC data. Thus, a method is presented to provide two-dimensional information for molecular weight (or D_m) as function of retention volume (or time) and behavior in the presence of ultrasonic agitation.

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