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USE OF CHROMATOGRAPHIC DATA TO DETERMINE THE MOLECULAR WEIGHT OF A SOLUTE ELUTED FROM A LIQUID CHROMATOGRAPHIC COLUMN

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SUMMARY

The diffusivities of 69 different compounds in a *n*-hexane ethyl acetate solvent mixture have been determined and a precise relationship between solute diffusivity, molecular weight and molar volume established. The band dispersion for each of the same solutes has also been measured employing a liquid chromatographic column designed to emphasize the resistance-to-mass transfer factor and minimize thermal effects resulting from the use of high pressures and high mobile phase velocities. The effect of the capacity ratio of a solute on the resistance-to-mass transfer factor is determined and the relationship between the bandwidth of a solute, its diffusivity and its molecular weight established. A procedure is outlined for the determination of the molecular weight of a solute from the measurement of its bandwidth, when eluted from a liquid chromatographic column, within an error of 13% for 90% of the solutes examined providing the density of the solute lies between 0.85 and 1.25 g/ml.

INTRODUCTION

Column technology has advanced considerably over the past decade and it is now not only possible to design columns with very high efficiency[1,2], but it is also possible to design columns to achieve a given separation in the minimum amount of time[3,4]. The minimum column radius can now be calculated from a knowledge of the instrument contribution to peak dispersion[5], and thus, provide minimum solvent consumption and maximum mass sensitivity. In contrast, the use of chromatographic data as an aid to solute identification has received minimum attention, the emphasis being placed on the use of appropriate spectroscopic systems directly associated with the liquid chromatograph, an expensive and cumbersome approach to the problem. An alternative solution is to use retention data such as separation ratios, α, and solute capacity factors, k', for identification purposes, together with such functions as the Kováts indices[6]. Such procedures depend on having data available from reference substances or from added standards which renders the identification procedure clumsy and time consuming. Furthermore, this approach is of very little use when a complex mixture of completely unknown solutes is being separated. The chromatographic parameter that could provide information about the nature of the solute and which, so far, has received only minor attention, is peak width. Measurement of peak width has been employed extensively to examine factors that affect column efficiency, but has only been used occasionally for peak identification purposes. Verzele *et al.*[7] used peak width measurements to estimate the molecular weight of some alkaloids and Shioya *et al.*[8] related solute molecular weight to the optimum mobile phase velocity in the analysis of peptides. The latter procedure could be extremely protracted as HETP (height equivalent to a theoretical plate) curves have to be obtained for each solute to be examined.

The major factor affecting peak width of solutes eluted from columns packed with large particles and operated at high linear mobile phase velocities is the resistance-to-mass transfer factor in the mobile phase as defined, for example, by the Van Deemter equation[9]. A number of workers have determined that the Van Deemter equation can accurately describe band dispersion in an LC column for practical mobile phase velocities up to about 1 cm/sec, *e.g.*, refs. 10, 11. Furthermore, according to Van Deemter, the resistance to mass transfer in the mobile phase is inversely proportional to the diffusivity of the solute in the mobile phase which, in turn, has been shown by a number of workers, to be related to the solute molecular weight and molecular size, *e.g.*, refs. 12–15. It follows that peak width measurements of a solute band taken at high mobile phase velocities could possibly provide a measurement of the molecular weight of the solute.

In this paper, the diffusivities of 69 solutes in a hexane–ethyl acetate solvent mixture are reported and the dispersion of the same solutes in a chromatographic column employing the same solvent mixture as the mobile phase determined at a high linear mobile phase velocity. In the first instance, the relationship between molecular weight and solute diffusivity is identified and secondly a relationship between peak width and molecular weight is established.

DETERMINATION OF THE DIFFUSION COEFFICIENT

Theory

The diffusion coefficient (diffusivity) of a solute can be determined by measuring the dispersion of the solute in a cylindrical tube[15]. According to the Golay equation[16] the variance per unit length, H, of a band passing through a tube is given by

$$H = \frac{2D_m}{u} + \frac{r^2u}{24\,D_m}$$

where D_m is the diffusivity of the solute in the mobile phase, u is the linear velocity of the mobile phase and r is the radius of the tube. If $u \gg D_m/r$, then the equation reduces to

$$H = r^2u/24D_m$$

or

$$D_m = r^2u/24H \qquad (1)$$

It follows that if the dispersion of a solute is measured in terms of the variance per unit length in a tube of known dimensions at a carefully measured linear velocity, then the diffusivity of the solute can be calculated.

Experimental

The apparatus consisted of a Perkin-Elmer Series 3B pump and a Valco injection valve with a 0.2-μl sample volume. The UV detector employed was the LC 85B, a detector designed to provide minimum dispersion, which when used in conjunction with the Valco valve ensured that the instrumental contribution to band dispersion was always less than 2% of the total dispersion being measured. The output from the chromatographic system was fed to a Bascom Turner recorder acquiring data at a rate of five data points per sec. The mobile phase employed was a mixture containing 5% (v/v) of ethyl acetate in *n*-hexane. The measurement of the diffusion coefficient from the dispersion of a solute band in a cylindrical tube has to be carried out under conditions where there can be no radial flow as this contributes to solute–solvent mixing and consequently renders diffusivity values incorrect. The importance of eliminating secondary flow has been emphasized by workers such as Grushka and Kikta[15] and Claessens and van den Berg[17] and the subject of radial flow has been treated theoretically by Tijssen[18].

To eliminate completely radial mixing induced by tube curvature, the tube was made to be absolutely straight. One end of the tube was connected directly into the Valco valve and the other directly into the LC 85B detector. The tube was also contained inside a length of plastic tube (1 cm I.D.) through which water was passed from an appropriate thermostat bath so that the inner tube was maintained at a temperature of 25 ± 0.2°C. The flow-rate employed was 0.5 ml/min. The mean radius of the tube was calculated from the retention volume of a solute which was measured directly, employing a microburette attached to the detector exit tube. It should be noted that as there was no stationary phase present, the retention volume of a solute was equivalent to the tube volume. The average of five replicate measurements was taken to determine the tube volume. The length of the tube was obtained by direct measurement and found to be equal to 365.4 cm and from the length and the tube volume the tube radius was calculated to be 0.0184 cm.

Each solute was dissolved in a sample of the mobile phase at an appropriate concentration commensurate with the extinction coefficient of the solute. A volume of 0.2 μl of the solute solution was injected into the tube and the profile of the eluted band recorded. Each determination was carried out in triplicate and if an individual measurement differed from the other two by more than 3%, further replicate determinations were carried out. Diffusivity values were calculated using eqn. 1. All the solutes examined were eluted as symmetrical peaks (the asymmetry factor less than 1.1 at 0.6065 of the peak height). The efficiency of the peak was taken as four times the square of the ratio of the retention time in seconds to the band width in seconds measured at 0.6065 of the peak height. The value of H was taken as the ratio of the tube length to the band efficiency. The linear velocity was taken as the ratio of the column length to the retention time. The diffusivities of 69 different substances covering a wide range of chemical types, were determined in this way and the results obtained together with the molecular weight of each solute are included in Table I.

In order to test the data obtained against established equations relating dif-

TABLE I

MOLECULAR WEIGHT (MW), DENSITY (*d*), DIFFUSIVITY, PEAK DISPERSION, TOGETHER WITH k' and k'_e VALUES FOR THE COMPOUNDS EXAMINED

Mobile phase: 5% (v/v) ethyl acetate in *n*-hexane. Temperature: 25 ± 0.2°C (water-thermostated). Diffusivity measurements: tube, r = 0.0184 cm, l = 365.4 cm; flow-rate, 0.5 ml/min. Peak dispersion measurements: packing, Partisil, 20-μm silica gel; column, r = 0.05 cm, l = 100 cm; flow-rate 362 μl/min (u = 0.98 cm/sec).

Compound	*MW*	*d* (*g/ml*)	*D* × *10*^{5} (*cm*^{2}*/sec*)	*HETP* (*cm*)	k'	k'_e
1 Benzene	78	0.874	4.196	0.02528	0.17	0.94
2 Benzonitrile	103	1.001	3.772	0.03302	2.21	4.30
3 *p*-Xylene	106	0.861	3.755	0.02627	0.12	0.87
4 Benzaldehyde	106	1.046	3.839	0.03281	1.98	3.91
5 Anisole	108	0.989	3.767	0.02945	0.56	1.60
6 Anthranil	119	1.183	3.524	0.03517	3.92	7.14
7 Acetophenone	120	1.028	3.550	0.03628	2.95	5.59
8 Nitrobenzene	123	1.207	3.732	0.03318	1.75	3.57
9 Benzyl chloride	126	1.103	3.423	0.02927	0.42	1.35
10 Naphthalene	128	1.145	3.694	0.02767	0.30	1.17
11 Phenyl-2-propanone	134	1.028	3.138	0.03883	5.64	10.05
12 *p*-Methylacetophenone	134	1.005	3.416	0.03805	3.00	5.61
13 2-Benzothiazole	135	1.248	3.294	0.03962	6.37	11.28
14 *o*-Nitrotoluene	137	1.168	3.557	0.03414	1.26	2.73
15 *p*-Dimethoxybenzene	138	1.053	3.487	0.03465	1.50	3.14
16 α,α,α-Trifluorotoluene	146	1.199	3.631	0.02834	0.40	1.37
17 4-Phenyl-3-buten-2-one	146	1.020	3.047	0.04106	7.38	12.95
18 Anethole	148	0.991	3.281	0.03331	0.60	1.67
19 Benzylacetone	148	0.989	3.020	0.04149	5.41	9.64
20 2-Methylbenzothiazole	149	1.203*	3.321	0.04078	6.30	11.12
21 Benzyl acetate	150	1.057	3.127	0.03820	2.35	4.58
22 Ethyl benzoate	150	1.051	3.055	0.03483	0.98	2.27
23 *p*-Tolyl acetate	150	1.049	3.059	0.03716	2.11	4.17
24 Biphenyl	154	1.041	3.123	0.02843	0.31	1.17
25 1-Chloro-3-nitrobenzene	157	1.534	3.349	0.03510	1.53	3.19
26 2-Methoxynaphthalene	158	1.013*	3.102	0.03382	0.79	1.97
27 2,4-Dichlorotoluene	161	1.280*	–	0.02865	0.23	1.06
28 1,3,5-Triethylbenzene	162	0.863	2.585	0.03085	0.06	0.76
29 *n*-Propyl benzoate	164	1.021	2.859	0.03557	0.79	1.97
30 Ethyl phenylacetate	164	1.031	2.839	0.03906	1.80	3.62
31 *p*-Diethoxybenzene	164	1.008	2.983	0.03575	0.89	2.12
32 2,3-Dimethoxybenzaldehyde	166	1.019*	2.942	0.04106	6.39	11.27
33 Carbazole	167	1.10	2.482	–	–	–
34 *m*-Dinitrobenzene	168	1.575	3.136	0.03687	11.99	20.49
35 2-Acetonaphthone	170	1.147*	2.966	0.04079	4.15	7.50
36 1-Nitronaphthalene	173	1.331	2.920	0.03750	2.04	4.01
37 Ethyl cinnamate	176	1.049	2.830	0.04004	1.64	3.40
38 4-Biphenylcarbonitrile	179	1.041*	2.812	0.03893	2.08	4.09
39 2′,5′-Dimethoxyacetophenone	180	1.126*	2.810	0.04204	8.03	14.02
40 Benzophenone	182	1.080	2.746	0.03830	1.47	3.11
41 2,4-Dinitrotoluene	182	1.521	2.812	0.03843	8.88	15.45
42 Bibenzyl	182	0.978	2.756	0.03272	0.25	1.08
43 Azobenzene	182	1.203	2.694	0.04080	2.64	5.05
44 1,2-Dimethoxy-4-nitrobenzene	183	1.348**	2.819	0.04120	18.56	31.43

TABLE I *(continued)*

Compound	*MW*	*d* *(g/ml)*	*D* × *10*^{5} *(cm*^{2}*/sec)*	*HETP* *(cm)*	*k'*	k'_e
45 *o*-Nitro-α,α,α-trifluorotoluene	191	1.281*	2.941	0.03760	6.84	11.94
46 Dibenzyl ether	198	1.036	2.617	0.03796	0.86	2.09
47 Phenyl benzoate	198	1.235	2.646	0.03764	1.10	2.47
48 *p*-Bromoacetophenone	199	1.647	3.143	0.03998	3.80	6.98
49 9-Cyanoanthracene	203	1.097*	2.510	0.04123	2.27	4.42
50 Benzil	210	1.23	2.497	0.04148	2.23	4.36
51 Benzyl benzoate	212	1.112	2.587	0.03811	1.13	2.54
52 1,2-Diphenoxyethane	214	1.098*	2.475	0.04020	1.29	2.78
53 2,5-Diphenyloxazole	221	1.152**	2.505	0.04757	5.40	9.55
54 Triphenylene	228	1.302	2.498	0.03800	0.78	1.97
55 *p*-Terphenyl	230	1.221**	2.422	0.03657	0.47	1.44
56 7H-Benz[*de*]anthracene-7-one	230	1.249*	2.484	0.04328	3.77	6.93
57 Diethyl phenylmalonate	236	1.095	2.250	0.04796	4.45	8.00
58 2-Naphthyl benzoate	248	1.160*	2.363	0.04030	1.34	2.87
59 Dipropyl phthalate	250	1.059	2.220	0.04842	4.09	7.47
60 Perylene	252	1.35	2.351	0.03892	0.97	2.28
61 Bis(2-phenoxyethyl) ether	258	1.125*	2.168	0.04958	6.66	11.74
62 Tridecylbenzene	260	0.881	2.055	0.03359	0	0.66
63 Dibutyl phthalate	278	1.043	2.024	0.05002	3.18	5.91
64 Hexachlorobenzene	285	2.044	2.604	0.03020	0.06	0.81
65 O,O-Diethyl O-*p*-nitrophenyl phosphorothioate	291	1.286*	2.149	0.04582	3.15	5.90
66 1,2,4,5-Dibenzopyrene	302	1.288*	2.180	0.04749	1.32	2.87
67 O,O-Diethyl O-[2-isopropyl-4-methyl-6-pyrimidyl] phosphorothioate	304	1.107*	1.922	0.05840	10.77	17.96
68 *m*-Quaterphenyl	306	1.206	1.890	0.04347	0.61	1.66
69 Dioctyl phthalate	390	0.981	1.635	0.05803	1.83	3.71
70 Didecyl phthalate	446	0.965	1.462	0.06208	1.55	3.24

* Measured.
** Calculated.

fusivity to other physical properties of the solute, the density of each compound at 25°C had to be acquired. The majority of density values were obtained directly from the literature (see Table I), others were calculated from densities measured at different temperatures, but the density of sixteen solutes had to be determined directly as the data was not readily available. Furthermore, some of these substances were only available in limited quantities so the following procedure for density measurement was adopted.

Five-centimeter lengths of flexible soft-glass tubing[19] were weighed on a microbalance and filled to a known length with water and weighed again; from this data the volume per unit length of the tubing was calculated. The substances of interest were then melted (if solid at room temperature) and allowed to be absorbed into a pre-weighed length of capillary tubing by surface tension. The substances were then allowed to solidify and cool, and the filled length was then measured. The tube was re-weighed and from a knowledge of the volume per unit length of the tube, the density was calculated. The density of all compounds determined in this way are also included in Table I.

TABLE II

THE EFFECT OF PRESSURE ON DIFFUSIVITY

Solvent: 5% (v/v) ethyl acetate in *n*-hexane. Solute: benzyl acetate.

Pressure (MPa)	*Diffusivity (cm^2/sec)*	
0.6	$3.10 \cdot 10^{-5}$	Data fitted to function
5.9	$2.96 \cdot 10^{-5}$	
7.1	$2.96 \cdot 10^{-5}$	$D_m = A + BP$
14.7	$2.77 \cdot 10^{-5}$	
24.0	$2.57 \cdot 10^{-5}$	Index of determination 0.997
31.0	$2.44 \cdot 10^{-5}$	Constant $A = 3.097 \cdot 10^{-5}$
37.0	$2.32 \cdot 10^{-5}$	Constant $B = -0.0214 \cdot 10^{-5}$
Change in diffusivity with pressure 0.69% per MPa		

Previous work has shown that both the viscosity of the mobile phase and the diffusivity of a solute is dependent on pressure[20]. As the precision of the molecular weight evaluation made by measuring the band width of a peak eluted from a packed column will depend on the diffusivity of the solute, then any dependence of diffusivity on pressure becomes important. The effect of pressure on diffusivity was measured using the same apparatus but employing the technique used by Katz *et al.*[20]. The end of the tube close to the detector was crimped thus, increasing the pressure in the tube. The peak dispersion of benzyl acetate was measured in triplicate over a range of pressures up to 36 MPa by progressively crimping the tube and the corresponding diffusivity values calculated. The results obtained are included in Table II and are shown in Fig. 3 as a linear curve relating diffusivity to pressure.

Discussion of results

There is a large number of equations in the literature, relating the diffusivity of a solute to various physical and molecular properties of both solute and solvent, four of which have already been referenced[12–15]. The data given in Table I was fitted to many of these equations but, with the exception of one (the equation that gave the best fit to the experimental data) the result of these correlations will not be given here. The values given in Table I are precisely measured and are available for any equations considered suitable to be tested. It was found that there were two molecular properties that were important to relate to the solute diffusivity for a given system operated at a given temperature. The first was the mean molecular radius of the solute which was taken as proportional to the cube root of the molecular volume and which, itself, was calculated from the ratio of the molecular weight to the solute density. The second factor, included in molecular volume but which had also a separate and additional contribution, was the molecular weight of the solute. Both these factors were included in the equation suggested by Arnold[12] to describe the diffusivity of a solute

$$D_m = A(1/M_1 + 1/M_2)^{1/2}/(V_1^{1/3} + V_2^{1/3})^2$$

where A is a constant under a given set of experimental conditions, M_1 is the molecular weight of the solute, V_1 is the molecular volume of the solute, M_2 is the molecular weight of the solvent and V_2 is the molecular volume of the solvent. It was found by trial and error that the following relationship accurately described the diffusivity in terms of the solute molecular properties

$$\begin{aligned} 1/D &= A + BV^{1/3}M^{0.5} \\ &= A + B\,M^{0.833}/d^{1/3} \end{aligned} \tag{2}$$

where A and B are constants for a given system and d is the density of the solute.

It is seen that if an accurate correlation is required, a knowledge of both the molecular weight and the density is necessary. However, as will be seen later, if we are dealing with substances that have a density close to unity then the reciprocal of the diffusivity can be related simply to the molecular weight raised to the power of the 0.833. The result of the correlation of the reciprocal of the diffusivity for the 69 different compounds to the product of the cube root of the molecular volume and the square root of the molecular weight is shown in Fig. 1. It is seen that a very close linear correlation indeed is obtained. The error involved in the correlation is shown in Fig. 2A and B. Fig. 2A is a histogram that relates the number of compounds associated with a particular error. In Fig. 2B the per cent of the compounds is plotted against their respective error.

It is seen that the linear relationship is held for 90% of all compounds within an error of less than 7%. 95% of all compounds had a maximum error of 8% and 98% a maximum error of 11%. The two substances that appeared to deviate most,

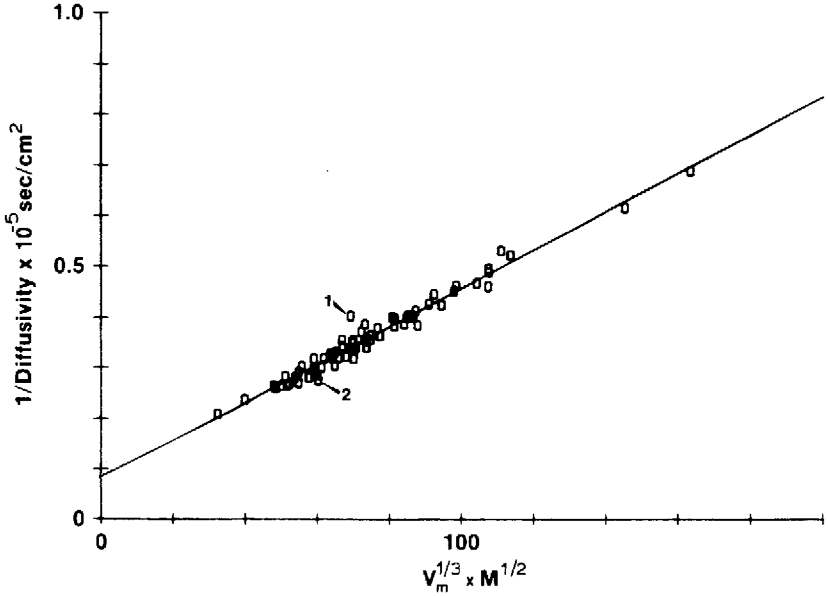

Fig. 1. Graph of the reciprocal of the diffusivity against the product of the cube root of the molar volume and the square root of the molecular weight. Tube: diameter, r = 0.0184 cm; length, l = 365.4 cm. Mobile phase: 5% ethyl acetate in n-hexane. Flow-rate: 0.5 ml/min. Temperature: 25°C (water-thermostated).

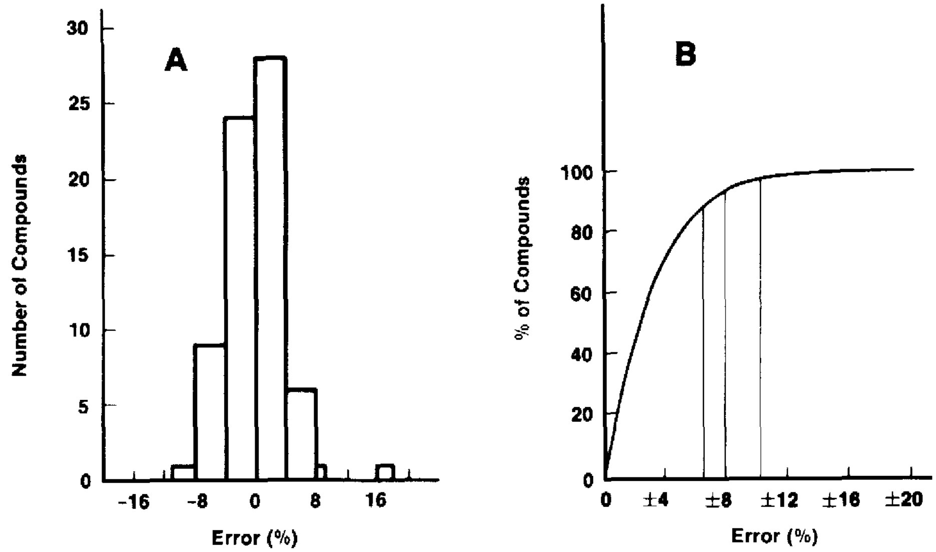

Fig. 2. Graphs demonstrating the distribution of the error (%) obtained from the linear regression of the reciprocal of the diffusivity against the product of the cube root of the molar volume and the square root of the molecular weight.

namely, carbazole and α,α,α-trifluorotoluene (see 1 and 2 in Fig. 1) had an error of 18% and 10%, respectively. It should be noted that 1,3,5-triethylbenzene also gave a significant error of 9% probably due to isomeric impurities. It was considered that the sample of carbazole used was likely to contain a significant amount of impurities and consequently was not employed in subsequent experiments.

It follows that if it is found possible to obtain a function of the dispersion of a solute eluted from a packed column that is linearly related to the reciprocal of its diffusivity, then it would be possible to predict, within given error limits, the molecular weight of any solute providing the density of the solute was known or alternatively its density was close to unity.

The effect of pressure on solute diffusivity will now be considered. The data in Table II relating diffusivity with absolute pressure was fitted to a linear function and the results included in Table II. It is seen that over the pressure range examined the diffusivity decreased linearly with pressure and furthermore it appears that the diffusivity changes by 0.69% per megapascal over the range examined. This result agrees well with that previously predicted from work describing the effect of pressure on viscosity and the relationship between viscosity and diffusivity[20]. In fact, the value predicted for the change in diffusivity of benzyl acetate with pressure was 0.80% per megapascal. It follows that as the diffusivity does change slightly with the absolute pressure, any change in column impedance may require recalibration for accurate diffusivity measurement. However, it should also be noted that the average column pressure can be taken as half the inlet pressure and consequently the change in diffusivity would be only 0.35% per MPa at the column inlet. It should also be pointed out that the change in solute diffusivity in a chromatographic column with pressure will depend on the temperature control of the column. Katz *et al.*[20] showed that

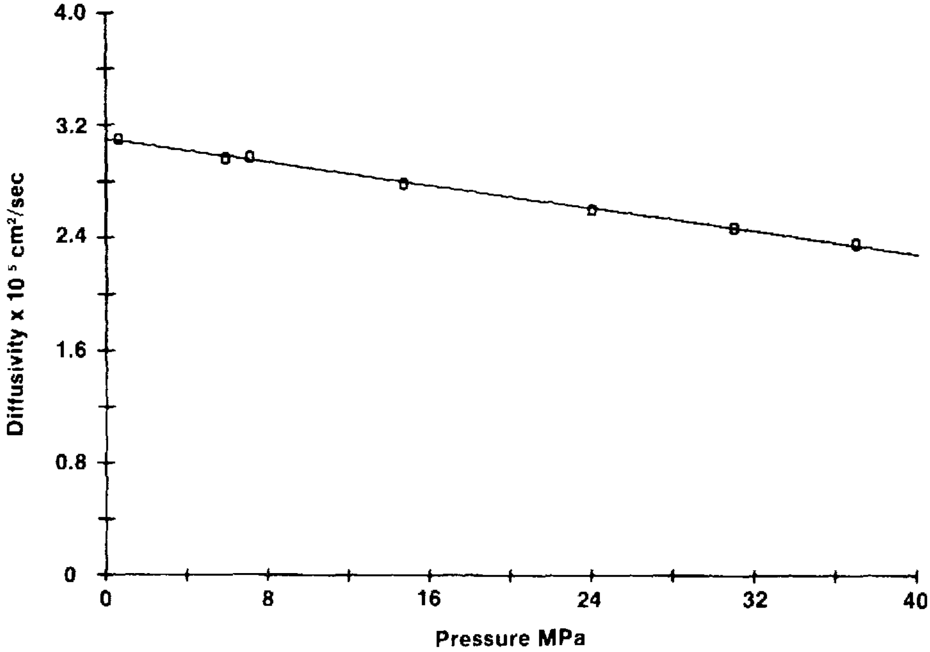

Fig. 3. Graph of the diffusivity of benzyl acetate against the absolute pressure.

temperature changes that result from viscous heating tend to compensate for the pressure effect on diffusivity and render the diffusivity independent of pressure.

RELATIONSHIP BETWEEN DISPERSION AND SOLUTE MOLECULAR WEIGHT

Theoretical

The equation derived by Van Deemter[9] to describe the dispersion of a solute band in a packed bed is as follows

$$H = 2\,\lambda d_{\mathrm{p}} + \frac{2\gamma D_{\mathrm{m}}}{u} + \frac{\mathrm{f_m}\,(k')d_{\mathrm{p}}^2\,u}{D_{\mathrm{m}}} + \frac{\mathrm{f_s}\,(k')d_{\mathrm{f}}^2\,u}{D_{\mathrm{s}}} \tag{3}$$

where, H, D_{m} and u have the meaning previously ascribed to them, λ and γ are constants, d_{p} is the particle diameter of the packing, d_{f} is the effective film thickness of the stationary phase and $\mathrm{f_m}\,(k')$ and $\mathrm{f_s}\,(k')$ are two functions of the capacity ratio, k'. Eqn. 3 can be put in a simplified form

$$H = A + \frac{B}{u} + Cu \tag{4}$$

where

$$A = 2\,\lambda d_{\mathrm{p}}$$
$$B = 2\gamma D_{\mathrm{m}}$$

$$C = \frac{\mathrm{f_m}\,(k')\,d_{\mathrm{p}}^2}{D_{\mathrm{m}}} + \frac{\mathrm{f_s}\,(k')\,d_{\mathrm{f}}^2}{D_{\mathrm{s}}} \tag{5}$$

At high linear mobile phase velocities ($u > 0.1$ cm/sec) $B/u \ll A$ and $B/u \ll Cu$, then

$$H = A + Cu$$

or

$$H - A = Cu$$

consequently

$$H - A = \frac{f_m\,(k')\,d_p^2\,u}{D_m} + \frac{f_s\,(k')\,d_f^2\,u}{D_s} \tag{6}$$

It has been suggested by Purnell and Quinn[21] that the most appropriate form of $f_m(k')$ for the resistance to mass transfer in the mobile phase is that derived by Golay[16] for capillary columns

$$f_m\,(k') = \frac{1 + 6k' + 11k'^2}{24(1 + k')^2} \tag{7}$$

and from the Van Deemter equation[9]:

$$f_s(k') = \frac{8\,k'}{\pi^2\,(1 + k')^2} \tag{8}$$

It should be pointed out, however, that $f_m\,(k')$, which is one factor that controls the magnitude of the resistance to mass transfer in the mobile phase, will contain two parts, one part pertaining to the resistance to mass transfer in the portion of the mobile phase that is moving and a second part that relates to the portion of the mobile phase contained in the pores that is static.

To date there is limited experimental evidence to indicate that the above functions for k' are the most appropriate. However, these functions can form a useful basis from which first order effects can be predicted. Sufficient and precise experimental data could confirm their general validity and would permit a more exact form of the functions to be developed. Thus, substituting from eqns. 7 and 8 for $f_m\,(k')$ and $f_s\,(k')$ in eqn. 6:

$$H - A = \frac{(1 + 6k' + 11k'^2)\,d_p^2\,u}{24\,(1 + k')^2\,D_m} + \frac{8k'\,d_f^2\,u}{\pi^2\,(1 + k')^2 D_s} \tag{9}$$

Eqn. 9 will be the basic equation that, with the relationship between diffusivity and molecular weight given by eqn. 2, will be employed to develop the relationship between the band width of an eluted solute and its molecular weight.

Experimental

The same apparatus was employed as that used for the measurement of diffusivity, namely, a Perkin-Elmer Series B liquid chromatograph, a Valco valve (sample volume 0.2 μl) and the LC 85B UV detector.

Due to the evolution of heat in packed columns operated at high linear mobile phase velocities[20], it was necessary to use a microbore column (1 mm I.D. and 100 cm long) appropriately thermostatted at a temperature of 25 ± 0.2°C. Microbore columns generate less heat than conventional columns as a result of the low volume flow-rate employed and furthermore, the heat that is generated is lost more rapidly to its surroundings due to its small cross-sectional area. To ensure that the resistance-to-mass transfer factor in the mobile phase was the predominant source of band dispersion, Partisil 20 silica was employed as the stationary phase which had a nominal particle diameter of 20 μm and an actual mean particle diameter of 17.5 μm. (The resistance-to-mass transfer term increases as the square of the particle diameter, *cf.*, eqn. 9.) The column was hand packed by tamping and each end was terminated by a scintered disk, 5-μm porosity, 1/16 in. O.D. The mobile phase employed was the same as that used in the measurement of diffusivity, *viz.*, 5% (v/v) ethyl acetate in *n*-hexane.

The same compounds previously employed in the measurement of diffusivity (with the exception of carbazole) were examined and HETP values were determined for each solute at a flow-rate of 362 μl/min which was equivalent to a linear velocity of 0.98 cm/sec. Samples of appropriate concentration, 0.2 μl in volume, were placed on the column and the efficiency of the resulting peak was taken as four times the square of the ratio of the retention distance to the band width measured at 0.6065 of the peak height. Each determination was repeated in quadruplicate and further replicate measurements were made if any individual result differed by more than 3% from the mean of the other three. The height of the theoretical plate or the variance per unit length was taken as the ratio of the column length to the column efficiency. The capacity factor, k' was also measured for each solute, and the dead volume taken as the retention volume of tridecylbenzene. The excluded capacity factor, k'_e, was taken as $1.664\,(k' + 1) - 1$ as the excluded volume of the column was assumed to be $V_0/1.664$[11].

The results obtained, that is, the values of H, k' and k'_e for each solute, are also included in Table I.

Results and discussion

In order to relate the value of H to the solute diffusivity and consequently to the solute molecular weight according to eqn. 9, certain preliminary calculations have to be made. First, an accurate value of the multipath term, A, has to be determined. Secondly, the pertinence of the Golay function of k' for the resistance-to-mass transfer in the mobile phase and the Van Deemter function of k' for the resistance-to-mass transfer in the stationary phase has to be established.

The stationary phase interactions on the silica gel surface must involve the static mobile phase in the pores and the layers of absorbed moderator on the silica surface. It follows that the diffusivity of the solute in the static and stationary phase, D_s, will be similar but not the same as the diffusivity of the solute in the mobile phase, D_m. Thus, it can be assumed to a first approximation that

$$D_s = \alpha D_m$$

where α is some constant probably close to unity. Thus, eqn. 9 becomes:

$$H - A = \frac{(1 + 6k' + 11k'^2)\, d_p^2 u}{24\,(1 + k')^2 D_m} + \frac{8k' d_f^2 u}{\pi^2\,(1 + k')^2\, \alpha D_m} \tag{10}$$

Determination of the value of the multipath term, A. Eqn. 10 can be used to evaluate A, the multipath term, from the data given in Table I. It has been shown that the multipath term is not dependent on solute diffusivity by the direct measurement of the multipath term from HETP curves determined with mobile phases of different viscosity[11]. However, additional dispersion effects other than those taken into account by the Van Deemter theory can also appear from a curve fitting procedure as a contribution to the value of A, the multipath term. Examples of extra column dispersion effects that would give higher A values are the effect of large sample volumes and channeling due to the inhomogeneity of bed permeability; the latter might well render the multipath term A *apparently* dependent on solute diffusivity. A well packed column should not have a multipath term greater than two particle diameters. The column employed in this work was dry packed and had values for the multipath term of over six particle diameters and thus, the apparent value of A could include diffusion dependent effects. *Ipso eo*, any method employed to determine A must accommodate any effect of diffusivity should it be there. Thus, a numerical value of A has to be arrived at employing data from solutes having significantly different diffusivities.

For solutes having a narrow range of k' [$f(k') \approx$ constant] separated on a given column at a given constant velocity, eqn. 10 reduces to

$$H = A + \frac{\sigma}{D_m} \tag{11}$$

where σ is a constant. A plot of H against $1/D_m$ for an appropriate series of solutes will consequently be linear and provide an intercept equivalent to A. The solutes selected are taken from Table I and are summarized in Table III. It is seen that the diffusivity of the solutes range from 1.46×10^{-5} to 3.83×10^{-5} cm^2/sec and the k' values all lie between 1.5 and 2.3. The graph of H against $1/D_m$ obtained is shown in Fig. 4 and the results of a curve fit of the data to a linear function included in Table III. It is seen that the linear relationship is indeed obtained and the intercept gave a value for A of 0.0144 cm. This value for A will be used in all subsequent calculations. The dependence of the resistance-to-mass transfer factor will now be considered.

Effect of the function of k′ on peak dispersion. Rearranging eqn. 10:

$$(H - A)D_m \Big/ \left[\frac{1 + 6k' + 11k'^2}{24\,(1 + k')^2}\right] = \psi = d_p^2 u + \frac{192 k' d_f^2 u}{\pi^2\,(1 + 6k' + 11k'^2)\alpha} \tag{12}$$

Eqn. 12 indicates that a graph relating ψ and k' should provide a smooth curve and ψ should achieve a constant minimum value at high values of k'. Values for ψ were calculated from the data given in Table I, and in Fig. 5 ψ is shown plotted against

TABLE III

THE RECIPROCAL OF SOLUTE DIFFUSIVITY AND PEAK DISPERSION VALUES FOR THE COMPOUNDS EMPLOYED IN THE DETERMINATION OF THE MULTIPATH TERM (k' RANGE: 1.5-2.3)

Compound	k'	$1/D \times 10^{-5}$ (sec/cm^2)	H (cm)
1 Benzaldehyde	1.98	0.261	0.03281
2 Benzonitrile	2.21	0.265	0.03302
3 Nitrobenzene	1.75	0.268	0.03318
4 *p*-Dimethoxybenzene	1.50	0.287	0.03465
5 1-Chloro-3-nitrobenzene	1.53	0.299	0.03510
6 Benzyl acetate	2.35	0.320	0.03820
7 *p*-Tolyl acetate	2.11	0.327	0.03716
8 1-Nitronaphthalene	2.04	0.342	0.03750
9 Ethyl phenylacetate	1.80	0.352	0.03906
10 Ethyl cinnamate	1.64	0.353	0.04004
11 4-Biphenylcarbonitrile	2.08	0.356	0.03893
12 9-Cyanoanthracene	2.27	0.398	0.04123
13 Benzil	2.23	0.401	0.04148
14 Dioctyl phthalate	1.83	0.612	0.05803
15 Didecyl phthalate	1.55	0.684	0.06208

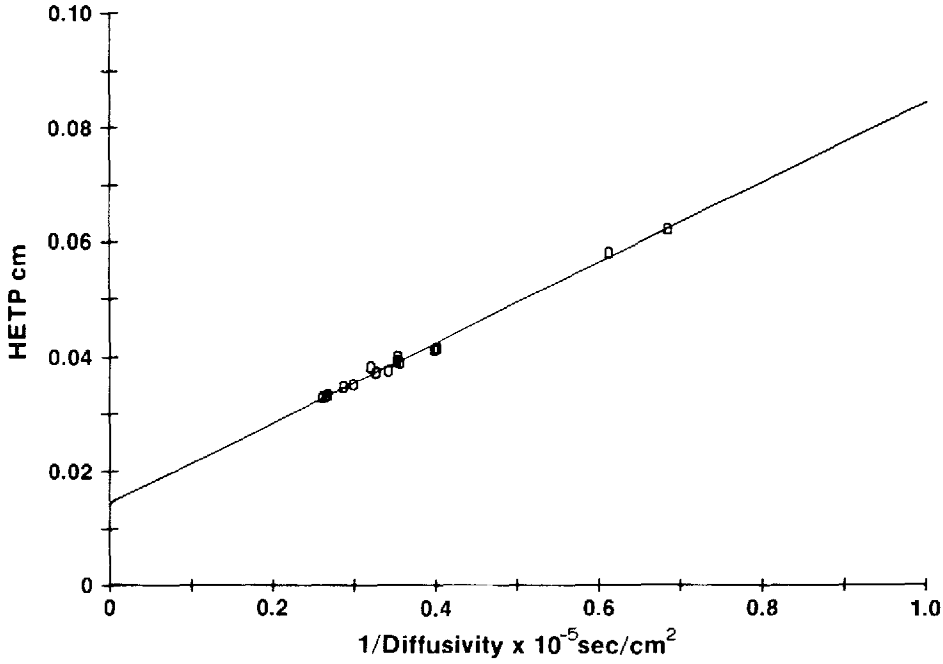

Fig. 4. Determination of the A term from the peak dispersion of different solutes. u = 0.98 cm/sec; $k' \approx 2.0$.

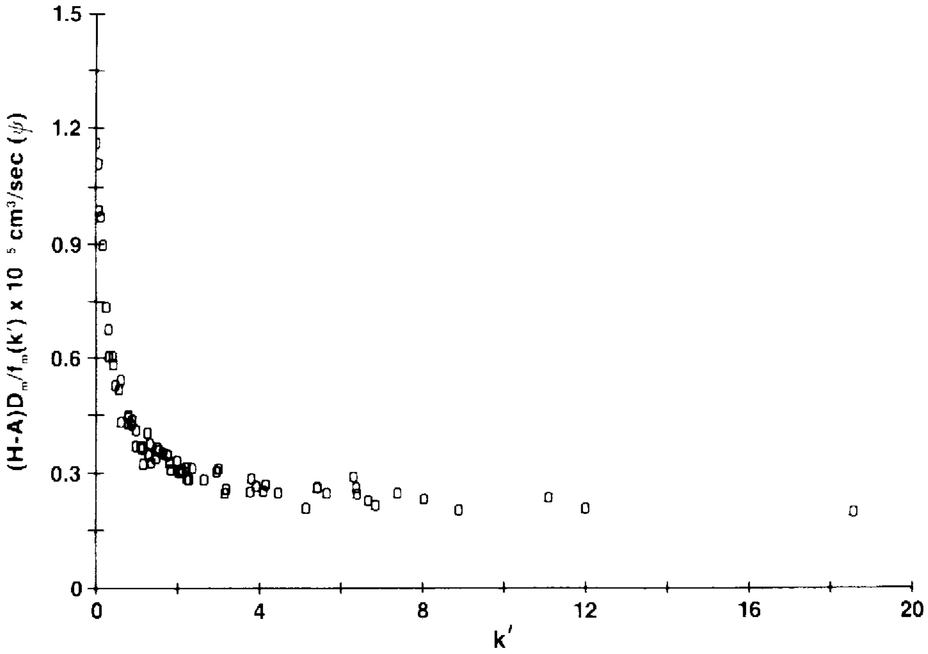

Fig. 5. Graph relating corrected peak dispersion, ψ, to the capacity factor, k'.

k'. It is seen that indeed a smooth curve is obtained with ψ tending to a constant at high values of k'. The smooth nature of the curve also confirms the linear dependence of $H - A$ on $1/D_m$. In Fig. 6 ψ is plotted against the function $k'/(1 + 6k' + 11k'^2)$ which, from eqn. 12, should provide a linear curve, the intercept being equivalent to d_p^2u and the slope equivalent to $192\ d_f^2u/\pi^2\alpha$. It is seen that a linear curve is obtained only at low values of $k'/(1 + 6k' + 11k'^2)$ (that is high values of k') however, the linear curve degenerates at large values of $k'/(1 + 6k' + 11k'^2)$ (that is at low values of k') and in fact, forms a parabola. It would appear that at low values of k' the relationship was incorrect whereas at high value of k' it gave predictable results.

The value of k' calculated from a dead volume measurement that included the pore volume could be questionable as the true definition of k' assumes that the dead volume of the column contains only the phase that is moving. This is generally true in gas chromatography for both packed and capillary columns but in liquid chromatography, due to the nature of silica gel, much of the mobile phase is contained in the pores and is indeed not moving but static. It follows that to maintain parity with the true concept of k', the excluded volume of the column should be employed in the calculation of all capacity values, *i.e.*, values for the capacity factor should be calculated from the excluded volume of the column and not from the fully permeated volume

$$k'_e = \frac{V_r - V_e}{V_e}$$

where V_r is the retention volume of the solute and V_e is the excluded volume of the column.

Using k'_e as an alternative to k' a new set of values for $k'_e/(1 + 6k'_e + 11 k'^2_e)$ was calculated for each value of ψ, and the results are shown as a curve relating ψ to $k'_e/(1 + 6k'_e + 11k'^2_e)$ in Fig. 7. It should be noted that the expected linear relationship is now obtained but the scale of ψ in Fig. 7 is expanded and thus, gives the impression of greater scatter than there is evident in Fig. 6. A linear curve fit to the data in Fig. 7 gave an intercept of $0.183 \cdot 10^{-5}$ and a slope of $1.24 \cdot 10^{-5}$. Employing the constants of 24 from the Golay equation and $8/\pi^2$ from the Van Deemter equation, value of 10.6 μm and 6.3 μm were found for d_p and d_f, respectively. The value for d_p is sufficiently far from the true value of d_p, *viz.*, 17.5 μm, to indicate that at least some of the theoretical values of the Golay constants may not be appropriate. The advantage of this approach, however, to ascertain the correct functions of k' to employ, is a consequence of it being based on equations theoretically derived and not solely an empirical fit.

Employing the constant values of the intercept and slope from Fig. 7 eqn. 12 can be put in this form:

$$24(H - A)D_m\,(1 + k'_e)^2/1 + 6k'_e + 11k'^2_e =$$

$$= 0.183 \cdot 10^{-5} + 1.24 \cdot 10^{-5} \cdot \frac{k'_e}{(1 + 6k'_e + 11k'^2_e)} \qquad (13)$$

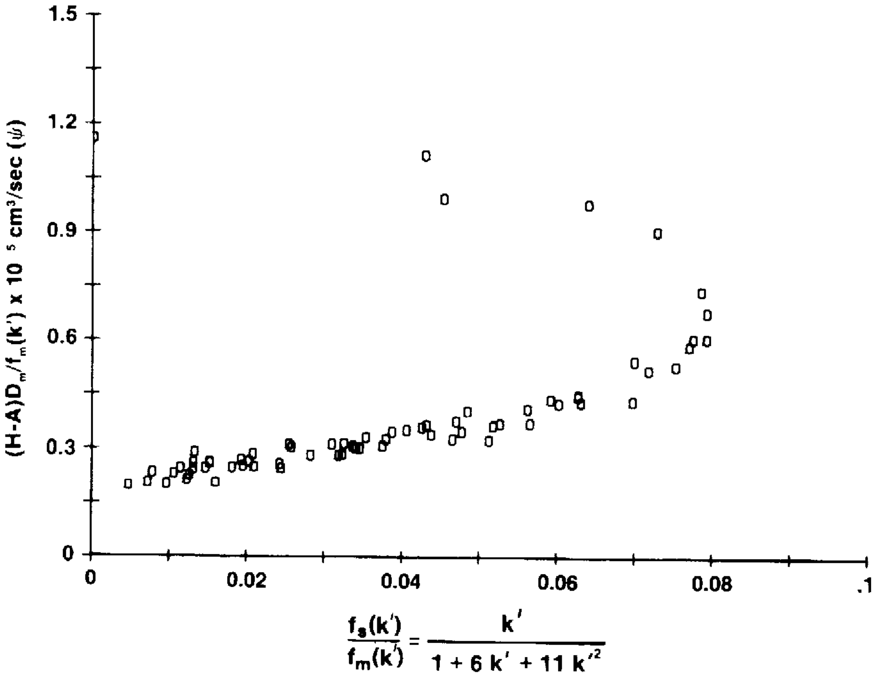

Fig. 6. Graph of $(H - A)D_m/f_m(k')$ against $f_s(k')/f_m(k')$. k' values were calculated from the retention time of the fully permeating unretained solute.

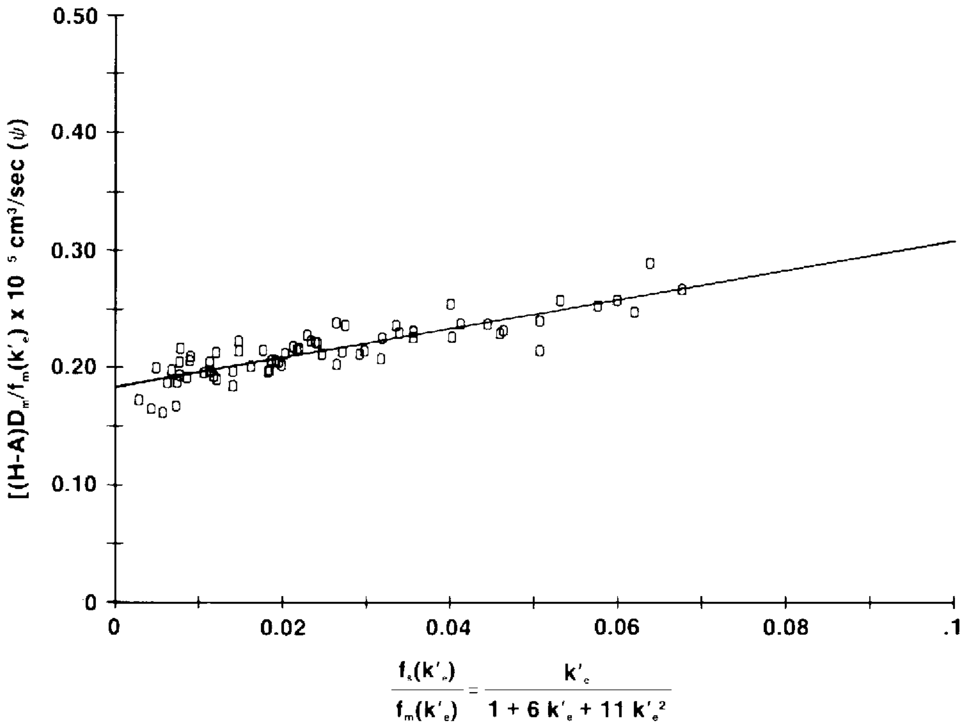

Fig. 7. Graph of $(H - A)D_m/f_m(k'_e)$ against $f_s(k'_e)/f_m(k'_e)$. k'_e values were calculated from the retention time of the fully excluded solute.

Knox[22] suggested an alternative approach which would lead to the same basic functions of k'_e and would be more simple to deal with, but would require a polynomial fitting procedure. Rearranging eqn. 10:

$$(H - A)D_m(1 + k'_e)^2 = \frac{(1 + 6k'_e + 11k'^2_e)\, d_p^2\, u_e}{24} + \frac{8k'_e d_f^2 u_e}{\pi^2 \alpha} \tag{14}$$

For a given column operated at a constant mobile phase linear velocity $d_p^2 u_e$ and $d_f^2 u_e/\alpha$ are constants and thus eqn. 14 can be put in the form:

$$(H - A)D_m(1 + k'_e)^2 = a + bk'_e + ck'^2_e \tag{15}$$

Now $(H - A)D_m(1 + k'_e)^2$ can be calculated from the data given in Table I and thus constants a, b and c can be found. However, the determination of a, b and c has to be carried out with some circumspection. If a curve fitting routine to a second order polynomial is attempted, the precise value of a is strongly effected by any small error in the high values of k'_e due to the second order term, ck'^2_e. Therefore, a, b and c should first be obtained by a curve fitting procedure of $(H - A)D_m(1 + k'_e)^2$ to a second order polynomial in k'_e for values of $k'_e < 2$. This will give precise values for a and b but not for c; a precise value of c will only be obtained if large values of k'_e are employed. The next step is to rearrange eqn. 15:

$$[(H - A)D_m (1 + k'_e)^2 - a]/k'_e = e = b + ck'_e \quad (16)$$

From eqn. 16 a linear fit of e to k'_e will give another value of b which will be close to that originally obtained and more precise value for c. This alternative approach is perfectly satisfactory to permit a precise relationship between plate height and diffusivity to be obtained but is basically merely a curve fitting procedure and thus more empirical than the former alternative.

RELATIONSHIP BETWEEN PEAK DISPERSION, SOLUTE DIFFUSIVITY AND SOLUTE MOLECULAR WEIGHT

Rearranging eqn. 13:

$$(H - A)(1 + k'_e)^2/(0.0076 + 0.0975\,k'_e + 0.084\,k'^2_e) \cdot 10^{-5} = Z = 1/D$$

From the data given in Table I and the established values of A, values of Z and $1/D$ were calculated and the results plotted as curves relating Z to $1/D$ in Fig. 8. It is seen that an excellent linear relationship is obtained substantiating the dependence of the resistance-to-mass transfer factor on the reciprocal of the solute diffusivity. The error

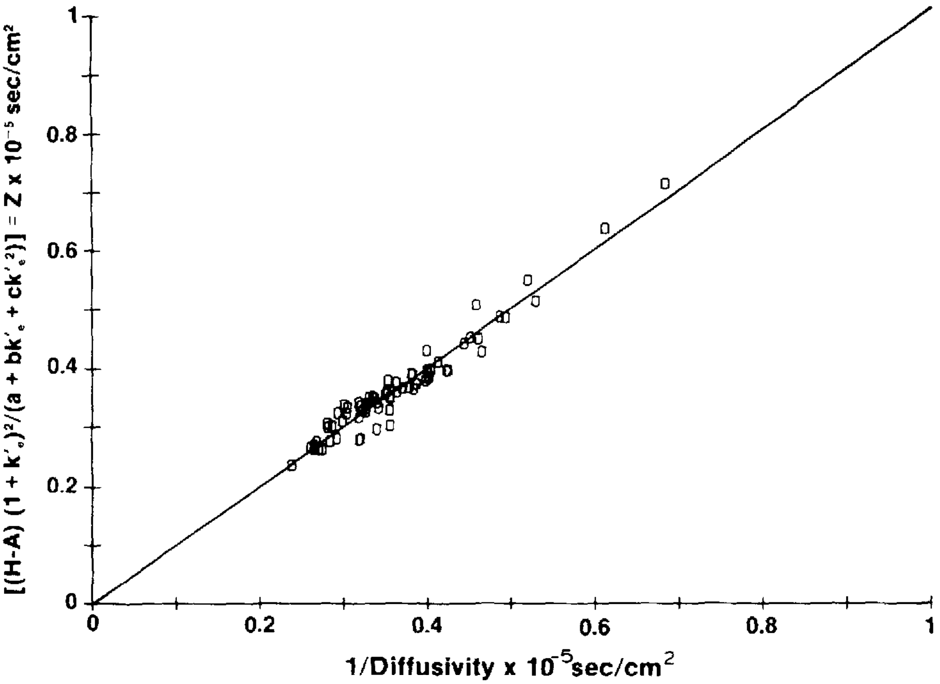

Fig. 8. Graph of the corrected peak dispersion $[(H - A)(1 + k'_e)^2/(a + bk'_e + ck'^2_e)] = Z$ against the reciprocal of the diffusivity.

expressed as a histogram relating the number of solutes against percentage error is given in Fig. 9A and the percentage of samples plotted against percentage error shown in Fig. 9B. It is seen that for 90% of the samples, the error is less than 9%. The relationship between diffusivity and molecular weight also includes the solute density and unfortunately this latter parameter will not be available for an unknown sample. However, a large proportion of the solutes of interest and separated today by liquid chromatography techniques and, in particular, those of biological origin have densities close to unity. Consequently, those solutes having densities lying between 0.85 and 1.25 g/ml (where the cube root of the density approaches unity) were selected from the 68 solutes examined (totaling 56 substances) and values for Z plotted against $M^{0.833}$ and the curves obtained shown in Fig. 10. It is seen that again a very good linear relationship is obtained. In Fig. 11A and B the number of samples and per cent of samples are plotted against percentage error. It is seen from Fig. 11B that over 90% of the samples involve an error of less than 9%, the greatest error being 16%. Finally, the values for the molecular weight obtained experimentally for each of the 56 solutes are plotted against the actual molecular weights in Fig. 12 and the percentage of samples plotted against percentage error in Fig. 13. It is seen that the molecular weight of a solute having a density between 0.85 and 1.25 g/ml can be obtained experimentally from peak width measurements for 90% of the compounds within an error of less than 13% (80% have an error less than 10%). It should be noted that the same compounds, 1,3,5-triethylbenzene and α,α,α-trifluorotoluene which had the greatest error in Fig. 2B, again have the extreme errors in Fig. 13B.

PROCEDURE FOR DETERMINING SOLUTE WEIGHTS FROM CHROMATOGRAPHIC DATA

The apparatus to be used for chromatographic molecular weight measurement must be similar to that employed in this work, namely a low dispersion detector such as the LC 85B, a low volume injection valve (*ca.* 0.2 μl) and a microbore column

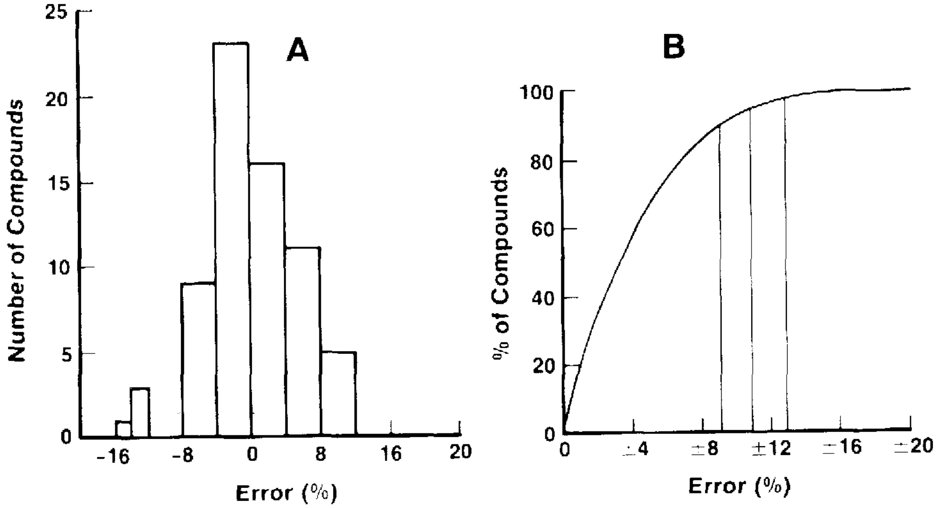

Fig. 9. Graph demonstrating the distribution of the error (%) obtained from the linear regression of the corrected peak dispersion, Z, against the reciprocal of the diffusivity.

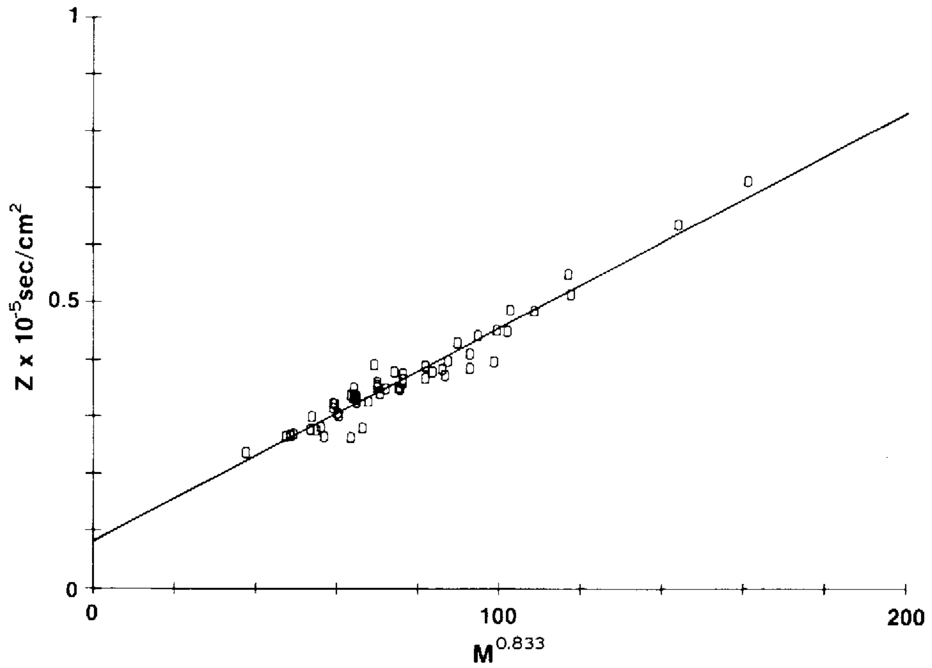

Fig. 10. Graph of the corrected peak dispersion, Z, against $M^{0.833}$.

about 1 m long and 1 mm I.D. packed with silica particles of about 20 μm in diameter. The column should be appropriately thermostatted, the mobile phase should be a 5% solution of ethyl acetate in *n*-hexane and a flow-rate employed that would provide a linear mobile phase velocity of about 1 cm/sec. Once the flow-rate is set it must be used throughout the calibration procedure and for all subsequent experiments. The chromatographic system should be calibrated using the calibration solutes given in Table IV. Solutes 8–14 may be chromatographed as a mixture but solutes 1–7 will have to be chromatographed individually as they will all be eluted close together.

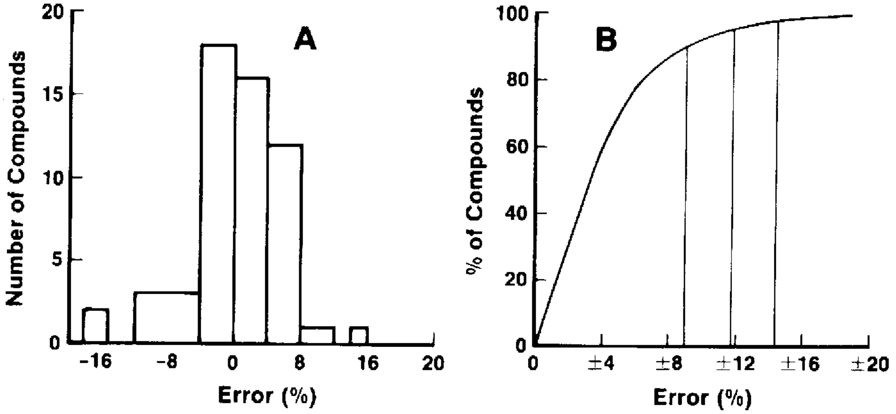

Fig. 11. Graph demonstrating the distribution of the error (%) obtained from the linear regression of the corrected peak dispersion, Z, against $M^{0.833}$.

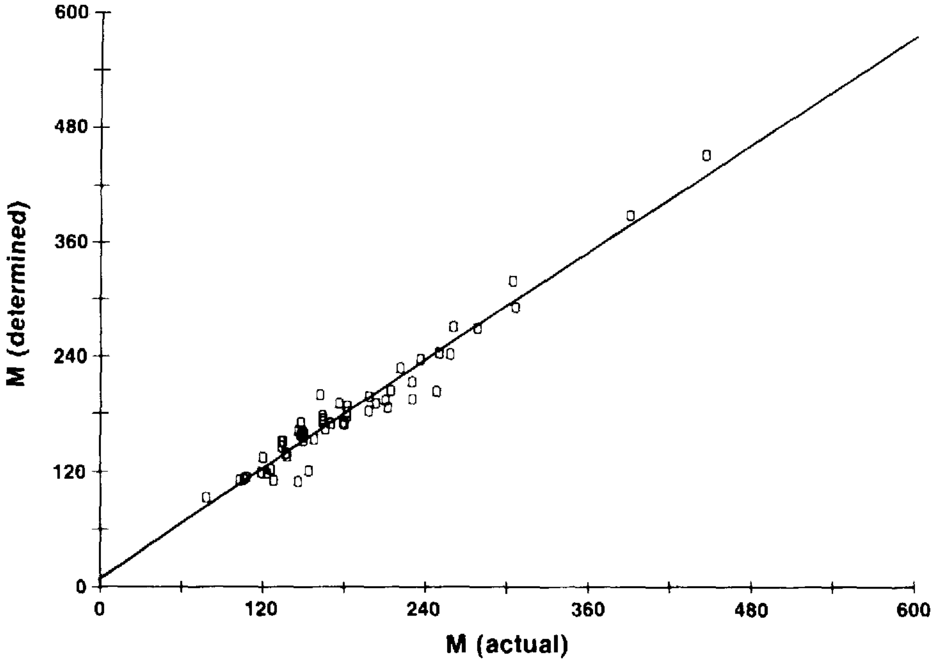

Fig. 12. Graph of the molecular weight experimentally determined against the actual molecular weight.

The mean of triplicate values for H and k'_e should be obtained to ensure adequate precision. The value of the multipath factor A must be first calculated by determining the intercept from the linear curve obtained from plotting H against $1/D_m$ (values of $1/D_m$ given in Table IV) employing the results from solute standards 1-7. Values of $(H - A)D_m\,(1 + k'_e)^2$ are then calculated for the results from solute standards 6-14 and curve fitted to the second order polynomial $a + bk'_e + ck'^2_e$ to determine the constants a, b and c. Finally, values of $(H - A)\,(1 + k'_e)^2/(a + bk'_e + ck'^2_e)$ are plotted against $M^{0.833}$ (values for M are also obtained from Table IV) to produce the final calibration curve. Thus, for any unknown solute having a plate height of H

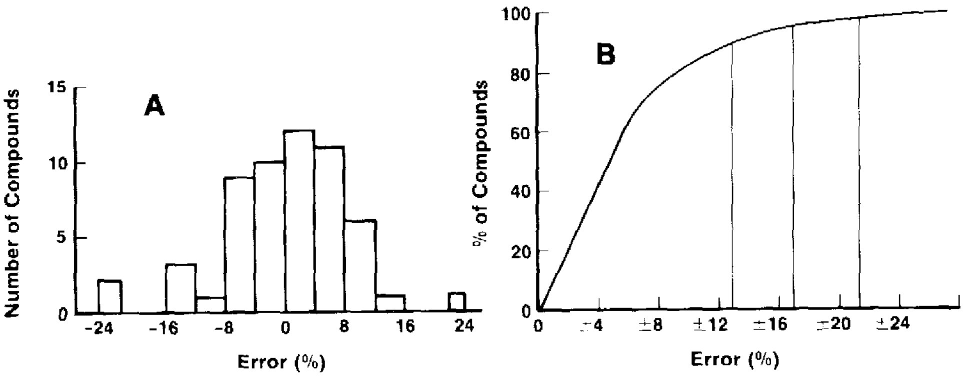

Fig. 13. Graph demonstrating the distribution of the error (%) obtained from the comparison of the molecular weight experimentally determined to the actual molecular weight.

TABLE IV

REFERENCE SUBSTANCES AND THEIR PERTINENT PHYSICAL PROPERTIES

Compound	k'_e	*D* × *10*^{5} *(cm*^{2}*/sec)*	*H (cm)*
1 Benzaldehyde	3.91	3.839	0.03281
2 *p*-Dimethoxybenzene	3.14	3.487	0.03465
3 *p*-Tolyl acetate	4.17	3.059	0.03716
4 4-Biphenylcarbonitrile	4.09	2.812	0.03893
5 Benzil	4.36	2.497	0.04148
6 Dioctyl phthalate	3.71	1.635	0.05803
7 Didecyl phthalate	3.24	1.462	0.06208
8 Benzene	0.94	4.196	0.02528
9 Anisole	1.60	3.767	0.02945
10 *o*-Nitrotoluene	2.73	3.557	0.03414
11 Benzyl acetate	4.58	3.127	0.03820
12 Acetophenone	5.59	3.550	0.03628
13 Benzylacetone	9.64	3.020	0.04149
14 2′,5′-Dimethoxyaceto-phenone	14.02	2.810	0.04204

and a capacity factor of k'_e, the value of $(H - A)(1 + k'_e)^2/(a + bk'_e + ck'^2_e)$ is calculated and the value of $M^{0.833}$ read off from the calibration curve and thus M determined.

CONCLUSIONS

The relationship between the molecular weight of a substance and its diffusivity in a given solvent has been established. It has also been shown that the resistance-to-mass transfer terms in the Van Deemter equation is proportional to the reciprocal of the solute diffusivity. Consequently, at high mobile phase velocities where the resistance-to-mass transfer effect predominates, the plate height of a solute is determined largely by the solute diffusivity and consequently the solute molecular weight. By establishing the magnitude of the multipath term of a given column and the function of the capacity factor that contributes to the magnitude of the resistance-to-mass transfer factor, the plate height of a solute can provide a measure of its molecular weight. The value obtained will have maximum error of 13% of the true value for over 90% of the solutes examined provided the density of the solute lies between 0.85 and 1.25 g/ml.

The basic principle of employing peak width measurements to provide appropriate values for the molecular weight of a solute is general and can be used in any chromatographic analysis provided that the sample volume injected into the column is small and no solute–solute interaction takes place. However, the results will be less accurate if the major factor effecting peak dispersion is not the resistance-to-mass transfer effect. The analytical column should be operated at as high a linear mobile phase velocity as possible and a calibration procedure carried out employing appropriate reference standards in the manner described previously. It should be emphasized, however, that the same phase system should be used and any solute should be eluted discretely and be completely separated from any other compounds before its width can be used for molecular weight assessment. The possibility of employing the same procedure to assess the molecular weight of a solute separated on a reverse phase column has also been briefly examined. Preliminary results indicate that a similar relationship between peak width and molecular weight of the eluted solute would be established and the same procedure applied. The measurement of the molecular weight of a substance separated on a reversed-phase column will be the subject of a future publication.

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