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Source of band broadening in liquid chromatographic–fast atom bombardment mass spectrometric systems with precolumn addition of viscous matrix to the mobile phase

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ABSTRACT

The factors affecting band broadening in liquid chromatographic–fast atom bombardment mass spectrometric (LC–FAB-MS) systems using precolumn addition of glycerol to the mobile phase were investigated and their relative importance evaluated. The integrated LC–MS system is subject to three sources of band broadening, namely the chromatographic system, the interface and the mass spectrometer. The individual variances associated with these components can be used to estimate the total variance of the system. The factors affecting broadening in the chromatographic system were identified by examination of the Van Deemter plots obtained for several types of compounds at glycerol concentrations ranging from 0 to 20%. The plots reveal that the C term is significantly affected by an increase in glycerol concentration and that the main factor affecting broadening is a change in the diffusion coefficient, D_m. The increase in the variance associated with the dead volume of the chromatographic system, as measured with a non-retained species, indicates that the increase in the viscosity of the mobile phase on addition of glycerol also results, to a lesser extent, in band broadening as a consequence of the change in flow dynamics within the system. Investigation of the factors affecting band broadening in the interface show that the main source of broadening is the wetting of the probe tip, which is far more important than the dead volume introduced by the transfer capillary. The band broadening induced by the mass spectrometer in the LC–FAB-MS system is essentially related to the scanning speed used for the analysis.

INTRODUCTION

The technique of fast atom bombardment mass spectrometry (FAB-MS) has developed considerably since its introduction by Barber *et al.* [1] in 1981. From the initial applications in which an analyte was dissolved in a viscous matrix (glycerol, thioglycerol, diethanolamine, nitrobenzyl alcohol, etc.) and exposed to bombardment by a beam of fast-moving neutral particles, the technique was modified to allow the introduction of aqueous solutions in a continuous-flow mode (CF-FAB) [2] and then to interface liquid chromatography with mass spectrometry (LC–FAB-MS) [3]. Although aqueous solutions can be introduced in the continuous-flow or dynamic mode by passing the mobile phase through a fused-silica capillary that is located in the hollow shaft of the FAB probe [2,4,5], it is still necessary that a viscous matrix be present in the mobile phase for ionization to occur [2,4]. The addition of the viscous

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matrix can be made before (precolumn) the chromatographic separation [3,6–8] or after the separation by use of postcolumn devices [8–11]. Even if the postcolumn addition of the viscous matrix is favored by some groups, in most instances precolumn mixing with the mobile phase is still utilized and mostly involves the use of glycerol.

Some of the effects of precolumn addition have been mentioned occasionally in the literature [3,8,12,13] but only one study has focused on this subject [8]. The addition of a viscous matrix to the mobile phase can significantly alter the chromatographic separation. If the viscous matrix is introduced into the mobile phase before chromatography occurs, its presence can change the conditions within the column and affect the separation. If mixing of the viscous matrix occurs after the chromatography, dead-volume effects can be introduced into the system, leading to peak broadening, especially if capillary columns are used [8]. In order to identify and quantify the effects on the chromatographic performance of the precolumn addition of a viscous matrix, a systematic study was recently undertaken [14] in which the chromatographic behavior of six compounds in three chemical classes was examined as a function of increasing glycerol content (0–30%) in the mobile phase.

The results obtained [14] demonstrated that the retention times and capacity factors of the analytes decreased for all compounds as the glycerol content in the mobile phase increased. It was also observed that the number of theoretical plates decreased in the system and that the normalized peak widths increased for all compounds when the concentration of glycerol was increased from 5 to 30%. However, at lower glycerol contents in the mobile phase ($< 5\%$) the effects on compounds were different, depending on their k' values. Analytes with smaller capacity factors showed a net decrease in the number of theoretical plates and an increase in the normalized peak widths, whereas for compounds with higher capacity factors such as peptides these two chromatographic indicators were almost invariant. Further, the data obtained in that study showed that an increase in the glycerol content of the mobile phase caused a net decrease in resolution and an increase in the separation impedance of the system. Hence, it was concluded that the overall effect of the precolumn addition of a high concentration of viscous matrix to the mobile phase is detrimental to the performance of the chromatographic system. The factors most likely responsible for this are changes in the distribution of the analyte due to modifications in the chemical composition of the mobile phase and band broadening induced by the increase in the viscosity of the mobile phase.

The objective of this work was, first, to identify the factors responsible for the chromatographic band broadening observed on precolumn addition of a viscous matrix to the mobile phase and to quantify their relative contributions, and second, to evaluate the broadening caused by other components of the LC–FAB-MS system such as the interface. The identification and quantification of the relative importance of the chromatographic and mass spectrometric factors governing the overall separation performance of these systems should facilitate their optimization and design.

EXPERIMENTAL

Instrumentation

The liquid chromatographic system consisted of a Perkin-Elmer Model 410

pump connected to a Rheodyne Model 7125 injector with a 6-μl sample loop. Detection was achieved by a Perkin-Elmer LC-90 variable-wavelength (254 or 280 nm) detector. The chromatographic columns [Spherisorb ODS-2, d_p = 5 μm, 125 mm × 4.6 mm I.D. (CSC, Montreal); Perisorb RP-18, d_p = 40 μm, used as a precolumn] used were maintained at 25°C by a water-jacket regulated by a Haake (Berlin-Steglitz, Germany) circulator. Experiments involving continuous-flow FAB (CF-FAB) were performed on a Kratos MS-50TCTA mass spectrometer equipped with a standard Kratos FAB source and using a laboratory-built continuous-flow probe which has been described previously [4,5]. The scanning conditions of the instrument were dependent on the experiment being conducted. Viscosity measurements on all mobile phases used were performed using a capillary viscosimeter.

Chemicals

The peptides met-enkephalin and bradykinin were obtained from Sigma (St. Louis, MO, USA). Substituted phenolic compounds, such as phloroglucinol and *p*-hydroxybenzoic acid, and organic acids, such as 3,5-dihydroxybenzoic, vanillic and trifluoroacetic acid, were purchased from Aldrich (Milwaukee, WI, USA). Glass-distilled glycerol (>99.0%) was obtained from BDH (Toronto, Canada). All compounds were used without further purification and the mobile phases were prepared using high-performance liquid chromatographic-grade acetonitrile, acetic acid and distilled, deionized water obtained with a Milli-Q purification system (Millipore, Bedford, MA, USA).

Preparation of mobile phases

The eluents were carefully prepared by mixing volumes of distilled, deionized water and appropriate organic modifiers. The mobile phases used for peptide analysis contained fixed proportions of trifluoroacetic acid (TFA) (0.1) and acetonitrile (ACN) (30) and the proportion of water was adjusted to complement the volume of glycerol (GLY) in the solution (ACN–H_2O–GLY–TFA = 30:70 − *x*:*x*:0.1). A similar procedure was utilized for the mobile phases involved in the analysis of low-molecular-weight phenolic compounds and organic acids. The ratio of acetic acid (AcOH) to acetonitrile was fixed at 1:10 and water was used to complement the volume of glycerol in the solution (ACN–H_2O–GLY–AcOH = 10:90 − *x*:*x*:1). Sufficient amounts of each mixture were prepared to ensure that all experiments would be conducted with the same mobile phases. In all instances, the solvents were filtered (0.45-μm filter) and degassed prior to use.

Chromatographic measurements

All chromatographic experiments were carried out at 25°C after the chromatographic system had equilibrated for at least 90 min. Precise values for the volumetric flow-rate were measured for each experiment. The retention of sodium nitrate was taken as the dead volume and the average linear velocity was calculated using the length of the chromatographic column. The number of theoretical plates (N) was estimated from the widths at half-height of the peaks. The Van Deemter plots were generated by measuring the theoretical plate height (H) with linear velocities over the range 0.02–7 mm/s.

RESULTS AND DISCUSSION

In a multi-component system such as LC–FAB-MS, the introduction of an analyte corresponds to the introduction of a signal which is submitted to several operators as it travels through the system. If the operators in the system induce broadening of the signal and the output signal has a Gaussian distribution, it can be assumed that each component will independently contribute to broadening [15]. Therefore, the total broadening of the signal can be estimated by the total variance, σ_t^2, which is given by the sum of the individual variances, σ_i^2, [15–19] as shown in Fig. 1. Thus, the total variance (σ_t^2) of the LC–FAB-MS system is represented by the equation

$$\sigma_t^2 = \sigma_{chr}^2 + \sigma_{int}^2 + \sigma_{spec}^2 \quad (1)$$

where σ_{chr}^2, σ_{int}^2 and σ_{spec}^2 refer to the variances associated with the chromatographic system, the interface and the mass spectrometer, respectively. Each of the three components of the system can be considered as a dispersion–dilution operator that will contribute to the broadening of the chromatographic band. Based on this assumption, one can attempt to identify the factors in each of the sub-systems that contribute to band broadening and evaluate their relative importance.

The total variance in a chromatographic system, σ_{chr}^2, can be expressed as the sum of the variances σ_c^2 and σ_{ex}^2:

$$\sigma_{chr}^2 = \sigma_c^2 + \sigma_{ex}^2 \quad (2)$$

which represent the broadening occurring in the column and the broadening induced by components external to the column. The external contributions usually affect the broadening of the chromatographic band by the introduction of dead-volume effects. In systems utilizing conventional LC columns (150 mm × 4.6 mm I.D., d_p = 5 μm), the extra-column dispersion of the analyte due to dead volumes in connectors and detectors is usually of the order of 30–60 μl [17,20], which is small compared with the elution volume of the analyte.

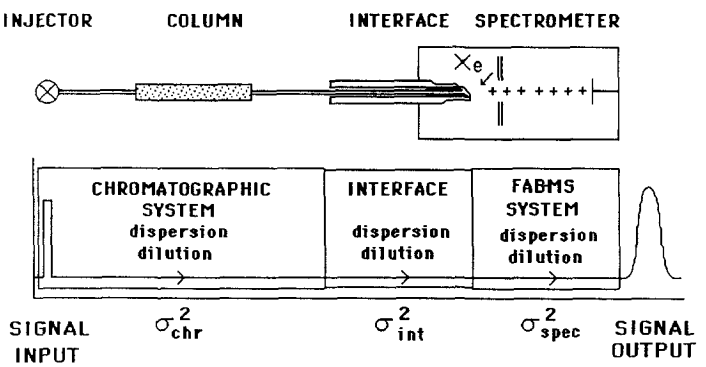

Fig. 1. Dispersion operators and associated variances, σ_{chr}^2, σ_{int}^2 and σ_{spec}^2, in an LC–FAB-MS system using precolumn addition of the viscous matrix.

The broadening that is induced within the chromatographic column is essentially due to the three kinetic effects, which are expressed as terms in the Van Deemter equation [21–24] that relates the height of a theoretical plate (H) to the average linear velocity of the mobile phase ($\bar{u}$). For the chromatographic system under analysis, the equation can be expressed [24] as

$$H = 2\lambda d_p + \frac{2\gamma D_m}{\bar{u}} + \frac{f_1(k')d_p^2\bar{u}}{D_m} + \frac{f_2(k')d_f^2\bar{u}}{D_s} \tag{3}$$

where λ and γ are constants, d_p and d_f represent the particle size and film thickness, D_m and D_s are the diffusion coefficients in the mobile and stationary phases and $f_1(k')$ and $f_2(k')$ are functions of the capacity factor k'. As the film thickness is usually considered to be small [23,25], the last term in eqn. 3 can be neglected, which leads to

$$H = 2\lambda d_p + \frac{2\gamma D_m}{\bar{u}} + \frac{(1 + 6\,k' + 11\,k'^2)d_p^2\bar{u}}{24(1 + k')^2 D_m} \tag{4}$$

where the last term on the right hand side of the equation is referred to as the C term. As the addition of a viscous matrix has been shown [14] to affect k' and D_m, it is interesting to determine the relative contributions of these two factors which appear in the C term of eqn. 4. Because the chromatographic variance σ^2_{chr} is related to H:

$$\sigma^2_{chr} = \frac{H\,t_r^2}{L} \tag{5}$$

where L is the length of the column, it is possible to identify the factors responsible for the decrease in efficiency that occurs in the chromatographic system when the concentration of a viscous matrix such as glycerol is increased.

A series of experiments were performed in order to evaluate the contribution of the kinetic factors to the band broadening which is observed when a viscous matrix is added to the mobile phase. In these experiments, Van Deemter plots were obtained for six compounds in three separate chemical classes as the concentration of glycerol in the mobile phase was increased from 0 to 30%. Fig. 2 shows typical plots obtained for the peptide met-enkephalin. The data in Fig. 2 indicate that as the concentration of glycerol is increased the C term in the Van Deemter equation steadily increases, as shown by the increase in the slopes with the glycerol content. A similar effect was observed for the peptide bradykinin. The same type of experiments were also conducted with several other analytes of lower molecular weight such as vanillic acid, 3,5-dihydroxybenzoic acid, *p*-hydroxybenzoic acid and phloroglucinol. Typical results obtained in those experiments are given in Fig. 3, where the Van Deemter plots at increasing glycerol concentration are shown for vanillic acid. As can be seen, there appears to be no significant differences in the C term for glycerol values below 3% but, as the values increase above 5%, the C term increases steadily as witnessed by an increase in the slopes of the plots. This trend is also observed with the other low-

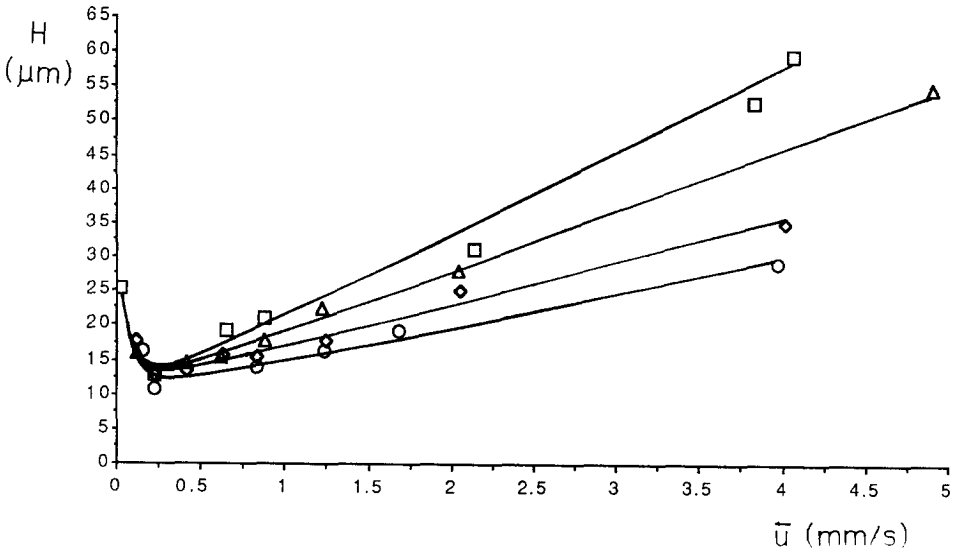

Fig. 2. Van Deemter plots obtained for met-enkephalin with increasing glycerol concentration (x)in the mobile phase: ○ = 0%; ◇ = 5%; △ = 10%; □ = 20%. Mobile phase: ACN–H_2O–GLY–TFA (30:70 − x:x:0.1).

molecular-weight compounds studied. Hence, the Van Deemter plots obtained indicate that there is a net decrease in system efficiency with an increase in the viscous matrix content in the mobile phase. As the C term of eqn. 4, which is affected by the change in glycerol concentration, depends on k' and D_m, it is possible to evaluate the relative importance of both of these factors.

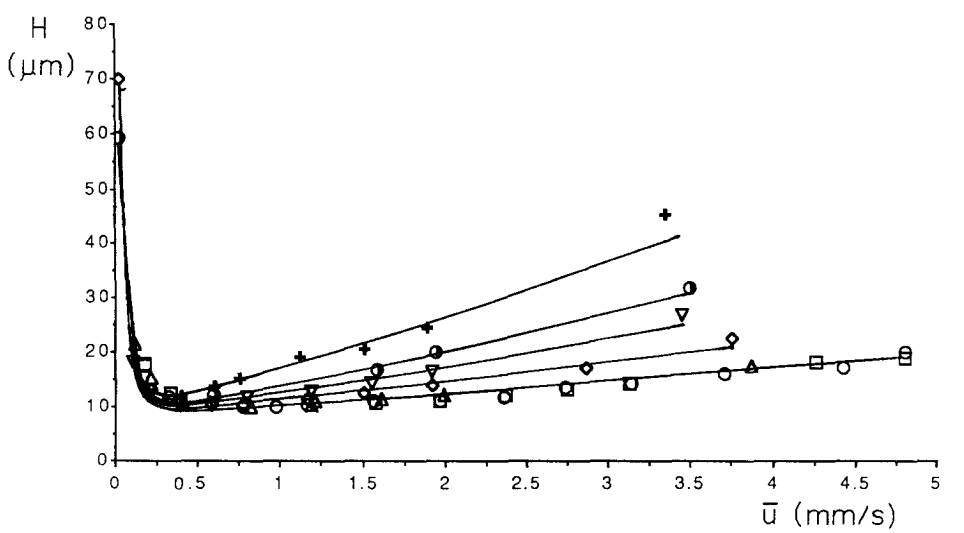

Fig. 3. Van Deemter plots obtained for vanillic acid with increasing glycerol concentration (x) in the mobile phase: ○ = 0%; □ = 1%; △ = 3%; ◇ = 5%; ▽ = 10%; ◑ = 20%; + = 30%. Mobile phase: ACN–H_2O–GLY–AcOH (10:90 − x:x:1).

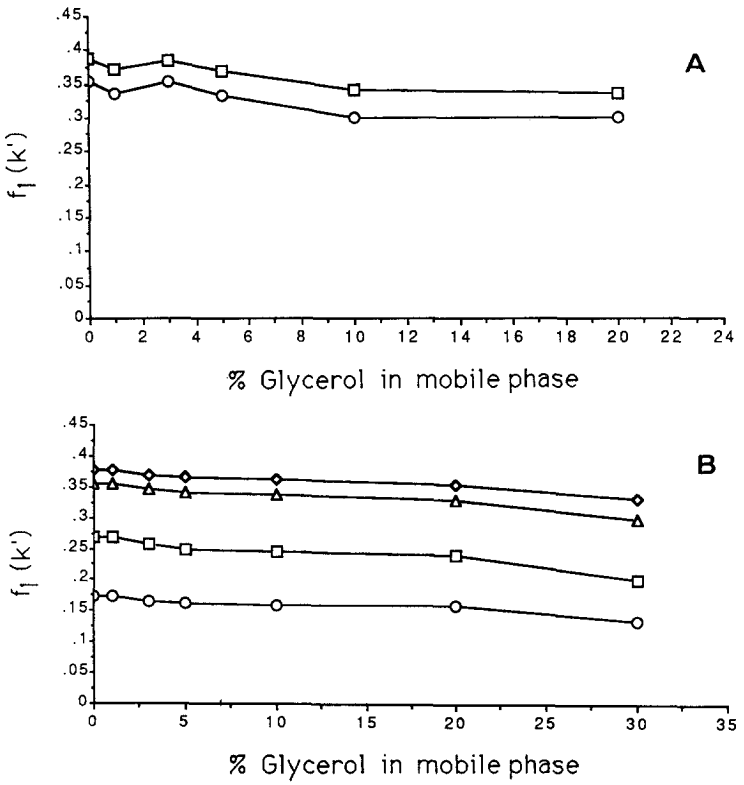

Fig. 4. Variation of $f_1(k')$ with glycerol content in the mobile phase. (A) □ = Bradykinin; ○ = met-enkephalin; (B) □ = *p*-hydroxybenzoic acid; △ = 3,5-dihydrobenzoic acid; ◇ = vanillic acid; ○ = phloroglucinol.

Results obtained in a previous study [14] concerning the effect on chromatographic behavior of the addition of a viscous matrix to the mobile phase have demonstrated that the capacity factors decrease when the content of glycerol is increased. This behavior is shown in Fig. 4A for the peptides met-enkephalin and bradykinin and in Fig. 4B for vanillic acid, 3,5-dihydrobenzoic acid, *p*-hydroxybenzoic acid and phloroglucinol, where the $f_1(k')$ functions, appearing in eqn. 4, are plotted against glycerol content in the mobile phase. The data can be rationalized by the fact that as the glycerol content increases the distribution of the analytes is changed. Glycerol is acting as an efficient organic modifier, the effect of which is to reduce the capacity factors. The isolated effect of the variation of the capacity factors should be to decrease the slopes of the Van Deemter plots, as the reduction in capacity factors induces a reduction in the $f(k')$ function as shown in Fig. 4.

The observed effect of glycerol content on the chromatographic system is not, however, what is predicted from the changes in the capacity factors. As the slopes of the Van Deemter plots increase with increasing concentrations of glycerol (Figs. 2 and 3), it appears that variations in the other factor involved, the diffusion coefficient (D_m), are significant. The data available for *p*-hydroxybenzoic acid allow an estimation of the variation in the diffusion coefficient using the Wilke–Chang equation [26],

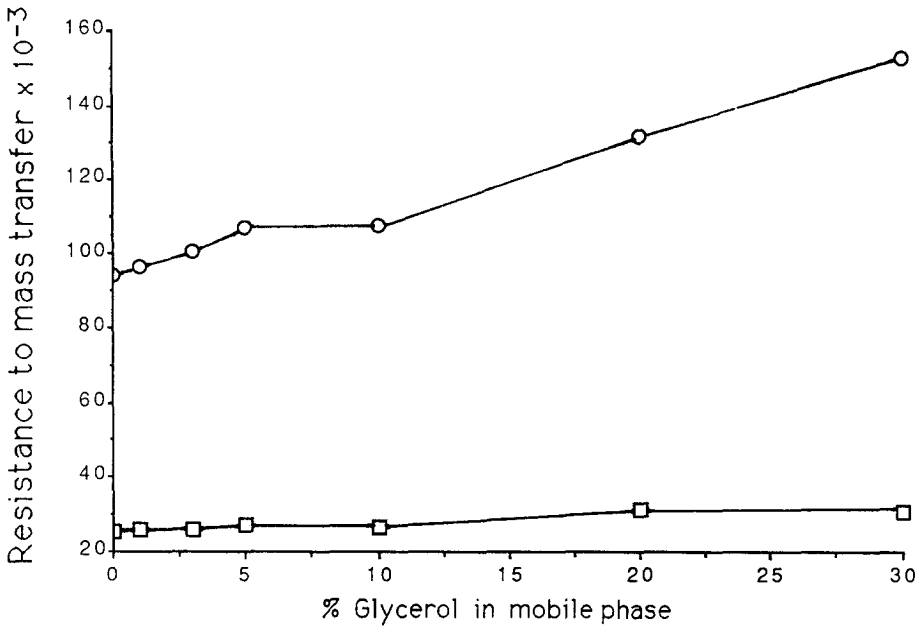

Fig. 5. Variation of the estimated diffusion coefficient, (○) $1/D_m$ and (□) $f_1(k')/D_m$, for *p*-hydroxybenzoic acid with glycerol concentration in the mobile phase. Units for *y*-axis are s.

and assuming that glycerol has an association constant comparable to that of methanol. Fig. 5 shows the variation that is expected in $1/D_m$ along with that of the C term in the Van Deemter plots. As observed, the variation of the C term is smaller than that of D_m owing to the opposite effects of $f(k')$ and D_m on the slope. However, the data obtained experimentally and by estimation of D_m clearly indicate that the variations observed experimentally are mainly due to variations in D_m which are caused by an increase in the viscosity of the mobile phase as the glycerol content increases. The variation of the viscosity of the mobile phase with the increase in the glycerol concentration was measured and the results are given in Table I. The data indicate

TABLE I

VARIATION OF THE VISCOSITY OF THE MOBILE PHASE WITH GLYCEROL CONTENT

Viscosity in cP.

Glycerol (%) (x)	ACN–H_2O–GLY–TFA (30:70 − x:x:0.1)	ACN–H_2O–GLY–AcOH (10:90 − x:x:1)
0	0.95	0.93
1	0.96	0.96
3	1.01	1.03
5	1.05	1.12
10	1.26	1.29
20	1.46	1.56
30	–	1.92

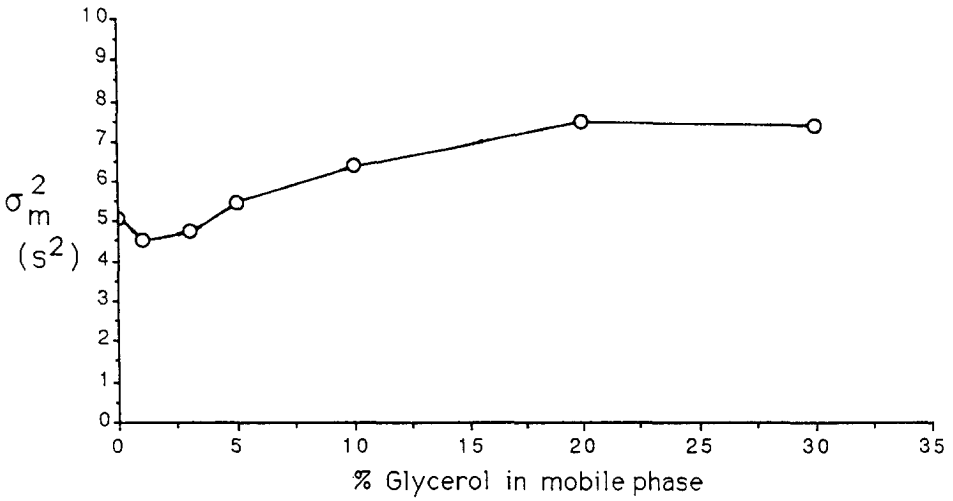

Fig. 6. Variation of the chromatographic variance, σ_m^2, associated with t_m (retention time of $NaNO_3$), with the concentration of glycerol in the mobile phase.

that the viscosity increases with the addition of glycerol and, therefore, D_m is reduced. For example, D_m as estimated for *p*-hydroxybenzoic acid decreases by 40% when the glycerol content increases from 0 to 30%. Therefore, the factor that is mainly responsible for the decrease in chromatographic efficiency of the system is the change in D_m which results from the increase in viscosity of the mobile phase. This increase in viscosity also produces an increase in the operating pressure of the system, therefore causing the separation impedance to be higher [14].

The findings that the addition of a viscous matrix such as glycerol affects the diffusion in the chromatographic system are also reflected in the variance σ_m^2 associated with a non-retained analyte. The variation of σ_m^2, which is associated with the increase in the viscous matrix content of the mobile phase, is shown in Fig. 6, which indicates that σ_m^2 is almost constant for glycerol contents below 3%, where changes in viscosity are small, and then increases with increasing concentration of glycerol. This demonstrates that convective diffusion processes become more important in the system and are responsible for a partial loss of chromatographic efficiency. The increase in the viscosity of the mobile phase modifies the flow dynamics in the system in accordance with eqn. 6.

$$\sigma_{tu}^2 = \frac{\pi r^4 LF}{24\ D_m} \tag{6}$$

The equation shows that the variance σ_{tu}^2 of the system varies inversely with D_m and this is reflected by the increase in σ_m^2 for a non-retained compound such as sodium nitrate. Hence, it would appear that the most important factor that influences the chromatographic broadening (σ_{chr}^2) in LC–FAB-MS systems using precolumn addition of glycerol are the changes in viscosity as shown by the increase in σ_m^2 and in the kinetics of mass transfer, as shown by the Van Deemter plots, that are induced by the increase in the viscosity of the mobile phase with glycerol content. The most

important contribution to broadening comes from the changes that occur in the mass transfer kinetics (σ_c^2) while broadening due to changes in flow dynamics (σ_{ex}^2) occurs but to a lesser extent.

In LC–FAB-MS systems, other components such as the interface and the mass spectrometer are also likely to act as dispersion operators. The interface that is used to couple the chromatographic system to the mass spectrometer consists essentially of a fused-silica capillary that is introduced in the hollow shaft of the CF-FAB probe between the column and the ion source. The capillary allows the transfer of the analytes to the heated surface of the FAB probe where fast atom bombardment occurs [2,4,5]. The dispersion operators in the interface will be the transfer capillary (fused silica, 1000 mm × 0.075 mm I.D.) and the liquid droplet which forms at the end of the probe tip (wetting). The total variance, σ_{int}^2, associated with the interface will be the sum of the variance of the transfer tube, σ_{tu}^2, and the variance σ_d^2 associated with the droplet formed on the probe tip, σ_d^2, as expressed by

$$\sigma_{int}^2 = \sigma_{tu}^2 + \sigma_d^2 \tag{7}$$

The contribution of the transfer capillary can be estimated using the Taylor–Golay relationship [16,22,27] when the linear velocity is sufficiently high. It can be seen from eqn. 6 that the variance σ_{tu}^2 is a function of the radius of the transfer tube (r), its length (L), the flow-rate (F) and the diffusion coefficient (D_m). In LC–FAB-MS systems, the volumetric dispersion caused by the transfer capillary is given in Table II, where σ_{tu} is shown as a function of capillary length, diameter and glycerol content for *p*-hydroxybenzoic acid. It is observed from Table II that a typical capillary of 75 μm, in the absence of glycerol, has a volumetric dispersion of 0.11 μl, and that this value can be reduced to 0.01 μl if the diameter of the tube is reduced to 25 μm. At 30% glycerol, the dispersion is only slightly increased to 0.14 and 0.02 for the same radii. Hence, the dispersion produced by the transfer capillary appears to be relatively small and its contribution should not significantly affect the total variance of the system unless the diameter of the transfer line is greater than 100 μm.

The other volumetric dispersion which occurs at the end of the probe and is referred to as a "memory effect" [8,20] is relatively difficult to evaluate as there are many phenomena occurring in the droplet that forms at the end of the probe tip (evaporation, diffusion, sputtering, mixing, etc.). One approach to estimate the dis-

TABLE II

DISPERSION CAUSED BY A CAPILLARY TRANSFER TUBE

Length × diameter (mm × μm)	Volume (μl)	σ_{tu} (0% glycerol) (μl)	σ_{tu} (30% glycerol) (μl)
1000 × 75	4.42	0.11	0.14
2250 × 50	4.42	0.08	0.09
1000 × 50	1.96	0.05	0.06
1000 × 25	0.49	0.01	0.02

TABLE III

EVALUATION OF VOLUMETRIC DISPERSIONS AT THE PROBE TIP

Dimensions		Volume (μl)	Dispersion (laminar)[a] (μl)	Dispersion (laminar)[b] (μl)	Dispersion (mixing) (μl)
Diameter (mm)	Thickness (mm)				
2	0.20	0.63	1.22	1.47	0.63
2	0.15	0.47	1.06	1.27	0.47
2	0.10	0.31	0.86	1.04	0.31
1	0.20	0.16	0.28	0.36	0.16
1	0.15	0.12	0.24	0.31	0.12
1	0.10	0.08	0.20	0.25	0.08

[a] Calculated at 0% glycerol.
[b] Calculated at 30% glycerol.

persion of the system is to concentrate on the droplet itself and consider it the major source of broadening. From this assumption, the droplet can be considered as a "connecting tube" and the variance associated with it can be obtained from the Taylor–Golay relationship [16,22,27]. Alternatively, the droplet can be considered as a mixing chamber, in which case the variance associated with it can be obtained from the equation [15,16,28,29]

$$\sigma_d^2 = \frac{V_d^2}{F^2} \tag{8}$$

If it is assumed that the composition of the liquid phase is constant within the droplet, the variance can be estimated. The values for the variance σ_d^2 that can be obtained using each of the models described are given in Table III.

The data in Table III show that the dispersions evaluated using the two approaches are within a factor of two of one another and that the laminar contribution is superior to the mixing effect. Further, it can be observed that the effect of the addition of glycerol increases the value of the laminar contribution. These two contributions allow the estimation of the magnitude of the dispersion caused by this operator. Hence, the dispersion created by the droplet at the tip of the FAB probe is between 0.63 and 1.47 μl when the concentration of glycerol is *ca.* 30%. These values appear as minimum values as other broadening phenomena are occurring at the tip. The differential vaporization of the components creates an enrichment of the less volatile components at the tip which produces an increase in the viscosity of the droplet [30]. This increase in viscosity reduces the diffusivity of the analyte, which results in a higher variance in the system, in agreement with eqn. 6. Therefore, a dispersion of the order of 1.47 μl appears probable under those circumstances.

The data in Tables II and III indicate that the contribution of the interface to the total broadening of the chromatographic band is mainly due to the formation of the liquid droplet at the end of the probe. This contribution is of the order of 1.5 μl for experimental conditions usually encountered in CF-FAB. Our experience with such

systems consisting of a 0.5-μl injector, a transfer capillary of 1000 mm × 75 mm I.D. and a tip 2 mm in diameter show that the elution volume under those experimental conditions is between 2 and 3 μl [4,5], depending on the amount of analyte injected on the content of glycerol and on the scanning speed of the mass spectrometer. These values are essentially the same as those reported by Caprioli *et al.* [2] using a similar system. The differences between the experimental and estimated values can be attributed to errors originating from the estimation of the thickness of the droplet or the diffusivity of the analyte. Alternatively, a small contribution of the mass spectrometer originating from the slow scanning speed used in these experiments can be considered. Thus, the measured dispersion implies that the interface can contribute significantly to the broadening of the chromatographic band, especially for narrow bands such as those found with capillary columns. These effects will be less important for conventional columns as the elution volume after splitting of the eluent can easily be of the order of 7–10 μl, which is much greater than extra-column contributions. With conventional columns, the broadening produced by the interface is comparable to that created by the decrease in efficiency observed with 30% of glycerol present in the mobile phase.

Finally, the signal can also be broadened by the mass spectrometer, σ^2_{spec}, which serves as the detector in the LC–FAB-MS system. The broadening that will be induced by the mass spectrometer is essentially related to the scanning speed of the instrument, which must be sufficiently rapid to reduce broadening of the signal. For compounds that elute in narrow bands, the cycle time should be of the order of 0.5–1 s [31,32] to maintain chromatographic integrity. This is well below the scan speeds usually used in LC–FAB-MS systems, which are of the order of 3–5 s and above.

CONCLUSIONS

The results presented clearly indicate that the precolumn addition of significant amounts of glycerol in LC–FAB-MS systems induces changes in the viscosity of the mobile phase and affects the distribution of the analytes between phases. Examination of the Van Deemter plots obtained for several analytes with different chemical structures shows that the C term in these plots increases with increasing glycerol content in the mobile phase. This increase is due to changes in the diffusivity of the analytes induced by an increase in the viscosity of the mobile phase. The reduction of the capacity factors with increasing glycerol content affects the system but to a much lesser extent. Hence, the source of chromatographic broadening in these systems can be attributed mainly to changes in diffusive processes and has been shown to increase the peak width by 30%. Other contributions, such as extra-column broadening, produce minor effects and can be neglected if dead volumes in the system are reduced.

The variances associated with other dispersion operators, such as the interface and the mass spectrometer, present in the LC–FAB-MS system have also been evaluated. The measured volumetric dispersion of 2.5 μl caused by the interface can be attributed mainly to effects occurring at the tip of the FAB probe, as the variance associated with the dead volume of the transfer capillary is found to be small. The dispersion at the tip of the probe will vary with glycerol content in the mobile phase, the thickness of the droplet on the tip, the temperature of the tip and the flow-rate in the system. The dispersion caused by the mass spectrometer is essentially related to

the scanning speed, which has to be sufficiently rapid to avoid broadening of the signal.

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REFERENCES

1 M. Barber, R. Bordolini, R. D. Sedgwick and A. N. J. Tyler, *J. Chem. Soc., Chem. Commun.*, (1981) 325.
2 R. M. Caprioli, T. Fan and J. S. Cottrell, *Anal. Chem.*, 58 (1986) 2949.
3 Y. Ito, T. Takeuchi, D. Ishii and M. Goto, *J. Chromatogr.*, 346 (1985) 161.
4 M. J. Bertrand, V. Benham, R. St-Louis and M. J. Evans, *Can. J. Chem.*, 67 (1989) 910.
5 M. J. Bertrand and V. Benham, in T. Theophanides (Editor), *Spectroscopy of inorganic Bioactivators, Theory and Applications*, Nato ASI Series, Kluwer, Dordrecht, 1989, pp. 349–377.
6 T. Takeuchi, S. Watanabe, N. Kondo, D. Ishii and M. Goto, *J. Chromatogr.*, 435 (1988) 482.
7 A. E. Ashcroft, *Org. Mass Spectrom.*, 22 (1987) 304.
8 S. Pleasance, P. Thibault, M. A. Moseley, L. J. Deterding, K. B. Tomer and J. W. Jorgenson, *J. Am. Soc. Mass Spectrom.*, 1 (1990) 312.
9 D. E. Games, S. Pleasance, E. D. Ramsey and M. A. Mc Dowall, *Biomed. Environ. Mass Spectrom.*, 15 (1988) 179.
10 M. A. Moseley, L. J. Deterding, J. S. M. de Wit, K. B. Tomer, R. T. Kennedy, N. Bragg and J. W. Jorgenson, *Anal. Chem.*, 61 (1989) 1577.
11 D. J. Bell, M. D. Brightwell, W. A. Neville and A. West, *Rapid Commun. Mass Spectrom.*, 4 (1990) 88.
12 M. A. Moseley, L. J. Deterding, J. S. M. de Wit and K. B. Tomer, R. T. Kennedy, N. Bragg and J. W. Jorgenson, *Anal. Chem.*, 61 (1989) 1577.
13 J. S. M. de Wit, L. J. Deterding, M. A. Moseley, K. B. Tomer and J. W. Jorgenson, *Rapid Commun. Mass Spectrom.*, 2 (1988) 100.
14 J.-P. Gagne, A. Carrier and M. J. Bertrand, *J. Chromatogr.*, 554 (1991) 61.
15 J. C. Sternberg, *Adv. Chromatogr.*, 2 (1966) 205.
16 M. Martin, C. Eon and G. Guiochon, *J. Chromatogr.*, 108 (1975) 229.
17 J. L. DiCesare, M. W. Dong and L. S. Ettre, *Introduction to High-Speed Liquid Chromatography*, Perkin-Elmer, Norwalk, CT, 1981.
18 J. J. Kirkland, W. W. Yau, H. J. Stoklosa and C. H. Dilks Jr., *J. Chromatogr. Sci.*, 15 (1977) 303.
19 H. H. Lauer and G. P. Rozing, *Chromatographia*, 14 (1981) 641.
20 D. Ishii, K. Asai, K. Hibi, T. Jonokuchi and M. Nagaya, *J. Chromatogr.*, 144 (1977) 157.
21 J. J. van Deemter, F. J. Zuiderweg and A. Klinkenberg, *Chem. Eng. Sci.*, 5 (1956) 271.
22 M. J. E. Golay, in D. H. Desty (Editor), *Gas Chromatography*, Butterworths, London, 1958, p. 36.
23 J. A. Jonsson, in J. A. Jonsson (Editor), *Chromatographic Theory and Basic Principles*, Marcel Dekker, New York, 1987, p. 27.
24 E. D. Katz, K. L. Ogan and R. P. W. Scott, *J. Chromatogr.*, 270 (1983) 51.
25 C. Gluckman, A. Hirose, V. L. McGffin and M. Novotny, *Chromatographia*, 17 (1983) 303.
26 C. R. Wilke and P. Chang, *AIChE J.*, 1 (1955) 264.
27 G. Taylor, *Proc. R. Soc. London, Ser. A*, 255 (1956) 67.
28 G. Guiochon and H. Colin, in P. Kucera (Editor), *Microcolumn High-Performance Liquid Chromatography*, Elsevier, Amsterdam, 1984, p. 1.
29 K.-P. Hupe, R. J. Jonker and G. Rozing, *J. Chromatogr.*, 285 (1984) 253.
30 M. J. Connoly and R. G. Orth, *Anal. Chem.*, 59 (1987) 903.
31 G. Guiochon and P. J. Arpino, *J. Chromatogr.*, 271 (1983) 13.
32 B. L. Karger and P. Vouros, *J. Chromatogr.*, 323 (1985) 13.