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Limits and effects of precolumn addition of thioglycerol in liquid chromatographic–fast atom bombardment mass spectrometric systems☆

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ABSTRACT

A series of experiments were conducted in order to determine the effect of the precolumn addition of a viscous matrix such as thioglycerol on the chromatographic performance of liquid chromatographic–fast atom bombardment mass spectrometric (LC–FAB-MS) systems. In those experiments, the concentration of thioglycerol in a mobile phase consisting of acetonitrile–water–trifluoroacetic acid was varied and important chromatographic parameters such as retention times, capacity factors, number of theoretical plates, peak widths, resolution and impedance of separation were monitored for analytes such as met-enkephalin, leu-enkephalin and *p*-hydroxybenzoic acid. The results obtained indicate that for concentrations of thioglycerol in the mobile phase below 3% most chromatographic indicators are only slightly affected. However, for concentrations of the viscous matrix above that value the capacity factors are significantly decreased, indicating that thioglycerol is behaving as an efficient organic moderator, and peak broadening becomes important, having a detrimental effect on the performance of the system. Van Deemter plots obtained for the analytes at concentrations of thioglycerol in the mobile phase of 0–15% reveal that the major effect of thioglycerol is to reduce the mass transfer efficiency in the chromatographic system at high linear velocities and concentrations of thioglycerol above 3%. Comparison of the effects of viscous matrices such as thioglycerol and glycerol on the chromatographic performance of LC–FAB-MS systems indicates that the chromatographic efficiency is almost independent of the matrix concentration when the systems are operated near their optimum linear velocities and that high matrix contents and high linear velocities can be used with little decrease in efficiency if the systems are operated at higher temperatures.

INTRODUCTION

The technique of fast atom bombardment mass spectrometry (FAB-MS) is now confirmed as a powerful tool in the mass spectral analysis of polar, thermally labile or involatile compounds. In its application [1], the analyte was dissolved in a viscous matrix such as glycerol, which was subsequently introduced into the ion source of the mass spectrometer and bombarded with a beam of high-energy (5–8 keV), fast-moving neutral species (FAB). High-energy Cs^+ ions can also be used as in liquid secondary ion mass spectrometry (LSIMS) and yield similar results, although some differences exist because the ion beam is generally more focused than the neutral beam and usually has a higher density of particles. The liquid matrix used in these desorption–ionization techniques provides a means by which the molecules on the surface can be replenished by molecular diffusion in the liquid, thus allowing for the presence of fresh material at the surface and partial elimination of secondary products issuing from the radiation damage caused to the solution. The role of the liquid matrix, however, is not simply to dissolve the analyte but it is also involved

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intimately in the ionization process. The mass spectra obtained in FAB-LSIMS are affected by the nature of the matrix [2,3] and strong matrix–analyte interactions exist in the solution [2,4–7].

FAB-MS has in recent years extended the scope of its application primarily as a result of the introduction of dynamic FAB or continuous-flow FAB (CF-FAB) [8,9]. The latter technique allows the continuous introduction of aqueous solutions into the mass spectrometer by delivering the mobile phase through a fused-silica capillary located in the hollow shaft of the FAB probe [10,11]. The advent of CF-FAB has not only increased the power of the technique but it has also allowed its interfacing with liquid chromatography (LC–FAB-MS) [12–25]. Severals approaches have been used in order to couple LC to CF-FAB systems. The most commonly used interface involves the direct coupling of conventional [12,13], microbore [14] or packed capillary columns [15–19, 24–26] with CF-FAB. The columns are connected with the FAB probe tip through a transfer fused-silica capillary (50–75 μm I.D.) and split-flow devices are used to reduce the flow through the interface below typically 10 μl/min when appropriate. In all of these LC–FAB-MS systems, it is necessary to add a viscous matrix to the mobile phase in order to optimize the ionization efficiency. Matrix concentrations ranging from 0 to 25% have been reported [8,12–16,21,22,27,28] and the optimum concentration required for proper ionization is usually a compromise between absolute sensitivity and signal-to-noise ratio. The addition of the viscous matrix can be done before the chromatographic separation (precolumn) [14,16,18,19,24,25] or after the separation (postcolumn) [12,16,17].

Although postcolumn addition has been suggested, in most instances precolumn addition is easier to achieve and offers advantages in conventional systems. It should be noted, however, that the precolumn addition of the matrix will significantly alter the polarity and viscosity of the mobile phase and therefore will affect the chromatographic process.

Although many studies have focused on the optimization of FAB probes and CF-FAB systems [8,9,26,27], few have dealt with the optimization of LC–FAB-MS systems [16,17,27–32]. Occasionally, some of the effects of precolumn addition on the chromatographic performance have been mentioned [8,16,17], but until recently not many systematic studies were focused on the optimization of the chromatographic systems [16,33,34]. Previous work in this laboratory [33,34] has shown that the precolumn addition of a viscous matrix such as glycerol over a wide range of concentrations affects most chromatographic indicators (R_s, k', E, t_R, $w_{1/2}$, N). The extent of the changes observed depends on the actual glycerol content in the mobile phase. The overall performance of the chromatographic system was seen to decrease when the concentration of glycerol in the mobile phase exceeded 3%, whereas minor decreases in performance were observed when the glycerol content was maintained below 3% for a wide range of flow rates. Further work conducted on the optimization of LC–FAB-MS systems using analytes from several chemical classes has revealed that the main source of chromatographic band broadening in these systems was related to the increase in the viscosity of the mobile phase and changes in the diffusivity of the analytes with glycerol content [33].

In seeking to broaden the scope of our previous studies and to evaluate the general boundaries within which precolumn addition of a matrix can be used, we undertook this work, which included the characterization of the effect of precolumn addition of various amounts of thioglycerol to a mobile phase consisting of acetonitrile–water–trifluoroacetic acid and the study of the kinetic optimization of the chromatographic process in the presence of such a matrix. The objectives of the study were (i) to quantify the effects of the addition of thioglycerol to the mobile phase over the concentration range 0–15% on most chromatographic indicators, (ii) to identify the source of broadening in LC–FAB-MS systems by kinetic studies and determine the optimum operational conditions to be used in these systems, (iii) to compare the effects of the addition of different matrices in order to establish general guidelines for precolumn addition and (iv) to determine the effect of an increase in the temperature of analysis on the performance of the chromatographic system. The results obtained indicate that general guidelines can be formulated, as was indicated by our earlier findings, and that the deleterious effects of precolumn addition can be compensated under selected conditions.

EXPERIMENTAL

Instrumentation

The LC system consisted of a Perkin-Elmer Model 410 pump connected to a Rheodyne Model 7125 injector with a 6-μl sample loop. Detection (280 nm) was effected with a Perkin-Elmer LC-90 variable-wavelength detector. The chromatographic column used [Spherisorb ODS-2, d_p = 5 μm, 125 mm × 4.6 mm I.D. (CSC, Montreal, Canada)] was maintained at constant temperature by a water jacket regulated by a Haake (Berlin-Steglitz, Germany) circulator. Viscosity measurements were performed with a ball viscosimeter (Haake).

Chemicals

The peptides met-enkephalin and leu-enkephalin were obtained from Sigma (St. Louis, MO, USA) and *p*-hydroxybenzoic acid and Glass-distilled thioglycerol (THIO) (>95%) were purchased from Aldrich (Milwaukee, WI, USA). The solvents used in the preparation of the mobile phases were distilled and deionized water (Milli-Q system; Millipore, Bedford, MA, USA), HPLC-grade acetonitrile (ACN) and trifluoroacetic acid (TFA), obtained from Aldrich. All compounds were used as received.

Preparation of mobile phase

The mobile phases were prepared by mixing appropriate volumes of distilled, deionized water and organic modifiers. The mobile phase contained fixed proportions of trifluoroacetic acid (0.05%) and acetonitrile (30%) and the amount of water added was adjusted to component the volume of thioglycerol added to the solution (ACN–water–THIO/TFA = 30:70 − *x*:*x*:0.05). Sufficient amounts of each mixture were prepared in order to ensure that all experiments would be conducted with the same mobile phase. In all instances, the solvents used were filtered (0.45 μm) and degassed prior to use.

Chromatographic measurements

All chromatographic experiments were conducted at 25 or 38.5°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 indicator and the average linear velocity of the mobile phase was calculated using the length of the chromatographic column. The number of theoretical plates (N) was calculated from the peak width at half-height. The Van Deemter plots were generated by measuring the theoretical plate heigth (H) with linear velocities in the range 0.1–7 mm/s, corresponding to flow-rates of 0.05–3 ml/min in our chromatographic system. Other measurements were made at a constant linear velocity.

RESULTS AND DISCUSSION

It is common practice in LC to add an organic modifier to the mobile phase in order to alter the chromatographic process and the retention characteristics of the analytes. The modifier is usually of low viscosity in order to obtain high chromatographic efficiency and minimize pressure build-up in the system. In LC–FAB-MS systems, however, the matrix added to the mobile phase is used to optimize ionization and is fairly viscous, leading to the use of rather unusual mobile phases in terms of chromatography. Recently, the use of thioglycerol as a matrix in LC–FAB-MS and FAB-MS has been

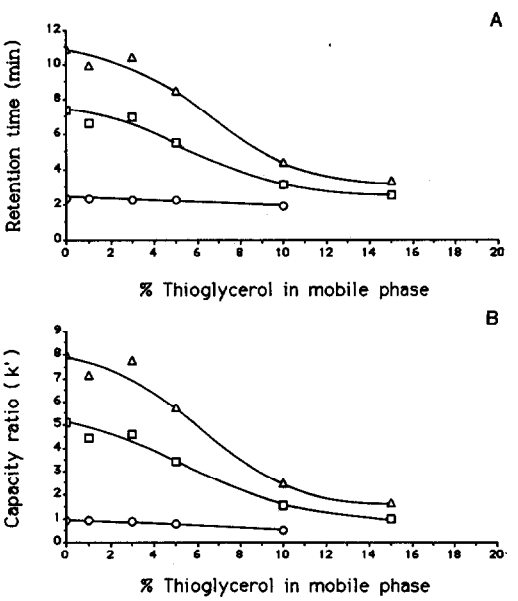

Fig. 1. Effect of concentration of thioglycerol in the mobile phase on (A) retention times and (B) capacity factors (k'), □ = Met-enkephalin; △ = leu-enkephalin; ○ = *p*-hydroxybenzoic acid.

reported to offer advantages over glycerol in the analysis of oligosaccharides, peptides and eicosanoic compounds [15,18,35,36], but its effects on the overall chromatographic performance were not described. The effect of an increasing concentration of this particular matrix in the mobile phase on the chromatographic properties of the analytes can be seen in Fig. 1*a* and *b*, which show the effect of the variation of the thioglycerol content in the mobile phase (0–15%) on the retention times and capacity factors of two peptides, met- and leu-enkephalin, and of a less retained analyte, *p*-hydroxybenzoic acid. As can be observed, higher contents of thioglycerol reduce the capacity factor of met- and leu-enkephalin by *ca.* 80%. This indicates that small increases in thioglycerol in the mobile phase alter the distribution of these compounds significantly. The reduction observed for *p*-hydroxybenzoic acid is *ca.* 40%, which is consistent with the much lower capacity factor of this compound, which is only sligtly retained. Hence, thioglycerol acts as an efficient organic moderator, behaving as other viscous matrices that have been studied [32]. Thioglycerol behaves as a less polar moderator than glycerol, causing greater changes in retention characteristics than glycerol for small changes in concentrations in the mobile phase. As for systems containing glycerol, however, essentially no change is observed when the added concentration of the matrix in the mobile phase is below 3%.

The chromatographic performance of the system was also monitored as the thioglycerol content in the mobile phase was increased. This was done by evaluating the relative variation in the normalized theoretical plate number (N/N_0) with and without thioglycerol present in the mobile phase. As shown in Fig. 2, the efficiency of the chromatographic system as measured by N/N_0 is only slightly affected when the thioglycerol content is below 3%, but above this value a reduction of up to 25% can be observed for the compounds studied. The reduction in efficiency observed for *p*-hydroxybenzoic acid is greater, possibly indicating dead volume effects for this less retained compound. The broadening induced by the addition of thioglycerol to the mobile phase can be evaluated by examining the variation of the normalized peak width ($w_{1/2}/t_R$) with thioglycerol content. The behavior of this chromatographic indicator with increasing thioglycerol content in the mobile phase is shown in Fig. 3, which indicates that peak broadening increases with increasing thioglycerol concentration for all the compounds studied, being greater for *p*-hydroxybenzoic acid, which correlates well with the findings that the decrease in efficiency is greater for the less retained compounds, indicating that instrumental dead volume diffusion is important.

The overall effect of the addition of thioglycerol to the mobile phase can be reflected by examining the behavior of two important chromatographic parameters, the resolution and the impedance of separation [37]. The influence of thioglycerol on the resolution is shown in Fig. 4 for the pair met- and leu-enkephalin. The resolution decreases almost linearly with increase in the thioglycerol content in the mobile phase, demonstrating the deleterious effects of the decrease in efficiency and increase in peak broadening on the separation power of the

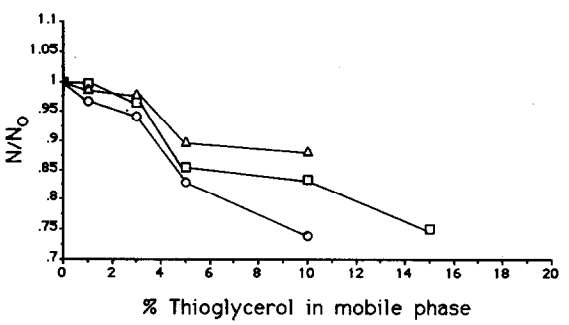

Fig. 2. Effect of concentration of thioglycerol in the mobile phase on the normalized number of theoretical plates, N/N_0. □ = Met-enkephalin; △ = leu-enkephalin; ○ = *p*-hydroxybenzoic acid.

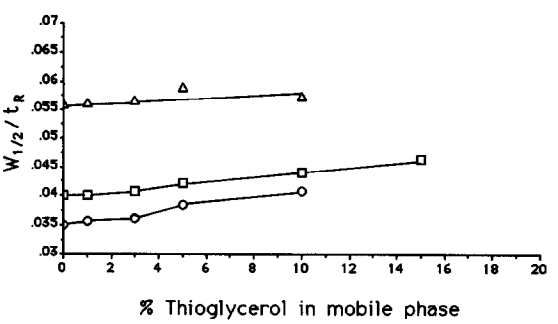

Fig. 3. Effect of concentration of thioglycerol in the mobile phase on the normalized peak width, $w_{1/2}/t_R$. □ = Met-enkephalin; △ = leu-enkephalin; ○ = *p*-hydroxybenzoic acid.

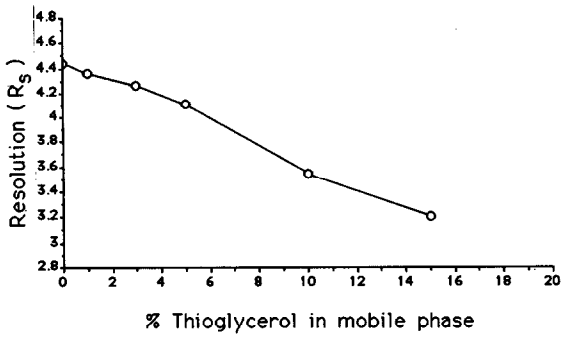

Fig. 4. Effect of concentration of thioglycerol in the mobile phase on the resolution, R_s, for met- and leu-enkephalin.

system. The decrease in performance of the chromatographic system can be measured using the separation impedance (E), which is related to the efficiency and to the physical properties of the system. The variation of the separation impedance (E) normalized to that in the absence of thioglycerol (E_0) can be observed in Fig. 5, which gives the normalized impedance (E/E_0) as a function of thioglycerol content in the mobile phase. The results clearly indicate that this parameter is more than doubled as the concentration varies from 0 to 15%. The sharp increase observed in E/E_0 for thioglycerol contents above 10% is indicative that the chromatographic system is perturbed under these conditions, which results in a dramatic decrease in performance. For thioglycerol contents below 3%, however, the impedance is stable, suggesting that the performance is retained.

In order to access the factors responsible for the decrease in chromatographic performance and obtain qualitative information on the system, Van

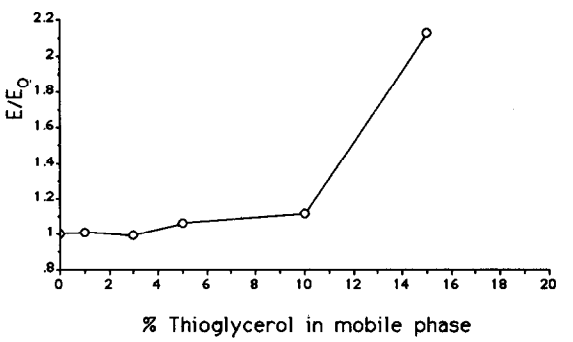

Fig. 5. Effect of concentration of thioglycerol in the mobile phase on the normalized separation impedance, E/E_0.

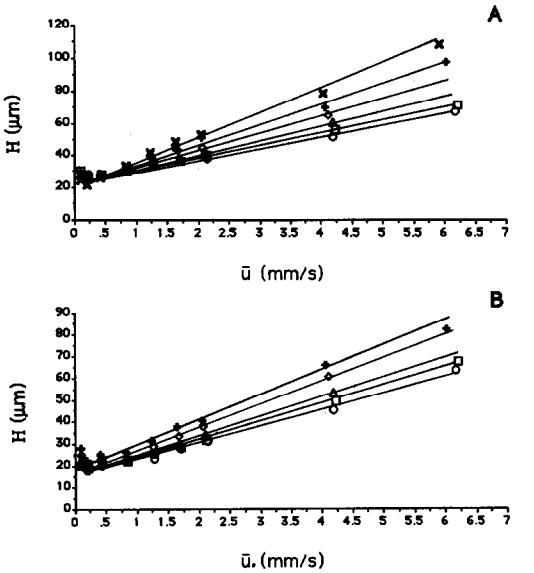

Fig. 6. Van Deemter plots for met-enkephalin at various thioglycerol concentrations in the mobile phase: ○ = 0%; □ = 1%; △ = 3%; ◇ = 5%; + = 10%; × = 15%. (A) Met-enkephalin; (B) *p*-hydroxybenzoic acid.

Deemter plots were generated with the thioglycerol content in the mobile phase varying in the range 0–15% and for linear velocities between 0.2 and 7 mm/s. The plots that were obtained under these experimental conditions using met-enkephalin and *p*-hydrobenzoic acid are shown in Fig. 6a and b, respectively. The data indicate that as the thioglycerol content is increased from 0 to 15% the slopes of the Van Deemter plots increase steadily and significantly. This means that the presence of thioglycerol affects the mass transfer kinetics in the system. It can also be observed that for both compounds the plots converge at lower linear velocities (0.2–0.6 mm/s), indicating that the system can be operated near optimum performance and that only small differences in chromatographic performance occur under these conditions as the concentration of thioglycerol in the mobile phase changes. This important effect, which has also been observed when glycerol was used as a viscous matrix [33], implies that it is possible to use precolumn addition or high concentrations of the viscous matrix and retain the chromatographic performance, provided that the system is operated near its optimum kinetic conditions.

The systematic reduction of the capacity factor, the normalized number of theoretical plates and the resolution occurring for all the different compounds studied clearly demonstrates that the presence of thioglycerol in the mobile phase produces significant changes in the kinetics of the chromatographic process. This is well supported by experimental evidence of the increase in broadening as demonstrated by the variation of the normalized peak width and the increase in the slope of the Van Deemter plots. The processes that occur within the chromatographic column can be represented by the three kinetic effects which are expressed as terms in the Van Deemter equation relating the height equivalent to a theoretical plate (H) to the average linear velocity of the mobile phase ($\bar{u}$) [38–40]. For the system under investigation, the Van Deemter equation has the form [40]

$$H = 2\lambda d_p + \frac{2\gamma D_m}{\bar{u}} + \left[\frac{f_1(k')d_p^2}{D_m} + \frac{f_2(k')d_f^2}{D_s}\right]\bar{u} \qquad (1)$$

where λ and γ are constants, d_p *and* d_f are the particle size and film thickness, respectively, D_m and D_s are the diffusion coefficients in the mobile phase and the stationary phase, respectively, and $f_1(k')$ and $f_2(k')$ are functions of the capacity factor. As the film thickness is considered to be small [39,41], eqn. 1 can be approximated by

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

which represents an expanded form of the more general Van Deemter equation

$$H = A + \frac{B}{\bar{u}} + C\bar{u} \qquad (3)$$

where A, B and C represent the contributions from eddy diffusion, longitudinal diffusion and mass transfer, respectively. As shown in Fig. 6, the term which is mostly affected by the presence of thioglycerol in the mobile phase is the mass transfer term (C), which involves $f_1(k')$, and the diffusion coefficient in the mobile phase, D_m.

It is possible from available data on *p*-hydroxybenzoic acid to evaluate D_m at different thioglycerol concentrations using the Wilke–Chang equation [42]. Knowledge of the $f_1(k')$ function and the estimated values for D_m should allow a qualitative

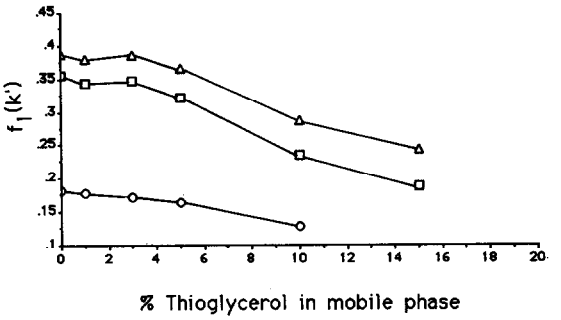

Fig. 7. Effect of concentration of thioglycerol in the mobile phase on $f_1(k')$. □ = Met-enkephalin; △ = leu-enkephalin; ○ = *p*-hydrobenzoic acid.

discussion of the relative contributions of both $f_1(k')$ and D_m to the mass transfer term in the Van Deemter equation. The variation in $f_1(k')$ with thioglycerol content is shown in Fig. 7 for met-enkephalin, leu-enkephalin and *p*-hydroxybenzoic acid. It can be observed that $f_1(k')$ decreases significantly for all compounds studied as the concentration of thioglycerol in the mobile phase is increased. This decrease in $f_1(k')$ should induce a decrease in the mass transfer term, but this is not observed experimentally (Fig. 6). This apparent discrepancy can easily be explained if it is assumed that D_m decreases with increase in thioglycerol content, therefore producing an opposite effect on the mass transfer term. This hypothesis can be verified by examination of Fig. 8, where the variations of $1/D_m$ and $f_1(k')/D_m$ with the thioglycerol content are shown. It is clearly seen that $1/D_m$ decreases as the thioglycerol content is increased and that the opposite variations of D_m and $f_1(k')$ result in a small increase in the mass

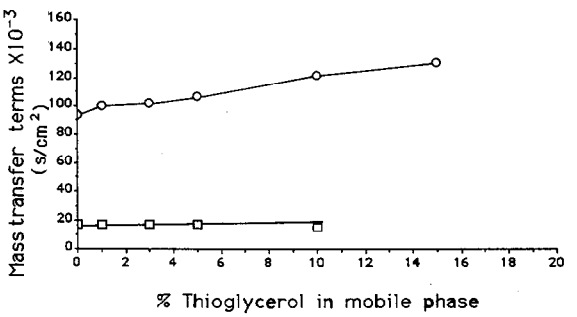

Fig. 8. Effect of concentration of thioglycerol in the mobile phase on the variation of (○) $1/D_m$ and (□) $f_1(k')/D_m$ for *p*-hydroxybenzoic acid.

transfer term with increase in the thioglycerol content. Therefore, the net effect of the presence of thioglycerol in the mobile phase is to reduce the efficiency of mass transfer in the system at higher thioglycerol contents or at higher linear velocities. However, the situation around the optimum linear velocity is different, because under these conditions the theoretical plate height becomes independent of the diffusion coefficient, as can be shown using the Van Deemter equation.

The conclusion that the theoretical plate height becomes independent of the diffusion coefficient D_m around the optimum linear velocity seems to be confirmed by the experimental data shown in Fig. 6, where only slight variations in H are observed near the optimum linear velocity. As the measured values of H represent the resultant contribution to broadening produced by the column and extra-column dead volume, the weak variation observed under optimum conditions can be attributed to the extra-column dead volume. This is confirmed by the data shown in Fig. 9, where the increase in broadening as measured by the variance of an unretained compound ($NaNO_3$) is seen to increase at higher thioglycerol concentrations. This effect will cause a vertical translation of the Van Deemter plots for high contents of thioglycerol, as can be observed by close examination of Fig. 6B. The finding that the chromatographic performance becomes independent of the thioglycerol content under optimized conditions implies that working at the optimum linear velocity will significantly increase the time of analysis. Higher linear velocities can be used to reduce the time of analysis with only a slight

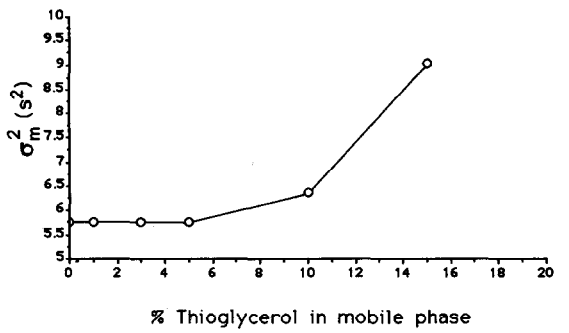

Fig. 9. Effect of concentration of thioglycerol in the mobile phase on the variance associated with the elution of an unretained compound.

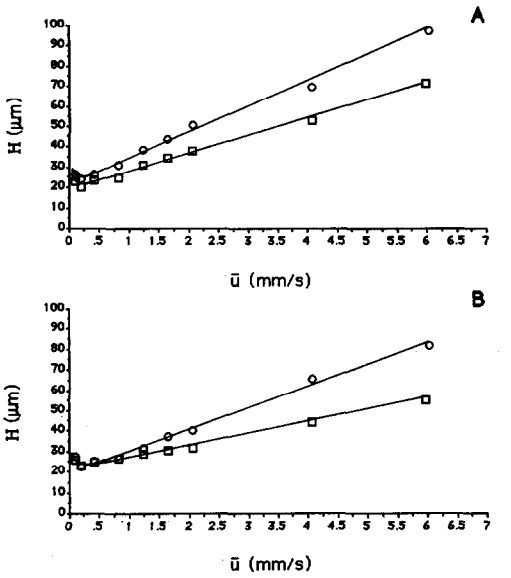

Fig. 10. Temperature dependence of Van Deemter plots for (A) met-enkephalin and (B) *p*-hydroxybenzoic acid at 10% thioglycerol in the mobile phase. ○ = 25°C; □ = 38.5°C.

deterioration in the chromatographic performance, but the results in this study indicate that this is possible only at thioglycerol concentrations below 3%. This is due to the fact that most chromatographic indicators are not significantly affected for thioglycerol contents up to that value. However, if the thioglycerol content is above 3%, the use of higher linear velocities will result in a severe deterioration of the chromatographic performance. The dependence of $f_1(k')$ on temperature and of D_m on both viscosity and temperature suggests that this parameter could possibly be used to nullify the adverse effect of high concentrations of thioglycerol in the mobile phase. In order to increase the chromatographic performance at elevated thioglycerol contents or high linear velocity, Van Deemter plots were generated at 38.5°C to verify the effect of temperature on the mass transfer term. The results are shown in Fig. 10, which compares the theoretical plate height (H) at 25 and 38.5°C for (A) met-enkephalin and (B) *p*-hydroxybenzoic acid in a mobile phase containing 10% of thioglycerol. As expected, the plot at 38.5°C indicates that the increase in temperature has the desired effect and allows the performance to be restored. The theoretical plate height measured at 38.5°C is similar to that

at 25°C with no thioglycerol present in the mobile phase. Hence higher linear velocities can be used with minor decreases in performance at high thioglycerol contents provided that the experiments are conducted at higher temperatures.

In order to obtain general guidelines for the precolumn addition of viscous matrices in LC–FAB-MS systems, it can be useful to compare the results obtained in the investigation of the effects of these matrices. Using the results of this study for thioglycerol and those from our previous work with glycerol [32,33], it becomes possible to compare the effects of both matrices on the chromatographic performance of LC–FAB-MS systems using precolumn addition and to identify similarities and differences in their behavior. For chromatographic indicators such as retention times, capacity factors, number of theoretical plates and resolution, the effect of the addition of a viscous matrix to the mobile phase is to reduce these parameters significantly at high contents of the matrix. Thioglycerol produces a more significant change in the capacity factors than glycerol for concentrations above 3%. For other chromatographic indicators such as normalized peak widths and impedance of separation, these parameters show an increase at higher contents of the matrix in the mobile phase, indicating that the viscous matrix increases broadening in the system and reduces the overall performance. For example, the presence of 15% of thioglycerol in the mobile phase doubles the separation impedance whereas 30% of glycerol is required to produce a similar effect. In terms of increasing the viscosity of the mobile phase, thioglycerol and glycerol are seen to have equivalent effects, as shown by the viscosity measurements given in Table I.

TABLE I

VARIATION OF THE VISCOSITY OF THE MOBILE PHASE WITH VISCOUS MATRIX CONTENT

Data taken from ref. 33.

Matrix content (%)	Viscosity (cP)	
	ACN–water–THIO–TFA (30:70–*x*:*x*:0.05)	ACN–water–GLY–TFA (30:70–*x*:*x*:0.1)
0	0.93	0.95
1	0.99	0.96
3	1.02	1.01
5	1.07	1.05
10	1.24	1.26
15	1.36	

The Van Deemter plots obtained for peptides, acidic compounds and phenols using three different mobile phases and two different matrices show typical characteristics that are believed to represent common trends: (i) all the systems studied seem to retain their chromatographic performance near optimum linear velocities (0.1–0.6 mm/s) with almost no dependence on the matrix concentration in the mobile phase in the range of concentrations studied (0–30%); (ii) the effect of the increase in the concentration of the viscous matrix systematically results in a decrease in the mass transfer efficiency in the chromatographic system for linear velocities greater than 1 mm/s; and (iii) an increase in the temperature of analysis significantly improves the efficiency of the system at high matrix contents and high linear velocities. These three characteristics imply that under the appropriate experimental conditions, it is possible to use a wide range of concentrations of the viscous matrix in the mobile phase while retaining the chromatographic efficiency, contrary to the general belief that high concentrations of the matrix will necessarily result in inadequate chromatographic performance. However, the increase in matrix content can induce broadening effects outside the chromatographic column, and effects mostly apparent in the interface (droplet) have been partly documented [17,33].

CONCLUSIONS

As previously observed for glycerol, the precolumn addition of thioglycerol as a viscous matrix in LC–FAB-MS systems results in alterations to the chromatographic process. This is confirmed by the observation that an increase in the thioglycerol content induces a decrease in the capacity factor, an increase in broadening, a decrease in resolution and a decrease in the chromatographic performance. At concentrations of thioglycerol in the mobile phase below 3%, these changes are minor. The effects of the presence of thioglycerol in the mobile phase on chromatographic performance are minimized, however, if the system is operated near its optimum conditions for average linear velocities. Under those

conditions, high concentrations of the viscous matrix can be used and the efficiency of the chromatographic system is retained. At high thioglycerol contents and high linear velocities, the detrimental effect of the added matrix is apparent but it can be reduced substantially by increasing the temperature of the system, which allows the analysis to be conducted in a reasonable time and at almost any concentrations of matrix in the mobile phase with essentially no decrease in chromatographic performance.

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REFERENCES

1 M. Barber, R. Bordoli, R. D. Sedgwick and N. J. Tyler, *J. Chem. Soc., Chem. Commun.*, (1981) 325.
2 J. L. Gower, *Biomed. Mass Spectrom.*, 12 (1985) 191.
3 W. D. Lehman, M. Kessler and W. A. Konig, *Biomed. Mass Spectrom.*, 11 (1984) 217.
4 G. M. Allmaier, *Rapid Commun. Mass Spectrom.*, 2 (1988) 74.
5 B. L. Bentz and P. J. Gale, *Int. J. Mass Spectrom. Ion Processes*, 878 (1987) 115.
6 J. Meili and J. Siebl, *Org. Mass Spectrom.*, 19 (1984) 581.
7 J. Visentini, J. Gauthier and M. J. Bertrand, *Rapid Commun. Mass Spectrom.*, 3 (1989) 390.
8 Y. Ito, T. Takeuchi, D. Ishii and M. Goto, *J. Chromatogr.*, 346 (1985) 161.
9 R. M. Caprioli, T. Fan and J. S. Cottrell, *Anal. Chem.*, 58 (1986) 2949.
10 M. J. Bertrand, V. Benham, R. St.-Louis and M. J. Evans, *Can. J. Chem.*, 67 (1989) 910.
11 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.
12 D. E. Games, S. Pleasance, E. D. Ramsay and M. A. McDowall, *Biomed. Environ. Mass Spectrom.*, 15 (1988) 179.
13 D. W. Hutchinson, A. R. Woolfitt and A. E. Ashcroft, *Org. Mass Spectrom.*, 22 (1987) 304.
14 A. E. Ashcroft, *Org. Mass Spectrom.*, 22 (1987) 734.
15 P. Boulenguer, Y. Leroy, J. M. Alonso, J. Montreuil, G. Ricard, C. Colbert, D. Duquet, C. Dewaele and B. Fournet, *Anal. Biochem.*, 168 (1988) 164.
16 S. Pleasance, P. Thibault, M. A. Moseley, L. J. Deterding, K. B. Tomer and J. W. Jorgenson, *J. Am. Soc. Mass Spectrom.*, 1 (1990) 321.
17 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.
18 D. B. Kassel, B. D. Musselman and J. A. Smith, *Anal. Chem.*, 63 (1991) 1091.
19 W. J. Henzel, J. H. Bourell and J. T. Stults, *Anal. Biochem.*, 187 (1990) 228.
20 L. J. Deterding, M. A. Mosely, K. B. Tomer and J. W. Jorgenson, *Anal. Chem.*, 61 (1989) 2504.
21 P. Dobberstein, E. Korte, G. Memerhoff and R. Pesch, *Int. Mass Spectrom. Ion Phys.*, 46 (1985) 185.
22 J. G. Stroh, J. C. Cook, R. M. Milberg, L. Brayton, T. Kihara, Z. Huang, K. L. Rinehart, Jr., and I. A. S. Lewis, *Anal. Chem.*, 57 (1985) 985.
23 P. S. Kokonen, W. M. A. Niessen, U. R. Tjaden and J. Van Der Greef, *Rapid Commun. Mass Spectrom.*, 5 (1991) 19.
24 A. Capiello, P. Palma, I. A. Papayannopoulos and K. Biemann, *Chromatographia*, 30 (1990) 477.
25 A. C. Barefoot, R. W. Reiser and S. A. Cousins, *J. Chromatogr.*, 474 (1989) 39.
26 S. A. Martin, C. E. Costello and K. Biemann, *Anal. Chem.*, 54 (1982) 2362.
27 T. Takeuchi, S. Watanabe, N. Kondo, D. Ishii and M. Goto, *J. Chromatogr.*, 435 (1988) 482.
28 K. Tomer and C. E. Parker, *J. Chromatogr.*, 492 (1989) 189.
29 P. Kokkonen, E. Schröder, W. M. A. Niessen, U. R. Tjaden and J. Van Der Greef, *J. Chromatogr.*, 54 (1990) 35.
30 R. M. Caprioli, in R. M. Caprioli (Editor), *Continuous Flow Fast Atom Bombarment Mass Spectrometry*, Wiley, Chichester, 1990, p. 1.
31 R. M. Caprioli, B. B. Dague and K. Wilson, *J. Chromatogr. Sci.*, 26 (1988) 640.
32 J. P. Gagné, A. Carrier and M. J. Bertrand, *J. Chromatogr.*, 554 (1991) 61.
33 J. P. Gagné, A. Carrier and M. J. Bertrand, *J. Chromatogr.*, 554 (1991) 47.
34 J. P. Gagné, A. Carrier and M. J. Bertrand, in *Proceedings of the 39th ASMS Annual Conference on Mass Spectrometry and Allied Topics, Nashville, TN, May 24–28, 1991*, ASMS, East Lansing, MI, p. 372.
35 U. Justesen and G. Bojesen, *J. Chromatogr.*, 562 (1991) 59.
36 L. Shampine, D. B. Kassel and R. J. Andereg, in *Proceedings of the 39th ASMS Annual Conference on Mass Spectrometry and Allied Topics, Nashville, TN, May 24–28, 1991*, ASMS, East Lansing, MI, p. 777.
37 P. A. Bristow and J. H. Knox, *Chromatographia*, 10 (1977) 279.
38 J. J. van Deemter, F. J. Zuiderweg and A. Klinkenberg, *Chem. Eng. Sci.*, 5 (1956) 271.
39 J. A. Jonsson, in J. A. Jonsson (Editor), *Chromatographic Theory and Basic Principle*, Marcel Dekker, New York, 1987, p. 27.
40 E. D. Katz, K. L. Ogan and R. P. W. Scott, *J. Chromatogr.*, 270 (1983) 51.
41 C. Gluckman, A. Hirose, V. L. McGiffin and M. Novotny, *Chromatographia*, 17 (1983) 303.
42 C. R. Wilke and P. Chang, *AIChE J.*, 1 (1955) 264.