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Effect of the addition of viscous matrices to the mobile phase on chromatographic performance in liquid chromatography–fast atom bombardment mass spectrometry

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ABSTRACT

The effect of the pre-column addition of a viscous matrix to the mobile phase in liquid chromatographic–fast atom bombardment mass spectrometric experiments was studied with respect to the chromatographic process. A series of experiments, designed to discriminate against the mass spectral components, were conducted with six compounds, ranging in mass from 100 to 1100 daltons and distributed into three chemical classes. Several chromatographic indicators such as retention times, capacity ratios, number of theoretical plates, peak widths, resolution and separation impedance were monitored as a function of the glycerol content of the mobile phase. The results obtained indicate that the retention times and the capacity ratios decrease with increasing glycerol content of the mobile phase. Increasing concentrations of glycerol also reduce the number of theoretical plates in the chromatographic system and generally have a detrimental effect on peak widths for glycerol contents above 5%. However, lower glycerol contents produce negative effects on compounds with smaller capacity ratios but not on compounds with higher capacity ratios such as peptides. Furthermore, the increase in glycerol concentrations reduces the chromatographic resolution for all classes of compounds studied and creates a significant increase in the separation impedance of the system, resulting in higher operation pressures. The overall effect of a viscous matrix in the chromatographic system can be rationalized in terms of the modification of the analyte distribution between phases and changes in the kinetics of the system created by an increase in mobile phase viscosity.

INTRODUCTION

Since its introduction in 1981 [1], fast atom bombardment mass spectrometry (FABMS) has been confirmed as a powerful technique to provide mass spectral data on polar, thermally labile and non-volatile compounds. The major innovation in FAB resides in the solvation or dispersion of the analyte in a liquid matrix rather than in the use of a fast neutral beam to produce the ions, since the latter originates from secondary-ion mass spectrometry (SIMS), which has a long analytical tradition [2,3]. The use of a liquid matrix provides a mean 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 issued from the radiation damage of the solution. Although exceptions have been reported [4–7], polar compounds will usually give rise, in the positive-ion mode, to intense

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parent molecular ions of the type $[M + H]^+$ under fast atom bombardment.

In recent years, the technique of FAB has evolved, and it is presently used in three types of analysis: static or conventional FAB [1,2], continuous-flow (CF)–FAB or dynamic FAB [8] and liquid chromatography (LC)–FAB-MS [9,10]. In static FAB, the analyte is first dissolved in glycerol or some other suitable viscous matrix and a few microliters are placed on the probe tip, which is made of brass or another material. In CF- or dynamic FAB, the analyte is continuously introduced into the ion source as an aqueous solution, typically containing glycerol. The solution flows to the probe tip through a fused-silica capillary which is introduced into the hollow shaft of the probe. The capillary is connected to the probe tip at one end, while the other end is connected to a solution reservoir or a pumping system. The solution reservoir or pumping system delivers the mobile phase through a loop injector into which the analytes can be introduced [8,11]. The flow-rates used in CF-FAB are typically below 10 μl/min, which is the maximum pumping capacity of most mass spectrometers [12], and the glycerol content in the mobile phase can vary from a few percent to as high as 25%. FAB-MS can also be used in conjunction with LC for the analysis of complex mixtures. Several investigators have reported work involving different LC–FAB-MS systems [9,10,13–21]. One approach involves the use of a moving belt, onto which fractions of the high-performance liquid chromatographic (HPLC) are deposited [13,14]. The belt is continuously cycled into the ion source of the mass spectrometer, where the sample spots are exposed to the fast atom beam. A second approach uses a capillary inlet device to connect a microbore HPLC column to a FAB source [9,15,16]. The interface comprises a porous stainless-steel filter onto which vaporization of the solvent and ionization of the analytes occur. Finally, LC–FAB-MS systems in which the CF-FAB probe is directly connected to the LC system have been reported [17–19]. In these systems, conventional [17,18] and microbore [19] columns have been used with split-flow devices. More recently, work with capillary LC columns coupled to FAB-MS has shown promising results [20-22].

The optimization of experimental conditions in LC–MS systems is usually dependent on two types of factors, *i.e.* those affecting the chromatographic separation and those affecting the mass spectral analysis. In LC–FAB-MS, it is necessary that a viscous matrix be used for ionization to occur. The addition of a viscous matrix to the mobile phase can significantly alter the chromatographic conditions depending on whether the addition occurs before (pre-column) or after (post-column) the chromatographic separation. The concentration of viscous matrix in the mobile phase that has been used for LC–FAB-MS experiments varies from 1 to 25%. The addition can be made before the separation [9,16,18,19] or after the separation using several post-column devices [17,21–23]. The pre-column addition of the viscous matrix can create substantial changes in the polarity and viscosity of the mobile phase, modifying the chromatographic conditions. Furthermore, it has been reported that the addition of a viscous matrix requires higher operating pressures in the chromatographic system. In order to eliminate these undesirable effects, it has been suggested that, using conventional LC columns [17,23], post-column addition of the FAB matrix can be a viable solution. With microbore and capillary columns, post-column addition of the matrix introduces peak broadening due to the presence of additional dead volumes. Coaxial addition of the matrix has been tried in order to limit the peak-broadening phenomenon in capillary systems [22–24]. However, preliminary results indicate that peak

broadening is still present as a result of dead volumes and special effects on the probe tip [21].

Some of the effects caused by the addition of glycerol to the mobile phase in LC–FAB-MS have been reported in several studies [9,17,21,22,24]. However, the effect of the addition of a viscous matrix on chromatographic performance has been the immediate object of only one study [21] in which results obtained by pre-column addition were compared with those obtained by coaxial post-column addition. In order to evaluate the overall influence of pre-column addition on chromatographic performance and other chromatographic indicators, a systematic study was undertaken in which the concentration of the matrix was varied from 0 to 30% and important chromatographic parameters were monitored for compounds of different chemical classes and molecular weights ranging from 100 to 1100 daltons. The conditions of the study were such that they allowed for discrimination against combined effects of the chromatographic system and the interface used in FAB-MS. Problems related to the integrated LC–FAB-MS system have been studied elsewhere [25].

EXPERIMENTAL

Instrumentation

The LC system used in this study consisted of a Perkin-Elmer Model 410 pump connected to a Rheodyne 7125 injector with a 6-μl sample loop. Detection was effected by a variable-wavelength (254 or 280 nm) Perkin-Elmer LC-90 detector. The chromatographic columns [Spherisorb ODS-2 particle diameter, (d_p) = 5 μm, 125 mm × 4.6 mm I.D. (CSC, Montreal, Canada); Perisorb RP-18, d_p = 40 μm, used as precolumn] used in this study were maintained at 25°C by a water jacket regulated by a Haake circulator (Haake, Berlin-Steglitz, Germany). Viscosity measurements of the mobile phases were performed with a capillary viscosimeter.

Chemicals

The peptides met-enkephalin and bradykinin used in this study were obtained from Sigma (St. Louis, MO, USA). Substituted phenolic compounds, such as phloroglucinol and *p*-hydroxybenzoic acid, and 3,5-dihydroxybenzoic acid, vanillic acid and trifluoroacetic acid (TFA) 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 HPLC-grade acetonitrile, acetic acid and distilled, deionized water (Milli-Q system, Millipore, Bedford, MA, USA).

Mobile phases

The chromatographic eluents used in this study were carefully prepared by mixing the appropriate volumes of distilled, deionized water and appropriate organic modifiers. The mobile phase used for the experiments with peptides contained fixed proportions of TFA (0.1%) and acetonitrile (ACN) (39%), 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 used 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 quantities 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) and degassed prior to use.

Chromatographic measurements

All chromatographic experiments were carried out at a nominal flow-rate of 0.8 ml/min. Precise values for the volumetric flow were measured for each injection. Whenever the composition of the mobile phase was changed, the chromatographic system was purged and allowed to equilibrate for at least 90 min prior to subsequent sample injection. The retention of sodium nitrate was taken as dead volume, and the capacity ratios (k') were calculated from the retention of the solutes. The number of theoretical plates per meter (N/m) was estimated from the widths at half-height of the peaks, and the impedance of separation was estimated from the relationship of Bristow and Knox [26].

RESULTS AND DISCUSSION

The effect of the addition of a viscous matrix to the mobile phase can be examined by monitoring the changes that occur in the major chromatographic indicators as the matrix concentration is increased in the mobile phase. The quantification of the changes in chromatographic parameters such as retention time, void volume, peak width, capacity ratio, number of theoretical plates, resolution and separation impedance that occur upon addition of the viscous matrix should allow an assessment of its global effect on the chromatographic system. The trends observed in these parameters with increasing content of the matrix may enable one to characterize the modifications that occur, and to identify those which are most important. In order to analyze the effects of the added matrix on the chromatographic system, it was decided initially to study glycerol, the most commonly used matrix in FAB, because correlations could be made with other data that are available in the literature. Furthermore, it was decided to use compounds of different structures and molecular weights so that a general overview of the effects could be obtained without bias. The six compounds chosen for the study were bradykinin, met-enkephalin, *p*-hydroxybenzoic acid, 3,5-dihydroxybenzoid acid, vanillic acid and phloroglucinol. The chromatographic behavior of these compounds with respect to the variation of the concentration of glycerol was studied over the concentration range 0–30%, which is the range of values reported in the literature.

The first parameter studied was the retention time, and its variation for the six compounds with increase in the glycerol content is shown in Fig. 1. It can be seen from Fig. 1 that in all cases the retention times decrease steadily and significantly with increased glycerol content. The retention of the peptides bradykinin and met-enkephalin, shown in Fig. 1A, is decreased by 50% as the glycerol content is increased from 0 to 20%. The variation observed for the acidic compounds (Fig. 1B) is of the order of 40%, while that of the less retained phenolic compounds is somewhat less. It is interesting and noteworthy that these results are in disagreement with those reported by Pleasance *et al.* [21], which indicate a decrease in retention for glycerol concentrations below 2% and an increase in retention for higher values. The fact that a

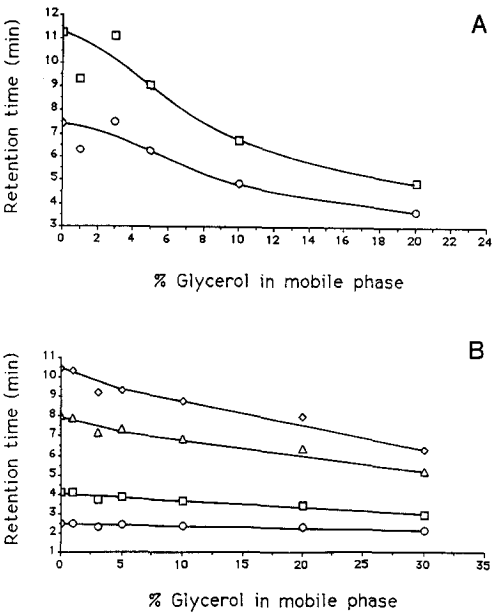

Fig. 1. Variation of the retention time with the glycerol content of the mobile phase. (A) □ = bradykinin; ○ = met-enkephalin in ACN–H_2O–GLY–AcOH (30:70-*x*:*x*:0.1). (B) ○ = phloroglucinol; □ = *p*-hydroxybenzoic acid; △ = 3,5-dihydroxybenzoic acid; ◇ = vanillic acid in ACN–H_2O–GLY–AcOH (10:90-*x*:*x*:1).

decrease in retention is observed for all the compounds in this study and that the decrease is related to the structure of the compounds [peptides (50%), acids (40%), phenols (20%)] strongly indicates that the presence of glycerol in the mobile phase affects the kinetics of the chromatographic process.

The changes in retention of the analytes can better be qualified by the variation of the capacity ratio (k') with the content of glycerol in the mobile phase. The variations in k' for the six compounds studied are shown in Fig. 2. In all cases it is observed that the capacity ratio (k') decreases with the increase in glycerol content of the mobile phase, contrary to the data published by Pleasance *et al.* [21], which indicate an increase in k' for glycerol values above 2%. The significant reduction observed in k' suggests that glycerol is acting as an efficient organic modifier in the mobile phase. As observed from Fig. 2A and B, compounds such as peptides which have important k' values are more affected by slight variations in the glycerol content, indicating changes in the eluotropic force of the mobile phase. The fluctuations observed occur mostly between values of 0 and 5%. Thus, the increase in the glycerol content of the mobile phase produces an effect similar to that which would be observed using an elution gradient.

The effect of the increase in glycerol on the number of theoretical plates per meter (N/m) and peak width can also provide information on the action of the viscous matrix on the chromatographic process. The results presented in Fig. 3 indicate that the chromatographic performance as measured by the number of theoretical plates decreases as the amount of glycerol in the mobile phase is increased. The data from

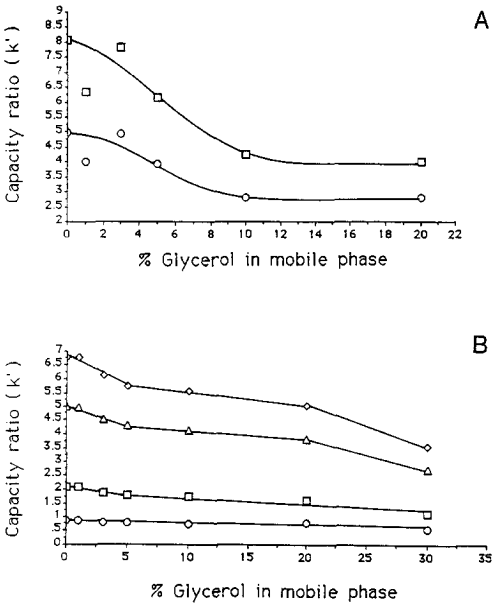

Fig. 2. Variation of the capacity ratio with the glycerol content of the mobile phase. (A) □ = bradykinin; ○ = met-enkephalin in ACN–H_2O–GLY–AcOH (30:70-*x*:*x*:0.1). (B) ○ = Phloroglucinol; □ = *p*-hydroxybenzoic acid; △ = 3,5-dihydroxybenzoic acid; ◇ = vanillic acid in ACN–H_2O–GLY–AcOH (10:90-*x*:*x*:1).

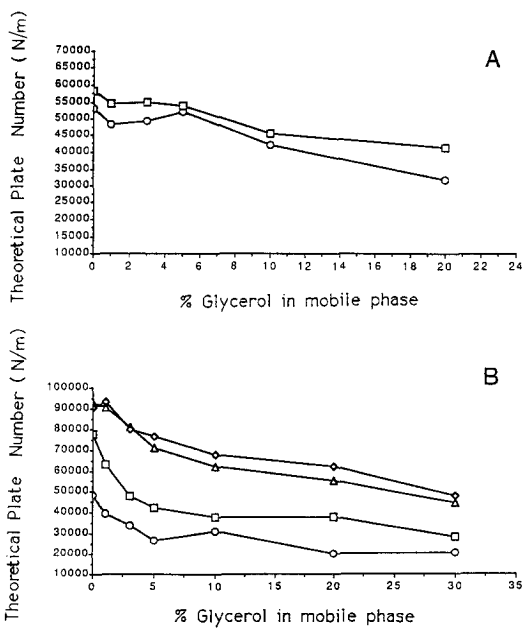

Fig. 3. Variation in the number of theoretical plates per meter with glycerol content in the mobile phase. (A) □ = bradykinin; ○ = met-enkephalin in ACN–H_2O–GLY–AcOH (30:70-*x*:*x*:0.1). (B) ○ = Phloroglucinol; □ = *p*-hydroxybenzoic acid; △ = 3,5-dihydroxybenzoic acid; ◇ = vanillic acid in ACN–H_2O–GLY–AcOH (10:90-*x*:*x*:1).

Fig. 3A indicate a 45% decrease in N/m for bradykinin as the glycerol content increases from 0 to 20%. The values are almost stable in the first 5% and then decrease as the content exceeds this value. This decrease is present for all compounds studied, and the maximum effect is observed with *p*-hydroxybenzoic acid where loss in efficiency is 64% when the glycerol content is 30%. As mentioned previously, changes are most evident in the initial 5% glycerol. For example, while an 8% reduction is seen for bradykinin at low content, an increase of 6% occurs for met-enkephalin. A similar situation can be observed for vanillic acid where a slight increase in efficiency is noticed in the first few percent of glycerol. Furthermore, the data from Fig. 3B indicate that compounds with a low capacity ratio are more readily affected by the presence of small amounts of glycerol. Phloroglucinol and *p*-hydroxybenzoic acid show a substantial reduction in efficiency (45%) when the content of glycerol varies from 0 to 5%, which suggests that the kinetic processes in the system are affected.

The variation in the efficiency of the chromatographic system can be caused by band broadening, but it appears that the effect is more subtle. The results shown in Fig. 4 represent the variation of the normalized widths at half height ($w_{1/2}$) with glycerol content for the compounds studied. Although the experimental peak widths are observed to decrease, the ratio $w_{1/2}/t_r$ (where t_r = retention time) is relatively stable for peptides when the glycerol content is below 5%, while the ratio is seen to increase at higher glycerol content, indicating that peak broadening is occurring. For the other compounds, the ratio increases rapidly with small glycerol contents and then less sharply at concentrations greater than 5%. The effect is greater for phenolic

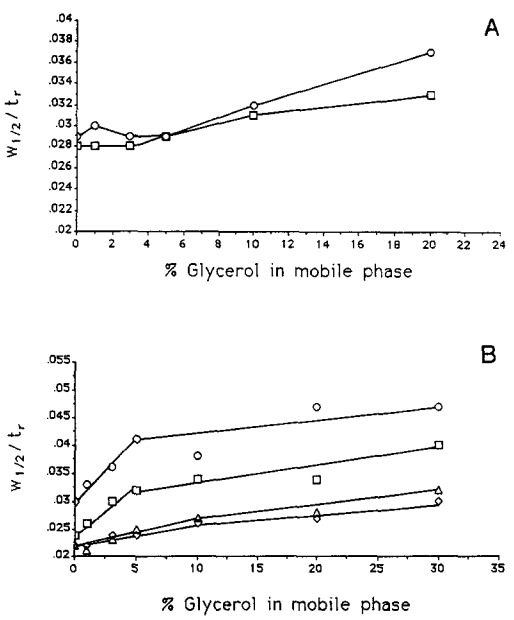

Fig. 4. Variation of the peak width with glycerol content in the mobile phase. (A) □ = bradykinin; ○ = met-enkephalin in ACN–H_2O–GLY–AcOH (30:70-*x*:*x*:0.1). (B) ○ = Phloroglucinol; □ = *p*-hydroxybenzoic acid; △ = 3,5-dihydroxybenzoic acid; ◇ = vanillic acid in ACN–H_2O–GLY–AcOH (10:90-*x*:*x*:1).

compounds having low k' values, which may suggest an increase in instrumental band width, since this contribution has a greater effect on analytes with shorter retentions [27].

This observation suggests that band broadening is related to the variation of the viscosity of the mobile phase. The viscosity of the mobile phase used for the analysis of peptides is given in Table I and is seen to vary from 0.95 to 1.05 as the glycerol content increases from 0 to 5%, while it varies from 0.93 to 1.12 over the same range for the mobile phase used for the other compounds. For content values above 5%, the relative variation in viscosity is similar for both systems and the band broadening is seen to increase in a similar fashion. A more specific study on the variation of the viscosity in this mixture has been conducted, and the results show that variations in the diffusion coefficient can explain most of the broadening observed in those systems [25]. Thus the reduction in efficiency that is observed for low-molecular-weight compounds at low glycerol content and for peptides at glycerol contents above 5% can be attributed to changes that are occurring in the kinetics of mass transfer in the chromatographic systems caused by a change in the diffusivity of the analyte upon change in the viscosity. For compounds with elevated k' values the variation in the retention almost compensates for the kinetic effects due to changes in viscosity below 5% and the efficiency appears stable in that region. For compounds with smaller k' the reduction in retention (Fig. 2) is not sufficient to compensate for the increase in diffusion processes with an increase in glycerol, and the system decreases in efficiency, as indicated by the substantial reduction in N/m.

In order to observe the net effect of an increase in the glycerol content of the mobile phase on chromatographic separation, the resolution has been calculated for two pairs of compounds, and the results are shown in Fig. 5. The variation of the resolution for the pair of peptides is seen to decrease in a similar way to the pair of acids when the glycerol content of the mobile phase is increased. In view of the effects that have already been observed on the retention times, the capacity ratios and the peak widths, the results obtained for the resolution reflect that the variations in those parameters produce an overall effect that results in a loss of the separation efficiency of the system. This reflects the modifications that are occurring in the distribution and kinetics of the system.

TABLE I

VARIATION OF THE VISCOSITY OF THE MOBILE PHASE WITH GLYCEROL CONTENT

Viscosity in cP.

Glycerol (%)	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

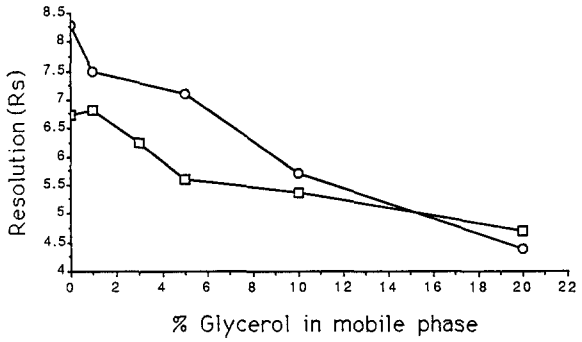

Fig. 5. Variation of the resolution with glycerol content of the mobile phase for two pairs: ○ = bradykinin–met-enkephalin; □ = 3,5-dihydroxybenzoic acid–vanillic acid.

The impedance of separation suggested by Bristow and Knox [26] is another way in which the variation of the overall performance of a chromatographic system can be evaluated. The impedance reveals the apparent viscosity which develops in a chromatographic system as a function of the viscosity of the mobile phase. The variation in the separation impedance of the chromatographic system with the glycerol content of the mobile phase is shown in Fig. 6. It is observed that the impedance is stable for concentrations of glycerol below 3% but increases rapidly as the glycerol content changes from 3 to 10%. This increase in the separation impedance reveals a general loss of performance by the system, which can be attributed to several phenomena caused by the increase in the viscosity of the mobile phase. The impedance demonstrates that the apparent viscosity of the system is increased by 2.5 by the addition of glycerol. This indicates that the presence of glycerol modifies significantly the initial characteristics of the system more than the two-fold variation in the mobile phase viscosity that it causes (Table I). The system is, thus, less efficient in the presence of a significant amount of glycerol ($> 5\%$), and increases in the operation pressure are to be expected, as has been observed experimentally by several other investigators.

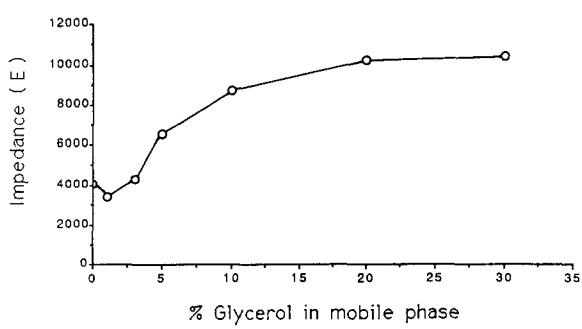

Fig. 6. Variation of the separation impedance with glycerol content of the mobile phase.

CONCLUSIONS

The pre-column addition of a viscous matrix such as glycerol can significantly alter the chromatographic conditions in systems used in LC–FAB-MS. The addition of glycerol shows effects on the retention times of the analytes, capacity ratios, number of theoretical plates, peak width, resolution and impedance of separation. In general terms, the trends are the same whether the compounds are peptides of high molecular weight or acids and phenols of lower molecular weight. The negative effects that the addition of glycerol has on the chromatographic system appear to be more important in compounds having smaller capacity ratios. The net effect of high glycerol contents on the chromatographic system is observed in terms of loss of efficiency and resolution and a significant increase in the separation impedance of the system, indicating a net deterioration in the chromatographic performance. At higher glycerol content, the main factor perturbing the system seems to be the increase in viscosity of the mobile phase that alters the mass transfer kinetics and the flow dynamics of the system. At lower concentrations of glycerol, even if the distribution is affected because glycerol is acting as an efficient organic modifier, the net effect on the chromatographic system, although not positive, is not necessarily detrimental. In fact, it would appear that below 3% glycerol in the mobile phase the chromatographic conditions are essentially stable and the presence of a viscous matrix does not appreciably interfere with the separation characteristics of the system. In those concentration values, although resolution is slightly decreased, the variation in retention induced by glycerol for compounds with a high capacity ratio tends to override the kinetic effects caused by the increase in the viscosity of the mobile phase. Thus optimization of LC–FAB-MS systems should require the minimal use of a viscous matrix or alternatively utilize a matrix with a lower viscosity inasmuch as it does not reduce mass spectral sensitivity.

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REFERENCES

1 M. Barber, R. Bordoli, R. D. Segdwick and A. N. J. Tyler, *Chem. Soc., Chem. Commun.*, (1981) 325.
2 M. Barber, R. S. Bordoli, G. J. Elliot, R. D. Segdwick and A. N. Tyler, *Anal. Chem.*, 54 (1982) 645A.
3 C. Fenselau and R. J. Cotter, *Chem. Rev.*, 87 (1987) 501.
4 Q. Zha and M. J. Bertrand, *Org. Mass Spectrom.*, 25 (1990) 435.
5 Q. Zha and M. J. Bertrand, *Proceedings of the 37th ASMS Annual Conference on Mass Spectrometry and Allied Topics, Miami, FL, May 21–26, 1989*, ASMS, East Lansing, MI, 1989, pp. 792–794.
6 Q. Zha and M. J. Bertrand, *Can. J. Appl. Spectrosc.*, 35 (1990) 141.
7 M. A. Baldwin, K. J. Welham, I. Toth and W. A. Gibbons, *Org. Mass Spectrom.*, 23 (1988) 697.
8 R. M. Caprioli, T. Fan and J. S. Cottrell, *Anal. Chem.*, 58 (1986) 2949.
9 Y. Ito, T. Takeuchi, D. Ishii and M. Goto, *J. Chromatogr.*, 346 (1985) 161.
10 K. Tomer and C. E. Parker, *J. Chromatogr.*, 492 (1989) 189.
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 P. J. Arpino and G. Guiochon, *J. Chromatogr.*, 185 (1979) 529.
13 J. G. Stroh, J. Carter Cook, R. M. Milberg, L. Brayton, T. Kihara, Z. Huang, K. L. Rinehart, Jr. and I. A. S. Lewis, *Anal. Chem.*, 57 (1985) 985.
14 P. Dobberstein, E. Korte, G. Mererhoff and R. Pesch, *Int. J. Mass Spectrom. Ion Phys.*, 46 (1985) 985.
15 T. Takeuchi, S. Watanabe, N. Kondo, D. Ishii and M. Goto, *J. Chromatogr.*, 435 (1988) 482.
16 P. Kokkonen, J. van der Greef, W. M. A. Niessen, U. R. Tjaden, G. J. ten Hove and G. van de Werken, *Rapid Commun. Mass Spectrom.*, 3 (1989) 102.
17 D. E. Games, S. Pleasance, E. D. Ramsey and M. A. McDowall, *Biomed. Environ. Mass Spectrom.*, 15 (1988) 179.
18 D. W. Hutchinson, A. R. Woolfitt and A. E. Ashcroft, *Org. Mass Spectrom.*, 22 (1987) 304.
19 A. E. Ashcroft, *Org. Mass Spectrom.*, 22 (1987) 734.
20 P. Boulenguer, Y. Leroy, J. M. Alonso, J. Montreuil, G. Ricart, C. Colbert, D. Duquet, C. Dewaele and B. Fournet, *Anal. Biochem.*, 168 (1988) 164.
21 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.
22 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.
23 D. J. Bell, M. D. Brightwell, W. A. Neville and A. West, *Rapid Commun. Mass Spectrom.*, 4 (1990) 88.
24 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.
25 J. P. Gagné, A. Carrier and M. J. Bertrand, *J. Chromatogr.*, 554 (1991) 47.
26 P. A. Bristow and J. H. Knox, *Chromatographia*, 10 (1977) 279.
27 J. L. DiCesare, M. W. Dong and L. S. Ettre, *Introduction to High Speed Liquid Chromatography*, Perkin-Elmer, Norwalk, CT, 1981.