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Origin of the decrease in chromatographic resolution induced by the addition of viscous matrices in liquid chromatographic–fast atom bombardment mass spectrometric systems

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ABSTRACT

The monitoring of the chromatographic resolution for three pairs of analytes separated in different chromatographic systems using mobile phases with varying concentrations of viscous fast atom bombardment matrices showed that it exhibits a steady decrease with increasing matrix content in the mobile phase. The decrease in resolution is observed in the partition and ion-pair chromatographic modes at both low and high matrix contents in the eluent for both conventional and capillary chromatographic systems using precolumn addition of glycerol and thioglycerol. Careful examination of the normalized efficiency, capacity factor and selectivity terms contributing to the resolution allowed the identification of the sources of the decrease in resolution in the presence of a matrix in the eluent. The efficiency and capacity factor terms show decreases with increasing matrix content in all systems. Subtle variations in the selectivity term observed in the presence of a viscous matrix can increase or limit the decrease in resolution. The variations observed for the efficiency term show similar trends in all systems studied and appear to be independent of the analyte or the chromatographic mode. However, the variations in capacity factor and selectivity induced by the presence of the viscous matrix are dependent on the nature of the analyte, the type of chromatography and the nature of the matrix.

INTRODUCTION

The technique of continuous-flow fast atom bombardment mass spectrometry (CF-FAB-MS) is a powerful tool that allows repetitive and rapid determinations of polar and labile compounds in aqueous media. In complex mixture analysis, however, this technique used alone can suffer from ionization suppression and insufficient resolution capability [1,2]. In order to reduce these limitations, coupling of this technique with liquid chromatography (LC–FAB-MS) is often a way to gain supplementary temporal resolution which allows the efficient operation of the mass spectrometric system. As the presence of a viscous matrix is necessary to optimize ionization in

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FAB-MS, premixing of the matrix with the chromatographic eluent is an easy method to admit involatile solvents in this coupled system. The inclusion of a viscous matrix in the mobile phase will, to some extent, affect the quality of the initial separation developed in the absence of the FAB matrix. In our previous studies on LC–FAB-MS systems [3–5], conducted with UV detection to allow the discrimination of chromatographic perturbations caused by the presence of a matrix in the mobile phase while excluding those induced by the mass spectrometric interface and operating parameters, it was observed that the presence of a matrix in the chromatographic eluent produces some alterations in the multiple chromatographic parameters that can affect the chromatographic system and reduce its performance [3–6].

This study pursued our investigation on the effect of the presence of FAB matrices in the mobile phase on the chromatographic separation. The changes in chromatographic resolution that result from precolumn addition of glycerol or thioglycerol to the chromatographic eluent in conventional and capillary liquid chromatographic systems used in LC–FAB-MS were specifically investigated. In this work, the effects on resolution produced by the presence of a FAB matrix in the mobile phase were monitored in partition and ion-pair chromatography. The results obtained show that the presence of a matrix, as noticed before, reduces the chromatographic resolution and affects both the physical and chemical processes taking place during the chromatographic separation. It was also observed that ion-pair chromatography seems to be much more sensitive than partition chromatography to the presence of a matrix in the liquid vector.

EXPERIMENTAL

Instrumentation

The conventional liquid chromatographic system used in this study consisted of a Perkin-Elmer Model 410 pump connected to a Rheodyne Model 7125 injector with a 6-μl sample loop. The UV detection system was a Perkin-Elmer LC 90 variable-wavelength detector operated at 280 nm. The conventional columns used (Spherisorb ODS-2, $d_p = 5$ μm, 125 mm × 4.6 mm I.D.) (CSC, Montréal, Canada) were maintained at 25°C by a water jacket regulated by a Haake (Berlin-Sterglitz, Germany) circulator. The capillary system used consisted of a Carlo Erba Phoenix-20 pump connected to a Valco C14W injector with a 60-nl sample loop. Detection at 280 nm was achieved with an ISCO μLC-10 variable-wavelength detector equipped with a 60-nl flow cell. The capillary columns were laboratory-made (180 mm × 0.25 mm I.D.) and packed with 5-μm particules (Spherisorb ODS-2). The temperatures of the injector, the capillary column and the detector flow cell were maintained at 25°C by a temperature equalization chamber supplied with the detector.

Chemicals

The peptides bradykinin, met-enkephalin and leu-enkephalin were obtained from Sigma (St. Louis, MO, USA). The acidic compounds 3,5-dihydroxybenzoic acid and vanillic acid were purchased from Aldrich (Milwaukee, WI, USA) along with thioglycerol (THIO) (<95%) and trifluoroacetic acid (TFA). Glass-distilled glycerol (GLY) (>99%) was obtained from BDH (Toronto, Canada). All compounds were used as received and the mobile phases were prepared using HPLC grade acetonitrile (ACN) and distilled, deionized water obtained with a Milli-Q purification system (Millipore, Bedford, MA, USA).

Mobile phases

The chromatographic systems used are summarized in Table I and are referred to in the text as systems A–C for conventional liquid chromatography and D for capillary liquid chromatography. The mobile phases were prepared as described previously [3]. In brief, the mobile phase was prepared by mixing well defined

TABLE I

CHROMATOGRAPHIC SYSTEMS INVESTIGATED

System	Column dimensions	Mobile phase[a]	Composition[b]	Model compounds
A	125 mm × 4.6 mm I.D.	ACN–H_2O–Gly–AcOH	10:(90 − x):x:1 ($x \leq 20$)	3,5-Dihydoxybenzoic acid, vanillic acid
B	125 mm × 4.6 mm I.D.	ACN–H_2O–Gly–TFA	30:(70 − x):x:0.1 ($x \leq 20$)	Met-enkephalin, bradykinin
C	125 mm × 4.6 mm I.D.	ACN–H_2O–Thio–TFA	30:(70 − x):x:0.5 ($x \leq 15$)	Met-enkephalin, leu-enkephalin
D	180 mm × 0.25 mm I.D.	ACN–H_2O–Gly–TFA	30:(70 − x):x:0.1 ($x \leq 10$)	Met-enkephalin, leu-enkephalin

[a] ACN = Acetonitrile; Gly = glycerol; Thio = thioglycerol; AcOH = acetic acid; TFA = trifluoroacetic acid.
[b] x = Proportion of matrix added to the mobile phase.

volumes of acetonitrile and organic acid and various volumes of water and viscous FAB matrices. The amount of water present was adjusted to complement the volume of liquid taking into account the proportions of added matrix (x in Table I). The composition of the mobile phases used in the experiments with conventional and capillary columns are given in Table I.

Chromatographic measurements

All chromatographic experiments are carried out at 25°C. Before the experiments conducted in the presence of a FAB matrix, each system was tested to evaluate its efficiency by injection of a standard solution of amylbenzene ($k' = 5$) eluted with acetonitrile–water (75:25, v/v). After the evaluation of the theoretical plate number, the mobile phase was changed and the system was allowed to equilibrate for at least 45 min. Measurements were made at average linear velocities around 1.5 mm/s. These conditions correspond to typical flow-rates of 0.8 ml/min for conventional columns and 2.5 μl/min for capillary columns. The retention of sodium nitrate was taken as a dead-time indicator (t_m) and the capacity factor (k') and the selectivity (α) were estimated from the retention of the solutes (t_r) in the usual way. The number of theoretical plates in the system (N) was calculated using peak widths at half-height ($W_{1/2}$) and the resolution (R_s) was calculated from the peak widths of the base (W_b).

RESULTS AND DISCUSSION

It is customary in LC–FAB-MS analysis to determine the optimum chromatographic conditions for separation prior to the inclusion of small proportions of viscous matrix in the mobile phase in order to optimize the sensitivity during the acquisition of mass spectral data. It is important, for this reason, to understand the effect that the presence of a matrix in the mobile phase will produce on the chromatographic separation so as to maintain the quality of separation. Fig. 1 shows the changes in resolution induced by the precolumn addition of glycerol or thioglycerol that occurred in the chromatographic systems used in this study. The addition of increasing amounts of matrix to the liquid vector induces a decrease in resolution under all chromatographic conditions used. It should be stressed that this addition has a deleterious effect on separation irrespective of the nature or concentration of the added matrix. As shown in Fig. 1A and B, this observation was confirmed in systems using both conventional and capillary columns. The de-

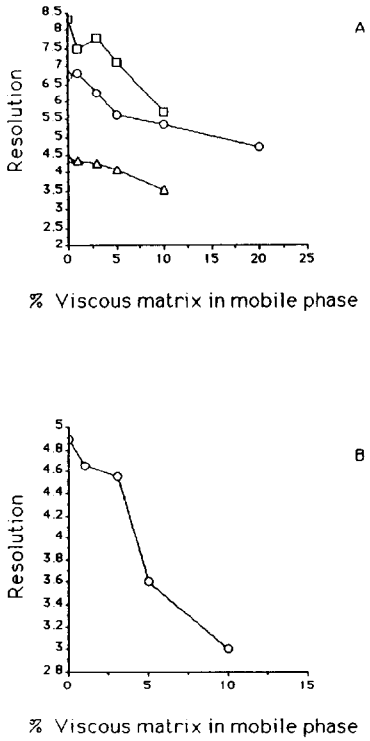

Fig. 1. Influences of FAB matrices on the resolution (R_s) achieved in (A) conventional and (B) capillary liquid chromatographic systems. ○ = System A; □ = system B; △ = system C; ○ = system D (see Table I).

crease in resolution caused by the presence of the matrix in the mobile phase can be minimized by using a low matrix content. However, for capillary columns the sharp decrease in resolution observed with matrix contents above 3% seems to indicate that capillary systems can be more sensitive than conventional systems to the presence of a matrix. It is therefore desirable to limit the addition of the matrix in the chromatographic system to a value that is less than 3% of the composition of the mobile phase.

The decrease in resolution previously demonstrated can originate from many sources. The widths of the chromatographic peak at the base (W_b) and the retention times (t_r) are the most important parameters in determining resolution, as shown in its operational definition given by the equation

$$R_s = \frac{2(t_{r_2} - t_{r_1})}{(W_{b_2} - W_{b_1})} \quad (1)$$

This important practical equation does not, however, give ample information on the physical or chemical processes involved in the separation. In order to obtain more insight into the physical and chemical processes that control the separation, a more descriptive equation has to be used, so as to reveal the true causes of the decrease in resolution that is observed under the present experimental conditions.

As the peak width depends on diffusional processes occurring in the column, the peak width expressed in terms of the number of theoretical plates (N) can be used as an indirect indicator of the physical processes taking place in the analytical system. On the other hand, the retention time can act as a probe for the chemical distribution of the analyte between the mobile and stationary phases. The capacity factor (k') and the selectivity coefficient (α), which are related to retention times, are suitable parameters that can be used in order to gain insight into the chemical equilibrium occurring in the chromatographic system. The three parameters N, k' and α can be combined to give the classical expression of resolution:

$$R_s = \frac{N^{1/2}}{4} \cdot \left(\frac{k'}{k'+1}\right) \cdot \left(\frac{\alpha - 1}{\alpha}\right) \quad (2)$$

The monitoring of the changes in N, k' and α induced by the presence of the matrix in the mobile phase can now be used in order to explore in more detail and to elucidate the specific effects altering the separation in the presence of glycerol or thioglycerol. This exploration may be instructive in providing a better understanding of the perturbation induced by the presence of the matrix in the chromatographic system. Further, it can also provide guidance in making better choices in selecting matrices and their concentrations in the mobile phase for LC–FAB-MS analysis.

In order to compare the relative importance of the contributions of parameters affecting resolution, it is desirable, in the present instance, to normalize all the data to a reference state. In this study, the 0% matrix content was chosen as the reference state as this state corresponds to the

usual conditions under which the method development will have been conducted. Normalization of all the diagnostic parameters was done by dividing the measurements acquired at a specific matrix content by the values obtained in the reference state. In this study, the normalized terms for the efficiency, the capacity factor and the selectivity contributing to the resolution will be designated by $\sqrt{N_x}/\sqrt{N_0}$, $f(k')_x/f(k')_0$ and $f(\alpha)_x/f(\alpha)_0$, respectively. This nomenclature will be used in presenting and discussing the data.

The effect of the addition of a FAB matrix in conventional liquid chromatography was initially studied for partition chromatography in system A using 3,5-dihydroxybenzoic acid and vanillic acid as model compounds eluted with ACN–H_2O–GLY–AcOH. Fig. 2 shows the variations in the normalized ratios with glycerol present in the mobile phase. The data in Fig. 2 indicate a decrease in the three normalized parameters as the content of glycerol is increased. However, the key factor that controls the observed decrease in resolution is the efficiency term, which decreases more than the other factors. For example, at a 20% glycerol content in the eluent the decreases in the normalized capacity factor and normalized selectivity terms are limited to less than 4% and 8%, respectively, of their initial values obtained in the absence of a matrix. These data indicate that the presence of a matrix in the eluent does not produce dramatic changes in the initial nature of the mobile phase, nor does it affect the mechanisms governing the separation and selectivity. In this system, the added glycerol replacing part of the water in the mobile phase seems to act in a very similar manner to water on the distribution of the analytes between the mobile and stationary phases. In contrast, the normalized efficiency term shows a decrease of 1% under the same boundary conditions. The large decrease in the normalized efficiency term for partition chromatography suggests that physical processes are more significantly affected than chemical processes occurring in the column.

The second chromatographic system was used to examine the effect of glycerol in ion-pair chromatography. In system B, met-enkephalin and bradykinin were chosen as reference compounds and were eluted with ACN–H_2O–GLY–TFA as the mobile phase. Fig. 3 shows the dependence of the three normalized parameters on the concentration of glycerol present in the mobile phase. It can be seen that the presence of glycerol influences the normalized efficiency, capacity factor and selectivity terms. The similar decreases observed in the ratios $\sqrt{N_x}/\sqrt{N_0}$, $f(k')_x/f(k')_0$ and $f(\alpha)_x/f(\alpha)_0$ with increasing content of matrix indicate that all parameters may contribute to the decrease in resolution, in contrast to the previous system studied, in which the efficiency term was the most active. The reduction in the normalized parameters, of the order of 10–20% at a 20% matrix content in the chromatographic eluent, suggests that ion-pair chromatography can be much more sensitive to the presence of the matrix than in partition chromatography. This also implies that in ion-

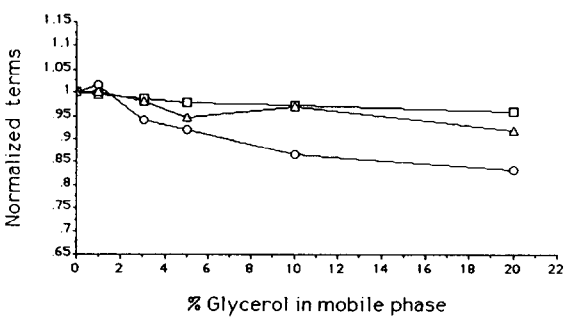

Fig. 2. Influence of glycerol on the normalized efficiency, capacity factor and selectivity terms in partition chromatography (system A). ○ = $\sqrt{N_x}/\sqrt{N_0}$; □ = $f(k')_x/f(k')_0$; △ = $f(\alpha)_x/f(\alpha)_0$.

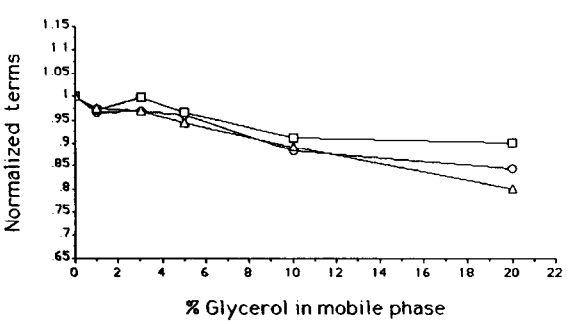

Fig. 3. Influence of glycerol on the normalized efficiency, capacity factor and selectivity terms in ion-pair chromatography (system B). ○ = $\sqrt{N_x}/\sqrt{N_0}$; □ = $f(k')_x/f(k')_0$; △ = $f(\alpha)_x/f(\alpha)_0$.

pair chromatography the presence of a matrix in the mobile phase will make the control of the chromatographic system more difficult since the physical and chemical processes in the system are altered.

In order to confirm the sensitivity of ion-pair systems to the presence of a matrix in the mobile phase, a second ion-pair chromatographic system consisting of leu-enkephalin and met-enkephalin in the presence of thioglycerol was studied. Fig. 4 shows, for system C, the changes induced in $\sqrt{N_x}/\sqrt{N_0}$, $f(k')_x/f(k')_0$ and $f(\alpha)_x/f(\alpha)_0$ with increasing content of thioglycerol in the mobile phase. The presence of thioglycerol produces a decrease in the $\sqrt{N_x}/\sqrt{N_0}$ term of the order of 10% at a 10% matrix content, as was similarly observed in systems A and B. This supports the idea that some common processes related to the parameter N are, in a systematic manner, affected in each system under study. The changes in the $f(k')_x/f(k')_0$, and $f(\alpha)_x/f(\alpha)_0$ terms, which are of the order of −30% and +15%, respectively, at a 15% matrix content in the mobile phase, confirm the sensitivity of ion-pair chromatography to the presence of a FAB matrix. The variations in $f(k')_x/f(k')_0$, and $f(\alpha)_x/f(\alpha)_0$ are different and more dramatic than previously observed in system B in the presence of glycerol. This shows that thioglycerol substantially perturbs the chemical distribution of the analyte in the chromatographic system in a fashion which is different to that of glycerol. In system C this results in an increase in selectivity in the presence of thioglycerol, contrary to the decrease in selectivity previously observed in the presence of glycerol in the other ion-pair system studied (system B).

In order to determine the influence of the presence of a FAB matrix on the results generated by packed capillary ion-pair liquid chromatography, the peptides met-enkephalin and leu-enkephalin were separated using ACN–H_2O–GLY–TFA as the mobile phase in which the glycerol content was increased to a maximum of 10% of the composition. The capillary column was flushed with 4 ml of HPLC-grade water, 2 ml of 0.1% TFA in water, 2 ml of 2-propanol, 2 ml of acetonitrile and 2 ml of HPLC-grade water prior to allowing the mobile phase ACN–H_2O–GLY–TFA to percolate through the column. Fig. 5 shows the changes observed in the $\sqrt{N_x}/\sqrt{N_0}$, $f(k')_x/f(k')_0$ and $f(\alpha)_x/f(\alpha)_0$ terms with the percentage of glycerol added to the mobile phase in capillary system D. In this system the normalized efficiency ratio $\sqrt{N_x}/\sqrt{N_0}$ shows a net decrease of the order of 22% at a 10% matrix content and the ratios $f(k')_x/f(k')_0$ and $f(\alpha)_x/f(\alpha)_0$ show a decrease of the order of 15%. The decrease observed in the two latter parameters in the capillary system is significantly higher than those measured in conventional systems A or B in the presence of glycerol. However, the decrease in all three parameters measured in system D are very similar to those observed in system B. It should be mentioned that using capillary systems it has been observed that the lifetime and usage of the column can affect the results obtained for the normalized

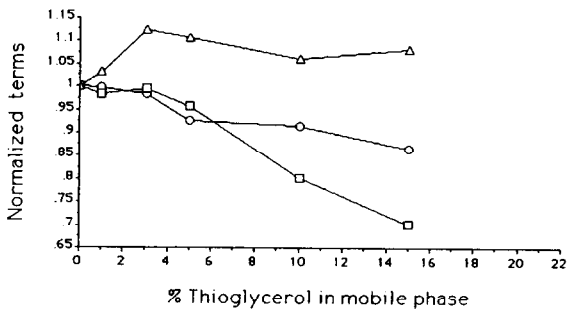

Fig. 4. Influence of thioglycerol on the normalized efficiency, capacity factor and selectivity terms in ion-pair chromatography (system C). ○ = $\sqrt{N_x}/\sqrt{N_0}$; □ = $f(k')_x/f(k')_0$; △ = $f(\alpha)_x/f(\alpha)_0$.

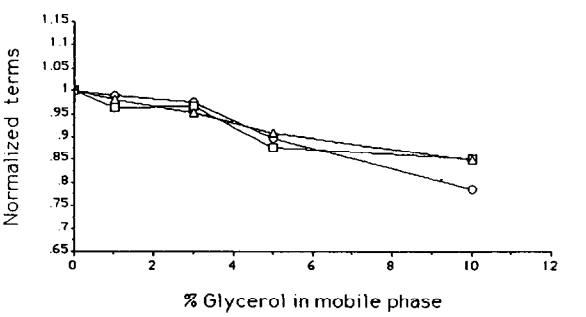

Fig. 5. Influence of glycerol on the normalized efficiency, capacity factor and selectivity terms in ion-pair chromatography (system D). ○ = $\sqrt{N_x}/\sqrt{N_0}$; □ = $f(k')_x/f(k')_0$; △ = $f(\alpha)_x/f(\alpha)_0$.

selectivity ratio. This parameter seems to be subject with time and number of analyses to irreversible adsorption of some compounds, which modifies the trends observed in the selectivity factor.

It therefore appears from monitoring of the chromatographic resolution for three pairs of analytes in three mobile phases containing different concentrations of FAB matrix that it steadily decreases with increasing matrix content in the mobile phase. The decrease in resolution is observed in the presence of glycerol or thioglycerol at both low and high matrix concentrations in conventional or capillary columns. This phenomenon is observed in partition and ion-pair systems and can be rationalized by analyzing the subtle processes that are related to each of the normalized ratios.

The degradation of the quality of the separation as measured by means of the normalized efficiency term ($\sqrt{N_x}/\sqrt{N_0}$) contributing to the resolution shows that the efficiencies of conventional and capillary systems are both decreased in the presence of a FAB matrix. The extent of the decrease seems to be independent of the nature of the FAB matrix, of the acid added to the mobile phase and of the initial proportions of ACN and H_2O present in the mobile phase. However, in capillary systems the decrease in efficiency is higher than that observed in conventional systems. There are four basic processes that control band broadening, which determines the efficiency in chromatographic systems: axial molecular diffusion, flow dispersion in a packed bed, mass transfer in the column and extra-column band broadening. In our experiments with conventional columns, the extra-column broadening was made negligible by using small connecting tubes and small injector and detector volumes. Also, the importance of longitudinal diffusion was maintained low by using average linear velocities of the order of 1.5 mm/s. As a result of choosing such experimental conditions, the efficiency of the conventional systems used should be under mass transfer control. In this instance, as shown in a previous study [4,5], the mass transfer process in the presence of a FAB matrix is governed by the extent of the change in the viscosity of the mobile phase, which is similar for all matrices studied. An increase in viscosity causes a lower diffusivity of the analyte [4], which results in a decrease in efficiency, as has been observed. In the capillary liquid chromatographic system used, an increase in viscosity also reduces the efficiency. However, as the capillary systems are very sensitive to dispersive effects in dead volumes [7–10], it is probable that extra-column broadening occurs in our capillary systems owing to the connexions between the column and injector and the column and detector, and this will decrease the efficiency in the capillary systems as measured by the ratio $\sqrt{N_x}/\sqrt{N_0}$ given in Fig. 5. From the break in the resolution curves of system D (Fig. 1B), it seems that dead volume effects become important when more than 3% of glycerol is present in the mobile phase. Decreases in resolution that may originate from changes in capacity factors and selectivity can also be rationalized. Considering the chromatographic systems studied, only system A, based on partition chromatography, seems to be controlled mainly by changes in the efficiency term. For the other systems, based on ion-pair chromatography, chemical alteration of the distribution of the analytes between the mobile and stationary phases appears to play an important role in the decrease in resolution.

The capacity factors and selectivity, which characterize the interactions responsible for retention, are dependent on the nature of the analytes, the nature of the stationary phase and the composition of the mobile phase. The variations observed in $f(k')_x/f(k')_0$ and $f(\alpha)_x/f(\alpha)_0$ as an increasing amount of viscous matrix is added to the mobile phase clearly indicate that the interactions between participants in the chromatographic equilibrium are changed. In the case of the $f(k')_x/f(k')_0$ term the steady decrease observed in the four systems monitored in the presence of increasing amounts of FAB matrix indicates that the analyte distribution is shifted towards the mobile phase in both the partition (system A) and the ion-pair (system B to D) chromatographic modes. The rationale for this observation is that glycerol and thioglycerol behave as organic moderators which increase the eluotropic force of the mobile phase, thus favoring the elution of the analytes. The data ob-

tained in the conventional systems indicate that thioglycerol is more efficient than glycerol in that role as a larger decrease in the $f(k')_x/f(k')_0$ term occurs in its presence.

The previous decrease observed in the $f(k')_x/f(k')_0$ term can also find a rational explanation on the basis of the molecular interactions occurring in the system. In the systems studied, the interactions between the analytes and the ODS moities are mainly dispersive and inductive, as the alkyl chain bonded to silica has a weak dipole moment [11]. Hence the addition of a polar matrix such as glycerol or thioglycerol should not significantly affect the dispersive interactions responsible for the retention of the analyte. In the present instance, the addition of a substance such as glycerol that has a lower dielectric constant than water ($\varepsilon_{glycerol} = 42.5$ and $\varepsilon_{water} = 78.5$ [12]) should lower the overall dielectric constant of the mobile phase [13–15]. As there is an inverse relationship between intermolecular interactions and the dielectric constant, the interactions between the solute and the mobile phase become stronger, which results in a decrease in analyte retention. This is clearly supported by the measured decrease in the normalized capacity factor term that occurs in all systems. However, it is noteworthy that each system (A, B and D) exhibits a different rate of decrease which reflects that role of the composition of the mobile phase and its interaction with the stationary phase in the perturbation caused by addition of glycerol.

The presence of a matrix in the mobile phase also affects the nature and the composition of the stationary phase. It is well known that bonded stationary phases adsorb constituents such as water, acetonitrile or alcohols from the mobile phase [16–20]. This adsorption would vary in accordance with the eluotropic strength of the eluent. Hence the addition of glycerol or thioglycerol should then favor the intrusion of these molecules in the stationary phase. It is to be expected that, because the matrices are efficient organic moderators, they will compete with water and, to an extent, with acetonitrile for adsorption. Therefore, any modification in the stationary zone that is participating in the separation can result in subtle and sometimes unpredicable changes in retention and selectivity, as has been observed in this work. However, some important changes in selectivity observed in the analysis of peptides appear to be related to the role and the presence of TFA in the mobile phase. This acid, which is added to mobile phases, has three distinct functions [21–25]: it promotes the protonation of peptides by affecting the pH, it is involved in ion pairing as the counter ion and it minimizes the interactions of the cationic residues in the molecule with the support by decreasing the ionization of the nonderivatized silanol groups. The addition of a FAB matrix to the ion-pair chromatographic systems will affect the distribution and mode of action of TFA. The presence of a polyhydroxylated matrix such as glycerol or thioglycerol can shield the silanol groups, making them less accessible to TFA. Further, the decrease in the dielectric constant of the mobile phase in the presence of a matrix should increase electrostatic interactions in the mobile phase, thus favoring ion pairing and affecting capacity factors and ultimately the selectivity of the system. In ion-pair systems, it was observed that the normalized selectivity term decreased in the presence of glycerol whereas it increased in the presence of thioglycerol. The increase in the normalized selectivity term in the presence of thioglycerol may be related to a differential competition for common sites. Both thioglycerol and met-enkephalin contain sulfur groups which are likely to interact in a similar way. Thus, as the matrix becomes more concentrated in the mobile phase it can saturate the retention sites and decrease the retention of met-enkephalin, which results in a decrease in the capacity factor. As leu-enkephalin is not affected to the same extent by this phenomenon because it does not contain sulfur, the net result is an increase in selectivity. Caution should be used, however, in attributing this effect strictly to thioglycerol, as this FAB matrix was only 95% pure in its commonly used form. It is possible, as stated previously, that impurities in the thioglycerol which was continuously percolated through the system were adsorbed irreversibly by the stationary phase and that this factor can also be involved in the changes observed.

CONCLUSIONS

It was demonstrated that a decrease in chromatographic resolution occurs in the presence of glycerol or thioglycerol in the mobile phase for peptides and phenolic compounds separated in both conventional and capillary chromatographic systems. The monitoring of the efficiency, the capacity factor and the selectivity terms contributing to chromatographic resolution permitted the identification of the decrease in capacity factors and in efficiency as constant sources causing the decrease in resolution in the presence of a FAB matrix. In the systems studied it was observed that selectivity can be affected in different ways and that changes in selectivity can increase or limit the decrease in resolution in the presence of a FAB matrix. The efficiency parameter were observed to exhibit similar changes in all systems studied and they were independent of the nature of the analyte and the matrix, whereas the variations observed in the capacity factor and selectivity term appear to be intimately related to the mode of chromatography and the nature of the analyte and of the matrix used. Impurities in the mobile phase may be involved in some of the changes in selectivity observed.

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