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Systematic Study of the Effects of Experimental Parameters on the Beam-induced Dehalogenation of Chlorpromazine in Liquid Secondary Ion Mass Spectrometry

R. Théberge, G. J. C. Paul and M. J. Bertrand*
Regional Center for Mass Spectrometry, Department of Chemistry, Université de Montréal, P.O. Box 6128, Stn A, Montréal, Québec, H3C 3J7, Canada

The effect of experimental parameters such as time of irradiation, analyte concentration, primary beam density, matrix selection and matrix additives on the beam-induced dehalogenation of chlorpromazine in liquid secondary ion mass spectrometry (LSIMS) was investigated. It was found that dehalogenation of chlorpromazine in glycerol increased with increasing time of irradiation, analyte concentration and primary beam density. These results were compared with those obtained using 4-chlorophenylalanine ethyl ester and the differences observed were rationalized in terms of compound surface activity. Evidence is given that matrix selection is the key experimental parameter affecting the extent of beam-induced dehalogenation of chlorpromazine in LSIMS. Of the eleven matrices used, the greatest extent of dehalogenation was observed in glycerol. Sulfur-containing matrices consistently exhibited a lower extent of dehalogenation than oxygen-containing aliphatic matrices, implying that sulfur is implicated in mitigating the reduction process. Dehalogenation was totally inhibited in 2-hydroxyethyl disulfide, 4-hydroxybenzenesulfonic acid and 3-nitrobenzyl alcohol. Similarly, the use of matrix additives such as 3-nitrobenzyl alcohol and trifluoroacetic acid was found to be useful in inhibiting the extent of dehalogenation occurring in glycerol.

INTRODUCTION

Although fast atom bombardment mass spectrometry (FABMS) is now past its first decade of existence,[1] the physico-chemical processes which occur to generate the ionic species that are observed in the spectrum are still not well understood.[2] One of the concerns of mass spectrometrists using FAB or liquid secondary ion mass spectrometry (LSIMS) is the possibility that artefacts can originate from beam-induced reactions of the sample. This phenomenon can significantly complicate spectrum interpretation or, conversely, lead to erroneous conclusions as to the nature of the sample.

In many cases, the ionic species generated under FAB/LSIMS conditions are the result of reductive processes involving the analyte. These processes can cause the intensities of $A + n$ peaks ($A = [M + H]^+$, $n \geqslant 1$) to be higher than the theoretical values calculated using natural abundances. These observations prompted the investigation of this phenomenon, particularly for peptides.[3,4] Cationic organic dyes can undergo beam-induced reduction which is typified by the addition of one of more hydrogen atoms to the molecular ion and gives rise to ions one or two mass units heavier than the molecular ion. These dyes are the most extensively studied systems.[5–10] In certain instances, a specific functional group of the analyte is known to be involved in the reductive process. Examples include the reduction of nitro[11] and azide[12] groups to the amine, reduction of aromatic oximes to imines,[13] dehydroxylation[14] (replacement of a hydroxy function by a hydrogen), deamination[15] (replacement of a primary amine by a hydrogen), reduction of disulfides to thiols[16] and dehalogenation[12,17–21] (replacement of a halogen by a hydrogen).

Dehalogenation reactions in mass spectrometry were first reported by Harrison and Lin,[22] who observed the replacement of aromatic halogens by hydrogen under chemical ionization (CI) conditions using hydrogen as the reagent gas. Dehalogenation has also been observed in ^{252}Cf desorption.[23] The importance of beam-induced dehalogenation in FAB/LSIMS resides in the fact that its occurrence can lead one falsely to conclude that a dehalogenated species is present in a given sample. This can have serious consequences in metabolic studies or compound identification.

Dung *et al.*[17] applied LSIMS to chlorpromazine in glycerol before and after irradiation with a mercury lamp in order to monitor the photodegradation of the compound. However, they did not comment on the wider significance of the dehalogenated ion present in the non-irradiated sample, i.e. that beam-induced dehalogenation was occurring. Sehti and co-workers[18] investigated the FAB-induced dehalogenation of a series of halogenated nucleosides. Using *C*- and *O*-perdeuteroglycerol as the matrix, they demonstrated that the hydrogen replacing the halogen originated from the matrix. The extent of dehalogenation was shown to be inversely proportional to the carbon–halogen bond strength. Using substituted nucleoside cyclophosphates, Schiebel *et al.*[12] showed that the dehalogenated product

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could be detected in the glycerol matrix with post-bombardment high-performance liquid chromatographic (HPLC) analysis. This finding indicates that the reaction could possibly occur (at least partially) in the condensed phase. The importance of matrix chemistry was demonstrated by Musser and Kelley,[19] who showed that dehalogenation of halonucleosides was totally inhibited when 3-nitrobenzyl alcohol was employed as the liquid matrix. This observation was linked to the calculated electron affinity of the matrix. The authors also detected the dehalogenated residue in the glycerol matrix using post-bombardment HPLC analysis. Edom *et al.*[20] studied the dehalogenation of chlorpromazine, showing that the dechlorinated ion arising during FAB analysis gave a daughter ion spectrum identical with that of the protonated unhalogenated analog, promazine. In a manner similar to Sehti and co-workers,[18] Edom *et al.*[20] demonstrated that the hydrogen replacing the halogen originated from the matrix by using perdeuterated glycerol.

However, despite these numerous reports of dehalogenation reactions occurring under FAB/LSIMS conditions, there has been little or no systematic study of the experimental factors that could potentially affect the extent of these reactions. The phenomenon is thus well known but poorly characterized. In order to systematically characterize the role of FAB/LSIMS parameters such as primary beam density, analyte concentration, matrix selection and matrix additives, a systematic LSIMS study was undertaken in which the individual parameters were varied. Chlorpromazine was chosen as the model compound because it undergoes pronounced dehalogenation[20] and it is soluble in a wide variety of matrices. To compare the results obtained with chlorpromazine, another compound known to undergo dehalogenation, 4-chlorophenylalanine ethyl ester, was also investigated.[21]

EXPERIMENTAL

All mass spectral data were obtained using a VG AutoSpec-Q spectrometer (VG Analytical, Manchester, UK) equipped with a cesium ion gun. The accelerating voltage was 8 kV and the mass resolution 1000. Magnet scans (5 s per decade) were used over the mass range 50–1000. Standard analysis conditions consisted of a beam energy of 22 keV and a beam density of 0.027 μA mm^{-2}, unless specified otherwise. The measurement of beam densities has been described elsewhere.[4] All mass spectral data were treated with the VG Opus data system. Electrospray ionization (ESI) mass spectra were recorded using a API III triple quadrupole mass spectrometer (Sciex, Thornhill, Ontario, Canada) operated with a cone voltage of 80 V.

Chlorpromazine and 4-chlorophenylalanine ethyl ester were used as hydrochlorides and the matrices employed were glycerol (Gly), thioglycerol (Thiogly), 3-nitrobenzyl alcohol (NBA), thiodiglycol (TDG), 3,4-dimethoxybenzyl alcohol (DMBA), 2-hydroxyethyl disulfide (HEDS), 2-hydroxyphenethyl alcohol (HPEA), 2-mercaptoethyl sulfide (MES), 1,4-anhydroerythritol (AET), 2-benzyloxypropane-1,3-diol (BOP) and 60% aqueous 4-hydroxybenzenesulfonic acid (HBSA) solution. All compounds were obtained from Aldrich Chemical (Milwaukee, WI, USA) and used without further purification. Solutions were prepared by dissolving the compound directly in the matrix and the concentration range was 0.006–0.097 M. Mass spectral analysis was carried out immediately following mixing by applying 2 μl of the solution on to a square probe tip (7 mm^2), which affords uniform irradiation of the sample.

RESULTS AND DISCUSSION

Effect of concentration

The positive-ion liquid secondary ion mass spectrum of chlorpromazine in glycerol is shown in Fig. 1. The main peaks observed are the protonated molecular ion at *m/z*

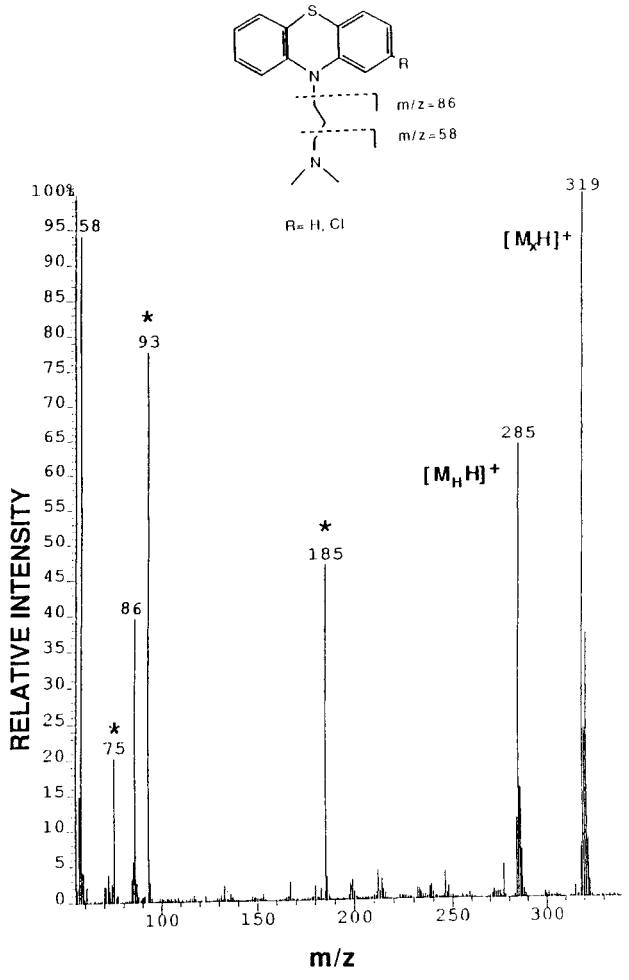
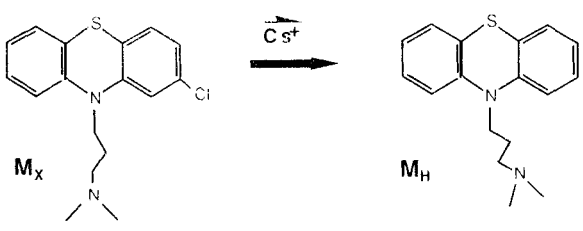


Figure 1. Positive liquid secondary ion mass spectrum of chlorpromazine in glycerol (0.037 M). Asterisks denote matrix peaks.



Scheme 1

319, characteristic fragments of the amine side-chain at *m/z* 86 and 58 and an ion at *m/z* 285 that corresponds to dechlorinated chlorpromazine. This ion is a consequence of beam-induced replacement of chlorine with hydrogen[20] as shown in Scheme 1.

The extent of dehalogenation is expressed in per cent as follows:

$$\text{Dehalogenation (\%)} = \frac{100(M_H + H]^+}{[M_H + H]^+ + [M_X + H]^+} \quad (1)$$

For chlorpromazine, $[M_H + H]^+$ represents the relative intensity of the *m/z* 285 peak and $[M_X + H]^+$ the relative intensity of the protonated molecular ion peak at *m/z* 319.

In the experiments conducted in this study, the extent of dehalogenation in glycerol was pronounced and found to be in the range of 16–60%. These values are the maximum and minimum percentage dehalogenation of chlorpromazine obtained when glycerol was the matrix under the different conditions used in this work.

The percentage dehalogenation for chlorpromazine in glycerol at various concentrations with time of analysis is shown in Fig. 2. The liquid secondary ion mass spectra were obtained with a primary beam energy of 22 keV and beam density of 0.027 μA mm^{-2}. These are the standard conditions used in this work unless specified otherwise. The apparent time dependence of the percentage dehalogenation can readily be seen. The percentage dehalogenation of chlorpromazine was found to increase significantly with the duration of beam irradiation. These results are in agreement with the findings of Edom *et al.*[20] The effect of increased concentration appears to be a concomitant increase in the percentage dehalogenation except for the highest concentration. The plot for a concentration of 0.097 M seems anomalous as it appears below that for 0.037 M. Further, the plot for the 0.097 M chlorpromazine–glycerol solution appears to indicate that the dechlorination maximizes after 3 min of beam irradiation and then decreases. These observations can be rationalized by considering the fragmentation behavior of chlorpromazine with time and concentration, and more specifically, the *m/z* 58 fragment, which is due to the cleavage of the C—C bond α to the dimethylamine group of the side-chain. This increased fragmentation of the amine side-chain with increasing concentration and time of irradiation can be seen in LSIMS, where *m/z* 285 and 319 ion abundances decrease whereas the *m/z* 58 ion abundance increases with increasing concentration and time of irradiation. This is substantiated by plotting the ratio of the intensity of the *m/z* 58 peak to that of the *m/z* 319 peak with respect to time at different concentrations, as shown in Fig. 3. Therefore, a correction factor was introduced in the percentage dehalogenation expression in order to take into account the increased fragmentation of *m/z* 285 and *m/z* 319 ions with time:

$$R(\%) = \frac{100[M_H + H]^+}{[M_H + H]^+ + [M_X + H]^+}\left(\frac{I_{58}}{I_R}\right) \quad (2)$$

where *R* represents the percentage dehalogenation corrected by the ratio of I_{58} (the actual intensity of the *m/z* 58 peak) to I_R (the average intensity of the *m/z* 58 peak at the lowest concentration, i.e. where the amine side-chain fragmentation is lowest). The correction factor compensates for the increased fragmentation of the amine side-chain which alters the calculated percentage dehalogenation by distorting the measured peak intensities of $[M_H + H]^+$ and $[M_X + H]^+$. This is due to the fact that M_H is most likely formed at the surface and thus more exposed to the beam, causing more rapid decomposition than the parent molecule, M_X. The corrected data for *R* are shown in Fig. 4 and indicate that,

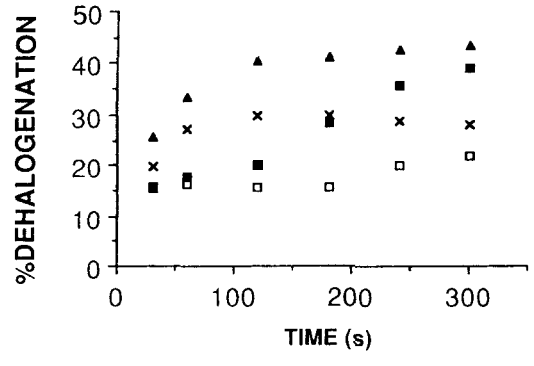


Figure 2. Percentage dehalogenation of chlorpromazine at various concentrations in glycerol as a function of time of analysis. Concentration: (□) 0.0068; (■) 0.011; (▲) 0.037; (×) 0.097 M.

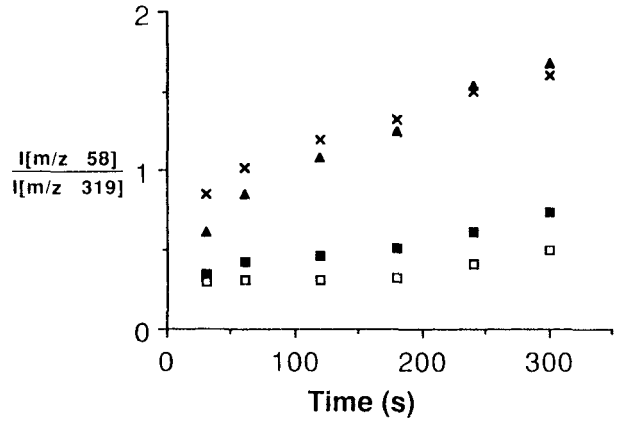


Figure 3. Ratio of relative intensity of the amino side-chain fragment *m/z* 58 peak to the intensity of the protonated molecular ion *m/z* 319 peak as a function of time of analysis and concentration. Concentration: (□) 0.0068; (■) 0.011; (▲) 0.037; (×) 0.097 M.

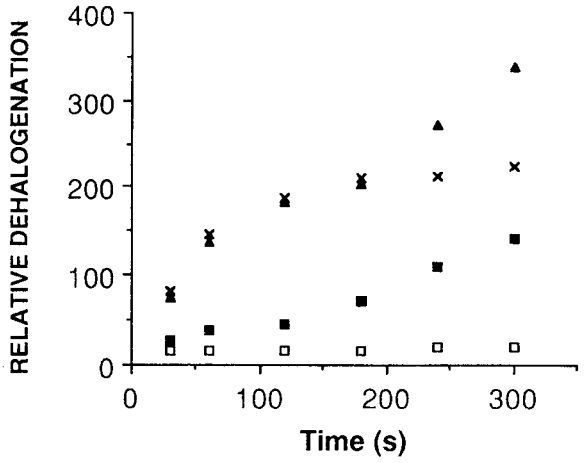


Figure 4. Relative dehalogenation of chlorpromazine at various concentrations in glycerol as a function of time of analysis. Concentration: (□) 0.0068; (■) 0.011; (▲) 0.037; (×) 0.097 M.

generally, dehalogenation increases with increasing time of irradiation and concentration. The fact that the plots representing the two higher concentrations coincide up to 3 min probably illustrates that the analyte is at its maximum surface concentration at 0.037 M. Of course, we are now dealing with relative values of dehalogenation, which merely serve the purpose of illustrating the importance of the amine side-chain fragmentation with respect to the dehalogenation process.

In order to compare the results obtained for chlorpromazine and allow perhaps more general conclusions to be drawn on the dehalogenation process, a second compound known to undergo dehalogenation was investigated. The effect of time on percentage dehalogenation for varying concentrations of 4-chlorophenylalanine ethyl ester in glycerol under standard conditions is shown in Fig. 5. The most striking feature of Fig. 5 is the similarity of the profiles for different concentrations. This is in marked contrast with the results

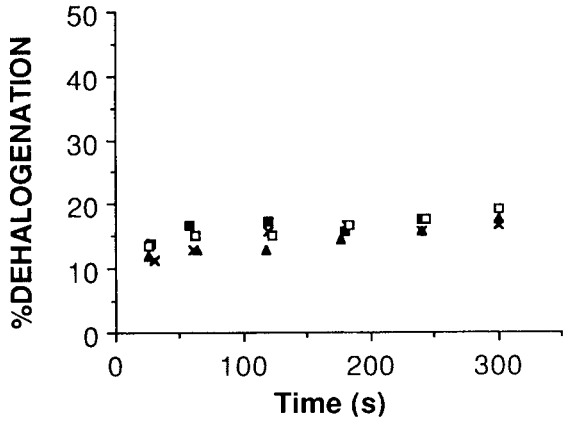


Figure 5. Percentage dehalogenation of 4-chlorophenylalanine ethyl ester at various concentrations as a function of time of analysis. Concentration: (□) 0.0068; (■) 0.011; (▲) 0.037; (×) 0.097 M.

obtained for chlorpromazine under similar conditions. The range of dechlorination values obtained was 15–50% for chlorpromazine and 12–18% for 4-chlorophenylalanine ethyl ester under standard conditions. If one assumes that similar dehalogenation processes are involved for the two analytes, a tentative explanation of the differences in percentage dehalogenation observed with varying time of irradiation and concentrations can be suggested on the basis of their respective surface activity and/or solvation in glycerol. Since chlorpromazine can be assumed to be more hydrophobic and thus more surface active, it is expected to be more affected by concentration changes relative to 4-chlorophenylalanine ethyl ester. Its propensity as compared with 4-chlorophenylalanine ethyl ester will be to migrate to the surface of the droplet where reactive species (radicals, ions and thermal electrons) are locally being generated by the bombarding cesium ions, hence increasing the probability of the analyte undergoing reaction. For opposite reasons, 4-chlorophenylalanine ethyl ester does not exhibit marked time or concentration effects since it is less surface active and better solvated. This appears to be confirmed by the presence of glycerol adducts in the spectra of 4-chlorophenylalanine ethyl ester, which are absent in the chlorpromazine mass spectra at all concentrations studied.

This kinetic rationalization of the differences observed between the two compounds is also in line with the trends observed in our experiments on the effect of beam flux on dehalogenation, which is discussed in the next section. The effect of surface activity has been demonstrated in a study of the beam-induced reduction of oximes to the corresponding imines.[13] It was found that an increased surface concentration of the analyte resulted in a greater abundance of the reduced species in the FAB mass spectrum. Surface activity was also invoked to rationalize the beam-induced generation of anomalous $[M - H]^+$ ions in the FAB mass spectra of cyclic acetals.[24]

Beam density effect

An increase in beam density results in a corresponding increase in the percentage dehalogenation of chlorpromazine as illustrated in Fig. 6. This phenomenon can be rationalized by the greater number of impacts per unit area per unit time, thus creating an increase in the concentration of reactive species such as ions, radicals and electrons formed which may be ultimately responsible for beam-induced reactions. The effect of flux on the extent of reduction of organic dyes shows the same trend.[10,25]

For 4-chlorophenylalanine ethyl ester, the extent of the flux effect also seems to be significantly reduced compared with chlorpromazine, as can be seen from Fig. 7. This difference in the beam density effect on the dehalogenation behavior of chlorpromazine and 4-chlorophenylalanine ethyl ester can be rationalized along the lines of the surface activity/solvation argument suggested in the previous section. Thus, the surface concentration of the compounds appears to be

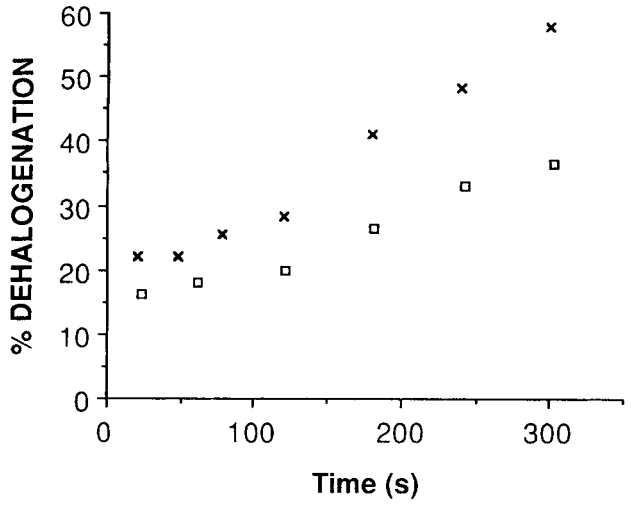


Figure 6. Percentage dehalogenation of chlorpromazine (0.0068 M in glycerol) at different beam densities as a function of time of analysis. Beam density: (□) 0.027; (×) 0.042 μA mm^{-2}.

an important factor affecting their dehalogenation behavior since the more surface-active compound, chlorpromazine, has a greater rate of dechlorination.

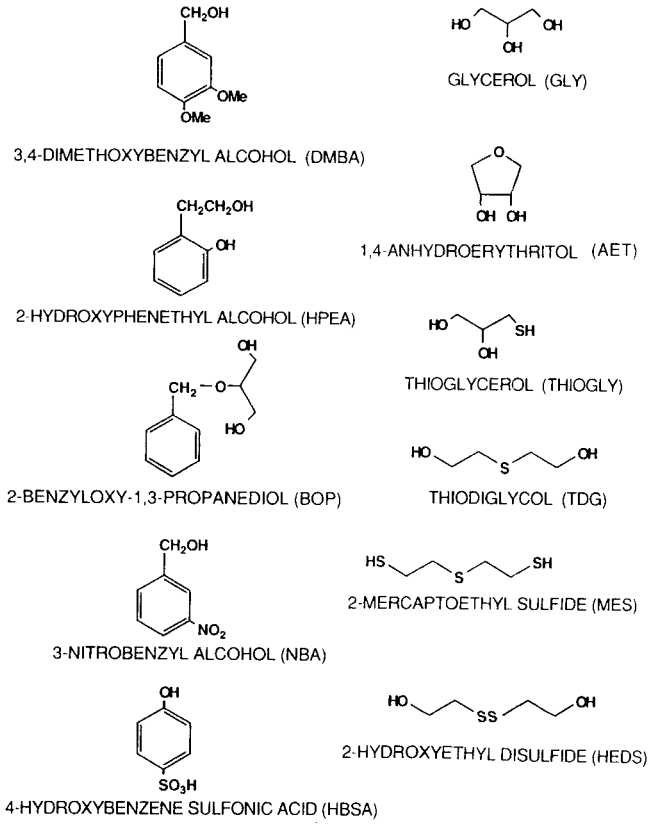


Figure 8. Structure of the liquid matrices.

Effect of matrices

Given the wide variety of liquid matrices available for FAB/LSIMS and the importance of matrix chemistry in effecting or inhibiting beam-induced reactions, the liquid secondary ion mass spectrum of chlorpromazine was obtained in eleven different matrices (Fig. 8) with standard beam conditions. The utilization of different matrices should give a greater insight into the dehalogenation process. The elaboration of a rough matrix structure–dehalogenation inhibiting tendency correlation might identify some important features of matrix chemistry. Three new matrix compounds not previously used in FAB/LSIMS studies were introduced in this work: AET, MES and BOP.

The resulting percentage dehalogenation values obtained are given in Table 1. From the data, it appears that the matrices can be grouped in three distinctive categories in terms of their ability to inhibit the dechlorination process. Gly and AET stand out as the least effective matrices of the eleven matrices used. Thiogly, TDG, MES, BOP, DMBA and HPEA form another

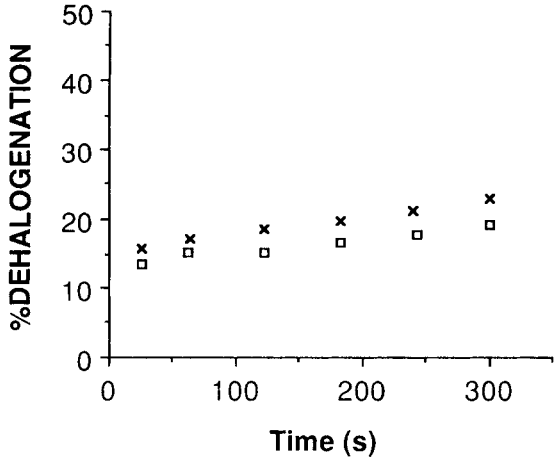


Figure 7. Percentage dehalogenation of 4-chlorophenylalanine ethyl ester (0.0068 M in glycerol) at different beam densities as a function of time of analysis. Beam density: (□) 0.027; (×) 0.042 μA mm^{-2}

Table 1. Effect of matrix selection on the dehalogenation of chlorpromazine at a concentration of 0.037 M[a]

Matrix	Dehalogenation (%)	Matrix	Dehalogenation (%)
Gly	34	HPEA	4
AET	17	MES	2
Thiogly	7	HEDS	0
TDG	7	HBSA	0
DMBA	5	NBA	0
BOP	4		

[a] Average of first 3 min of analysis.

group where dehalogenation is substantially reduced with respect to glycerol and AET but is still present. The last group consists of NBA, HBSA and HEDS. The spectra obtained with these matrices show complete suppression of the dechlorination process. These results suggests that the dehalogenation of chlorpromazine can be drastically reduced by matrix selection. Similarly, 4-chlorophenylalanine ethyl ester did not undergo dehalogenation in the last category of matrices.

It has been proposed that radicals, electrons, ions and excited species are produced in the matrix under FAB/LSIMS conditions. Of these species, radicals[26] and/or electrons[5,27] are thought to be involved in reduction processes. Sehti and co-workers[18] invoked the resemblance of beam-induced dehalogenation with that of radiolysis studies where reactions are initiated by the solvated electrons. Williams *et al.*[27] suggested a mechanism to account for the occurrence of dehalogenation in the FAB/LSIMS of organic compounds. The premise of this proposed mechanism is that keV particle impacts cause the production of electrons (via ionization of the sample or matrix) and that these electrons can be effective reducing agents once they have reached thermal energies. Subsequent capture of these beam-generated electrons by the analyte ultimately causes reductive dehalogenation. Hence, the use of matrices having a capability to scavenge such thermal electrons (or other reactive species) should inhibit reductive processes such as dehalogenation. This capacity can probably be related to the electron affinity of the matrix. Of course, the possibility that reactive species generated by the bombardment process other than electrons might contribute to dehalogenation cannot be ruled out.

Since there is a wealth of data on the electron affinity behavior of aromatic compounds, we can rationalize our results with the aromatic matrices on that basis to support the dehalogenation mechanism based on beam-generated thermal electrons.[27] The dehalogenated ion of chlorpromazine is present in mass spectra obtained with DMBA, BOP and HPEA. These molecules consist of aromatic rings with electron-donating groups which should reduce the electron-scavenging capability of the molecule. The dehalogenation process is completely inhibited in NBA and HBSA, molecules that contain aromatic rings with electron-withdrawing groups which should increase the electron-scavenging capabilities of the molecule. The above interpretation is, of course, limited, but in the absence of electron affinity (or other) data it is not possible to offer a rationalization of a higher order. Kebarle and Chowdhury[28] have shown that the electron affinity of an aromatic compound substituted with an electron-withdrawing substituent will be greater than that for a compound which is substituted with an electron-donating substituent. It is important to note that electron affinities (a gas-phase property) appear to follow the same general trend as polarographic half-wave reduction potentials (a condensed-phase property).[29,30]

The ability of NBA to inhibit reduction processes in FAB/LSIMS, including dehalogenation, is well documented.[9,10,19,31,32] Miller *et al.*[32] suggested that this property of NBA is related to the fact that the matrix can act as an electron sink and thus mitigate chemical damage. Interestingly, a recent report[33] indicated that even for NBA the reduction-inhibiting capability was limited. It should be stressed that these results were obtained with organometallic compounds. The reduction-inhibiting efficiency of HBSA (the matrix itself is a 60% aqueous HBSA solution) has previously been compared and found to be similar to that of NBA in a study of the reduction of disulfide bridge-containing peptides.[34]

However, a dehalogenation mechanism based on beam-generated thermal electrons is of limited usefulness for rationalizing the data obtained with the aliphatic matrices, since little is known about their electron affinities or the correlation between their structural features and that physical property. Further, low-level theoretical calculations for the electron affinities of Gly and Thiogly give large negative values, which implies rather convincingly that these molecules are unlikely to scavenge electrons.[19] The lower extent of dehalogenation observed in sulfur-containing matrices may be explained by the bombardment-induced formation of thiyl radicals, which are known to have oxidizing properties.[35] Sulfur compounds are known to be involved in the protection mechanism for biological systems subjected to ionizing radiation or other forms of free radical damage.[36] That sulfur plays a role in inhibiting the dehalogenation process appears undeniable since MES, has three sulfur atoms and no other heteroatom, exhibits the lowest non-zero dehalogenation value of all the matrices used in this study. In fact, if one considers the aliphatic matrices used in this study, sulfur-containing matrices (Thiogly, TDG, MES, HEDS) consistently exhibit considerably lower dehalogenation values than the matrices where sulfur is absent (Gly, AET).

The results obtained in this study correlate with the reported reduction-inhibiting properties of the respective aliphatic matrices. The highest extent of dehalogenation obtained when Gly is the matrix corroborates what one generally finds in the literature, namely that Gly is the matrix in which reductive processes have the greatest tendency to occur.[8–10] The structural similarities between AET and glycerol explain the high percentage dehalogenation value obtained with the former matrix. Watson and Musselman[37] noted the diminished tendency of reductive processes to occur in Thiogly compared with Gly. The propensity of TDG to reduce the occurrence of beam-induced reductions (including dehalogenation) relative to Gly has been reported for a series of substituted thiopyrilium and pyrillium salts.[11] A study[9] on the effect of matrices in the beam-induced reduction of organic dyes revealed that HEDS is the most effective aliphatic matrix in inhibiting reduction. In terms of the ability of the matrix to inhibit reductive processes, our results are generally in agreement with those of Kyranos and Vouros,[9] who found that for the reduction of organic dyes the order was NBA > HEDS > Thiogly > Gly, whereas we found that the dehalogenation inhibiting ability of the matrices to be NBA, HBSA, HEDS > DMBA, HPEA, BOP, MES > TDG, Thiogly > AET, Gly. The two trends are essentially the same apart from HEDS. This can be rationalized by the suggestion that the propen-

sity of organic dyes to be reduced in this matrix is greater than that of chlorpromazine to undergo dehalogenation.

In the event, however, that Gly must be used as the liquid matrix, dehalogenation can be reduced using doping agents such as TFA and NBA. The results in Table 2 corroborate this as dehalogenation is reduced by the insertion of TFA or NBA in Gly. The usefulness of TFA as an effective additive to inhibit reduction in the FAB/LSIMS analysis of disulfide-bridged peptides has been reported by Visentini *et al.*[38] The reduction-inhibiting effect of TFA was rationalized by invoking its electron/radical scavenging properties. Interestingly, addition of TFA to a glycerol–chlorpromazine solution caused the color of the solution to change from clear to magenta. It has been shown that TFA possesses light-dependent oxidative electron-transfer capabilities.[39] Further, the effect of TFA doping on the oxidation of organic compounds in ESI mass spectrometry has been investigated.[40] It was found that solvent systems containing TFA yielded the radical cation of polyaromatic hydrocarbons in the ESI mass spectrum. It therefore appears that TFA has oxidative properties as a solvent or solvent additive. It should be mentioned that the inhibiting effect of the additives, particularly TFA, is somewhat transient and decreases significantly after 2 min. The greater effectiveness of NBA as a dehalogenation-inhibiting additive is probably related to its lower vapor pressure and more effective electron/radical-scavenging capacity.

With a 50:50 Gly–NBA solution, dehalogenation was not observed in the first 3 min of analysis. This allowed the following experiment to be performed. A 0.037 M solution of chlorpromazine in Gly (1 μl) was subjected to 2 min of beam irradiation. The sample was withdrawn from the mass spectrometer and 1 μl of NBA was added. On re-analysis of this sample, a sizable peak corresponding to the dechlorinated ion of chlorpromazine was observed. Since dechlorination of chlorpromazine was shown not to occur in 50:50 NBA–Gly solutions under cesium ion bombardment, one is led to conclude that the observed ion at *m/z* 285 can only be explained as a residue generated by the irradiation of the Gly solution prior to NBA addition. The result of these experiments suggests that dechlorination could, at least in part, occur in the condensed phase. This is further substantiated by the fact that no dechlorination of the type observed in FAB/LSIMS, $[MH - Cl + H]^+$, has been reported in the ammonia and methane Cl mass spectra of chlorpromazine.[41] However, it is understood that the use of Cl data to rule out a gas-phase mechanism in FAB/LSIMS rests on the assumption that the conditions are similar. The ESI mass spectrum of chlorpromazine obtained in a water–methanol–HCl solution showed no significant extent of dehalogenation. The absence of dehalogenation in the ESI mass spectrum of chlorpromazine further implicates the beam-induced nature of the process.

Despite mounting evidence indicating that matrix chemistry is the primary parameter which influences beam-induced reductions, there has been little effort to relate the physical properties of the matrix to the observed extent of reduction. For rationalizing beam-induced reductive processes, matrix physical properties such as electron affinity and reduction potential would be most useful. The physical properties of matrices used in FAB/LSIMS have been collected.[42] However, it was admitted that the relative obscurity of some important matrix compounds have left important gaps in the tabulation. It is very possible that the answer does not lie in the physical properties of the individual matrix molecules *per se* and is in fact much more complex, given the dynamic nature of the FAB/LSIMS technique, involving various kinetic and thermodynamic factors that are highly system dependent. Although the present work by no means alleviates this lack, it does further support the contention that matrix chemistry is of overwhelming importance in minimizing undesirable beam-induced processes. Further, it is shown that steps can be taken to effect such a minimization.

Table 2. Effect of doping agents on the dehalogenation of chlorpromazine in glycerol (0.037 M)[a]

Matrix	Dehalogenation (%)	Matrix	Dehalogenation (%)
Gly	34	Gly–1% TFA	10
Gly–1% NBA	9	Gly–5% TFA	9
Gly–3% NBA	2	Gly–10% TFA	8
Gly–10% NBA	1	Gly–15% TFA	4
Gly–15% NBA	0		

[a] Average of the first 2 min of analysis.

CONCLUSION

The FAB/LSIMS parameters which influence the dehalogenation of chlorpromazine have been characterized. Increasing the beam density, concentration of the analyte and time of irradiation resulted in an increase in dehalogenation for chlorpromazine in glycerol. The markedly different effects of time, concentration and beam density on the dehalogenation of chlorpromazine and 4-chlorophenylalanine ethyl ester in glycerol were observed and rationalized in terms of their respective surface activity and extent of solvation by the glycerol matrix.

The effect of the matrix on the extent of observed dehalogenation was related to its electron/radical-scavenging properties. In some cases, proper matrix selection totally inhibited the process of beam-induced dehalogenation. It has been shown that HBSA and HEDS can be as effective as NBA in this respect. This broadens the scope of 'reduction-inhibiting' matrices available to the mass spectrometrist. Three new matrices were used in this study. The ability of BOP and AET to inhibit the dehalogenation of chlorpromazine was coherent with that of structurally similar compounds such as HPEA and Gly, respectively. The new matrix compound MES showed the lowest non-zero dehalogenation value. This result is intriguing as MES is the only matrix to contain three sulfur atoms without

any other heteroatom. The role of sulfur in inhibiting dehalogenation is not clear, but it seems indisputable as sulfur-containing matrices substantially diminish the occurrence of dehalogenation relative to glycerol. When added to glycerol, doping agents such as NBA and TFA markedly reduced dehalogenation. The role of these additives could be very useful when glycerol cannot be circumvented as the matrix of choice.

Generally, the bulk of our results are in agreement with recently published work[10] investigating the effect of FABMS parameters on the reduction of organic dyes, namely that beam density and matrix selection are important parameters in affecting the occurrence of reduction processes. Our results confirm the overwhelming importance of matrix chemistry where a minimization of reductive processes and thus artifacts is desired.

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