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INTEGRATED PAPER

Fast Atom Bombardment Mass Spectrometry: Matrix Effects on Ion Formation and Fragmentation

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Matrix effects on the competitive formation and extents of fragmentation of $M^{+\cdot}$ and $[M+H]^+$ ions produced under fast atom bombardment (FAB) conditions, and on the formation of complex $[M+matrix+H]^+$ and $[M+matrix-H]^-$ ions under FAB conditions have been described using various organic compounds. The use of hydrophilic matrices such as glycerol and thioglycerol resulted in the preferential formation of $[M+H]^+$ to $M^{+\cdot}$ ions compared to the use of hydrophobic matrices such as *m*-nitrobenzyl alcohol and *o*-nitrophenyloctyl ether. The use of alcoholic-hydrophilic matrices such as glycerol and pentamethylene glycol resulted in the preferential formation of $[M+H]^+$ to $M^{+\cdot}$ ions compared to the use of thiol-containing hydrophilic matrices such as thioglycerol and dithiothreitol. The latter matrix effect was explained on the basis of the distinction of hydrogen-bonding ability between hydroxyl -OH and thiol -SH groups, and it was proposed that the hydrogen-bonding interaction between the hydroxyl group(s) of matrix and the basic site(s) of analyte molecules in matrix solution, which was named as a *'quasi-preformed state'*, is advantageous for the formation of $[M+H]^+$ to $M^{+\cdot}$ ions. It was confirmed that the fragmentation of $[M+H]^+$ and $M^{+\cdot}$ ions produced under FAB conditions occurs independently to each other and that the extent of fragmentation of $M^{+\cdot}$ ions is often comparable to that of fragmentation of $M^{+\cdot}$ ions formed at 70 eV or above in the electron impact ionization method. The extent of fragmentation of $M^{+\cdot}$ ions under FAB conditions depended upon the matrix materials used. Further, the extent of fragmentation of $[M+H]^+$ ions under FAB conditions was larger than that of fragmentation of $[M+H]^+$ ions formed by the isobutane chemical ionization method. The complex ions, $[M+matrix+H]^+$ and $[M+matrix-H]^-$, were formed by the use of a certain prenylated flavone and thiol-containing matrices, while the complex ions were not formed by the use of the flavone lacking a prenyl group or glycerol matrix. The suitability of matrix materials for positive- and negative- ion FAB mass spectrometry has been discussed.

1. Introduction

The technique of fast atom bombardment mass spectrometry (FAB-MS), using glycerol (G) as a liquid matrix, was first reported by Barber *et al.* (received 19 February 1981, accepted 10 April 1981)[1] and Williams *et al.* (received 3 April 1981).[2] Although the use of matrix is essential to the FAB analysis of organic compounds, the introduction of matrix materials into the FAB technique has an interesting historical background.

The FAB technique using fast neutral atoms, typically of Ar of 2~8 keV, was initially developed by the group of Vickerman, Bordoli, and Barber of the University of Manchester Institute of Science and Technology, as a new ion source in the place

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of static secondary ion mass spectrometry (SIMS), and then the so-called solid FAB technique was successfully applied to bio-organic compounds such as amino acids,[3] methionyl-lysyl-bradykinin, oligosaccharides, peptides, glycosides, and others.[4] These pioneering papers of Vickerman *et al.*[3] and of Barber *et al.*[4] were received on 23 December 1980 and on 19 January 1981, respectively. The idea leading to the use of matrix has been described in an epoch-making report of Barber *et al.*, received on 25 February 1981 and accepted on 3 July 1981.[5] They have described that "the FAB ion source can accommodate liquids", "the oil produced a 'non-fading' spectrum without modified sample preparation", and "we have, however, largely overcome this effect by judicious use of solvent and support systems, paying particular attention to the viscosity and volatility of the medium from which the sample is deposited on the stage." The word 'medium' in the last sentence seems to be consistent with the concept of liquid matrix. They have noticed empirically that low vapor pressure oily samples and the common pumping fluids such as apiezon and santovac 5 lasted in the measurement time, as described later.[6] Immediately after the epoch-making paper was published, the FAB technique using glycerol matrix was applied to the peptide sequence analysis and structure determination of bleomycins by Barber and co-workers[7,8] and it was pointed out by Barber *et al.* and other groups that the addition of surfactants[6] such as triton X 100 and mono-[2-(4,4-dimethylpentyl)-5,12,12-trimethyl-1-octyl]-2,2,3,3,4,4,-hexafluoroglutarate or oxalic acid[9] in glycerol solution is important to increase the molecular related ion yields.

Although many workers had used G matrix for their FAB and liquid SIMS studies in the early stage,[10] it was pointed out that the material G is not a universal matrix.[11] In fact, to prepare various different matrix materials and to select a suitable matrix for a given compound are essential for superior FAB and liquid SIMS experiments, as summarized by some workers.[12] Some criteria for the choice of matrix have been described,[12a,b] although a definite criterion for the estimation of matrix capabilities to ionize analytes and to spatter ions formed has not yet been presented. It is an exciting theme to find such a criterion, and it should be presented on the basis of the mechanism of ion formation under FAB conditions and of the extensive experience.

We have reported so far on the matrix preparation,[12d,13] matrix effects,[14] fragmentation,[15] matrix-initiated degradation,[16] ion formation mechanism,[17] and collision-induced dissociation (CID) of adduct ions[18] in the FAB-MS using liquid matrix. It was always important for the success of those studies to select suitable matrices for given compounds and purposes. In particular, the matrix effects were of interest in connection with the FAB ionization processes. That is, the extent of fragmentation of $M^{+\cdot}$ ions formed[14] and the rate of formation of $M^{+\cdot}$ and $[M+H]^+$ ions[17a,c,e] markedly varied with the change of matrix. For the understanding of FAB ionization mechanism(s), it is essential to examine the matrix effects on the ion formation and fragmentation under FAB conditions. In this paper, we will describe the contents on the matrix effects reported so far, together with the latest related results. The detailed mechanism of ion formation under FAB conditions will be described in the sequel to this report, in the near future.

2. Experimental

The FAB experiments were performed on a JEOL JMS-DX303 double-focusing mass spectrometer equipped with a JMA-DA5100 data system (JEOL Ltd, Tokyo, Japan). The fast-atom argon or xenon beam used was generated from Ar^+ or Xe^+ ions which were accelerated to 5 kV. The FAB gun emission current was 10 mA. The liquid matrices used were glycerol (G), thioglycerol (TG), pentamethylene glycol (PMG), *m*-nitrobenzyl alcohol (NBA), *o*-nitrophenyloctyl ether (NPOE), diethanolamine (DEA), triethanolamine (TEA), dimethylsulfoxide (DMSO), a 1 : 3 (v/v) or 1 : 2 (v/v) mixture of dithiothreitol (DTT) and TG referred to as DTT/TG13 or DTT/TG12,[12d] a 1 : 1 (v/v) or 2 : 3 (v/v) mixture of G and TG (G/TG11 or G/TG23), and a 1 : 4 (v/v) mixture of sulfolane (SULF) and TG (SULF/TG14). Sample of *ca.* 40 μg was taken up into the tip of a 10 μl micropipet (Drummond Sci. Co., Pennsylvania, USA), and then matrix of 5 μl was introduced to the micropipet through capillary action. For the experiments, approximately 2 μl of the matrix solution was loaded onto a stainless steel tip.

The electron impact ionization (EI) and chemical ionization (CI) experiments were performed on the above JEOL JMS-DX303 system attached an EI and CI ion sources, respectively. The CI reagent gas used were isobutane, and the gas pressure, as read by a gauge attached to the pumping line for the ion source, was typically at 1.5×10^{-5} Torr. The B/E-constant linked-scanning experiments for metastable ion (MI) and collision-induced dissociation (CID) spectra were done without and with helium gas collisions in the first field-free region, respectively, using the above JMS DX-303 mass spectrometer. Tandem CID mass spectra were obtained with a JEOL HX110/HX110 four-sectors machine. The pressure of the collision gas was adjusted to reduce the parent ion beam intensity by 50%.

3. Results and Discussion

3.1 Matrix effects

As shown in positive ion FAB mass spectra of morusin (**1**)[19] and trolox (**2**)[20] obtained with DTT/TG12 and NBA matrices (Figs. 1 and 2), we can observe two matrix effects that the peak intensity ratios of $I(M^{+\cdot})/I([M+H]^+)$ (matrix effect *I*) and the extents of fragmentation of $M^{+\cdot}$ and $[M+H]^+$ ions (matrix effect *II*) are affected by the change of matrix from DTT/TG12 to NBA. The assignments of some fragments for **1** and **2** are shown in Scheme 1. The matrix effects *I* and *II* can be related to the mechanism of competitive formation of $M^{+\cdot}$ and $[M+H]^+$ ions and the internal energy contents of $M^{+\cdot}$ and $[M+H]^+$ ions produced under FAB conditions, respectively. In this section, we will describe those matrix effects in detail. In all of the FAB spectra, an asterisk indicates the peaks originating from the matrix used. The structures of various matrix materials will be shown in the later Section 4.

3.1.1 Competitive formation of $M^{+\cdot}$ and $[M+H]^+$ ions

Matrix effect I

It has been reported by some workers that positive ion FAB spectra of certain oily samples and organic compounds give predominant peaks corresponding to odd electron molecular ions, $M^{+\cdot}$, as well as protonated molecules $[M+H]^+$, even when liquid matrix

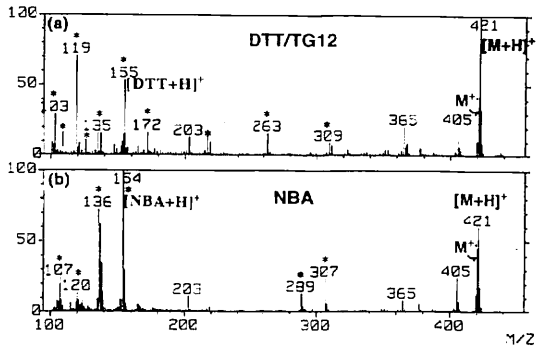


Fig. 1. Positive ion FAB mass spectra of morusin (**1**) obtained with (a) DTT/TG12 and (b) NBA matrices.

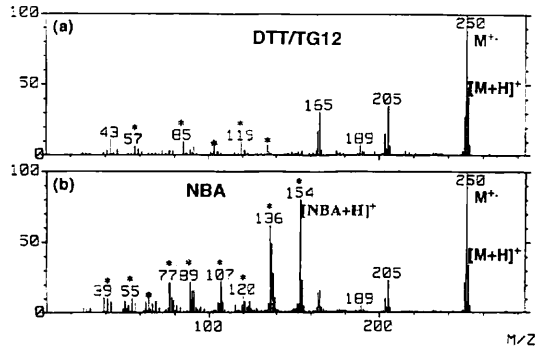
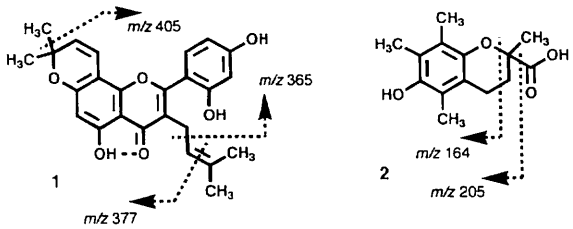


Fig. 2. Positive ion FAB mass spectra of trolox (**2**) obtained with (a) DTT/TG12 and (b) NBA matrices.



Scheme 1.

is used,[5), 6), 9), 13c), 17c), e), 21)] although the detailed mechanism has not yet been presented. It has been known in connection with matrix effect *I* that the change of matrix from relatively hydrophilic systems such as G, PMG, TG, and DTT/TG13 to hydrophobic systems such as NBA and NPOE increases the peak intensity ratios of $I(M^{+\cdot})/I([M+H]^+)$ for many different compounds.[17a), c), e), 22)] The ratios for the compounds examined with DTT/TG13 (NBA) were 1.35 (3.01) for moracin M, 4.43 (9.92) for mulberrofuran B, 4.76 (12.5) for gancaonin I, 1.80 (3.57) for dihydropyran derivative, 1.60 (2.44) for licoricidin, 0.63 (1.92) for *N*-allylaniline, 2.35 (4.42) for safrol, 0.11 (0.44) for *p*-bromoacetanilide, 0.12 (0.34) for antipyrine, 0.11 (0.39) for sulphatiazole, 0.83 (1.89) for medicarpin, 1.39 (2.56)

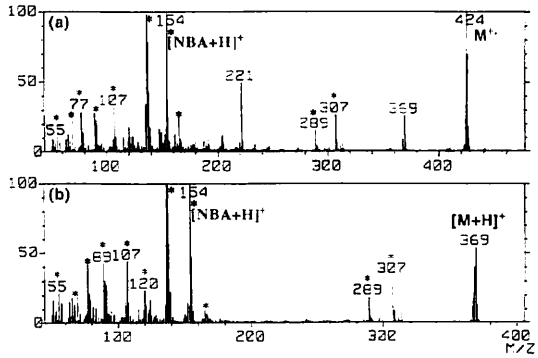
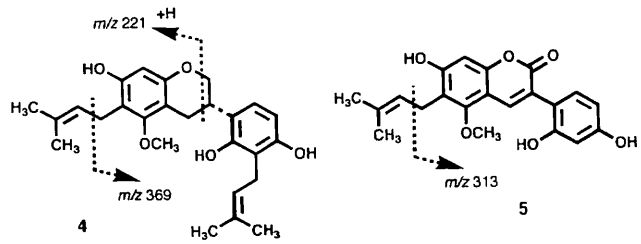


Fig. 3. Positive ion FAB mass spectra of (a) licoricidin (4) and (b) glycycoumarin (5) obtained with NBA matrix.



Scheme 2.

Table 1. Relative Intensity Ratios, $I([M+H]^+)/I(M^+)$, Obtained from the FAB Mass Spectra Measured before and after the Acetylation of Trolox (2) and Simple Aniline Compounds Using DTT/TG13 Matrix

Compounds	$I([M+H]^+)/I(M^+)$	
	Before	After
Trolox	0.33 (67.2)	0.96 (444)
o-Carboxyaniline	2.27 (276)	17.8 (265)
m-Carboxyaniline	2.52 (81.8)	27.9 (676)
p-Carboxyaniline	2.47 (310)	22.9 (588)
p-Methoxyaniline	3.88 (683)	3.83 (1098)
m-Aminobenzyl alcohol	1.56 (295)	6.30 (156)
3-Hydroxyl-4-methoxyaniline	1.94 (310)	5.75 (811)

Values in parenthese are absolute intensities of $[M+H]^+$ ions as printed in the FAB spectral data.

for kazinol O, 0.95 (2.78) for gancaonin R, 1.16 (2.78) for mulberrofuran A, 0.41 (1.11) for cudraflavone A, 0.30 (0.87) for kuwanon C (3), 0.68 (1.72) for mulberrofuran G, and 1.61 (3.13) for calcomoracin. The chemical structures of analytes used have been described elsewhere.[17a), c), e), 19)]

Effects of carbonyl group(s) on $[M+H]^+$ formation

Although the ratios obtained above suggest that the use of NBA is advantageous for $M^{+\cdot}$ ion formation, it is important to recognize that the formation of $M^{+\cdot}$ and $[M+H]^+$

ions is mainly governed by rather the analyte structural characteristics such as the presence or absence of proton acceptors, and the gas-phase thermochemical properties such as proton affinity (PA), gas-phase basicity (GB), and ionization potential (IP) than those properties of matrix. In fact, the GB is a most important factor for $[M+H]^+$ formation, and it governs the proton transfer reaction for $[M+H]^+$ formation under FAB conditions.[23] Further, the presence of carbonyl group(s) as a significant proton acceptor in analyte molecules acts advantageously for the formation of $[M+H]^+$ and hinders the $M^{+\cdot}$ formation.[17c), e), 21g)] It has been known from a molecular orbital calculation that the carbonyl oxygen has a significant proton acceptability compared with hydroxyl and ether oxygens.[24] Figure 3 is the FAB spectra of licoricidin (**4**)[19] lacking carbonyl group and glycycoumarin (**5**)[19] having a carbonyl group obtained with NBA matrix. The structures of these compounds are shown in Scheme 2. Further, the introduction of carbonyl group(s) into simple phenol and aniline compounds due to acetylation markedly increased the ratios of $I([M+H]^+)/I(M^{+\cdot})$ and resulted in enhanced emission of $[M+H]^+$ ions. The results obtained for some compounds are summarized in Table 1. The mechanism of $[M+H]^+$ formation under FAB conditions can be explained by the proton-transfer reactions in gas-phase as follows,[12d), 23b~d), 25)]

$$B+B \rightarrow [B+H]^+ + [B-H]^- \quad (1.1)$$

$$M+[B+H]^+ \rightarrow [M+H]^+ + B \quad (1.2)$$

$$M+B \rightarrow [M+H]^+ + [B-H]^- \quad (2.1)$$

$$M+B \rightarrow [M-H]^- + [B+H]^+ \quad (2.2)$$

where M and B represent gas-phase analyte and matrix molecules, respectively. It can be understood from the reactions (1.1)~(2.2) how important properties the PA and GB of analyte and matrix molecules are. The detailed mechanism for the formation of various molecular-related ions such as $M^{+\cdot}$, $[M+H]^+$, $[M+Na]^+$, and others under FAB conditions will be described elsewhere.

Influence of solvation on ion formation

On the other hand, it is also important to consider a solution chemistry such as solvent/solute interaction, as pointed out by Dass *et al.*[21h)] In fact, the solubility of analyte molecules into a liquid matrix, which relates to solvations such as hydrophilic and hydrophobic interactions between M and B molecules, is a most important criterion for the choice of matrix, and the success of FAB experiments is often governed by the solubility. Further, the importance of solution chemistry on the ion formation under FAB conditions has been pointed out by some workers.[10j), 26)] Caprioli has described that the FAB ionization process is sensitive to subtle ionic changes in a matrix solution and the FAB technique is useful for the direct study of solution chemistry.[10j)]

The matrix effect I here seems to reflect the solution chemistry related to intermolecular interactions between M and B molecules. Figures 4 and 5 are partial FAB spectra of compounds **1** and **2**, respectively, obtained with the matrix systems that are alcoholic-hydrophilic such as G and PMG (group 1), thiol-containing hydrophilic such as TG and DTT/TG12 (group 2) and hydrophobic such as NBA and NPOE (group 3) in nature. The spectra were normalized in the intensity of ions at *m/z* 421 for **1** and *m/z* 250 for **2** as the base peak. The $I([M+H]^+)/I(M^{+\cdot})$ ratios calculated from the spectra are summarized in Table 2. The ratio for **2** with NPOE matrix was estimated by considering

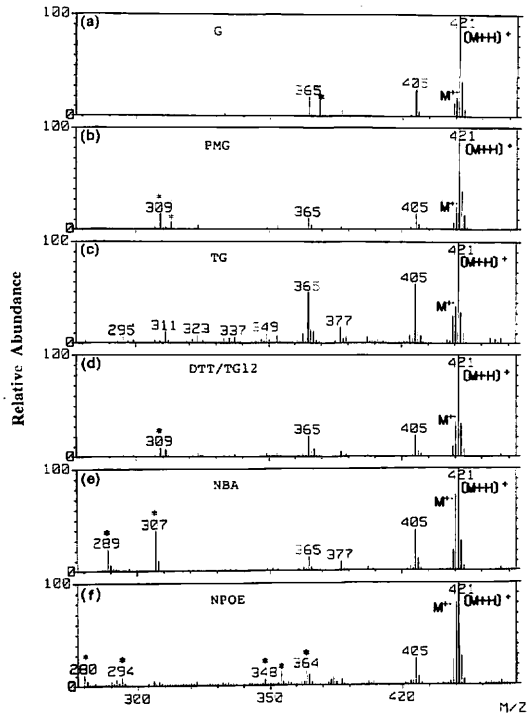


Fig. 4. Positive ion partial FAB mass spectra of morusin (1) obtained with (a) G, (b) PMG, (c) TG, (d) DTT/TG12, (e) NBA, and (f) NPOE matrices.

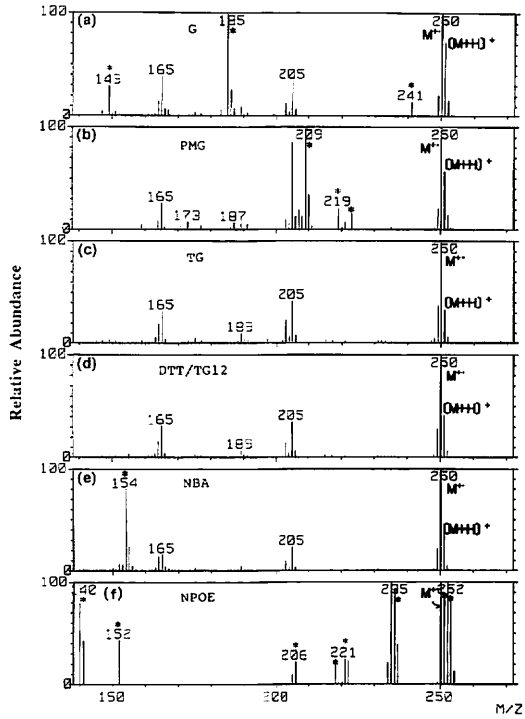


Fig. 5. Positive ion partial FAB mass spectra of trolox (2) obtained with (a) G, (b) PMG, (c) TG, (d) DTT/TG12, (e) NBA, and (f) NPOE matrices.

Table 2. Relative Intensity Ratios, $I([M+H]^+)/I(M^{+\cdot})$, Obtained from the FAB Mass Spectra of Morusin (**1**) and Trolox (**2**), Using Matrix Systems That Are Alcoholic-Hydrophilic (Group 1), Thiol-Containing Hydrophilic (Group 2), and Hydrophobic (Group 3) in Nature

Compounds	$I([M+H]^+)/I(M^{+\cdot})$					
	Group 1		Group 2		Group 3	
	G	PMG	TG	DTT/TG12	NBA	NPOE
Morusin	5.26	4.76	2.78	2.94	1.33	1.23
Trolox	0.71	0.57	0.32	0.41	0.34	0.15

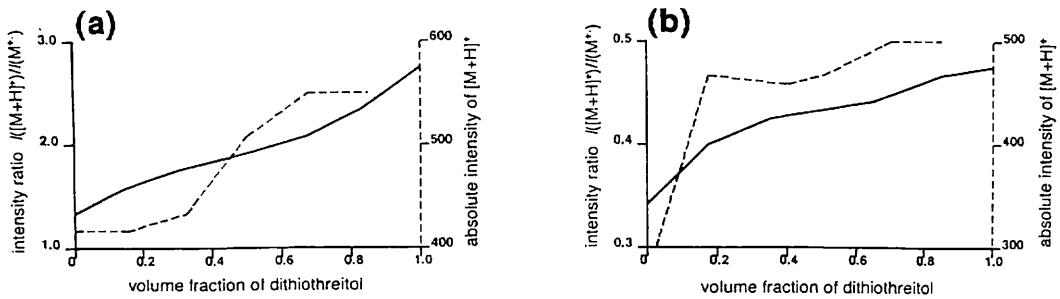


Fig. 6. Variations of the $I([M+H]^+)/I(M^{+\cdot})$ ratio (solid line) and absolute intensity of $[M+H]^+$ ion (dotted line) for (a) morusin (**1**) and (b) trolox (**2**) with the volume fraction of DTT matrix in the mixture of DTT and NBA.

the FAB spectrum of NPOE, because the ion peaks at *m/z* 251 and 250 for **2** were overlapping with the peaks originating from NPOE. The order in the ratio, group 1 > group 2 > group 3, indicates that the use of hydrophilic matrices is advantageous for $[M+H]^+$ ion formation compared with hydrophobic matrices, except for the ratio for **2** with NBA, and that the use of alcoholic-hydrophilic matrices is relatively advantageous for $[M+H]^+$ formation rather than thiol-containing hydrophilic matrices. The ratio obtained here for **2** with NBA matrix was larger than the previous result of 0.19,[17e] although the reason why the peak intensity fluctuates is not clear. This indicates how subtle the ion formation processes under FAB conditions are. The former indication, groups 1 and 2 > group 3, can be understood from the situation that hydroxyl (–OH) and thiol (–SH) groups in the hydrophilic matrix molecules act as a proton donor according to the reactions (1.1)~(2.2). Figures 6(a) and (b) represent the relationship between volume fraction of DTT and intensity ratio $I([M+H]^+)/I(M^{+\cdot})$ for **1** and **2**, respectively, obtained with mixtures of DTT and NBA. For both cases, the intensity ratio obviously increased with increasing volume fraction for the hydrophilic matrix DTT. Further, the absolute intensity of $[M+H]^+$ ions for **1**and **2** also increased with increasing volume fraction of DTT, as shown in Fig. 6. This may be due to the proton donating property of the hydroxyl (–OH) and thiol (–SH) groups in DTT matrix. The effect of the volume fraction on the extent of fragmentation of $M^{+\cdot}$ and $[M+H]^+$ ions of **1** and **2** will be described in the later section.

The latter indication, group 1 > group 2, however, seems strange, because it is believed that relatively acidic matrices DTT and TG act as a proton donor to the analyte

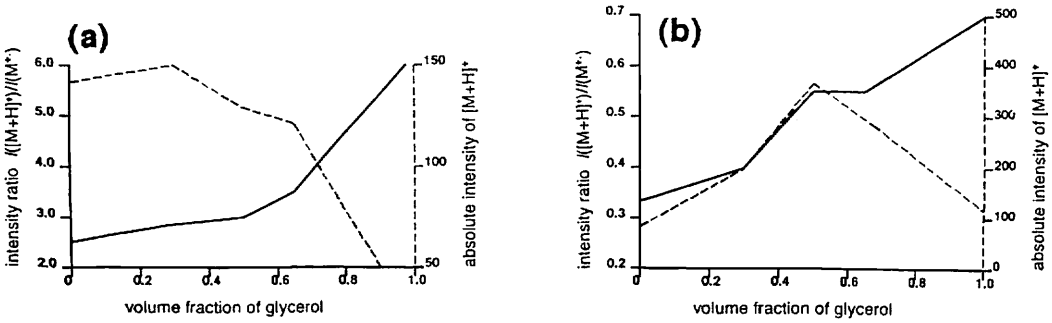


Fig. 7. Variations of the $I([M+H]^+)/I(M^{+\cdot})$ ratio (solid line) and absolute intensity of $[M+H]^+$ ion (dotted line) for (a) morusin (**1**) and (b) trolox (**2**) with the volume fraction of G matrix in the mixture of G and TG.

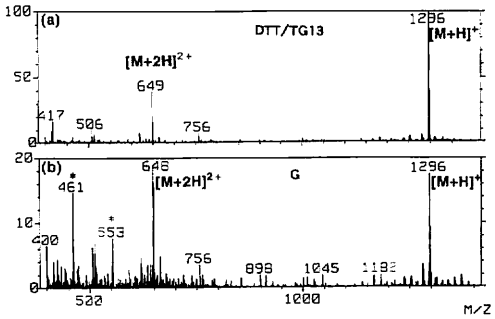


Fig. 8. Positive ion FAB mass spectra of angiotensin I (**6**) obtained with (a) DTT/TG13 and (b) G matrices.

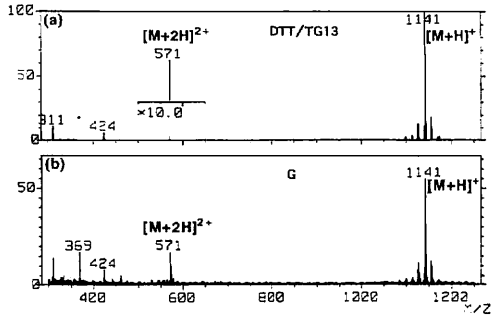
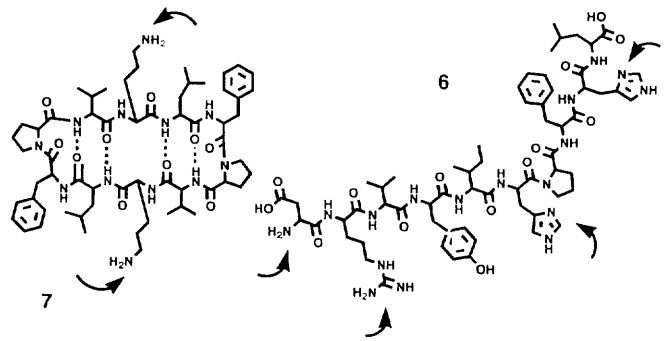


Fig. 9. Positive ion FAB mass spectra of gramicidin S (**6**) obtained with (a) DTT/TG13 and (b) G matrices.

molecules M compared to neutral matrices PMG and G. In fact, thiol-containing compounds are stronger acids than their oxygen analogs, and the dissociation energy of the S–H bond (~80 kcal/mol) is much less than that of the O–H bond (~100 kcal/mol).[27] Regarding this, we have obtained two interesting phenomena. Using mixtures of G and TG the intensity ratio $I([M+H]^+)/I(M^{+\cdot})$ for **1** and **2** increases with increasing volume fraction of G, as shown in Fig. 7,[17e] although the spectra obtained with the mixtures often fluctuated in the intensity ratio and absolute intensity. It is a subtle work in practice to estimate quantitatively the peak intensity of ions produced under FAB



Scheme 3.

conditions. Further, the intensity ratios of doubly-charged to singly-charged ion peaks, $I([M+2H]^{2+})/I([M+H]^{+})$, in the FAB spectra of angiotensin I (**6**) and gramicidin S (**7**) increased with the change of matrix DTT/TG13 to G, as shown in Figs. 8 and 9.[17e] The sites of protonation must be on the arginine and/or histidine residues, and/or N-terminus for **6** and the ornithine residues for **7**, as shown by the arrow in Scheme 3. The ratios, $I([M+2H]^{2+})/I([M+H]^{+})$, obtained with DTT/TG13 (G) were 0.41 (1.06) for **6** and 0.03 (0.27) for **7**. These two phenomena are of interest in connection with the solute/solvent interaction in solution. How should we explain these phenomena?

A most important distinction between –SH and –OH groups is the ability of hydrogen-bonding interaction to analytes in solution. The hydrogen bonds of thiols are not nearly as strong as those of alcoholic hydroxyl groups, so that it is expected that the hydrogen-bonding interaction between analyte M and thiol-containing matrix B-SH, B-SH···M, as a solvation state is a very weak compared to the interaction between M and alcoholic matrix B-OH, B-OH···M. As described above, on the other hand, the formation of $[M+H]^{+}$ ions was enhanced by the presence of carbonyl group(s) as a significant proton accepter or a basic site in the analyte molecules M. The hydrogen-bonding solvation generally differ from so-called "preformed ion" state[9), 23a), 26b), 28)] which is represented by an ionic state, $M+AH \rightarrow MH^{-}\cdots A^{-}$, in solution with addition of acids AH, so that the two phenomena described above seem to be suggestive of which the hydrogen-bonding interaction between the hydroxyl group(s) of the matrix and the basic site(s) of the analyte, B-OH···M, is advantageous for the formation of $[M+H]^{+}$ to $M^{+\cdot}$ ions. Such a hydrogen-bonding interaction may be measured as the orientation energy, resulting from mutual orientation of the molecules due to electrostatic effects in solution. In fact, the interaction energy between the carboxy oxygen of acetone and the hydroxyl group of ethanol has been estimated as *ca.* 2.0 kcal/mol.[29)] Although the state of hydrogen-bonding solvation for the $[M+H]^{+}$ formation was first described by Nakata,[30)] we have recently called as a *'quasi-preformed state'* for the $[M+H]^{+}$ formation.[17c)] The significance of the hydrogen-bonding interaction in matrix solution for effective $[M+H]^{+}$ formation under FAB conditions has been recently stressed by Nakata *et al.* again.[22)]

Consequently, the formation of $M^{+\cdot}$ and $[M+H]^{+}$ ions under FAB conditions occurs competitively according to the gas-phase thermochemical properties such as PA, GB,

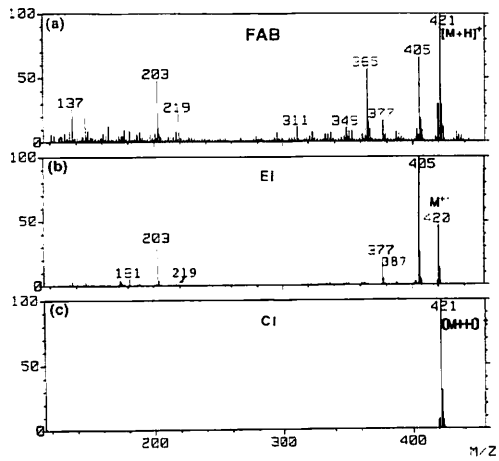


Fig. 10. Positive ion mass spectra of morusin (**1**) obtained by (a) FAB with SULF/TG14 matrix, (b) 70 eV/EI, and (c) isobutane CI methods.

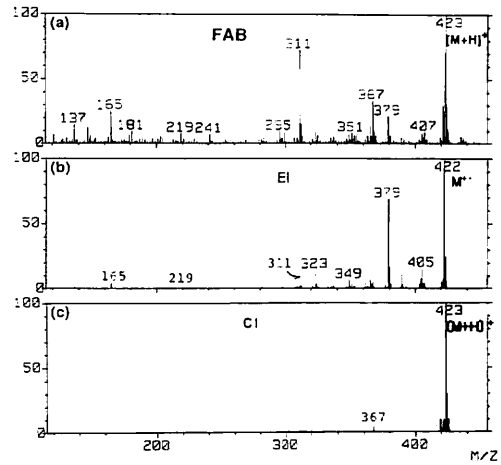


Fig. 11. Positive ion mass spectra of kuwanon C (**3**) obtained by (a) FAB with SULF/TG14 matrix, (b) 70 eV/EI, and (c) isobutane CI methods.

and IP of the analytes and the analyte/matrix solvation states, and the matrix effect *I* may come from the solvation states in matrix solution rather than the gas-phase behavior. It is important to recognize, further, that the ion formation under FAB conditions is governed by both gas-phase thermochemistry and solution chemistry.

3.1.2 Extents of fragmentation of $M^{+\cdot}$ and $[M+H]^+$ ions

The FAB spectra of organic compounds give considerable informative fragment peaks, as well as molecular related ion peaks. As shown in Figs. 4 and 5, however, the relative intensity and appearance of the fragment peaks are affected according to the matrix used. This suggests that the extents of fragmentation (or internal energy contents) of molecular related ions produced under FAB conditions depend on matrix materials. This matrix effect *II* is of interest in connection with the mechanism of FAB ionization.

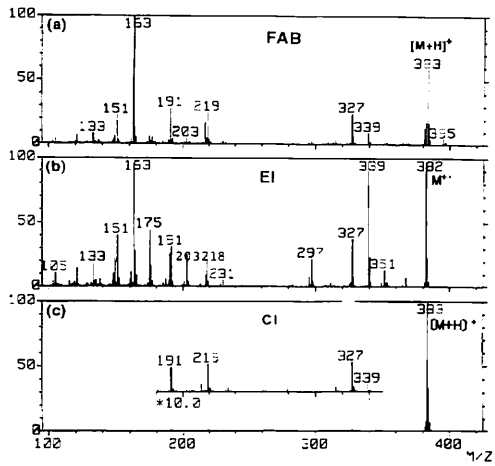


Fig. 12. Positive ion mass spectra of trimethoxychalcone (8) obtained by (a) FAB with SULF/TG14 matrix, (b) 70 eV/EI, and (c) isobutane CI methods.

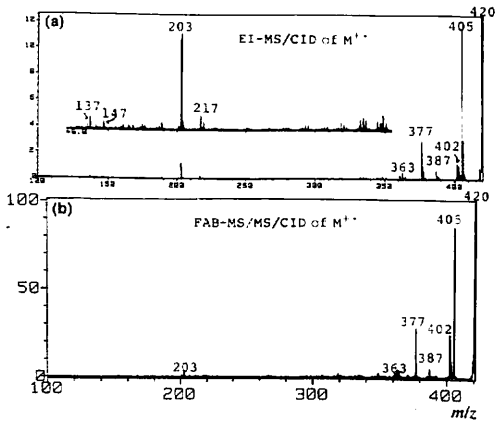


Fig. 13. CID spectra of $M^{+\cdot}$ ions at *m/z* 420 of morusin (1), produced under (a) 70 eV/EI and (b) FAB conditions, by using B/E-constant linked scanning technique with (a) two-sectors and (b) four-sectors machines.

MS/MS spectra of $M^{+\cdot}$ and $[M+H]^+$ ions

In order to examine the extents of fragmentation of $M^{+\cdot}$ and $[M+H]^+$ ions produced under FAB conditions, the FAB spectra of **1**, **3** and trimethoxychalcone (**8**),[19] obtained with SULF/TG14 matrix were compared with the corresponding 70 eV EI and isobutane CI mass spectra (Figs. 10, 11, and 12).[15a), c)] The FAB spectrum of **1** is very similar in the fragments at *m/z* 405, 387, 377, 349, 295, 279, 219, 203, 181, 165, 147, and 137 to the EI spectrum, while the CI spectrum does not show any fragment peaks. The FAB spectrum of **3** is similar in the fragments at *m/z* 379, 323, 311 and 165 to the EI spectrum, and is similar in a fragment at *m/z* 367 to the CI spectrum. Although the origin ($M^{+\cdot}$ or $[M+H]^+$) of those fragments in their FAB spectra is roughly distinguishable from the comparison described above, strictly speaking, the fragmentation of $M^{+\cdot}$ and $[M+H]^+$ ions under FAB conditions should be separately examined each other. For this purpose, the technique of tandem mass spectrometry (MS/MS)[31] was used.[14), 15b), c)]

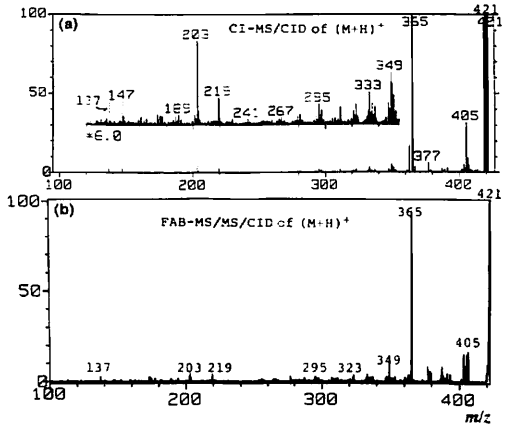


Fig. 14. CID spectra of $[M+H]^+$ ion at *m/z* 421 of morusin (**1**), produced under (a) isobutane CI and (b) FAB conditions, by using B/E-constant linked scanning technique with (a) two-sectors and (b) four-sectors machines.

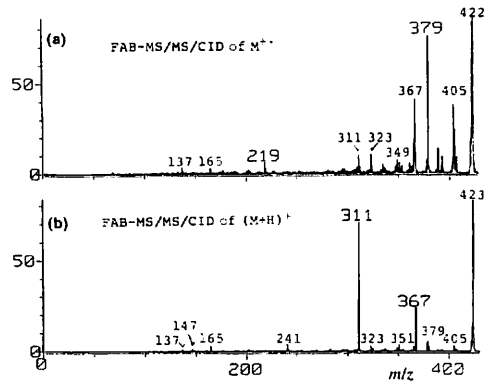


Fig. 15. CID spectra of (a) $M^{+\cdot}$ ion at *m/z* 422 and (b) $[M+H]^+$ ion at *m/z* 423 of kuwanon C (**3**), produced under FAB conditions, by using B/E-constant linked scanning technique with a four-sectors machine.

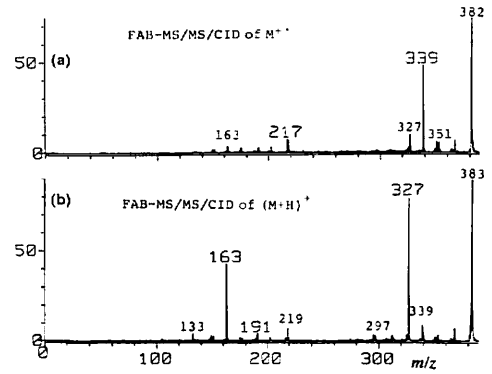


Fig. 16. CID spectra of (a) $M^{+\cdot}$ ion at *m/z* 382 and (b) $[M+H]^+$ ion at *m/z* 383 of trimethoxychalcone (**8**), produced under FAB conditions, by using B/E-constant linked scanning technique with a four-sectors machine.

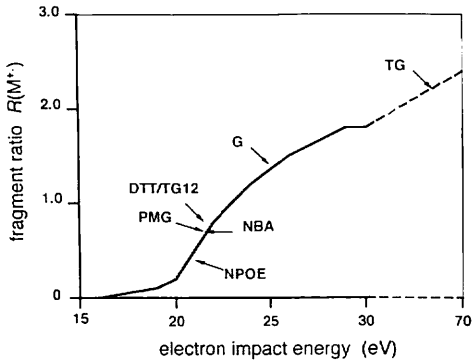


Fig. 17. The fragment ratio $R(M^{+\cdot})$ *versus* electron impact energy (IE) for morusin (**1**). Arrows represent the $R(M^{+\cdot})$ values obtained from the FAB mass spectra.

The MS/MS spectra of $M^{+\cdot}$ and $[M+H]^+$ ions of **1**, produced under FAB conditions, were compared with the CID spectra of $M^{+\cdot}$ ion formed by 70 eV EL and $[M+H]^+$ ion formed by isobutane CI, respectively, as shown in Figs. 13 and 14. The respective FAB-MS/MS/CID spectrum is very similar in the fragment pattern to the corresponding EI- or CI-MS/CID spectrum, so that we can describe that the $M^{+\cdot}$ and $[M+H]^+$ ions produced under FAB conditions result in the EI-like and CI-like fragmentations, respectively, although the structure and fragmentation of $M^{+\cdot}$ and $[M+H]^+$ ions produced under FAB conditions may generally differ from those of $M^{+\cdot}$ in EI and $[M+H]^+$ in CI conditions.

As described above, using the MS/MS technique we can pick up the fragments originated from $M^{+\cdot}$ and $[M+H]^+$ ions separately from the FAB spectra of organic compounds, and the respective fragment pattern of the FAB spectra can be compared with that of the EI or CI spectra. Further, the MS/MS spectra of $M^{+\cdot}$ and $[M+H]^+$ ions of **3** and **8**, produced under FAB conditions, are shown in Figs. 15 and 16, respectively. The fragments originated from $M^{+\cdot}$ ions in the FAB spectra, which are comparable with the EI spectra, are at *m/z* 405, 402, 387, and 377 for **1**, at *m/z* 379, 365, and 323 for **3**, and at *m/z* 367, 351, and 339 for **8**. The fragments originated from $[M+H]^+$ ions in the FAB spectra, which are comparable with the CI spectra, are at *m/z* 365 for **1**, at *m/z* 367 for **3**, and at *m/z* 327 for **8**.

Matrix effect II

In order to examine the extents of fragmentation of $M^{+\cdot}$ and $[M+H]^+$ ions produced under FAB conditions, the fragment ratios R $(M^{+\cdot})$ and R $([M+H]^+)$ are defined as follows,

$$R(M^{+\cdot}) = \Sigma I(m_i)/I(M^{+\cdot}) \quad (i=1,2,\cdots) \tag{3}$$

$$R([M+H]^+) = \Sigma I(m_j)/I([M+H]^+) \quad (j=1,2,\cdots) \tag{4}$$

where $I(m_i)$ represents the relative abundance of i-th fragment originated from $M^{+\cdot}$ ion and $I(m_j)$ the relative abundance of j-th fragment originated from $[M+H]^+$ ion. For the compounds here, such fragment ions have been already selected as described above. The values of the fragment ratios, $R(M^{+\cdot})$ and $R([M+H]^+)$, evaluated from the FAB spectra of **1** in Fig. 4 are summarized in Table 3. As described above, it is difficult to estimate rigorously the fragment ratios, because the peak intensity often fluctuates according to the state of matrix solution coated on the sample holder tip,[14b] *e.g.*, analyte

Table 3. Fragment Ratios, $R(M^+)$ and $R([M+H]^+)$, Obtained from the FAB Mass Spectra of Morusin (**1**), Using Various Matrix Systems

Fragment ratio	Matrix					
	G	PMG	TG	DTT/TG12	NBA	NPOE
$R(M^+)$	1.40	0.72	2.24	0.75	0.70	0.45
$R([M+H]^+)$	0.19	0.10	0.49	0.20	0.14	0.10

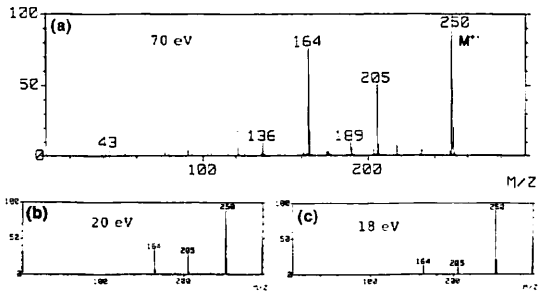


Fig. 18. Positive ion EI mass spectra of trolox (**2**) obtained at the impact energies of (a) 70 eV, (b) 20 eV, and (c) 18 eV.

concentration, amount of matrix solution, fast atom irradiation time, and other preparation procedures of matrix solution. It was a marked tendency for **1**, **3**, and **8**, however, that the use of G and/or TG matrix resulted in the extensive fragmentation of $M^{+\cdot}$ and $[M+H]^+$ ions compared with the use of NBA and/or NPOE matrix.[14b] This is an evidence for that the mechanism of ion formation and the origin of internal energy of the ions formed under FAB conditions closely associate with the matrix molecules.

In order to correlate the extent of fragmentation of $M^{+\cdot}$ ion produced under FAB conditions with electron impact energy (IE), the $R(M^{+\cdot})$ value calculated from the EI spectrum of **1** was plotted against the IEs of 15~30 eV and 70 eV, as shown in Fig. 17. The $R(M^{+\cdot})$ values in Table 3, obtained from the FAB spectra of **1**, were located on the R-IE curve, as indicated by arrows. The $R(M^{+\cdot})$ values for **1** with G, PMG, TG, DTT/TG 12, NBA, and NPOE correspond to the impact energies of 24~25, 21~22, 30~70, 21~22, 21~22, and 20~21 eV, respectively. In the same manner, the R $(M^{+\cdot})$ values for **3** with G, TG and NBA could be corresponded to 24~25, 21~22, and 21~22 eV, respectively. Further, the $R(M^{+\cdot})$ values for **8** with TG and NBA correspond to 18~19 and 16~17 eV, respectively.[14b] It is surprising that the extents of fragmentation of $M^{+\cdot}$ ions in the FAB spectra of **1** and **3** (Figs. 10 and 11), obtained with SULF/TG14 matrix, correspond to those of $M^{+\cdot}$ ions formed at the electron impact energy of 70 eV or above.

As shown in Fig. 5, trolox (**2**) gives a preferential peak of $M^{+\cdot}$ ion and characteristic fragments at *m/z* 205, 203, 165, and 164 in the FAB spectra, while the EI spectra show characteristic fragments at *m/z* 205 and 164 (see Fig. 18). Using the fragments at *m/z* 205 and 164, the $R(M^{+\cdot})$ values for **2** obtained with TG and NBA correspond to the electron impact energies of 20 and 18 eV, respectively.

On the other hand, it is of interest in connection with the origin of internal energy to compare the extents of fragmentation of $[M+H]^+$ ions produced under FAB and CI

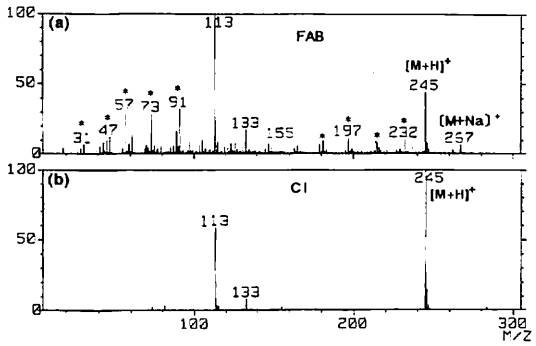


Fig. 19. Positive ion mass spectra of uridine (**9**) obtained by (a) FAB with TG matrix and (b) isobutane CI methods.

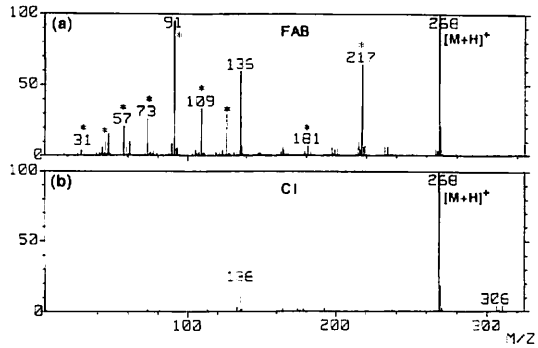


Fig. 20. Positive ion mass spectra of adenosine (**10**) obtained by (a) FAB with TG matrix and (b) isobutane CI methods.

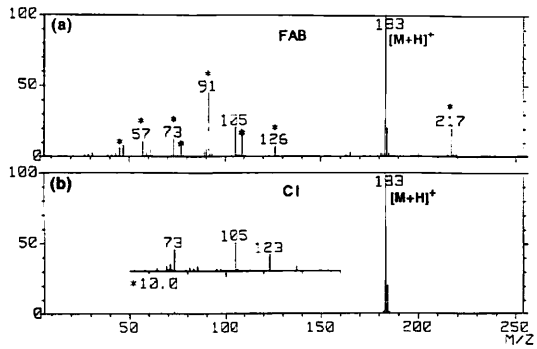
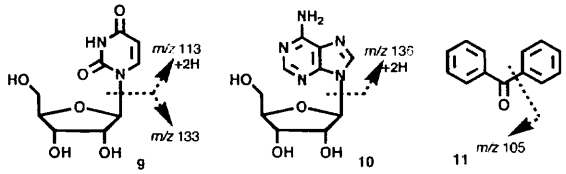


Fig. 21. Positive ion mass spectra of benzophenone (**11**) obtained by (a) FAB with TG matrix and (b) isobutane CI methods.

conditions, although FAB may be quite different from CI in the ion formation processes and the internal energy distribution of $[M+H]^{+\cdot}$ ions formed. As can be seen in Figs. 10 and 11, the fragments at *m/z* 365 for **1** and at *m/z* 367 for **3** originated from $[M+H]^+$ ions produced under FAB conditions have relatively high intensity, while the corresponding fragments in the CI spectra are very weak in the intensity. This means that for those compounds the internal energy content of $[M+H]^+$ ions produced under FAB conditions is larger than that of $[M+H]^+$ ions formed by the CI process. This tendency



9 10 11

Scheme 4.

can be seen in the FAB (with TG) and isobutane CI spectra of uridine (**9**), adenosine (**10**), and benzophenone (**11**) (see Figs. 19~21), though the extent of fragmentation of $[M+H]^+$ ions produced under FAB conditions generally depends upon analyte and matrix used. The fragments at *m/z* 133 and 113 for **9**, at *m/z* 136 for **10**, and at *m/z* 105 for **11** were selected for the comparison, and it was confirmed using the B/E technique that those fragments were originated from the $[M+H]^+$ ions. The assignments of those fragments are shown in Scheme 4. The extent of fragmentation of $[M+H]^+$ ions in CI-MS is governed by its internal energy ΔE which is supplied to analyte M by a proton transfer reaction, *i.e.*,

$$RH^+ + M \rightarrow [M+H]^+ + R \qquad (5)$$

where RH^+ represents a reagent ion such as $C_4H_9^+$ in isobutane CI. The internal energy of $[M+H]^+$ ions formed is given by the difference of PAs of M and R, *i.e.*,

$$\Delta E = PA(M) - PA(R) \qquad (6)$$

Since the reaction (5) is essentially the same as a reaction (1.2) for FAB ionization, we can now apply the reaction (6) to the case of FAB processes. In the case of FAB processes, matrix ion species $[B+H]^+$ play a role of reagent ion and the relation corresponding to (6) is as follows:

$$\Delta E' = PA(M) - PA(B) \qquad (7)$$

Consequently, the distinction of the extents of fragmentation between FAB and CI spectra may be understood by the difference of internal energies, *i.e.*,

$$\delta\Delta E = \Delta E' - \Delta E = PA(R) - PA(B) \qquad (8)$$

The PAs (kcal/mol) for the isobutane system,[32] NBA and G[23b] are 195.9, 194, and 209, respectively. Although the PA of TG has not been evaluated, the PAs of thiol compounds are greater than those of their alcohol analogs.[32] For these values, the difference of (8) is approximately given by $\delta\Delta E \leq 0$. This is not consistent with the experimental results obtained above, and it is suggested that the $[M+H]^+$ ion formation and fragmentation under FAB conditions can not be explained by the CI processes and that the internal energy of $[M+H]^+$ ions produced under FAB conditions should be originated from other processes as well as the thermochemical process (5). This is an important problem which should be solved in future.

The influence of the volume fraction of DTT, in the mixtures of DTT and NBA, on the extents of fragmentation of $M^{+\cdot}$ and $[M+H]^+$ ions of **1** and **2** was examined. The respective fragment ratio, $R(M^{+\cdot})$ or $R([M+H]^+)$, was plotted against the volume fraction of DTT (Fig. 22). In order to estimate the ratio $R(M^{+\cdot})$ for **2**, the fragment ion at *m/z* 165 was employed as a fragment originated from $[M+H]^+$ ion. For both compounds, the extents of fragmentation increased with increasing the volume fraction of DTT. We now can say that the use of NBA matrix produces a milder condition for the internal

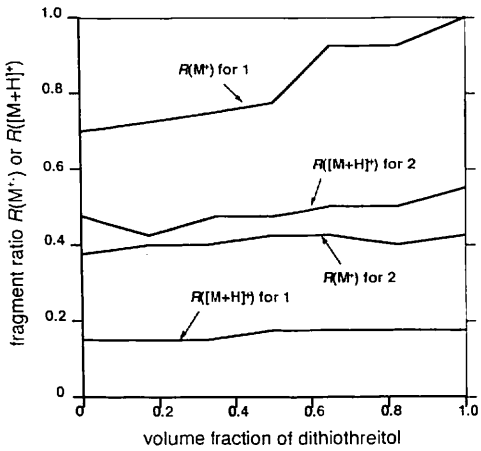


Fig. 22. Variations of the fragment ratios $R(M^{+\cdot})$ and $I([M+H]^+)$ for morusin (**1**) and trolox (**2**) with the volume fraction of DTT matrix in the mixture of DTT and NBA.

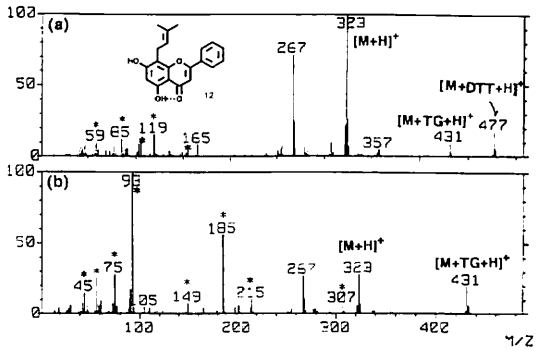


Fig. 23. Positive ion FAB mass spectra of 8-prenylated chrysin (**12**) obtained with (a) DTT/TG12 and (b) G/TG11 matrices.

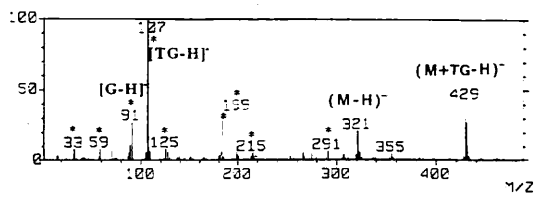


Fig. 24. Negative ion FAB mass spectrum of 8-prenylated chrysin (**12**) obtained with G/TG11 matrix.

energy contents of $M^{+\cdot}$ and $[M+H]^+$ ions formed.

The facts of the formation of $M^{+\cdot}$ ions, which requires relatively high electronic excitation, and the considerable fragmentation of $M^{+\cdot}$ and $[M+H]^+$ ions under FAB conditions imply that the FAB technique is no longer referred to as a "soft ionization", in the sense of the ionization and fragmentation processes, even when a liquid matrix is used.

3.1.3 Formation of $[M+B+H]^+$ ions

While the use of thiol-containing matrices such as TG, DTT and dithioerythritol

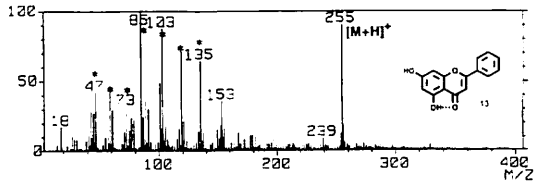


Fig. 25. Positive ion FAB mass spectrum of chrysin (**13**) obtained with G/TG11 matrix.

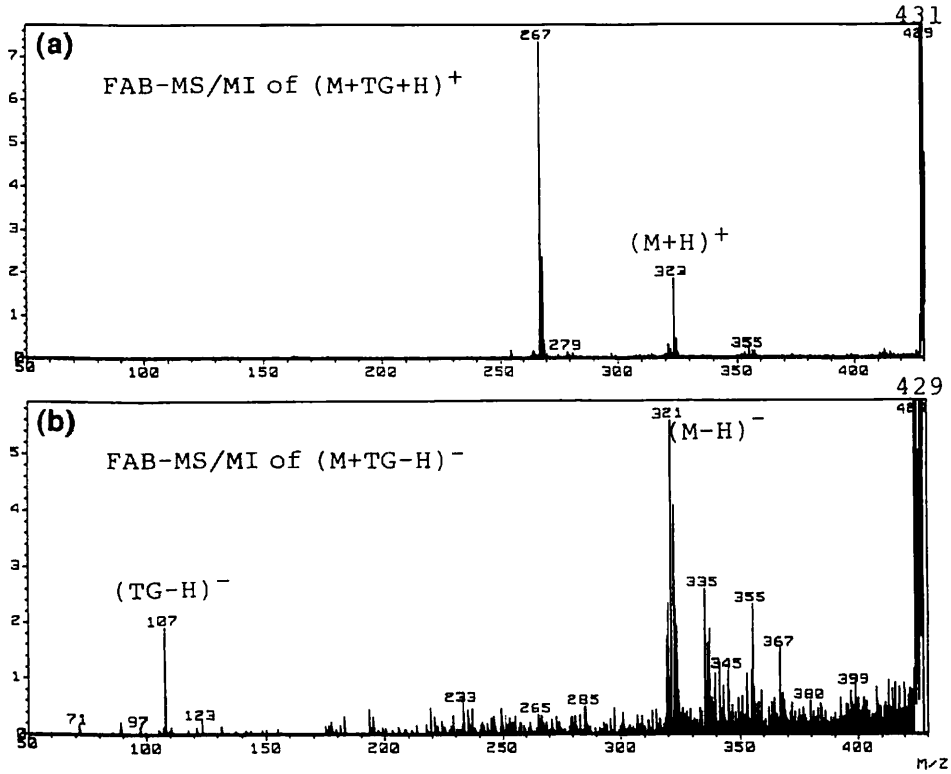
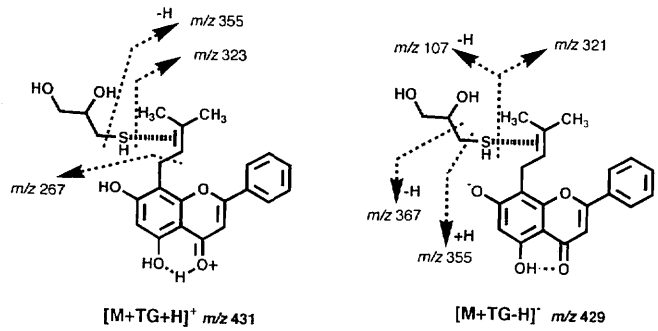


Fig. 26. Metastable ion spectra of the complex ions (a) $[M+TG+H]^+$ at *m/z* 431 and (b) $[M+TG-H]^-$ at *m/z* 429 for 8-prenylated chrysin (**12**), produced under FAB conditions with G/TG11 matrix.

(DTE) often induces the degradation[16] and reduction reactions,[33] an interesting matrix effect is observed by using DTT/TG12 matrix. Figure 23 is the FAB spectra of 8-prenylated chrysin (**12**),[15e] obtained with DTT/TG12 and G/TG11 matrix systems. The FAB spectra showed the peaks corresponding to the complex ions of $[M+DTT+H]^+$ at *m/z* 477 and $[M+TG+H]^+$ at *m/z* 431, while the $[M+G+H]^+$ ion was not observed. Further, the FAB spectra of **12** obtained with NBA, DMSO, and DTDE matrices did not show the complex ions, while the FAB spectrum with DEA showed the peak of $[M+DEA+H]^+$ ion. The negative ion FAB spectrum of **12** with G/TG11 matrix gave the peaks corresponding to $[M-H]^-$ ion at *m/z* 321 and a complex ion $[M+TG-H]^-$ at *m/z* 429, while the spectrum did not show $[M+G-H]^-$ ion peak, as shown in Fig. 24. The



Scheme 5.

matrix effect here suggests that the compound **12** can bind or interact to thiol-containing matrices DTT and TG, and to an amide matrix DEA, not alcoholic matrices such as G, NBA, and DTDE. In order to examine the structure dependence of the complex ion formation, the FAB spectra of chrysin (**13**) lacking a prenyl group were obtained with DTT/TG12 (see Fig. 25) and G/TG11 matrices, but those spectra did not show the complex ion peaks. The results obtained suggest that thiol-containing matrices DTT and TG bind, at least in part, to the prenyl group of **12**, although it is not clear whether the interaction is a covalent or non-covalent bond. The metastable ion (MI) spectra of $[M+TG+H]^+$ and $[M+TG-H]^-$ ions of **12** are shown in Fig. 26. The flavone skeleton of prenylated flavonoids is so stable that the degradation of prenyl group(s) occurs characteristically.[34] The MI spectrum of $[M+TG+H]^+$ ion showed two intense fragment peaks at *m/z* 323 and 267 indicating the loss of TG and $(TG+C_4H_8)$ from the complex ion, respectively. The MI spectrum of $[M+TG-H]^-$ ion showed the fragments originating from the degradation and loss of TG, and a matrix $[TG-H]^-$ ion at *m/z* 107, whereas the MI spectrum of $[M+TG+H]^+$ ion did not show the matrix peak of $[TG+H]^+$. This may be due to the charge location in the positive and negative ions. From the MI spectra, possible fragmentation pathways for the $[M+TG+H]^+$ and $[M+TG-H]^-$ ions are shown in Scheme 5.

Harada *et al.*[35] reported the formation of the complex ions, $[M+B+H]^+$, in the system of oligosaccharides and amide matrices under liquid SIMS conditions, and they described that the CID spectra of $[M+B+H]^+$ ions often reflect the sequence of the saccharides.[35b] Further, Tondeur *et al.* reported the formation of $[M+B+H]^+$ ions in the system of monosaccharides and matrices of DEA and G under FAB conditions.[36] The formation of $[M+B+H]^+$ ions is an interesting phenomenon in connection with the problem of the intermolecular interactions in gas-phase,[37] so that further study is necessary to clarify the structure of $[M+B+H]^+$ ions.

As described above, the introduction of liquid matrix into the FAB technique have brought about some interesting and basic phenomena with respect to the ion formation and fragmentation, as well as the practical values in the field of mass spectrometry. Lastly, we will describe on the suitability of matrix to various compounds and some unusual matrix materials reported so far.

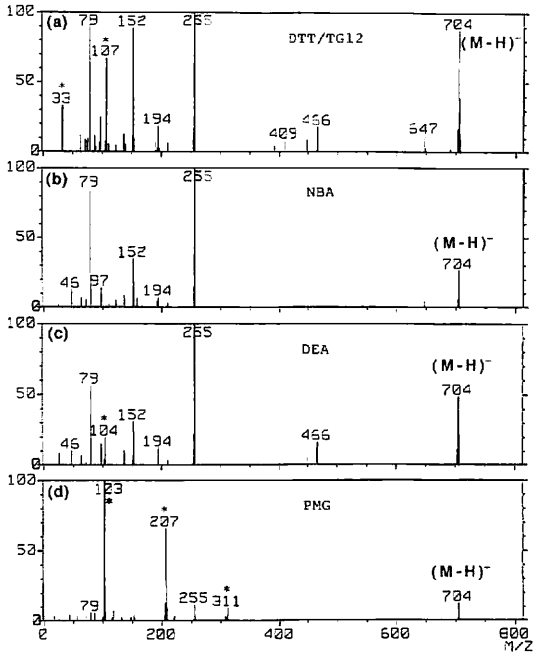


Fig. 27. Negative ion FAB mass spectra of L-α-phosphatidyl-*N*-monomethylethanolamine, dipalmitoyl (**14**) obtained with (a) DTT/TG12, (b) NBA, (c) DEA, and (d) PMG matrices.

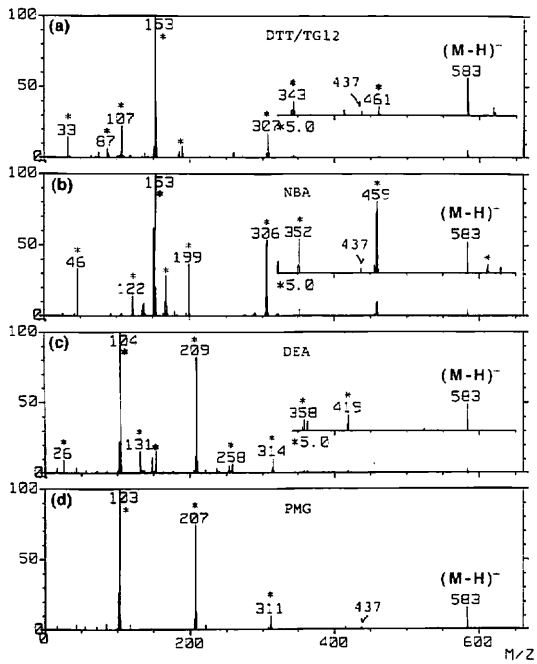


Fig. 28. Negative ion FAB mass spectra of ouabain (**15**) obtained with (a) DTT/TG12, (b) NBA, (c) DEA, and (d) PMG matrices.

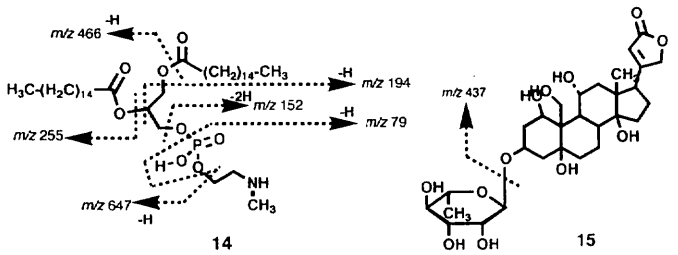
3.2 Suitability of matrix

It is difficult to select *a priori* a most suitable matrix for a given compound, since the definite criterion for the suitability has not been established. The capability or suitability of matrix to various compounds can be evaluated by obtaining actually the FAB spectral data, so that we have to obtain the FAB spectra of a given compound by using various different matrix materials. The actual suitability of matrix can be evaluated by the quality of the FAB spectra obtained. The quality of the FAB spectra will be evaluated by means of ratios of the relative intensities of molecular related ions, $I(M^{+\cdot})$, $I([M+H]^+)$, and $I([M+Na]^+)$, or fragment ion peaks, $I(m_j^+)$, to those of matrix ion, $I([B+H]^+)$, or matrix fragments, $I(b^+)$. This corresponds to a criterion for the choice of matrix, called 'unobstructive to analysis'[12] which means that the ion peaks originating from matrix itself must be as unobstructive as possible in the FAB spectra obtained. In fact, the capabilities of conventional matrix systems DTT/TG12, NBA, a 3 : 1～5 : 1 (v/v) mixture of DTT and DTE referred to as magic bullet (MB),[38] NPOE, G/TG23, and a 1 : 1 (v/v) mixture of G and NBA (G/NBA11) were evaluated by using both the ratios $I([M+H]^+)/I([B+H]^+)$ or $I([M+H]^+)/I(b^+)$ and the absolute intensities of $[M+H]^+$ ions for various compounds, and it was pointed out that both DTT/TG12 and NBA systems should be used as the first choice matrix for the positive ion FAB analysis of unknown compounds.[12d] In the same manner, the suitability of some matrix materials for the negative ion FAB analysis will be evaluated using glycosides and other compounds in the next subsection.

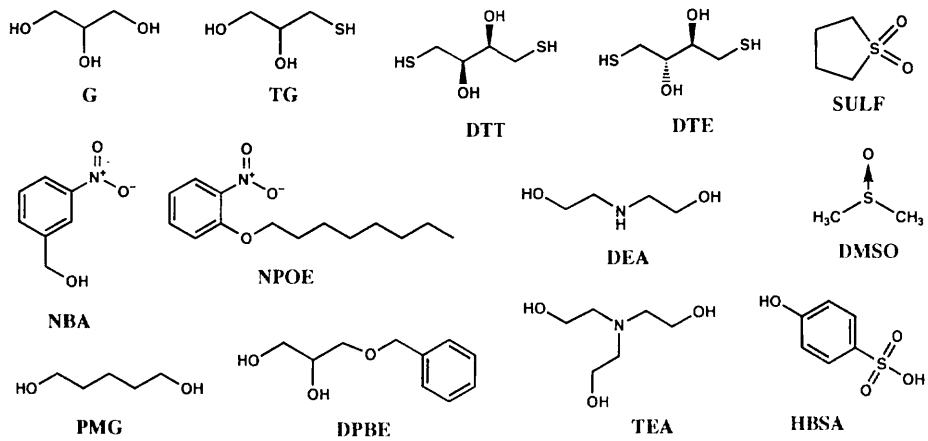
3.2.1 Suitability of matrices for negative ion FAB-MS

It is considered that the proton transfer reaction (2.2) is essential for the formation of $[M-H]^-$ ions under FAB conditions. In fact, the amine matrices DEA[36), 39)] and TEA,[40)] which are relatively high in the PA,[23b)] have been often used for the negative ion FAB analysis. Isobe *et al.*[41)] have used a 1 : 1 (v/v) mixture of hexamethylphosphoric triamide (HMPA) and G, which is very high in the potential as a proton accepting matrix system, for the negative ion FAB analysis of some natural products. We have obtained the order of the suitability of matrices for the negative ion FAB analysis of glycosides, *i.e.*, PMG > DEA > TEA.[42)] We here apply some matrix systems, DTT/TG12, NBA, PMG, DEA and/or TEA, to evaluate the suitability for the negative ion FAB analysis of organic compounds.

The negative ion FAB spectra of L-α-phosphatidyl-N-monomethylethanolamine, dipalmitoyl (**14**) and ouabain (**15**), obtained with DTT/TG12, NBA, DEA, and PMG matrices, are shown in Figs. 27 and 28, respectively. Some fragments are assigned as shown in Scheme 6. The reference peaks used for matrices DTT/TG12, NBA, DEA, and PMG were *m/z* 107 ($[TG-H]^-$) or *m/z* 153 ($[DTT-H]^-$), *m/z* 153 (NBA^-), *m/z* 104 ($[DEA-H]^-$), and *m/z* 103 ($[PMG-H]^-$), respectively. The ratios of relative intensities of $[M-H]^-$ ions to the reference peaks, obtained for **14** and **15**, were 1.30 and 0.05 for DTT/TG12, 1.44 and 0.05 for NBA, 2.46 and 0.04 for DEA, and 0.12 and 0.16 for PMG, respectively. The orders of the suitability of matrices for **14** and **15** were DEA > NBA > DTT/TG12 > PMG and PMG > DTT/TG12 ≥ NBA > DEA, respectively. In the same manner, further estimation for the matrix suitability was done by using other compounds, *N*-palmitoyl-DL-dihydrosphingosine (**16**), L-α-phosphatidic acid, dipalmitoyl



Scheme 6.



Scheme 7.

(**17**), *N*-palmitiyl-D-sphingomyelin (**18**), rutin (**19**), digitonin (**20**), and GM1 (**21**). The orders of the suitability of matrices were NBA > PMG > DEA > DTT/TG12 for **16**, NBA > DEA > PMG > DTT/TG12 for **17**, DTT/TG12 > NBA > DEA > PMG for **18**, PMG > DEA > TEA > NBA > DTT/TG12 for **19**, DEA ≥ NBA > DTT/TG12 > PMG for **20**, and TEA > DEA > PMG for **21**.

Although it has been believed empirically that TEA and DEA are quite useful as matrices for negative ion FAB-MS,[36), 39), 40), 42)] the results obtained here indicate that the empirical suitability does not have a definite basis and that it is difficult to find a definite tendency for the suitability of matrices to given compounds here. Further, TEA and DEA can often react with analyte molecules, forming covalent adducts such as [M+b], where b represents a matrix fragment.[43)] Consequently, it seems to be necessary to reconsider various factors regarding the ion formation under FAB conditions before we present a definite criterion for the choice of matrix.

3.2.2 Various different matrix materials

Although various different matrix materials have been reported and summarized so far,[12)] further, some unusual materials have been used to obtain prominent mass spectral data. Antimony trichloride ($SbCl_3$) was used for liquid SIMS of pyrene.[21c)] Dithiodiethanol (DTDE)[44)] or its mixture with NBA were used for liquid SIMS of protected peptides[45)] or for the FAB analysis of non-polar compounds.[13b)] The liquid SIMS spectra

of many substituted polynuclear aromatic hydrocarbons and xanthopterin were successfully obtained using sulfuric acid (H_2SO_4) matrix.[21g] The FAB spectra of the clusters of sulfuric acid have been reported by Sharp *et al.*[46] A glycerol derivative, 2,3-dihydroxypropylbenzylether (DPBE), has been used for the FAB analysis of cobyrates of which the molecular ions can not be detected by using G and TG matrices.[47] An interesting material, 4-hydroxybenzenesulfonic acid (HBSA), as a reduction-inhibiting matrix was introduced by Visentini *et al.*[48] They have used the material for the FAB analysis of deaminoarginine-vasopressin and other peptides and nucleosides, and have described that HBSA is a good solvent possesses physical properties that are similar to those of G, and is as efficient as NBA in inhibiting reduction. The structures of various matrix materials are shown in Scheme 7.

As described above, the suitability or selectivity of matrices is quite complicated and diverse according to the nature of analytes. This seems come from the complexity of solution chemistry, so that much experience and extensive data are needed for understanding the relationships between the ion formation and matrix solution chemistry.

4. Conclusions

It is meaningful for the fundamental and practical aspects to examine the matrix effects on the appearance of ion formation and fragmentation under FAB conditions. The matrix effects obtained here presented the information regarding the competitive formation of $M^{+\cdot}$ and $[M+H]^+$ ions, the interaction between analyte and matrix molecules in solution, the internal energy contents of $M^{+\cdot}$ and $[M+H]^+$ ions formed, and the complex formation between analyte and matrix molecules in gas-phase. These matrix effects can be summarized as follows:

i) A hydrogen-bonding interaction between basic site(s) of analyte and matrix hydroxyl group(s) in matrix solution plays an essential role for the preferential formation of $[M+H]^+$ to $M^{-\cdot}$ ions. This led to a concept of *'quasi-preformed state'* for the $[M+H]^+$ formation.

ii) The extent of fragmentation of $M^{+\cdot}$ ions produced under FAB conditions often corresponded to that of fragmentation of $M^{+\cdot}$ ions formed at the electron impact energy of 70 eV or above, and it was dependent on the matrix material used. The $[M+H]^+$ ions produced under FAB conditions were larger in the extent of fragmentation than the $[M+H]^+$ ions formed by isobutane CI conditions. These results implied that the FAB processes possess a hard ionization characteristic such as EI method, as well as the characteristics of soft ionization methods such as CI and other desorption techniques.

iii) The formation of adduct ions, $[M+\text{matrix}+H]^+$ and $[M+\text{matrix}-H]^-$, which may be due to intermolecular interaction showed the structure dependence of analyte and matrix molecules. This matrix effect is of interest in connection with the study of noncovalent interaction or molecular recognition between organic molecules in gas-phase. Further, it was pointed out that the ion formation under FAB conditions is governed by both gas-phase and solution-phase chemistry.

The capability of matrix materials for negative ion FAB analysis was estimated using various organic compounds, but it was difficult to find a most suitable matrix and

a definite criterion for the choice of matrix. It was pointed out, further, that various factors regarding the ion formation under FAB conditions should be reconsidered for the criterion for the choice of matrix. This will be clarified on the basis of the mechanism(s) of ion formation under FAB conditions in the near future.

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Keywords
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