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INTEGRATED PAPER

Fast Atom Bombardment Mass Spectrometry: Ionization Mechanism[†]

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Some ideas have been presented to understand ion formation under matrix fast atom bombardment (FAB) conditions. A state of hydrogen-bonding hydrophilic solvation, named "quasi-preformed state", has been introduced to explain the ion formation reflecting condensed-phase, and the both "quasi-preformed state" and a state of "preformed ion" have been defined on the basis of the Bjerrum's proposition for ion-pairs in solution. Some examples of preformed ion have been described. The extents of electronic excitation leading to the formation of molecular ions $M^{+\cdot}$, under matrix FAB conditions, have been examined by detecting FAB-induced fluorescence from a thin layer of alkali-halides (LiCl, NaCl, KCl, and CsCl), and by using some compounds of which ionization energies are known. The results obtained indicate that matrix FAB conditions are sufficient in energy for $M^{+\cdot}$ formation (by about 10 eV electronic excitation) and are insufficient for M^{2+} formation (by about 20 eV electronic excitation). The mechanisms of the formation of various ions, $[M+H]^+$, $[M+Na]^+$, $[M-H]^+$, $M^{+\cdot}$, $M^{-\cdot}$, and $[M-H]^-$, under matrix FAB conditions have been described. A model for FAB ionization, named "cavity" model, has been introduced to deal quantitatively with the rate of ion formation or ion yields from matrix surface. The "cavity" model requires that the ion formation occurs in both processes, i) the collision cascade reflecting condensed-phase and ii) the ion/molecule reactions reflecting gas-phase. The influence of fast atom species (He, Ar, and Xe) on the ion yields which may directly relate to the size of cavity or crater has been examined by comparing with the spectral patterns in the gas-phase FAB mass spectra of *m*-nitrobenzyl alcohol. It has been roughly estimated that a "cavity" formed with Ar or Xe beam contains several 100 molecules.

1. Introduction

Matrix fast atom bombardment (FAB)[1)] and liquid secondary ion (LSI)[2)] are ranked among the most useful and easy ionization methods in mass spectrometry, and these methods have been reviewed by some workers from the standpoints of liquid matrix,[3)] chemical aspect,[4)] and ionization mechanism.[5)] Matrix FAB (LSI) mass spectrometry has been interestingly applied to the physicochemical and function analysis such as thermodynamic/kinetic properties in solution,[6)] charge-transfer complexation,[7)] isomer characterization,[8)] and molecular recognition,[9)] as well as the molecular weight determination. It is of importance to recognize that various different applications are based on the diversity of ion formation under matrix FAB conditions. The diversity of matrix FAB ionization comes from the situation, of which FAB mass spectra often reflect the

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[†] This is the sequel to the previous paper (ref. 3c).

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gas-phase processes like a chemical ionization (CI) and/or solution chemistry like an acid-base equilibrium according to the nature of analyte and matrix compounds. Therefore, it is significant to understand the FAB ionization processes for the practical ways, as well as the basic interest.

In the present paper, the ion formation mechanism(s) under FAB conditions would be described on the basis of the reports presented so far.[10] A concept of the state of hydrogen-bonding hydrophilic solvation named "quasi-preformed state", which differs from a state of "preformed ion", is introduced to explain diverse ionization features reflecting condensed-phase. The "preformed ion" and "quasi-preformed state" will be defined by the Bjerrum's means of ion-pairs in solution. With respect to the formation of $M^{+\cdot}$ ions, the electronic excitation of analyte molecules under matrix FAB conditions will be discussed on the basis of the detections of fluorescence under solid FAB conditions and multiply-charged ions M^{z+} ($z=1$–3) under electron ionization (EI) and gas-phase FAB conditions. The formation of various molecular-related ions will be described: positive molecular ions $M^{+\cdot}$, protonated molecules $[M+H]^+$, dehydride molecules $[M-H]^+$, metal cationized molecules $[M+\mathbf{c}]^+$ ($\mathbf{c}$=Li, Na, K, $\cdots$), non-covalent bonding complex molecules $[M+L]^+$ and $[M+L+H]^+$ (L: ligand), negative molecular ions $M^{-\cdot}$, deprotonated molecules $[M-H]^-$, and others. A model for matrix FAB ionization, named "cavity" model, is introduced with an explanation of the term "cavity". The cavity model was first introduced by the author to explain the preferential formation of molecular ions $M^{+\cdot}$ of α-tocopherol **1**, which is oily and hydrophobic in nature, under FAB conditions without a liquid matrix.[10k] The ion yields sputtered from matrix surface and the cavity size will be discussed on the basis of the matrix FAB experiments with different fast atoms He, Ar, and Xe.

2. Experimental

All the mass spectrometric 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 argon or xenon atom was generated from $Ar^{+\cdot}$ or $Xe^{+\cdot}$ ions which were accelerated to 5 kV. The FAB gun emission current was 10 or 20 mA. The liquid matrices used were 1 : 1~1 : 3 (v/v) mixtures of dithiothreitol (DTT) and thioglycerol (TG) referred to as DTT/TG, glycerol (G), pentamethylene glycol (PMG), *m*-nitrobenzyl alcohol (NBA), *o*-nitrophenyl octyl ether (NPOE), deuteroglycerol (G-d_3), and diethanolamine (DEA). Further detailed experimental procedures have been described elsewhere.[10]

3. Results and Discussion

3.1 Preformed ion and quasi-preformed state in solution

It has been believed by some workers that the existence of a precharged form of analyte molecules in matrix solution, which is often called "preformed ion", is favorable for the molecular-related ion yields under FAB conditions.[11] Such a sample preparation to form "preformed ions" is performed by adding an organic acid, AH, or base, *b*, into matrix solution consisting of matrix solvent, B, and analyte molecules, M, and is called reverse-derivatization,[12] so it is expected that solution equilibria would be achieved as follows;

$$\mathrm{M+B+AH} \rightleftarrows \mathrm{B+M\cdots H^{+}\cdots A^{-}} \rightleftarrows \mathbf{(M+H)^{+}}\mathrm{+A^{-}+B} \qquad (1)$$

$$\mathrm{M+B+}b \rightleftarrows \mathrm{B+|M-H|^{-}\cdots H^{+}\cdots}\, b \rightleftarrows \mathbf{(M-H)^{-}}+b\mathrm{H^{+}+B} \qquad (2)$$

For an equilibrium (1), the extent of the formation of preformed ion $(M+H)^+$ could be quantitatively described by the equilibrium constant K_{DI} which is represented as follows;

$$K_{DI}=[(M+H)^+][A^-][B]/[M][B][AH] \qquad (3)$$

The concentration of preformed ion, $[(M+H)^+]$, in matrix solution would depend on the liquid phase basicity of M, LB(M), and the concentration of preformed ion, $[(M-H)^-]$, may depend on the liquid phase acidity of M, LA(M). However, this theoretical expectation is often disappointed when the sample preparation results in a lowering of the surface activity of a given matrix solution[12] and the intermolecular interaction between M and B is stronger than that between M and AH, or b and H^+. In fact, a favorable effect to enhance the ion yields does not always occur by the addition of acid or base, and the ion formation under FAB conditions is governed by rather solvation such as hydrophilic or hydrophobic interaction between M and B than the addition of acid or base.[10e] The addition of aqueous acid or base into matrix solution seems to be effective for an increasing of the sputtering yields coupled with a lowering of the viscosity of matrix solution. As already reported,[10e] the presence of carbonyl group(s) as a significant proton acceptor in analyte molecules was favorable for the formation of $[M+H]^+$ ions and inhibited the formation of $M^{+\cdot}$ ions. Furthermore, the use of hydrophilic and/or protic matrices such as DTT/TG, G, and PMG significantly increased the intensity ratio $I([M+H]^+)/I(M^{+\cdot})$, compared with the hydrophobic matrices such as NBA and NPOE.[3c]

From the circumstantial evidences above, we could present a concept of "quasi-preformed state" for $[M+H]^+$ formation, which means a hydrogen-bonding solvation between matrix hydroxyl group(s), b-OH ($\equiv$B), and the proton-accepting site(s) of analytes M in matrix solution as follows;

$$\mathrm{M+B} \rightleftarrows b\text{-}\mathrm{OH\cdots M} \rightleftarrows \mathbf{(M+H)^{+}}\mathrm{+(B-H)^{-}} \qquad (4)$$

In the case of the analyte and matrix molecules have active hydrogen(s), m-OH ($\equiv$M), (not strong acidic groups) and basic site(s), respectively, the solution equilibrium may be represented as follows;

$$\mathrm{M+B} \rightleftarrows m\text{-}\mathrm{OH\cdots B} \rightleftarrows \mathbf{(M-H)^{-}}\mathrm{+(B+H)^{+}} \qquad (5)$$

In the both equilibria (4) and (5), here we could present following definitions:

a) the quasi-preformed states, b-OH $\cdots$ M and m-OH $\cdots$ B, are in the states of hydrogen-bonding solvation, and

b) the preformed ions, $(M+H)^+$ and $(M-H)^-$, are in the free ionic states in solution.

The presence of quasi-preformed states, b-OH $\cdots$ M and m-OH $\cdots$ B, in solution may be supported by the interaction energy between the carboxyl oxygen of acetone and the hydroxyl group of ethanol, (*ca.* 8.37 kJ/mol), which has been spectroscopically measured as an orientation energy resulting from mutual orientation of the molecules by electrostatic effects.[13] Since the alcoholic hydroxyl group(s) of matrix materials is of hydrogen-bonding and weak in acidity, the equilibrium constant for the formation of preformed ions $(M+H)^+$, K_{DI}, must be very small than that for the formation of quasi-preformed state b-OH $\cdots$ M, K_{sl}, *i.e.*,

$$K_{\mathrm{sl}} \gg K_{\mathrm{Dl}} \tag{6}$$

where

$$K_{\mathrm{sl}} = [b\text{-OH} \cdots \mathrm{M}]/[\mathrm{M}][\mathrm{B}] \tag{7}$$

$$K_{\mathrm{Dl}} = [(\mathrm{M}+\mathrm{H})^{+}][(\mathrm{B}-\mathrm{H})^{-}]/[\mathrm{M}][\mathrm{B}] \tag{8}$$

Although, in the same manner, the concentration of preformed ions $(\mathrm{M}-\mathrm{H})^{-}$ in (5) would be very small than that of quasi-preformed state m-OH $\cdots$ B, if the analyte molecules have strong acidic moieties such as carboxyl, sulfonic, and phospho groups, the preformed ions $(\mathrm{M}-\mathrm{H})^{-}$ may occur preferentially by the electrolytic dissociation in matrix solution.[10m)]

Definition by means of the Bjerrum's proposition The concepts of "preformed ion" and "quasi-preformed state" could be defined by using the critical length q between an ion and one other ion of opposite charge as an ion-pair,[10m)] introduced by Bjerrum[14)];

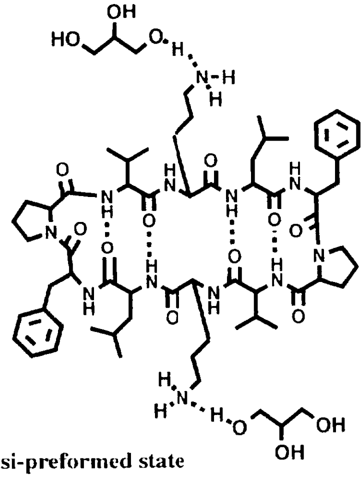
$$q = (|z_i z_j| \mathrm{e}^2)/(2\varepsilon k T) \tag{9}$$

where z_i, z_j, e, ε, k, and T represent charge number of i-th ion, charge number of j-th ion, elementary electric charge, dielectric constant of medium, Boltzmann's constant, and absolute temperature of solution, respectively. The critical length q means a distance d between ions of which the electrostatic potential energy between z_ie and z_je is equal to thermal energy $2kT$. The exact value of q is unimportant. The point is that the length q was introduced to distinguish between associated ion-pairs $(\mathrm{A}^{+}\mathrm{X}^{-})$ and free ions $(\mathrm{A}^{+} \cdots \mathrm{X}^{-})$ in solution. The length q might be represented by an intermediate $(\mathrm{A}^{+} \cdots \mathrm{X}^{-})$. Using the Bjerrum's proposition (9), the "preformed ion" could be defined as a case of which the distance d is longer than the length $q (d > q)$ which means free ions. On the other hand, the "quasi-preformed state" could be defined as a case of which the distance d is shorter than the length $q (d < q)$ which means in the state of associated ion-pairs. The ion-pairs $(\mathrm{A}^{+}\mathrm{X}^{-})$ behave like a neutral species. Schematic illustration for the "preformed ion" and "quasi-preformed state" is shown in Scheme 1.

Examples of quasi-preformed state The importance of quasi-preformed states for protonation reaction could be seen in the formation of doubly protonated molecules $[\mathrm{M}+2\mathrm{H}]^{2+}$ of angiotensin I and gramicidin S under FAB conditions with glycerol matrix.[10e)] The results obtained there suggested that a hydrogen-bonding interaction between the alcoholic hydroxyl group(s) of glycerol and the basic-site(s) of analyte compounds in solution plays an important role in the formation of $[\mathrm{M}+2\mathrm{H}]^{2+}$, as well as the formation of $[\mathrm{M}+\mathrm{H}]^{+}$ ions. For a cyclic peptide gramicidin S, such a quasi-preformed state in glycerol solution might be represented as Scheme 2. It has been well known, in the

quasi-preformed state ($d<q$)　　preformed ion ($d>q$)

Scheme 1.



Scheme 2.

study of maximum charge state of multiply protonated molecules $[M+nH]^{n+}$ formed by electrospray ionization (ESI),[15] that the protonation reaction is strongly correlated to the number of basic-residues and the conformation of analytes in solution. Gramicidin S has two significant basic residues, $-(CH_2)_3NH_2$, which are favorable in steric for protonation in solution, so that the $[M+2H]^{2+}$ ion may be formed by reflecting the quasi-preformed state in matrix solution (Scheme 2). For the formation of protonated species under FAB conditions, consequently, it is important to consider quasi-preformed states in solution even when an acid is added into matrix solution.

Various types of preformed ion It is a striking characteristic of FAB mass spectra to often give the peak of complex or adduct ions such as ammonium and/or alkali-metal cationized species, $[M+\mathbf{c}]^+$ ($\mathbf{c}=NH_4$, Na, K, *etc.*). These complex ions could be formed according to the strength of binding interaction between analytes M and cations $\mathbf{c}^+$ in gas-phase and/or solution. The complexation phenomenon under FAB conditions had been used for the order determination of binding selectivity between macrocyclic ligands and alkali-metals in solution.[9a), 9b)] We also have studied the complexation between crown ethers and alkali-metals, and directly compared the peak intensities of $[M+Na]^+$ and $[M+K]^+$ ions with corresponding stability constants ($\log K$) in solution.[10c)] The results obtained suggested that the peak intensities of $[M+Na]^+$ and $[M+K]^+$ ions in the FAB mass spectra would reflect the concentration of the complexes $(M+Na)^+$ and $(M+K)^+$ formed in matrix solution, and we called the complexes as preformed ions. Positive-ion FAB mass spectra of crown ethers, 12-crown-4 (12C4), 15-crown-5 (15C5), and 18-crown-6 (18C6) obtained without and with added an equimolar solution of NaCl and KCl are shown in Figs. 1, 2, and 3, respectively. The FAB mass spectra of these compounds without added the solution showed intense peaks corresponding to $[M+H]^+$ and $[M+NH_4]^+$ ions. The ammonium ion of adduct $[M+NH_4]^+$ ions may be originating from an impurity in matrix material, as shown in the case of valinomycin (Fig. 6). The relative abundances of $[M+\mathbf{c}]^+$ ions and stability constants ($\log K$)[16)] for crown ethers and alkali-metal ions are summarized in Table 1. It could be expected that

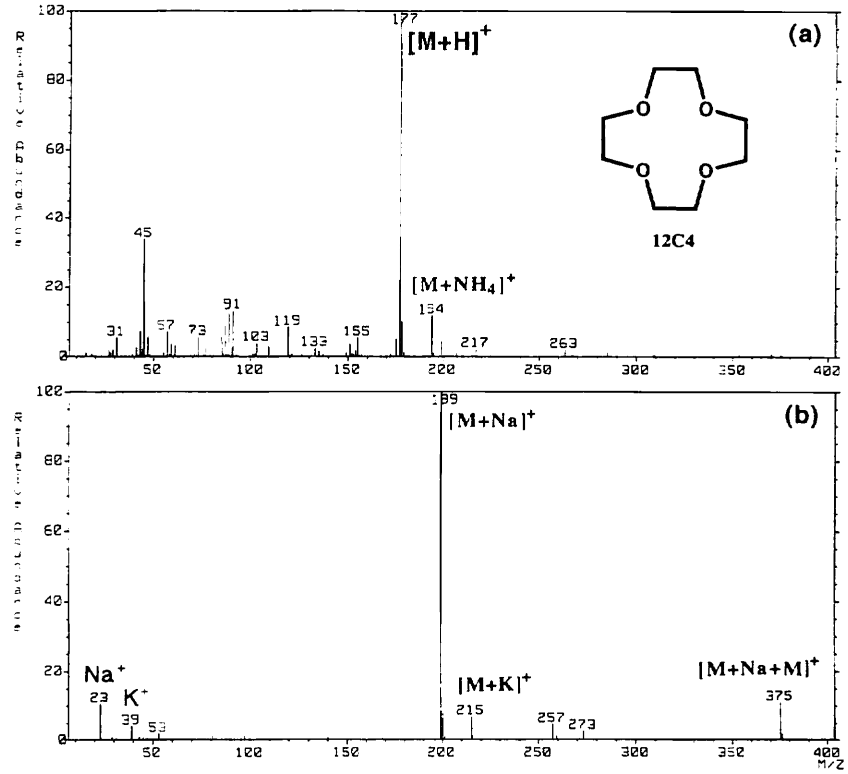


Fig. 1. FAB mass spectra of 12-crown-4 (a) without and (b) with added an equimolar NaCl and KCl solution.

the complexation in matrix solution occurs selectively according to the cation diameter (1.94Å for Na^+ and 2.66Å for K^+) and the hole diameter (1.2–1.5Å for 12C4, 1.7–2.2Å for 15C5, and 2.6–3.2Å for 18C6).[16c] The reason why the relative abundances for $[M+Na]^+$ and $[M+K]^+$ ions of 12C4 do not reflect the order of stability constants $Na^+ < K^+$ is that the stability constant for 2 : 1 complex as a "sandwich" structure, (12C4 ··· Na^+ ··· 12 C4), is larger than that for 1 : 1 complex, (12C4 ··· Na^+). The intense peak of $[M+Na]^+$ ion may be mainly originating from the 2 : 1 complex in solution. In fact, the FAB mass spectrum of 12C4 alone gave a peak corresponding to $[M+Na+M]^+$ ion at *m/z* 375, which is an evidence that the complexation occurs to form preformed ions in matrix solution (Scheme 3). Furthermore, the peak abundances of Na^+ and K^+ ions seem to reflect rather the peak abundances of $[M+Na]^+$ and $[M+K]^+$ ions than the cation concentration in solution. This suggests that the peaks of metal ions Na^+ and K^+ are originating from the loss of analyte molecule M from the adduct ions $[M+\mathbf{c}]^+$, as well as the direct sputtering of free ions Na^+ and K^+ in matrix solution.

Another type of the complexation in solution may be due to the host-guest interaction between host cyclodextrins (CDs) and guest ligands.[17] The torus-shaped oligosaccharides consisting of six (α-CD), seven (β-CD), and eight (γ-CD) glucose units could selectively include through hydrophobic interaction the guest ligands L, according to a match of size between the host and the ligands. The cavity diameters are 4.5Å for α-CD,

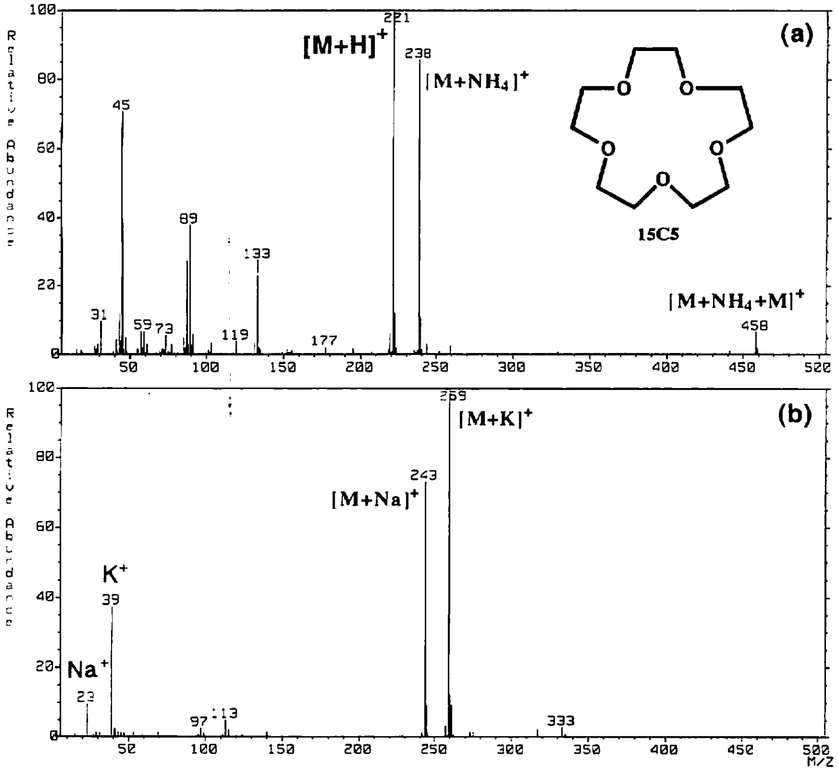


Fig. 2. FAB mass spectra of 15-crown-5 (a) without and (b) with added an equimolar NaCl and KCl solution.

7.0Å for β-CD, and 8.5Å for γ-CD. Kurono *et al.* first reported the detection of complexes between β-CD and some ligands such as nileprost, propranolol, and nipradilol, using FAB method.[9d)] Here we examined the complex formation between CDs and acetylcholine (AC^+) or glycyl-glycyl-L-leucine (GGL) under FAB conditions with DTT/TG matrix. Positive-ion FAB mass spectra of α-CD, β-CD, and γ-CD obtained with adding an aqueous solution of acetylcholine iodide (AC^+I^-) are shown in Fig. 4. These spectra clearly showed the peaks at *m/z* 1,118, *m/z* 1,280, and *m/z* 1,442 that correspond to complex ions of $[\alpha\text{-CD}+AC]^+$, $[\beta\text{-CD}+AC]^+$, and $[\gamma\text{-CD}+AC]^+$, as well as the protonated molecules $[M+H]^+$. A possible structure of the complex ions formed based on the hydrophobic interaction is shown in Scheme 4. A similar complexation between resorcin[4]arene and acetylcholine in a neutral aqueous solution, based on the hydrophobic and/or cation-π interactions, has reported in the study of acetylcholine receptors.[18)] The complexation of CDs with another ligand, a peptide GGL, also occurred under FAB conditions. The FAB mass spectrum of β-CD showed a definite peak that correspond to complex $[M+H+GGL]^+$ ion, as shown in Fig. 5. It should also be noted that acetylcholine is a preformed ion in itself which is coming from the electrolytic dissociation of ionic compounds, A^+X^-, in a matrix B, as follows;

$$B+A^+X^- \rightarrow B+A^+ +X^- \qquad (10)$$

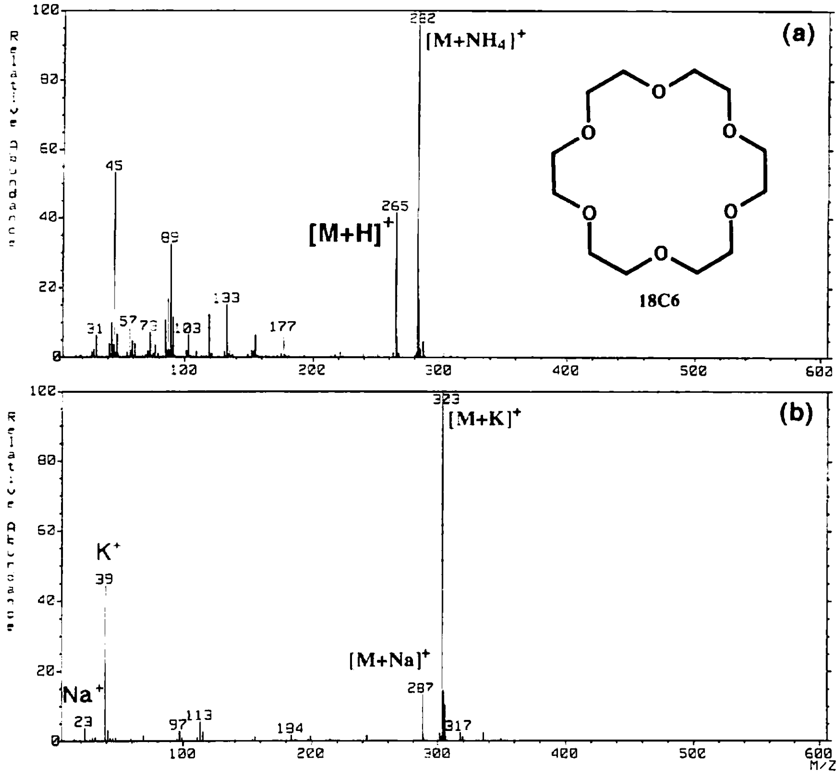


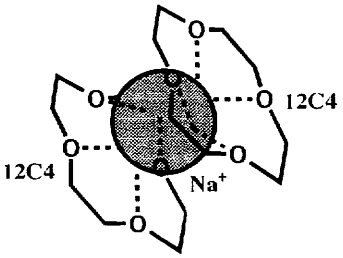
Fig. 3. FAB mass spectra of 18-crown-6 (a) without and (b) with added an equimolar NaCl and KCl solution.

Table 1. Relative Abundances of $[M+c]^+$ Ions on Addition of an Equimolar Solution of NaCl and KCl, and Stability Constants (log K) for Crown Ethers and Cations

Crown ether	Relative abundance (%)[a]				log K	
	H^+	NH_4^+	Na^+	K^+	Na^+	K^+
12C4	—	—	100	7	1.70	1.74
	(100)	(12)	(5)	(—)		
15C5	—	—	73	100	3.24	3.43
	(100)	(86)	(3)	(3)		
18C6	—	—	13	100	4.35	6.08
	(42)	(100)	(5)	(2)		

[a] Top rows, values with salt addition; values in parentheses, without salt addition.

Since a cyclic depsipeptide valinomycin is an ionophore which can interact with cations such as NH_4^+, Na^+, K^+, and Rb^+ in solution,[19] it may form preformed ions in matrix solution. In fact, the FAB mass spectrum of valinomycin showed an intense peak corresponding to an ammonium cationized molecule $[M+NH_4]^+$ without added salt such as NH_4Cl (Fig. 6). But, valinomycin did not show the peak of complex ions with AC^+ and GGL in the FAB mass spectra, though the spectra are not shown. This may be due to the selectivity between hosts and guests.



Scheme 3.

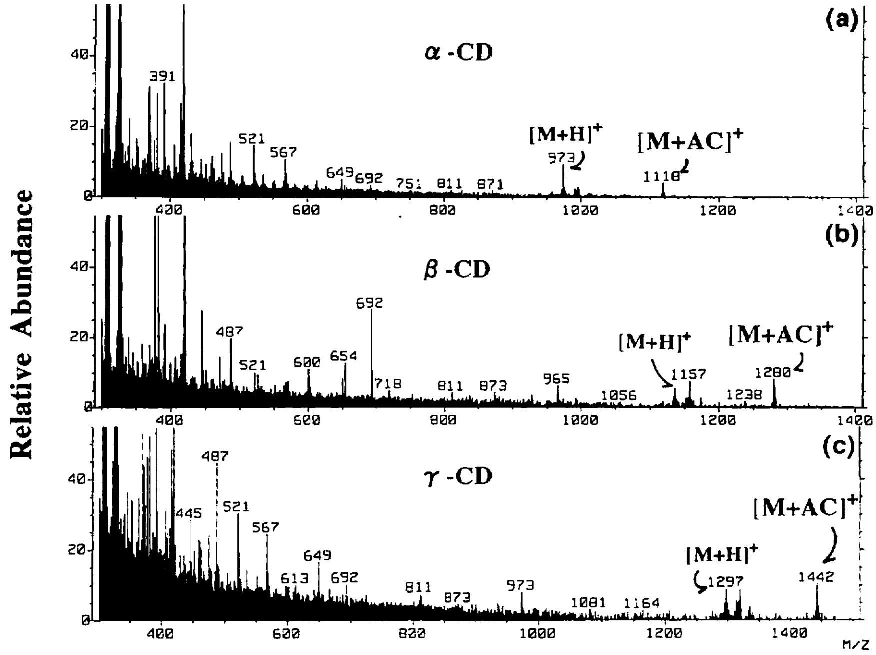
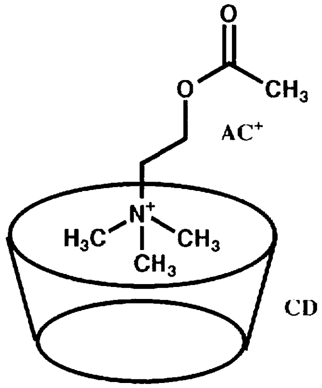


Fig. 4. FAB mass spectra of (a) α-cyclodextrin, (b) β-cyclodextrin, and (c) γ-cyclodextrin, with added acetylcholine iodide (AC^+I^-).

Although some types of preformed ion were described above, it should be considered that the complex ions detectable in FAB mass spectra could be formed in both gas-phase and solution even when an analyte formed the preformed ions in matrix solution, and that the extent of formation of preformed ions strongly depends on the binding energy between guest analytes and host ions, the acidity of organic acids, and the electrolytic dissociation constant of ionic compounds in solution.

3.2 Electronic excitation

Although the preferential formation of molecular ions $M^{+\cdot}$ under FAB conditions was a rather unusual phenomenon, now it has been supported by some reports[10), 20)] that it is a fairly usual phenomenon and that the formation of $M^{+\cdot}$ and $[M+H]^+$ ions occurs competitively according to the presence/absence of appropriate basic sites in analyte molecules. Since the formation of $M^{+\cdot}$ ions of organic compounds requires an electronic



Scheme 4.

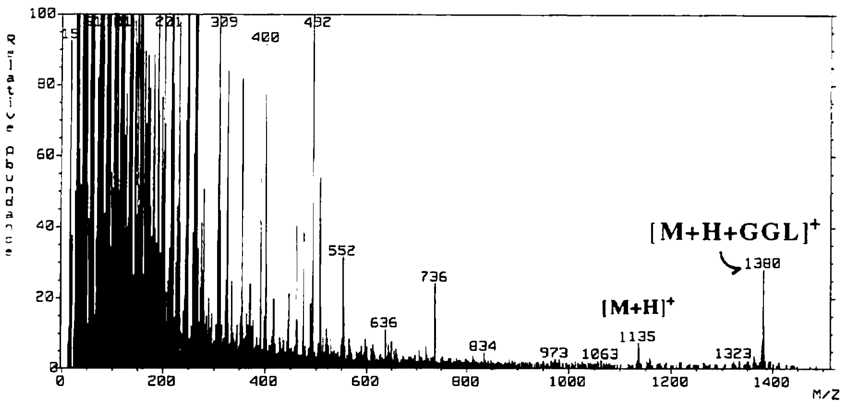


Fig. 5. FAB mass spectrum of β-cyclodextrin with added glycyl-glycyl-L-leucine.

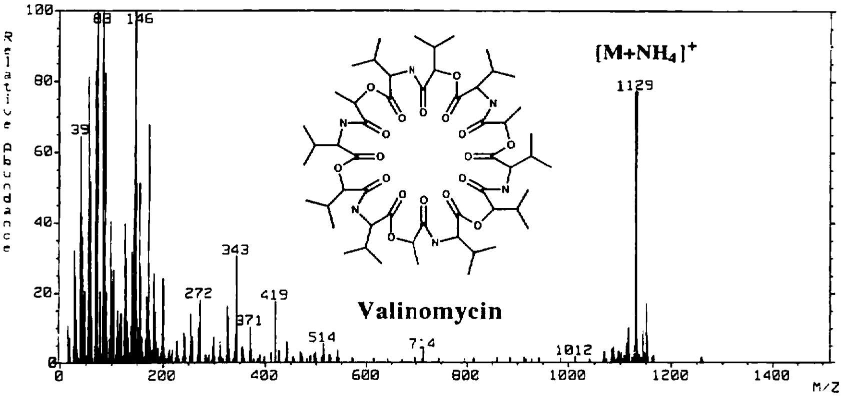


Fig. 6. FAB mass spectrum of valinomycin without added salt NH_4Cl.

excitation of about 10 eV, it is interesting in connection with the origin of internal energy of $M^{+\cdot}$ ions formed how FAB brings about such electronic excitation of organic compounds.

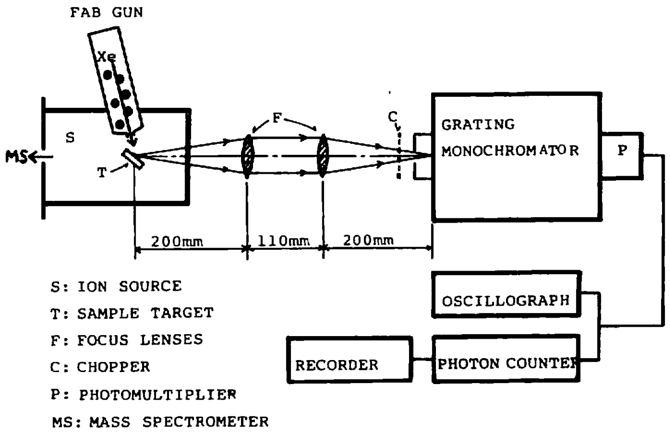


Fig. 7. Schematic layout of the experimental system.

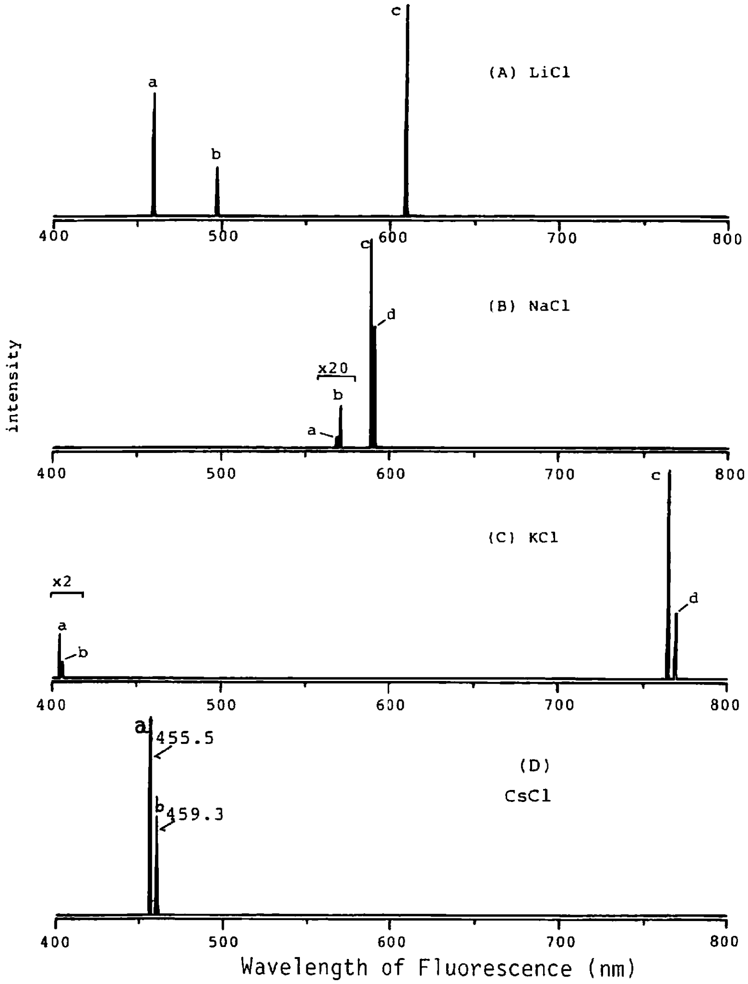


Fig. 8. Fluorescence spectra of (A) LiCl, (B) NaCl, (C) KCl, and (D) CsCl.

Direct confirmation of electronic excitation by using FAB-induced fluorescence In order to confirm the electronic excitation under FAB conditions, the observation and analysis of fluorescence emitted from the solid thin-layer of LiCl, NaCl, KCl, and CsCl coated onto a FAB target were done by using 6 keV Xe beam.[10d] The fluorescence was observed with the intrinsic colors red for LiCl, orange for NaCl, purple for KCl, and light blue for CsCl. A schematic layout of the experimental system and the fluorescence spectra obtained are shown in Figs. 7 and 8, respectively. The assignments of each peak, wavelength (nm) and transition energy levels (cm^{-1}) based on the data of atomic

Table 2. Wavelength (nm) and the Corresponding Transition Energy Levels (cm^{-1}; in parentheses) of FAB-Induced Fluorescence Observed for LiCl, NaCl, KCl, and CsCl

Peak	Wavelengths of observed fluorescence			
	LiCl	NaCl	KCl	CsCl
a	460.29 (36623→14904)	568.27 (34549→16956)	404.42 (24720→0)	455.54 (21947→0)
b	497.17 (35012→14904)	568.82 (34549→16903)	404.72 (24701→0)	459.32 (21766→0)
c	610.36 (31283→14904)	588.99 (16973→0)	766.49 (13043→0)	
d		589.59 (16956→0)	769.90 (12985→0)	

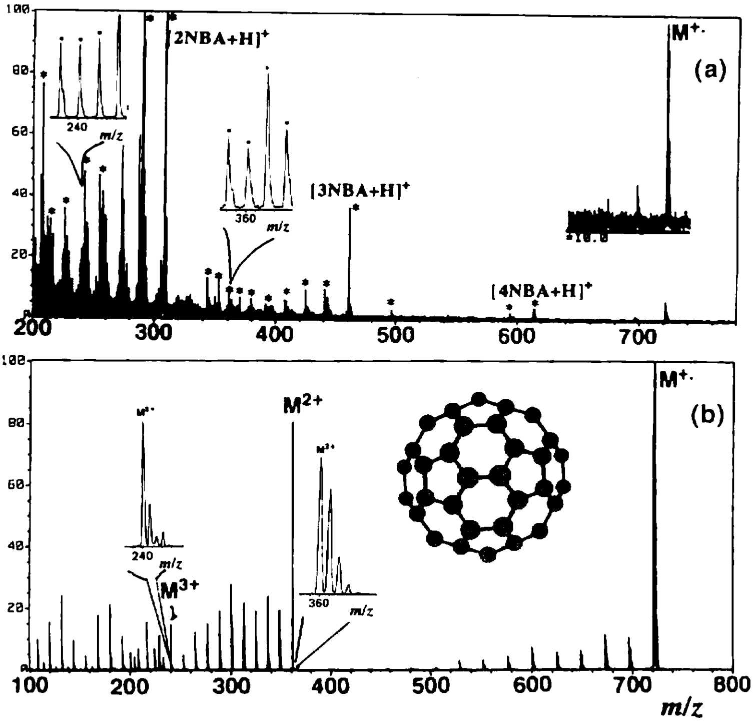


Fig. 9. FAB mass spectra of fullerene C_{60} by (a) matrix FAB and (b) gas-phase FAB techniques using 5 keV Ar beam.

transition and experimental transition probabilities for spectral lines from an arc discharge of elements,[21] are summarized in Table 2. Since the transition energy levels of 36,623 cm^{-1} for LiCl, 34,549 cm^{-1} for NaCl, 24,720 cm^{-1} for KCl, and 21,947 cm^{-1} for CsCl could be converted to 4.54 eV, 4.28 eV, 3.06 eV, and 2.72 eV, respectively, it is suggested that alkali chlorides were electronically excited by 6 keV Xe bombardment up to at least 4.54 eV. Although this is a direct evidence for electronic excitation under FAB conditions, the energy is insufficient to form the molecular ion $M^{+\cdot}$ of organic compounds.

Electronic excitation under matrix FAB conditions In order to estimate the extent of electronic excitation under matrix FAB conditions, we tried to detect multiply-charged molecular ions, M^{z+} ($z=1$–3), of pyrene, coronene, and fullerene C_{60} which have the known ionization energies (IE)[10j]; *i.e.*, pyrene: 7.4 eV for $M^{+\cdot}$, 21.8 eV for M^{2+}, and 38.7 eV for M^{3+},[22] coronene: 7.4 eV for $M^{+\cdot}$, 21.0 eV for M^{2+}, and 37.1 eV for M^{3+},[22] and C_{60}: 7.6 eV for $M^{+\cdot}$, 19.9 eV for M^{2+}, and 36.9 eV for $M^{3+\cdot}$.[23] All the compounds showed only the $M^{+\cdot}$ ions in their FAB mass spectra obtained with 5 keV Ar beam and NBA matrix, while both 70 eV/EI and gas-phase FAB (5 keV Ar) mass spectra showed the peaks corresponding to $M^{+\cdot}$, M^{2+}, and M^{3+} ions. The results obtained suggested that in the case of C_{60} the matrix FAB process could cause electronic excitation of at least 7.6 eV, but less than 19.9 eV. The FAB mass spectra of C_{60} obtained with (a) NBA matrix and (b) gas-phase FAB technique using 5 keV Ar beam are shown in Fig. 9.

Furthermore, some α-(1-pyrenyl)benzyl esters, which form preferential $M^{+\cdot}$ ions

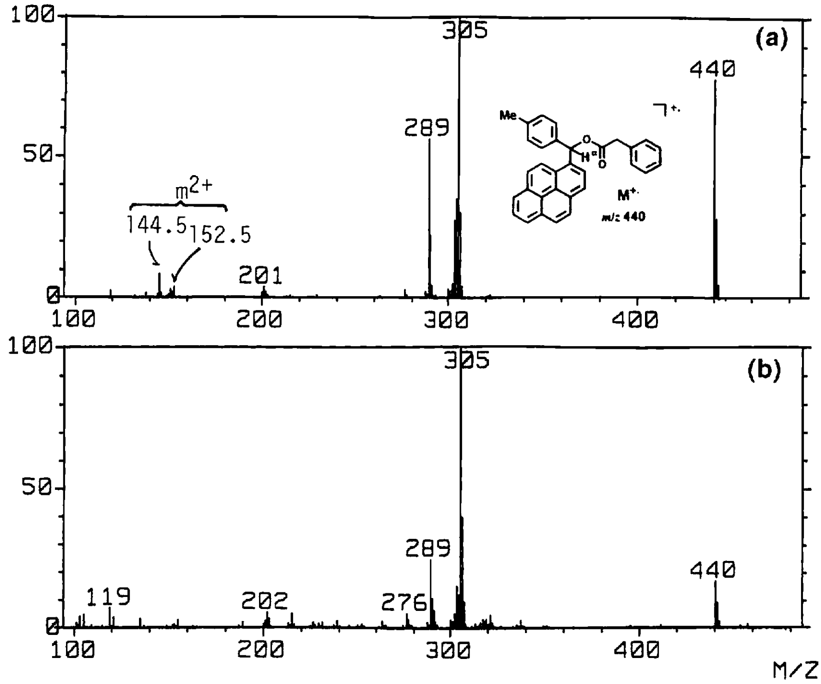


Fig. 10. Mass spectra of α-(1-pyrenyl)-*p*-methylbenzyl phenylacetate by (a) 70 eV/EI and (b) matrix FAB techniques.

under matrix FAB conditions, did not show any doubly-charged ions in the FAB mass spectra, whereas the 70 eV/EI mass spectra clearly showed the peaks corresponding to doubly-charged fragment ions.[10i] The EI and FAB mass spectra of α-(1-pyrenyl)-*p*-methylbenzyl phenylacetate are shown in Fig. 10. Therefore, it could be concluded that matrix FAB conditions are sufficient in energy for the formation of $M^{+\cdot}$ ions which requires about 10 eV electronic excitation, and are insufficient for the formation of multiply-charged molecular and fragment ions, M^{z+} and m^{z+} ($z=2, 3$), which requires relatively high electronic excitation of about 20 eV or above.

Relating to the electronic excitation under FAB conditions, it is of interest to consider the appearance of a number of fragment ions in the FAB mass spectra of organic compounds. In the previous paper,[3c] it was described that the extents of fragmentation of $M^{+\cdot}$ ions produced under matrix FAB conditions could be corresponded to those produced under 21–22, 24–25, or 30–70 eV/EI conditions, and that the extents of fragmentation of $[M+H]^+$ ions produced under matrix FAB conditions were always greater than those produced under isobutane chemical ionization (CI) conditions. The results described above suggest that the origin of internal energy to form fragment ions from the $M^{+\cdot}$ and $[M+H]^+$ ions is not only electronic excitation and proton transfer reaction, but also vibrational excitation caused by collision cascade in a condensed-phase and by the following processes such as collisions between analyte and matrix molecules in a gas-phase, because the vibrational excitation may affect directly degradation of the molecular-related ions. Such a consideration will be described in the introduction of cavity model subsequently.

3.3 FAB characteristic ions and analyte structures

$[M+H]^+$ formation It has been known that the ion formation and ion yield of non-ionic compounds under matrix FAB conditions strongly depend on the structure of analyte molecules, as well as the nature of liquid matrices. In particular, the influence of acetylation on the formation and yield of $[M+H]^+$ ions was significant.[10e] Using relatively simple phenol and aniline compounds, the ratios of relative abundances of $[M+H]^+$ ions to those of $M^{+\cdot}$ ions, $I([M+H]^+)/I(M^{+\cdot})$, and ion yields were evaluated from the FAB mass spectra measured before and after the acetylation of these compounds.

Table 3. Peak Abundance Ratios of $[M+H]^+$ Ions to $M^{+\cdot}$ Ions Obtained from the FAB Mass Spectra Measured before and after the Acetylation of Some Simple Compounds Using DTT/TG Matrix

Compounds (RMM)	$I([M+H]^+)/I(M^{+\cdot})$	
	Before acetylation	After acetylation
Trolox (250)	0.33 (67.2)	0.96 (444)
o-Carboxyaniline (137)	2.72 (276)	17.8 (265)
m-Carboxyaniline (137)	2.57 (81.8)	27.9 (676)
p-Carboxyaniline (137)	2.47 (310)	22.9 (588)
p-Methoxyaniline (123)	3.88 (683)	3.83 (1098)
m-Aminobenzyl alcohol (123)	1.56 (295)	6.30 (156)
3-Hydroxy-4-methoxyaniline (139)	1.94 (310)	5.75 (811)

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

The values determined are summarized in Table 3. The acetylation of these compounds markedly increased the ratios and enhanced the yields of $[M+H]^+$ ions, except for a few cases. These effects may originate from both reasons that the carbonyl group as a significant proton acceptor[24)] plays a role in the preferential formation of $[M+H]^+$ over $M^{+\cdot}$ ions and the methyl group contributes to surface activity, though it is difficult to decide where protonation occurs.

Without the addition of acids as a proton donor, the formation of $[M+H]^+$ ions could be explained by the proton-transfer reactions (11) and (12.2) as follows;

$$M+B+A_{fast} \rightarrow [M+H]^+ + [B-H]^- + A \qquad (11)$$

$$M+B+A_{fast} \rightarrow [B+H]^+ + [M-H]^- + A \qquad (12.1)$$

$$M+[B+H]^+ \rightarrow [M+H]^+ + B \qquad (12.2)$$

where A_{fast} represents the fast atoms. In these reactions, (11) and (12.1) may come from solvation states that correspond to the equilibria (4) and (5), respectively. The reaction (12.2) as a secondary process may occur in gas-phase like a CI process. To see a major process for the formation of $[M+H]^+$ ions in the above reactions, an experiment was conducted by using a deuterated matrix, deuteroglycerol (G-d_3), and an analyte methyl stearate which is fat-soluble or hydrophobic in nature. Since methyl stearate has a carbonyl group as a proton acceptor, it could be expected that the formation of

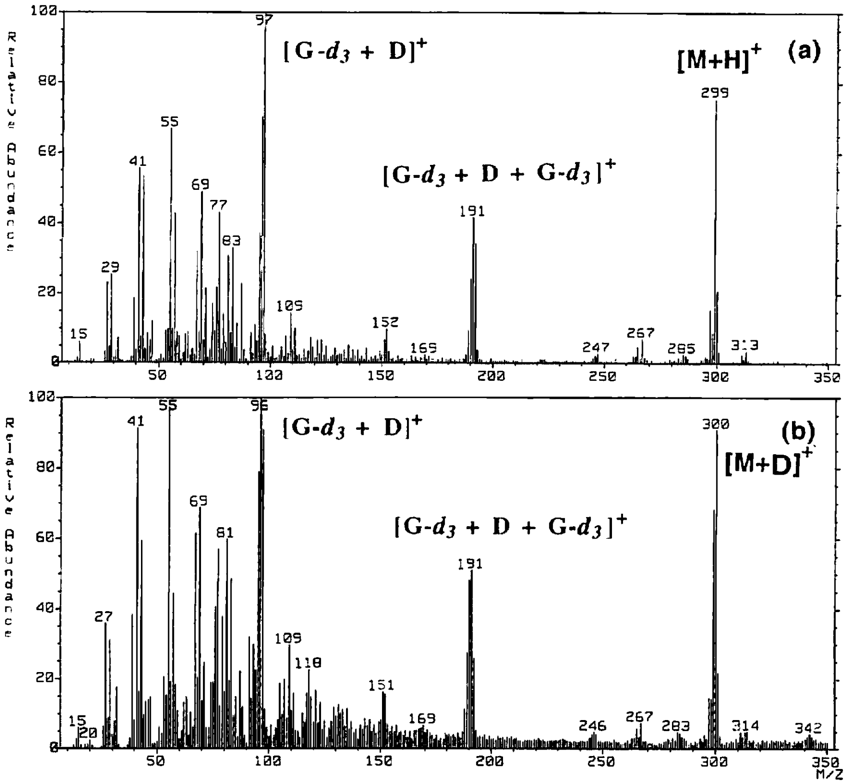
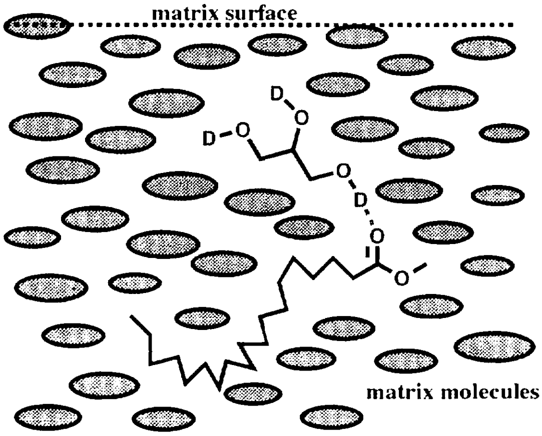


Fig. 11. FAB mass spectra of methyl stearate with a deuteroglycerol matrix, whereby (a) analyte was merely putted on the matrix surface and (b) analyte was mixed intimately with the matrix.



Scheme 5.

deuteronated molecule $[M+D]^+$ occurs under FAB conditions with G-d_3 matrix. Figure 11 is the FAB mass spectra of methyl stearate, obtained with two methods: i) the analyte powder was merely putted on the matrix surface and ii) several 10 micrograms of the analyte was mixed intimately with the matrix in a micropipet. Figure 11a with the method i) showed the peaks corresponding to $[M+H]^+$ ion at *m/z* 299 and $[G\text{-}d_3+D]^+$ ion at *m/z* 97. The proton for the $[M+H]^+$ formation may originate from a D/H exchange on the matrix surface in ionization cell. The result obtained suggests that a deuteron transfer from the reagent ions $[G\text{-}d_3+D]^+$ to analyte molecules M does not occur in gas-phase. On the other hand, Fig. 11b with the method ii) showed an intense peak of deuteronated molecule $[M+D]^+$ at *m/z* 300 with the protonated molecule $[M+H]^+$. This suggests that it is required for the formation of $[M+D]^+$ ion the analyte molecules are lying in the lower layer of matrix surface, not on the surface, as shown in Scheme 5. Consequently, a major process for the formation of $[M+H]^+$ ions under matrix FAB conditions is the reaction (11) which may reflect solvation states.

$[M+Na]^+$ formation Positive-ion FAB mass spectra of complex analytes such as glycosides, oligosaccharides, and other highly polar compounds often show characteristic adduct ion peaks that correspond to sodium and/or ammonium cationized species, $[M+Na]^+$ and/or $[M+NH_4]^+$, without addition of salts such as NaCl and NH_4Cl.[10c] The adduct ion formation may originate from the salt impurity in matrix solution and the relatively strong cation affinity of analyte molecules. The peak abundances of the cationized species in FAB mass spectra may reflect a binding ability or affinity of analyte molecules to cations, though such a binding ability or affinity is not the quantity defined by thermodynamics. Comparing the peak abundances of $[B_1+\mathbf{c}]^+$ and $[B_2+\mathbf{c}]^+$ ions in the FAB mass spectra of 1 : 1 (v/v) mixtures of matrices B_1 and B_2 with added salt **c**Cl (**c**=Li, Na, Cs, and NH_4), the order of cation affinity for various matrix materials has been estimated as follows;[10h]

Li^+: DEA > G > DTT > TG > NBA
Na^+: DEA > G > DTT > TG > NBA
Cs^+: DEA > NBA > G > DTT > TG

NH_4^+: G>DTT>NBA>TG>DEA

The complexation to form adduct ions $[B+c]^+$ depends strongly on the cation size and structural nature of matrix B. Although the collision-induced dissociation (CID) spectra of $[B+c]^+$ ions suggested that the CID patterns reflect the coordination structures and binding energy of the $[B+c]^+$ ions,[10h)] the order of cation affinity estimated could not be rationalized. To see structure characteristics of analytes for the formation of $[M+Na]^+$ ions, the influence of acetylation has been examined by employing the *o*-, *m*-, and *p*-isomers of simple phenol and aniline compounds.[10f)] In order to estimate the peak abundances, we employed the abundance of matrix ion $[DTT+Na]^+$ as a reference peak, *i.e.*, $I([DTT+Na]^+)$. The ratios of relative abundances of $[M+Na]^+$ ions to $I([DTT+Na]^+)$ were determined from the FAB mass spectra measured before and after the acetylation of the compounds, with added NaCl, as summarized in Table 4. The FAB mass spectra of nitrophenol isomers did not show the $[M+Na]^+$ ions. This could be explained by which nitro group is a significant electron acceptor rather than a cation acceptor. Except for the nitrophenol, the effect of acetylation on the formation of $[M+Na]^+$ ions is particularly noteworthy in all the *o*-isomers used here. This might be coming from an isomeric effect in which the -isomers acetylated give more favorable conformations for multisite binding with an Na^+ ion than *m*- and *p*-isomers. The carbonyl oxygen in the acetyl group presents a cation binding site, as well as the isomeric effect. A typical example using an analyte, salicylic acid phenyl ester, is shown in Fig. 12. In Fig. 12(c), a positive influence of methylation on the $[M+Na]^+$ formation may be due to an inductive effect originating from the electron-donating property of the methyl group.

From the results obtained in Table 4, the structure characteristics required to bind Na^+ ion could be presented as Scheme 6. Furthermore, it could be considered that binding and releasing of Na^+ ion occur by a conformational change with the rotation of the single bond between phenolic-oxygen and carbonyl-carbon in the acetyl group, $PhO\text{-}COCH_3$, in gas-phase or solution (Scheme 7). This is of interest in connection with the cation transport phenomena through cell membranes.[10l)]

$[M-H]^+$ Formation Although the formation of $[M-H]^+$ and $M^{+\cdot}$ ions under matrix FAB conditions was unusual phenomena before, now it could be believed that molecular-related ions, $[M-H]^+$, $M^{+\cdot}$ and $[M+H]^+$, form competitively according to the nature of analytes and matrix used.[25)] The formation of dehydride molecules $[M-H]^+$

Table 4. Peak Abundance Ratios of $[M+Na]^+$ Ions to $[DTT+Na]^+$ Ions Obtained from the FAB Mass Spectra Measured before and after the Acetylation of Some Simple Compounds, with Added NaCl

	o-	*m*-	*p*-
Carboxyphenols	4.55 (0.06)	1.02 (0)	0.02 (0.03)
Carboxyanilines	0.71 (0.50)	0.43 (0)	0.09 (0.10)
Nitrophenols	0 (0)	0 (0)	0 (0)
Acetylphenols	2.70 (—)	—	0.14 (0.34)
Acetylaniline	1.64 (—)	—	—
Methoxyphenol	—	—	0.72 (0)
Methoxyanilines	4.17 (—)	—	2.05 (0.02)

( ) Before acetylation.

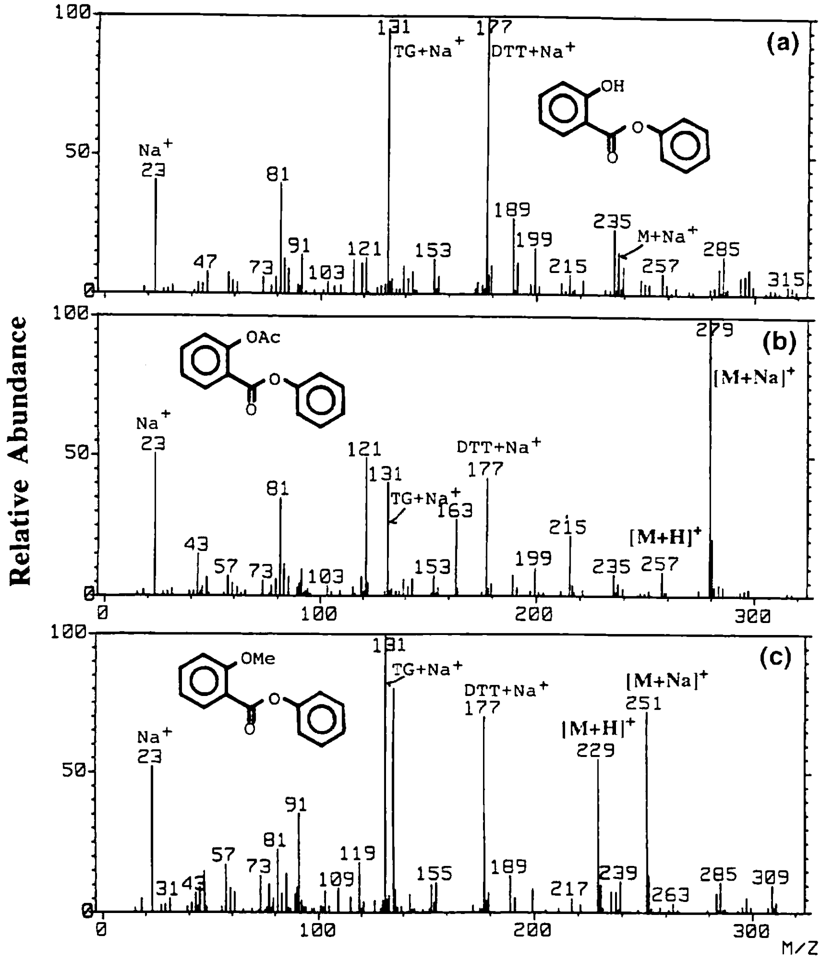
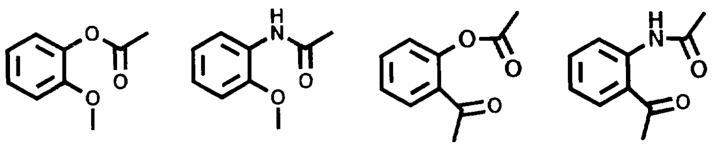


Fig. 12. FAB mass spectra of (a) salicylic acid phenyl ester, (b) acetylsalicylic acid phenyl ester, and (c) methylsalicylic acid phenyl ester, with added NaCl.

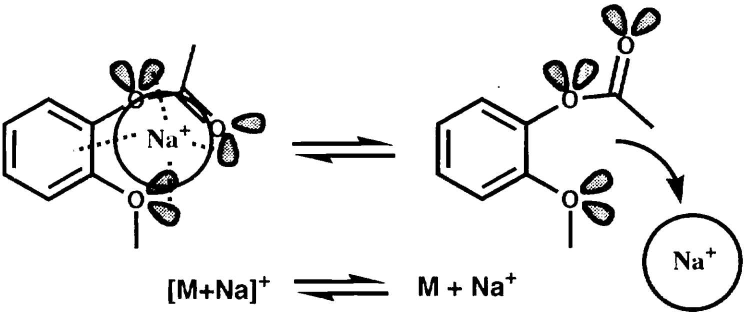


Scheme 6.

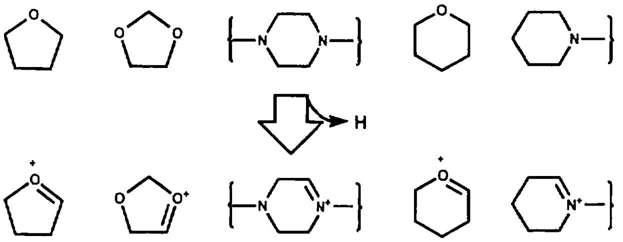
relates strongly to the presence of liquid matrices, and it has been suggested that the reaction to form $[M-H]^+$ ions proceeds by interactions between analyte and matrix (or matrix ions) as a result of FAB-induced processes in a condensed-phase.[26] A likely reaction is the hydride abstraction like a CI process in which a matrix ion $[B+H]^+$ in (12.1) plays a role of reagent ion as follows;

$$M+[B+H]^+ \rightarrow [M-H]^+ + BH_2 \text{ (or } B+H_2) \qquad (12.3)$$

This is a secondary process in gas-phase which disagrees with the consideration by Paul



Scheme 7.



Scheme 8.

et al.[26), 27)] From a beam-induced accumulation phenomenon that the peak abundance of $[M-H]^+$ ions increased with time of FAB beam irradiation, they have concluded that the hydride abstraction occurs by FAB-induced process in the condensed-phase. Therefore, another process could be presented to form $[M-H]^+$ ions, which may come from a relatively high-energy process, as follows;

$$M+B+A_{fast} \rightarrow [M-H]^+ + B + H^- + A \qquad (13)$$

This seems to be a reaction reflecting hydride-donating property of analytes rather than the hydride abstraction due to reagent ions. This is suggested from the fact that analyte compounds which are liable to form $[M-H]^+$ ions under matrix FAB conditions have structure characteristics.[26)] Such structures could form the $[M-H]^+$ ions by releasing a hydride H^-, as shown in Scheme 8.

$M^{+\cdot}$ formation Under matrix FAB conditions, the positive molecular ions $M^{+\cdot}$ seemed to form for analyte molecules lacking in the basic groups as proton acceptor.[10e)] As described in Subsection **3.2**, polycyclic aromatic hydrocarbons (PAHs) such as pyrene and coronene are such typical analytes. For the formation of $M^{+\cdot}$ ions under matrix FAB conditions, some suggestions have been already obtained as follows[10a)];

(1) the liquid matrix plays any essential roles,
(2) the addition of electron acceptor such as *p*-benzoquinone does not enhance $M^{+\cdot}$ formation,
(3) one-electron transfer between analytes does not occur,
(4) the abundant $M^{+\cdot}$ formation cannot be explained by three different mechanisms, *i.e.*, the direct collision between analytes and fast atoms, the formation of a charge-transfer complex between analyte and matrix, and the charge exchange reaction between analytes and fast atom ions such as $Xe^{+\cdot}$.

Further, the charge exchange reaction between analytes and matrix ions such as $B^{+\cdot}$, which was a powerful possibility for the $M^{+\cdot}$ formation,[10i), j)] was recently denied from the results obtained under neat liquid FAB conditions without matrix.[10k)] The neat analyte of α-tocopherol which is a viscous oily substance shows an intense peak corresponding to the $M^{+\cdot}$ ion and little shows the $[M+H]^+$ ion in the FAB mass spectrum obtained without liquid matrix.[28)] For such a system, an earliest collision interaction between surface analyte molecules M_{surf} and fast atoms A_{fast} and the following collision cascade interactions between analyte molecules M in a condensed-phase are essential to form ionic species. Since the collision interaction between M_{surf} and A_{fast} as an earliest event would result in a similar phenomenon with the gas-phase FAB processes,[29)] the molecular ions $M^{+\cdot}$ could form by the high-energy collision. However, the gas-phase FAB mechanism does not explain the abundant $M^{+\cdot}$ formation because of the low flux of the fast atom beam.[30)] In fact, the abundance of ions formed by gas-phase FAB was a few orders of magnitude lower than that by neat liquid FAB.

A most likely event with respect to the abundant ion formation seems the collision cascade as a secondary event which is a relatively high-energy consecutive collision in the analyte condensed-phase. It seems to be clear that a major process is not collisions between analytes and fast atoms, but consecutive collisions between analytes which may lead to the phase explosion (explosive or very rapid vaporization) of a local surface layer.[31)] For the analyte of α-tocopherol, this means that ionization leading to the abundant $M^{+\cdot}$ formation occurs by the consecutive collisions between analytes in the condensed-phase and/or the following collision interactions in a dense gas-like phase, as follows[10k)];

$$M_{surf}+A_{fast} \rightarrow M^{*} \quad \text{or} \quad M_{fast}+A \tag{14.1}$$

$$M^{*} \quad \text{or} \quad M_{fast}+M \rightarrow M^{+\cdot}+e^{-}+M_{fast} \tag{14.2}$$

where M^* and M_{fast} represent an excited analyte and a high-translational analyte, respectively. This is a collision-induced ionization. According to the experimental results described above (Figs. 9 and 10), the collision processes (14.1) and (14.2) are sufficient in energy for the $M^{+\cdot}$ formation, and are insufficient for the formation of multiply-charged molecular and fragment ions. For matrix FAB conditions, the process (14.2) corresponds to collision interactions between matrix and analyte molecules. Such collision interactions bring about the competitive formation of $[M-H]^+$, $M^{+\cdot}$, and $[M+H]^+$ ions.

$M^{-\cdot}$ and $[M-H]^-$ Formation It is a usual phenomenon that negative-ion FAB mass spectra of organic compounds show a preferential peak corresponding to deprotonated molecules $[M-H]^-$. It has been reported by the author[10g)] that an important condition for which the reaction successfully proceeds to the formation of $[M-H]^-$ ions under matrix FAB conditions is the presence of liquid matrix as a proton acceptor, as well as the presence of acidic or active hydrogen(s) in analyte molecules. In particular, the use of higher matrices in proton acceptability is favorable for the formation of $[M-H]^-$ ions.[10m)] The order of proton acceptability, which differs from gas-phase basicity and proton affinity, was evaluated as follows[10g)];

H^-: DEA > PMG > DTT > NBA

In the absence of significant acidic groups such as carboxyl group(s) in analyte

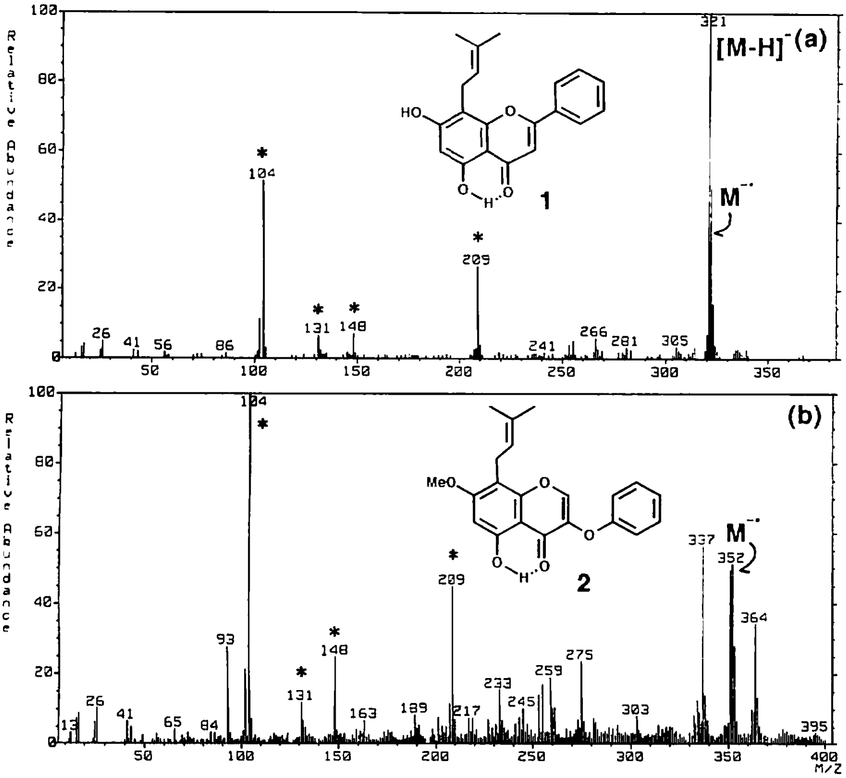


Fig. 13. Negative-ion FAB mass spectra of (a) 8-prenylchrysin and (b) compound 2, with DEA matrix.

molecules, on the other hand, matrix FAB mass spectra often give a preferential peak corresponding to $M^{-\cdot}$ ions.32) The electrons attached to analytes may be originating from secondary free electrons formed in the reaction (14.2). The formation of $M^{-\cdot}$ and $[M-H]^-$ ions under matrix FAB conditions may occur competitively according to such natures of analyte as electron affinity (EA), basicity, and structures. In fact, it has been suggested that nitroaromatic isomers (*o*-, *m*-, and *p*-) are distinguishable from to each other by using the abundance ratios, $I([M-H]^-)/I(M^{-\cdot})$, evaluated from negative-ion FAB mass spectra.32d) In this case, the presence of nitro group(s) as a significant electron acceptor is essential for the formation of $M^{-\cdot}$ ions.

The influence of phenolic hydroxyl group(s) on the formation of $M^{-\cdot}$ and $[M-H]^-$ ions of some flavonoid compounds interestingly could be examined by using negative-ion FAB mass spectrometry. Negative-ion FAB mass spectrum of 8-prenylchrysin (1),33) having two hydroxyl groups at 5- and 7-positions, showed an intense peak corresponding to $[M-H]^-$ ion at *m/z* 321 (Fig. 13a). Asterisk indicates the peaks originating from DEA matrix. Since the proton of hydroxyl group at 5-position may be restricted by an intramolecular hydrogen-bonding with carbonyl oxygen at 4-position, it could be considered that deprotonation mainly occurs in the hydroxyl group at 7-position. This could be supported from the FAB mass spectrum of a compound (2)34) of which the –OH group at 7-position was protected by methylation, as shown in Fig. 13b. The FAB mass spectrum showed an intense peak corresponding to $M^{-\cdot}$ ion at *m/z* 352, as well as $[M-H]^-$ ion at *m/z* 351. Almost the same abundances of $M^{-\cdot}$ and $[M-H]^-$ ions in this spectrum may not be due to enhancement of $M^{-\cdot}$ formation, but due to decreasing of the rate of $[M-H]^-$ formation owing to the methylation. The same tendency, in which the ratio $I([M-H]^-)/I(M^{-\cdot})$ decreases by the methylation of phenolic hydroxyl groups, was observed in the FAB mass spectra of morusin and its methyl esters,[35] though the spectra are not shown.

The important factors for the formation of $M^{-\cdot}$ ions under matrix FAB conditions, therefore, are the lack of acidic groups such as carboxyl, sulfonic, phospho, and hydroxyl groups, and the presence of electron acceptors such as nitro group, halogens, and any oxygen- and sulfur-containing groups having the positive values of EA, whereas the presence of acidic groups and the lack of electron acceptors are essential factors for the formation of $[M-H]^-$ ions. While the formation of $[M-H]^-$ ions can be explained by a dissociative proton-transfer reaction (12.1), the formation of $M^{-\cdot}$ ions could be explained by an electron attachment reaction coupled with the reaction (14.2) as follows;

$$M+e^- \rightarrow M^{-\cdot} \qquad (15)$$

This electron attachment reaction as a secondary process may occur in a gas-phase formed under matrix FAB conditions.

3.4 Cavity model

Various different models Various models for the ion formation have been presented so far to understand individual events under matrix FAB and LSIMS conditions. Recently, Sunner who has widely studied on the ionization mechanism(s) of LSIMS a little referred to the term "*cavity*" to account for another keyword "*selvedge*".[5b] The term "selvedge" can be defined as 'the interface region between solid and gas phases'[36] or 'a temporary state which is intermediate between condensed and gas phases'.[37] Although Sunner *et al.* have presented two ionization models for FAB/LSIMS methods, "gas collision"[38] and "phase explosion",[39] the ideas were essentially in agreement with the model of "ion/molecule reactions" with matrix ions in the selvedge or reagent gas plasma region[40] which is presented by the reaction (12.2). According to the models by Sunner *et al.*, such a region could be characterized by a high-temperature, high-density, and high-pressure dense gas-like phase in which a number of collisions occur. The ion formation occurs through the ion/molecule reactions due to frequent collisions in the dense gas-like phase.

On the other hand, another type model named "preformed ion" has also been believed by some workers as described in Subsection **3.1**. Various types of preformed ion were described there. Differing from the models by ion/molecule reactions, it is a significant character of the "preformed ion" model that FAB mass spectra obtained would reflect solution phase equilibria such as acid/base reaction, hydrophilic/hydrophobic solvations, and ionic dissociation.

Todd[41] and Paul *et al.*[25] have presented a model for the secondary ion emission from matrix surface. According to their consideration, major events to form molecular-related ions occur in the condensed-phase or solution as beam-induced processes, though the "preformed ion" model is not supported. Furthermore, the ion formation is governed by the condensed-phase behaviors and the kinetics of reactions near, but below, the matrix surface. This means that major processes occur in the step of collision cascade occurring immediately before the formation of dense gas-like phase and after the collisions between a matrix molecule in the surface layer, B_{surf}, and a fast atom A_{fast} or a fast atom ion. It is fortunate that advanced model presented by Todd and Paul *et al.* is consistent with the idea of quasi-preformed state in solution described in Subsection **3.1**.

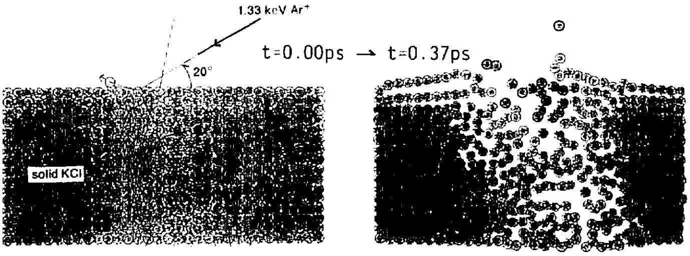
Although the models described above could explain each ionization phenomenon, there is no consistency in the understanding of the complexity and diversity of ion formation under matrix FAB and LSIMS conditions. The "cavity" model presented here is an extended model which contains various ideas by some workers described above.

What is the cavity? The term "*cavity*" is originating from the phenomenon of bubble formation as a spontaneous nucleation in superheated liquids.[42] The bubble formation in superheated liquids is a kind of bumping, and a bubble formed as the critical size is meso-scopic in size (with a radius of about 45Å) and contains several hundred molecules.[43] The term "bubble" is often referred to as "cavity".[44] The vapor pressure p inside the cavity can be given as

$$p = p_0 + 2\gamma/r \tag{16}$$

where p_0, γ, and r represent the hydrostatic pressure, the surface tension, and the radius of cavity, respectively. The point is that the surface tension which is a macroscopic physical property is applicable to such a small system, which is often called as meso-scopic system consisting of several 10–100 molecules. An important role of surface tension is to crush the cavity formed in liquids or to recover a liquid (condensed-phase) state. The rate and processes of cavity formation within an octane-like fluid heated at 358–514 K have been demonstrated by using a molecular dynamics simulation.[45] According to the simulation, the cavity formation in the fluid could occur at 15 ps later a homogeneous state.

On the other hand, a concrete image for cavity formation could be seen in the illustration based on a two-dimensional molecular dynamics calculation of the sputtering of solid KCl by 1.33 keV Ar^+ beam,[46] as shown in Scheme 9. The image could be recognized as a crater which is a hole in the matrix surface layer made by the phase explosion. The time-scale for such a cavity formation is of ~ps, while that for a collision in the collision cascade processes is of ~fs.[5b] As has been presumed by Shiea *et al.*,[47] the size of a cavity may be of the order of 100Å. The initial translational energy (keV) of a fast atom, A_{fast}, will be dispersed through the collision cascade and will utilized to form



Scheme 9.

a "cavity". Therefore, a "cavity" will be formed in a matrix surface layer 10–100 molecules in thickness, with the time-scale of ~ps. The dense gas-like cavity formed would consist of matrix and analyte molecules having relatively high translational energy, B_{fast} and M_{fast}, because of the elastic collisions between A_{fast} and B_{surf}. Since several ions and neutral species in a "cavity" formed will be sputtered into the vacuum region and several others will return to the condensed phase again, the "cavity" will vanish with the time scale of ~ps and the original plane surface will come out by the surface tension. The return of the ion and neutral species to the condensed-phase is supported by the accumulation phenomena that the peak abundance of $[M-H]^+$ and $[M+3H]^+$ ions formed by beam-induced reactions increases with the time of beam irradiation.[26),48)] The sequential processes described above for a "cavity" will be completed with the time-scale of several ps.

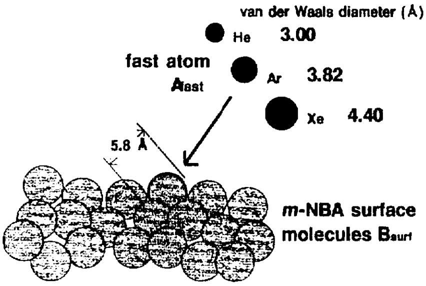
Condensed-phase and gas-phase reflected processes As described above, the cavity formation could be divided into two processes except for the earliest event of two-body collision between A_{fast} and B_{surf}, as follows;

(1) the collision cascade processes initiated by the earliest two-body collision,

(2) the secondary collision processes in a dense gas-like phase, named "cavity", consisting of positive-ion, negative-ion, and neutral species having high translational energy.

Since the collision cascade processes may occur with frequent collisions between matrix and analyte molecules in the condensed-phase, this seems to be a most important process for the ion formation. In fact, the collisional events in the cavity formed will directly lead to the vanishment of the dense gas-like phase. These processes (1) and (2) proposed here seem to be convenient for the understanding of the facts that FAB and LSI mass spectra often reflect either condensed-phase or gas-phase, according to the nature of analytes. The collision cascade as a high energy process could explain the abundant formation of fragment ions in the FAB and LSI mass spectra, by which the process (1) may result in collisional activation, as well as the competitive formation of $[M-H]^{+\cdot}$, $M^{+\cdot}$, and $[M+H]^+$ ions. On the other hand, the process (2) may enable analyte molecules to protonate as a soft-ionization reaction (12.2).

Cavity size and ion yields It could be expected that the ion yields from the matrix surface is directly affected by a cavity size which means the number of matrix and analyte molecules, $\boldsymbol{n}$, in a "cavity". The ion yield of i-th ion species by the "cavity", $\boldsymbol{y}_i$,



Scheme 10.

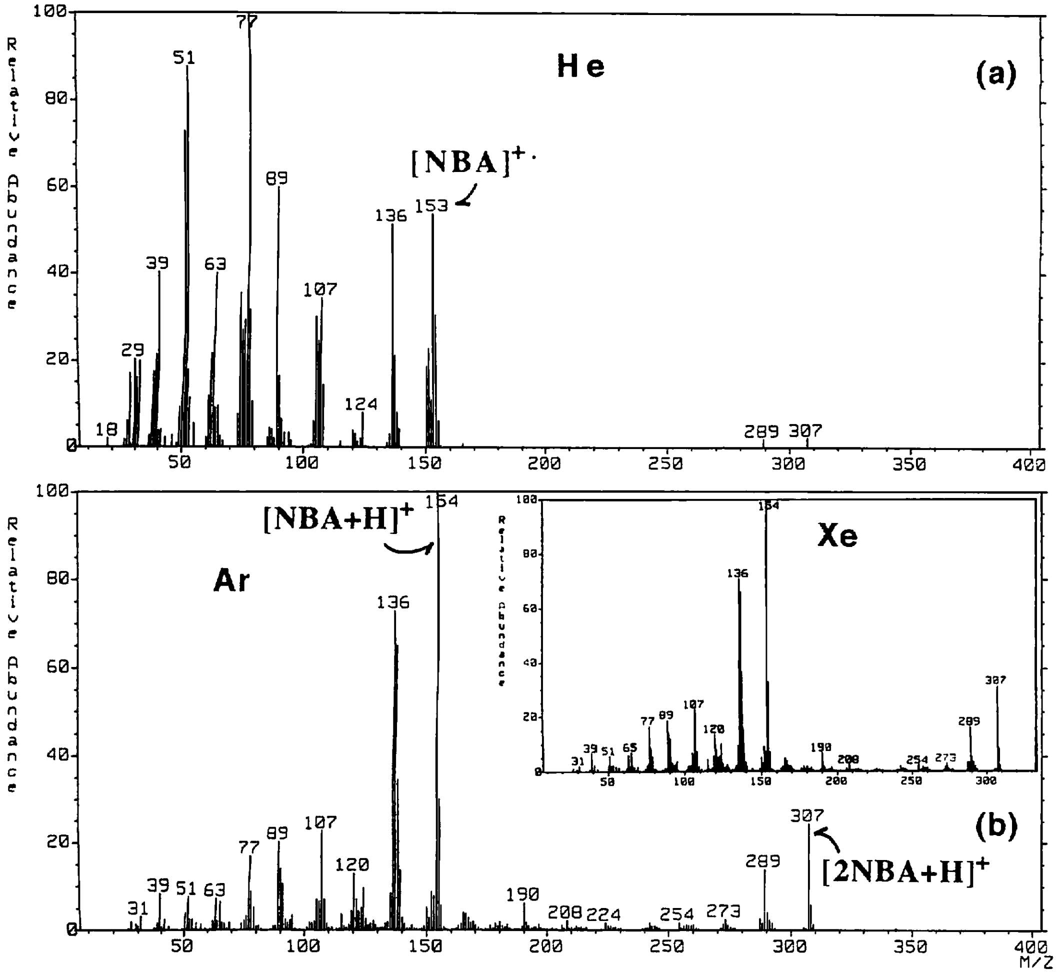


Fig. 14. FAB mass spectra of *m*-nitrobenzyl alcohol with 6 keV (a) He and (b) Ar beams. The inset represents FAB mass spectrum with 6 keV Xe beam.

could simply be given by

$$y_i = n \times w_i \tag{17}$$

where w_i represents the probability for i-th ion formation. Although it is difficult to determine the number n, the formulation for the rate of ion formation under matrix FAB conditions is in progress on the basis of the collision theory.

In order to examine the influence of fast atom species on the ion yields, positive-ion FAB mass spectra of NBA were obtained with He, Ar, and Xe beams. This experiment seemed to be useful for the consideration of cavity size, because the use of different atoms in mass affects the extent of elastic or inelastic collision[49] which is directly relating to a region of collision cascade. As is understood from the sizes of a NBA molecule and fast atoms (Scheme 10), an earliest event in matrix FAB experiments is a two-body collision between A_{fast} and B_{surf} like a gas-phase FAB process. When a fast

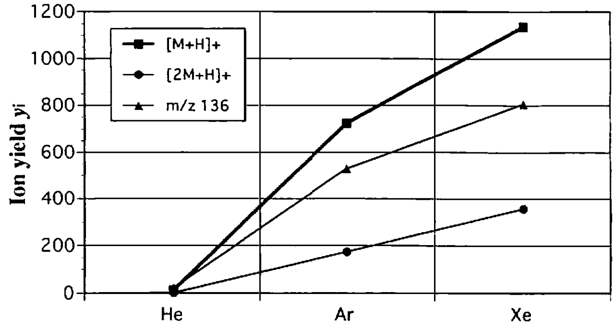


Fig. 15. Ion yields of the ions $[M+H]^+$ at *m/z* 154, $[2M+H]^+$ at *m/z* 307, and a fragment at *m/z* 136, with 6 keV He, Ar, and Xe beams.

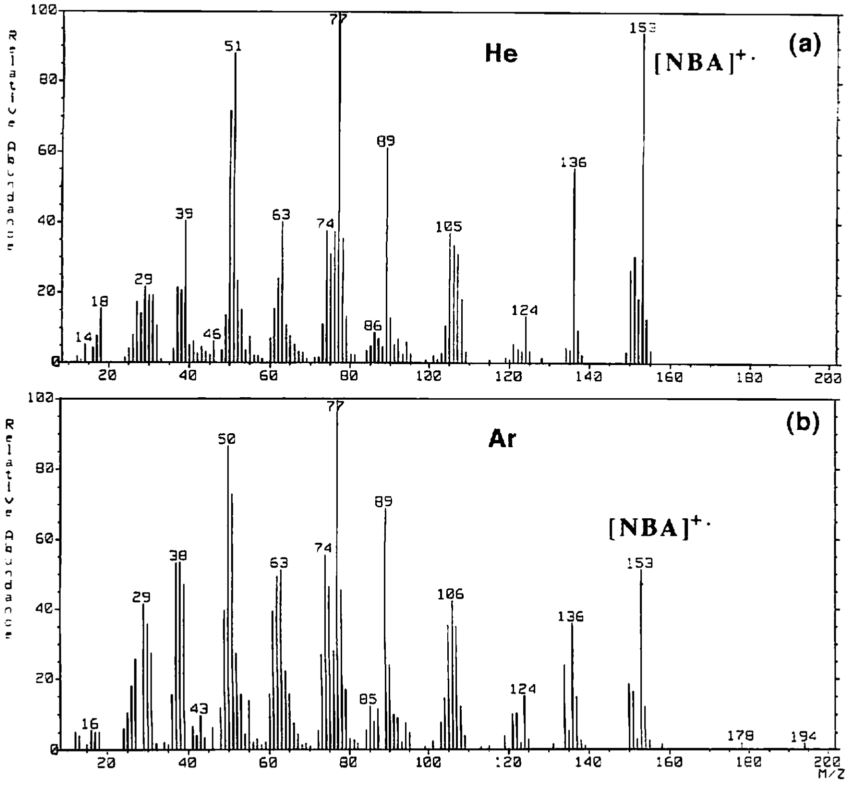


Fig. 16. Gas-phase FAB mass spectra of *m*-nitrobenzyl alcohol with 6 keV (a) He and (b) Ar beams.

atom of mass m_1 with the primary energy E_0 (keV) collides against a NBA molecule of mass m_2, the extent of elastic collisions which means the primary energy E_0 is transferred to the translational energy of a NBA molecule increases with increasing the mass of fast atoms. Therefore, it is expected that the ion yield with Xe beam is larger than that with Ar or He beam. Figure 14 represents the FAB mass spectra of NBA obtained with He, Ar, and Xe beams (6 keV). Interestingly, the FAB mass spectrum with He beam showed a preferential peak that corresponds to $M^{+\cdot}$ ion at *m/z* 153, while the spectra with Ar and Xe beams mainly showed the peak of $[M+H]^+$ ion at m/z 154. The ion yields against different atoms are shown in Fig. 15. The results obtained were qualitatively in agreement with the expectation from the collision theory.[49)]

The appearance of $M^{+\cdot}$ ion in the FAB mass spectrum with He beam suggests that the He primary energy E_0 is mainly utilized to electronically excite the NBA molecules in the surface layer, not to form a "cavity". This is suggested from the fact that the FAB mass spectrum with He beam is in good agreement with the gas-phase FAB mass spectrum of NBA.[29)] The gas-phase FAB mass spectra of NBA with He and Ar beams are shown in Fig. 16. Since the gas-phase FAB process brings about the lower ion yields of a few orders than matrix FAB process and the ionization occurs by single collisions, it could be understood that the matrix FAB conditions with He beam here little result in many-body processes leading to the formation of $[M+H]^+$ ions in the cavity. This suggests that only a few surface molecules corresponding to $\boldsymbol{n}$ in Eq. (17) take part in the ion formation and that the cavity formation does not occur under matrix FAB conditions with He beam. Form the ion yields of $[M+H]^+$ ions with Ar (58 times of He) and Xe (90 times of He), it could be estimated that a "cavity" formed with Ar and Xe beams may contains several 100 molecules (58 or $90 \times$ a few molecules). This is never overestimation, compared with the neutral yield of 10^3 molecules from glycerol surface.[50)]

4. Conclusion

In order to understand each ionization phenomenon occurring under matrix FAB conditions, the introduction of some concepts and the experiments were performed as follows;

i) The concepts of "preformed ion" and "quasi-preformed state" in solution were newly defined on the basis of the Bjerrum's proposition for ion-pairs.

ii) Examples of "preformed ion" and "quasi-preformed state" were presented experimentally.

iii) Electronic excitation to form M^{z+} ($z=1$–3) ions of analytes under matrix FAB conditions were examined, and it was concluded that matrix FAB conditions were sufficient in energy for $M^{+\cdot}$ formation and were insufficient for M^{z+} ($z=2, 3$) formation.

iv) Formation of various molecular-related ions, $[M+H]^+$, $[M+Na]^+$, $[M-H]^+$, $M^{+\cdot}$, $M^{-\cdot}$, and $[M-H]^-$, under matrix FAB conditions was described.

v) A model for matrix FAB ionization, named "cavity" model, was introduced with the origin of the term "cavity" which is formed in the surface layer of matrix solution as a crater with meso-scopic size.

vi) The cavity size, the number of molecules by a cavity, was estimated by measuring the ion yields from matrix surface bombarded with He, Ar, and Xe beams.

In spite of the great efforts of many researchers, the detailed mechanism of ion formation under FAB conditions remains unknown owing to the diversity and complexity of phenomena. This is coming from nonequilibrium and dynamic events with the various different time-scales, many-body collisions, chemical and physical processes in gas-phase and condensed-phase. A promising method to approach to the ionization phenomena is molecular dynamics (MD) calculations which could simulate the elementary processes occurring under matrix FAB conditions. In fact, the MD simulations have succeeded in understanding the sputtering events from solid surface[51] and the processes of matrix-assisted laser desorption.[52] Needless to say, further experimental and theoretical efforts are necessary to apply matrix FAB mass spectrometry to various fields of chemical, physical, and biological sciences.

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