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Reduction in Liquid Secondary Ion Mass Spectrometry. Comparison of the Fission Fragment and Liquid Secondary Ion Mass Spectra of Organic Dyes

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Mass spectral characterization of the intact chromophore of organic dyes, frequently prepared as salts, is relatively facile with desorption ionization. The mass spectra obtained, however, may show the presence of chemically altered forms of the chromophore. Comparison of the fission fragment (FF) and liquid secondary ion mass spectra (liquid SIMS) of a series of dyes reveals enhanced $(C + 1)^+$ and $(C + 2)^+$ intensities in the liquid SIMS spectra of some of the materials examined. This enhancement appears to be related to reduction reactions occurring in the liquid matrix of the SIMS experiments which result in dye species weighing 1 and 2 amu more than the original chromophore. The absence of the liquid matrix in the FF experiments precludes such reactions, and hence the $(C + 1)^+$ and $(C + 2)^+$ intensities observed in the FF spectra are those calculated from the natural abundances of the ^{13}C and ^{15}N isotopes incorporated in the dye molecules. While the mechanism(s) by which reduction takes place have not been established, the role of the matrix may be in providing radical species that facilitate reduction.

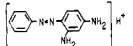
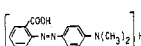
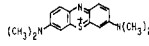
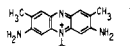
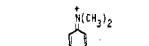
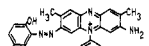
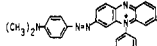
Aromatic dyes constitute a class of chemicals that have until quite recently largely eluded characterization by mass spectrometry. These species are usually prepared as salts of inorganic bases and are thus ionic and in general involatile. In addition, they are fragile and are easily pyrolyzed by conventional techniques of sample preparation and presentation for mass spectral analysis. Only in the most favorable cases can molecular weights of cationic dyes be determined from their electron impact (EI) mass spectra by identifying the products of thermal reduction, dealkylation, and Hoffman degradation (1). The field desorption (FD) spectra of some cationic and anionic dye species published by several laboratories show ions characteristic of the chromophore weight

(1-3), but the technique has not proved universally useful for dye analysis. With the popularization of sputtering techniques for the analysis of organic materials, however, mass spectral characterization of such compounds has become nearly routine. A number of laboratories are involved in these studies and are publishing spectra for a growing number of dyes (4-6).

As the body of literature grows it becomes obvious that chemical reactions occur during the desorption process and are recorded in the mass spectra. Evidence for dehalogenation has been observed in the spectra of a number of compounds of biological interest (7, 8). Reduction has also been shown to take place, particularly in the liquid secondary ion mass spectrometry (liquid SIMS) or fast atom bombardment (FAB) experiments (9, 10). Evidence for reduction of dye species in liquid SIMS has been noted by Pelzer et al. (11). To explain the enhanced $(C + 1)^+$ and $(C + 2)^+$ intensities observed in the liquid SIMS spectra of some dyes, he invoked oxidation/reduction reactions occurring in the liquid matrix. The extent of the enhancement could be correlated with the ease of reduction of the dye, as measured by polarographic reduction potentials. The mechanism by which reduction takes place in these experiments has not been established. Cerny and Gross suggest multiple protonation, followed by one- and two-electron reductions (12). Others have suggested that radicals, produced in abundance in the liquid matrix of the SIMS experiments, may play a role (13, 14).

Evidence for reduction in the form of enhanced $(C + 1)^+$ and $(C + 2)^+$ intensities has not been observed in the field desorption (1) or fission fragment (FF) (6, 15) mass spectra of organic dyes. While both the liquid SIMS and FF techniques depend on particle bombardment to produce ions from the sample, one obvious difference between them is the absence of the liquid matrix in the FF experiments. Thus comparison of the mass spectra produced by the two techniques provides a means of probing the role of the matrix in the liquid SIMS experiments.

Table I. Organic Dyes Investigated in This Study

Dye	Cation Weight	Color Index Number	Supplier
 Chrysoidin	213	11270	Aldrich
 Methyl Red Hydrochloride	270	---	Aldrich
 Methylene Blue	284	52015	MCB
 Safranin O	315	50240	Aldrich
 Crystal Violet	372	42555	Sigma
 Basic Blue 16	470	12210	Pfaltz & Bauer
 Janus Green	475	11051	MCB

To study the behavior of aromatic dyes under different conditions of analysis, a joint investigation among three laboratories employing different experimental techniques was undertaken. The dyes were chosen so as to provide cationic species with a range of functional groups and reduction potentials. In two of the laboratories, sample ions were produced by liquid SIMS. Mass analysis in one case was obtained with quadrupole mass filters; in the other, a magnetic sector was used. On the third instrument, a time-of-flight mass spectrometer, bombardment with californium fission fragments was used to produce ions from the sample. We report here the results of the comparative study and the conclusions drawn from our observations.

EXPERIMENTAL SECTION

Chemicals. Seven dyes were chosen for analysis in this study. Table I contains information pertinent to each of the samples examined. No further purification of the as-received dyes was attempted. Because chemical purity in commercial dyestuffs varies widely, samples of the dyes from a specified lot and shipment were shared among the three groups participating in the study. Thus any impurities or artifacts in the samples as a result of synthesis or packaging were common to all three experiments.

The dye samples were dissolved in a solvent for application to the sample stage. Liquid SIMS requires sample presentation in a viscous matrix of low volatility from which the sample ions and neutrals are sputtered. In both the quadrupole and magnetic sector experiments, glycerol was employed. For the FF experiment each dye was dissolved in methanol and electrospayed on the target. Since the methanol evaporates in the course of the electrospay, ion production by fission fragment bombardment occurs from a dry sample. This method of sample preparation has been described in detail elsewhere (16).

Instrumentation. The instruments on which the experiments were conducted have all been described previously. They consist of a Finnigan triple stage quadrupole (TSQ) mass spectrometer, equipped with a discharge ionization source (17), a Kratos MS-50 magnetic sector mass spectrometer, equipped with an Ion Tech FAB source (18), and a time-of-flight mass spectrometer built at The Rockefeller University, which employs a californium (^{252}Cf)

fission fragment source (19). The experimental conditions employed with each instrument are described below.

Liquid SIMS in a Quadrupole Mass Filter Instrument. Several micrograms of the dye to be studied were dissolved in glycerol on a glass slide. A drop of the solution was applied to the end of the SIMS probe, a copper stage with a 45° bevel. The discharge chamber was filled with argon at a pressure of 0.5 torr and the discharge was struck, creating 5 keV ions and neutrals of the rare gas. A current of several microamperes was measured at the probe. For this comparative study, liquid SIMS spectra were acquired with Q1 and Q2 in the rf only mode, and Q3 in the rf/dc mode. Thus mass filtering occurred only in Q3. The spectra were recorded once per second and stored on the standard Incos Data System with which the TSQ is equipped. For the collision-induced dissociation (CID) experiments, the parent ion was mass-selected by Q1 and its daughter ions mass analyzed by Q3. Q2 was filled with argon at a pressure of several millitorr and collisional dissociation of the parent ions was carried out at an energy of 10 eV. Operation of the TSQ in this mode has been fully documented (20, 21).

Liquid SIMS in a Magnetic Sector Mass Spectrometer. A few micrograms of each dye were dissolved in methyl alcohol and the solution was deposited on the SIMS probe, which had been coated with glycerol. The probe was then inserted in the Kratos MS-50 high-resolution magnetic sector mass spectrometer vacuum system. Xenon neutrals and ions from an Ion Tech FAB source operated at 8 keV were focused on the probe, and mass spectra from the sputtered materials were collected and stored by the DS-55 data system with which the instrument is equipped.

Fission Fragment Bombardment in a Time-of-Flight Mass Spectrometer. The Rockefeller University fission fragment ionization mass spectrometer and its operation have been described in detail previously (19). The dye samples were electrospayed to produce a solid film with a thickness corresponding to 10 μg on the 1 cm^2 area of the aluminized polyester sample foil. The ^{252}Cf source had a strength of 20 μCi giving a fission fragment flux through the sample foil of 2000 fission fragments/s. Spectrum acquisition times ranged from 1 to 30 min.

RESULTS AND DISCUSSION

Comparison of the spectra obtained for the six dyes studied suggests that they divide into two categories: those for which the liquid SIMS and FF spectra are similar and those for which they are different. Figure 1 shows spectra obtained for Chrysoidin by each of the three techniques. Of particular interest is the region of the spectrum that includes the cation weight, 213 amu. The ratio of $(\text{C} + 1)^+$ to C^+ is nearly the same in all three spectra, irrespective of the experimental method, and is equal to the value predicted from natural isotopic abundances of ^{13}C and ^{15}N .

Figure 2 compares the spectra for Janus Green obtained by the three techniques. Inspection of the FF spectrum in the region of cation weight, 475 amu, shows that the ratio of $(\text{C} + 1)^+$ to C^+ is again that predicted from natural isotopic abundances. However, for liquid SIMS spectra the ratios found are much larger than can be explained by the isotopic abundances.

Comparative data for the remaining five dyes included in this study are shown in Tables II–VI. We have listed the intensities of ions observed in each spectrum relative to the intensity of the intact molecular cation, since it is the ratios $(\text{C} + 1)^+:\text{C}^+$ and $(\text{C} + 2)^+:\text{C}^+$ that are of particular interest here.

The relative intensities of the C^+ , $(\text{C} + 1)^+$, and $(\text{C} + 2)^+$ ions obtained from the seven dyes using the three different experimental techniques are compared in Table VII. The FF spectrum for each of the dyes studied contains an intense ion at the cation weight, and ions at $(\text{C} + 1)^+$ and $(\text{C} + 2)^+$ with intensities that can be accounted for by the natural abundance of isotopes in the molecules. Indeed, the values of the measured FF $(\text{C} + 1)^+:\text{C}^+$ ratios agree with the values calculated from the natural isotope abundances (Table VII, column 3) to better than 25% for all seven compounds, while

Table II. Relative Intensities of Ions in the Mass Spectra of Methyl Red Hydrochloride m/z (C^+) 270

m/z	fission fragment	liquid SIMS	
		quadrupole	magnetic sector
277		0.04	0.01
272	0.06	0.15	0.07
271	0.22	0.23	0.30
270	1.00	1.00	1.00
269	0.19	0.25	0.20
268	0.36	0.35	0.20
253	0.10	0.20	
252	0.13	0.65	0.07
251	0.08	0.12	0.02
238		0.20	0.01
226	0.05	0.13	
224	0.11	0.29	0.03
214	0.04	0.08	
213	0.13	0.08	
209	0.03	0.33	0.02
208	0.04	0.23	0.02
185		0.39	0.07
181	0.04	0.41	0.02
148	0.08	0.65	0.06
137	0.14	0.72	0.01
136	0.34	0.72	0.04
135	0.60	1.46	0.17
134	0.14	0.64	0.05
133	0.10	0.57	0.03
122	0.15	0.42	0.03
121	0.21	1.22	0.06
120	0.56	1.43	0.10
119	0.69	1.17	0.09
118	0.12	0.49	0.02
106	0.10	0.40	0.03
105	0.26	0.63	0.03
104	0.15	0.51	0.03
93	0.17	2.73	0.13
77	0.35	1.30	

the $(C + 2)^+ : C^+$ ratios agree within a factor of 2 or 3. The discrepancies that are apparent generally arise because the $(C + 2)^+$ ion peak is superimposed on the broad tail of the intense C^+ ion peak. This contribution to the $(C + 2)^+$ intensity has not been subtracted in the present measurements. Agreement between the measured and calculated $(C + 1)^+ : C^+$ ratios is also observed in the liquid SIMS spectra of four of the dyes: Chrysoidin, Methyl Red Hydrochloride, Safranin O, and Crystal Violet.

In contrast, the liquid SIMS spectra of Methylene Blue, Basic Blue 16, and Janus Green all exhibit intensities of $(C + 1)^+$ and $(C + 2)^+$ relative to C^+ that are significantly greater than the values calculated from isotopic abundances. As previously suggested, reduction of the dye cations by radicals could account for the unusually large $(C + 1)^+ : C^+$ and $(C + 2)^+ : C^+$ ratios observed in the liquid SIMS spectra of these dyes. Since our results show that these reduction reactions do not occur in the fission fragment ionization/desorption process, we suggest that the source of the reactant radicals in the liquid SIMS process is the glycerol solvent.

Reduction does not occur with all the compounds in the liquid SIMS experiments, however, suggesting that other factors must also be operative in the experiments. The extensive work of Berg (22) on polarography of dyestuffs indicates that the reduction potentials of many organic dyes range from just over 0.1 V to nearly 1 V, depending on the dye structure. To compare ease of reduction in the dyes examined in these studies, we have obtained pulsed voltam-

Table III. Relative Intensities of Ions in the Mass Spectra of Methylene Blue m/z (C^+) 284

m/z	fission fragment	liquid SIMS	
		quadrupole	magnetic sector
298	0.03	0.02	0.04
287		0.11	0.90
286	0.06	0.39	1.79
285	0.18	1.60	1.90
284	1.00	1.00	1.00
283		0.05	0.24
277			0.29
272		0.08	0.47
271	0.01	0.31	1.03
270	0.02	0.58	0.69
269	0.03	0.50	0.37
268	0.11	0.48	0.26
256		0.12	0.26
255		0.12	0.32
254	0.01	0.23	0.24
253		0.10	0.24
252	0.02	0.12	0.16
243		0.09	0.71
242	0.01	0.33	0.69
241	0.02	0.31	0.69
240	0.02	0.15	0.24
228		0.09	0.37
227	0.01	0.16	0.39
226	0.01	0.15	0.39
225	0.01	0.15	0.66
224		0.20	0.10
223		0.09	0.30
213		0.10	0.47
201		0.04	0.21
200		0.06	0.40
199		0.19	1.31
198		0.11	6.05
185		0.09	2.42
167		0.08	0.40
93		2.75	8.00

Table IV. Relative Intensities of Ions in the Mass Spectra of Safranin O m/z (C^+) 315

m/z	fission fragment	liquid SIMS	
		quadrupole	magnetic sector
317	0.03	0.07	0.13
316	0.18	0.31	0.44
315	1.00	1.00	1.00
314	0.11	0.06	0.09
313	0.05	0.04	0.03
301	0.03	0.08	0.07
300		0.06	0.04
299		0.07	0.03
277		0.01	0.36
239	0.03	0.07	0.04
238	0.04	0.07	0.02
237	0.03	0.05	
224	0.01	0.05	0.02
223	0.01	0.03	0.04
222	0.01	0.04	
210	0.02	0.07	0.01
185		0.33	1.27
93		0.36	5.10

metric reduction potentials for each of them. These data are given in Table VIII. Some of the dyes have multiple reduction potentials; we have listed here only the lowest of the values. The values for Chrysoidin and Methylene Blue are in good

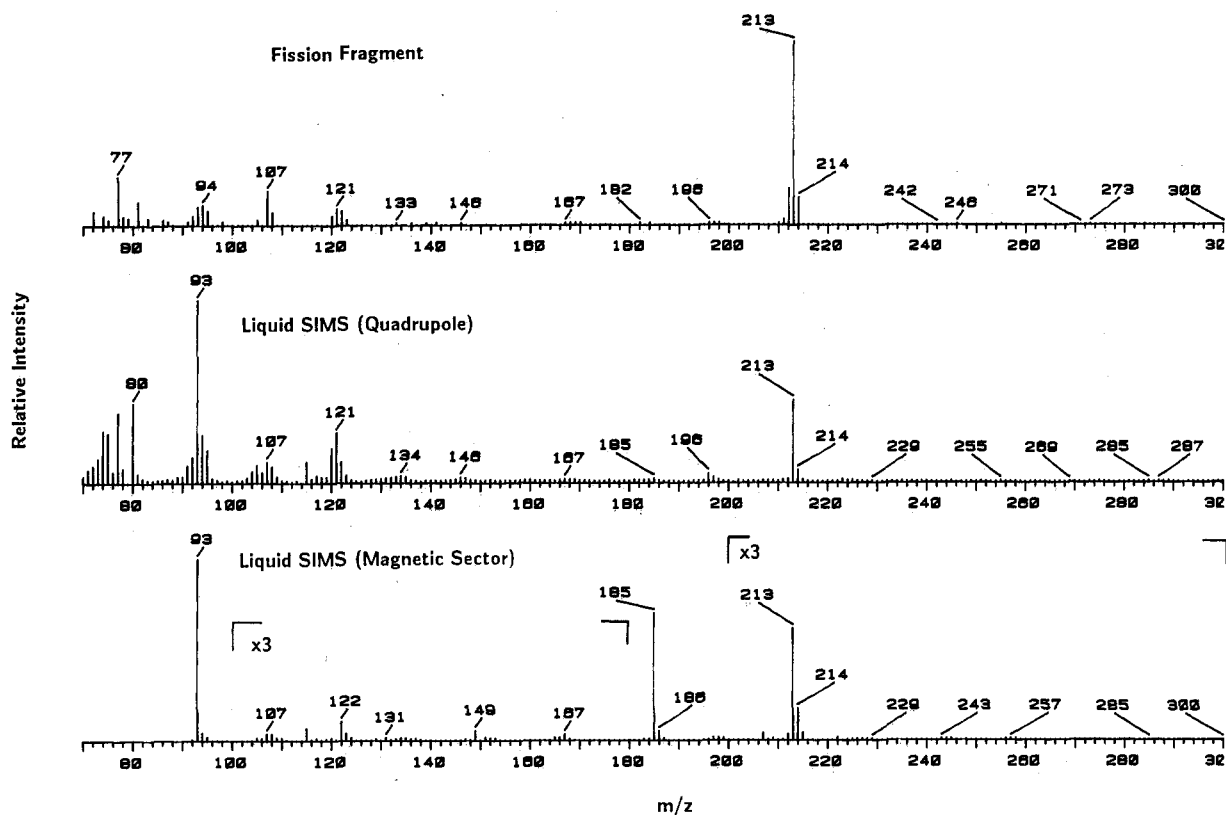


Figure 1. Comparison of Chrysoidin mass spectra obtained by the three experimental techniques.

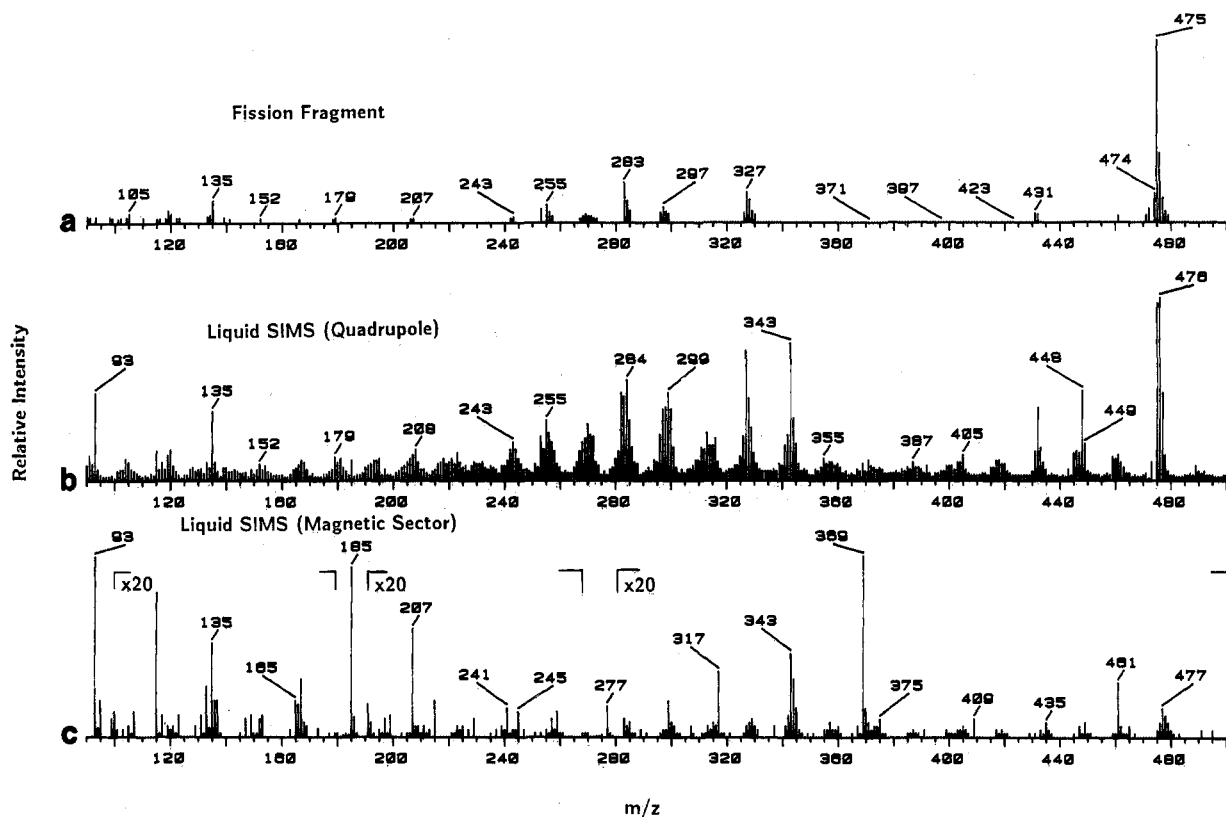


Figure 2. Comparison of Janus Green mass spectra obtained by the three experimental techniques.

agreement with those published by Berg. His value for Crystal Violet is somewhat higher than what we obtained, but the discrepancy does not alter the argument.

Inspection of these data shows that Methylene Blue, Basic Blue 16, and Janus Green, the dyes for which liquid SIMS analysis gave enhanced $(C + 1)^+ : C^+$ and $(C + 2)^+ : C^+$ ratios, have lower reduction potentials than do the other four dyes.

Two criteria thus appear to be important in determining whether or not reduction will take place during energetic particle bombardment: first, a reducing agent must be present and, second, the process must be energetically feasible. If the energy requirement for reduction cannot be met, liquid SIMS and FF mass spectra of a particular dye will be quite similar in the region of cation weight. If the energy criterion can be

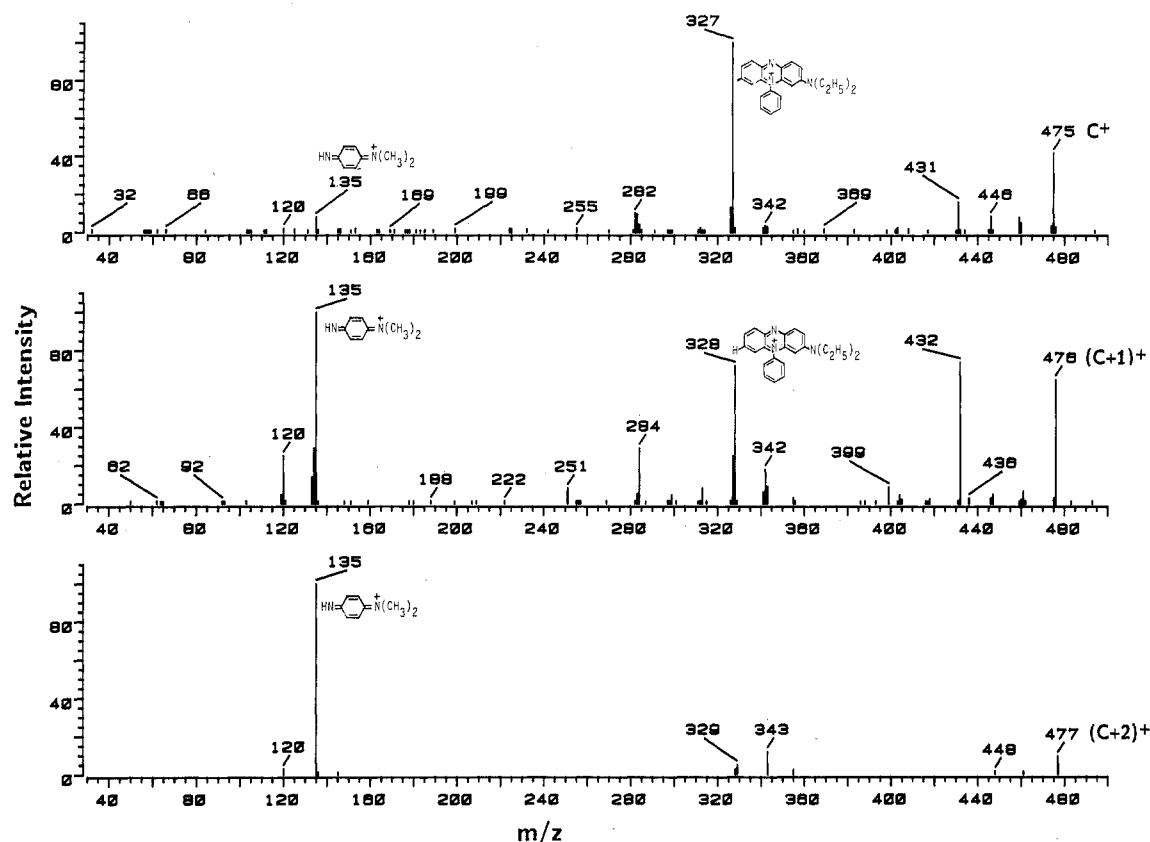


Figure 3. Collision-induced dissociation (CID) studies of oxidized and reduced Janus Green cations.

Table V. Relative Intensities of Ions in the Mass Spectra of Crystal Violet m/z (C^+) 372

m/z	fission fragment	liquid SIMS	
		quadrupole	magnetic sector
374	0.08	0.06	0.14
373	0.31	0.25	0.35
372	1.00	1.00	1.00
371	0.06		0.06
358	0.05	0.11	0.08
356	0.12	0.46	0.09
342	0.03	0.08	0.03
340	0.04	0.16	0.02
329	0.02	0.08	0.05
328	0.03	0.09	0.02
277		0.04	0.01
253		0.13	0.07
239		0.10	0.02
237		0.12	0.02
185			0.15
134		0.06	0.04
93		0.07	0.23

met, reduction will take place in the liquid SIMS experiments, and the spectra will reflect the presence of reduced dye cations in having intense ions weighing 1 and 2 amu more than the oxidized cation.

Ions corresponding to the intact cation are not, of course, the only ions observed in the particle bombardment spectra of organic dye molecules. Unimolecular decomposition of intact cations results in fragment ions, which are also observed in both the FF and liquid SIMS spectra. These ions can be extremely useful for deducing the structures of the complex dyestuffs. For the four dyes that did not undergo reduction, the FF and liquid SIMS spectra contain nearly identical

fragment ions. Even the relative intensities are comparable in the FF and liquid SIMS (magnetic sector) spectra. The relative intensities of fragment ions are greater in liquid SIMS spectra obtained with the TSQ, presumably owing to the longer flight time of ions through this instrument as compared with the time-of-flight or magnetic sector instruments.

The FF and liquid SIMS spectra of the dyes that undergo reduction shown in Figure 2 (parts a and b) and tabulated in Tables V and VI are not identical in the cation weight region nor in the fragment ion region at lower mass. We suggest that these apparent differences in fragmentation result from dissociation of the abundant $(C+1)^+$ and $(C+2)^+$ ions in the liquid SIMS experiments. To investigate differences in dissociation of the three intact Janus Green cations m/z 475, 476, and 477, the collisional dissociation spectra of these species, shown in Figure 3, were obtained on the Finnigan TSQ. CID of m/z 475 produced the same species observed as fragment ions in the FF spectrum of Janus Green shown in Figure 2a. Reduction of this quaternary ammonium cation obviously changes its structure and bond strengths, and these changes are reflected in the CID spectra of m/z 476 and 477. The m/z 477 ion cleaves almost exclusively at the N-N bond of the azo linkage, with charge retained by the (dimethylamino)aniline fragment, m/z 135. Collisional dissociation of m/z 476 appears to occur by several routes. One pathway produces m/z 328, probably reduced m/z 327, the predominant ion in the m/z 475 CID spectrum. Another cleaves the azo bond, yielding m/z 135, as did m/z 477. Since m/z 475, 476, and 477 are all present in the liquid SIMS experiments, the survey scans of Janus Green shown in Figure 2 (parts b and c) must be composites of the fragment ions produced by dissociation of the three cations.

CONCLUSION

While FF mass spectrometry and liquid SIMS produce spectra from organic dyestuffs that are similar, some differences are apparent. Dyes that are relatively easily reduced

Table VI. Relative Intensities of Ions in the Mass Spectra of Basic Blue 16 m/z (C^+) 470

m/z	fission fragment	liquid SIMS	
		quadrupole	magnetic sector
472	0.17	1.25	1.80
471	0.44	2.00	1.60
470	1.00	1.00	1.00
448	0.13	0.50	0.10
443		0.50	0.10
442	0.07		
420	0.57		0.10
372	0.63	7.75	0.40
369		1.96	?
358	0.59	10.00	0.40
344	0.27	5.50	0.20
330	0.11	2.00	0.10
329	0.11	2.50	0.10
328	0.12	2.25	0.10
327	0.10	1.50	0.10
315	0.31	8.75	0.80
314	0.42	4.25	0.30
299	0.31	3.00	0.20
298	0.27	2.00	
288	0.15	2.25	
287	0.35	1.75	
277		8.17	?
271	0.38		
270	0.32	1.75	
185		45.02	?
93		61.15	?

Table VII. Comparison of the Cation Regions of the Dye Mass Spectra Obtained by the Three Experimental Techniques

Dye	Peak Identity, m/z	Calculated	Fission Fragment	Liquid SIMS (Quadrupole)	Liquid SIMS (Mag. sector)
Chrysoidin	C^+ 213	100	100	100	100
	$(C+1)^+$ 214	15	15	15	28
	$(C+2)^+$ 215	1	1	4	6
Methyl Red Hydrochloride	C^+ 270	100	100	100	100
	$(C+1)^+$ 271	18	22	23	30
	$(C+2)^+$ 272	2	6	15	7
Methylene Blue	C^+ 284	100	100	100	100
	$(C+1)^+$ 285	19	19	160	190
	$(C+2)^+$ 286	6	6	39	179
Safranin O	C^+ 315	100	100	100	100
	$(C+1)^+$ 316	24	18	31	44
	$(C+2)^+$ 317	3	3	7	13
Crystal Violet	C^+ 372	100	100	100	100
	$(C+1)^+$ 373	29	31	25	35
	$(C+2)^+$ 374	4	8	6	14
Basic Blue 16	C^+ 470	100	100	100	100
	$(C+1)^+$ 471	35	44	200	160
	$(C+2)^+$ 472	6	17	125	180
Janus Green	C^+ 475	100	100	100	100
	$(C+1)^+$ 476	36	38	103	234
	$(C+2)^+$ 477	6	13	52	462

yield liquid SIMS spectra which have $(C+1)^+ : C^+$ and $(C+2)^+ : C^+$ ratios too large to be accounted for by ^{13}C and ^{15}N isotopes. The abundant ions corresponding to $(C+1)^+$ and $(C+2)^+$ appear to be reduced forms of the original cation, probably produced by radicals formed in the matrix by the energetic particle bombardment. The absence of the liquid

Table VIII. Reduction Potentials for Several Organic Dye-stuffs^a

dye	pulsed voltammetric reduction potential, V
Chrysoidin	-0.605
Methyl Red Hydrochloride	-0.395
Methylene Blue	-0.205
Safranin O	-0.535
Crystal Violet	-0.445
Basic Blue 16	-0.335
Janus Green	-0.275

^aThe voltammograms, obtained by Bioanalytical Systems, Inc., were run with approximately 0.5 mM solutions prepared in 1/99 ethanol/[0.067 M phosphate buffer at pH 7.0].

matrix in the FF experiments means that reduction cannot occur by this mechanism; hence $(C+1)^+$ and $(C+2)^+$ are observed in the FF spectra at the relative intensities predicted from natural isotopic abundances.

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Registry No. Chrysoidin, 532-82-1; methyl red hydrochloride, 63451-28-5; methylene blue, 61-73-4; safranin O, 477-73-6; crystal violet, 548-62-9; basic blue 16, 4569-88-4; janus green, 4618-88-6.

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Fast Atom Bombardment–Tandem Mass Spectrometry Studies of Organo–Alkali Metal Ions of Small Peptides

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Tandem mass spectrometry (TMS) combined with fast-atom bombardment (FAB) ionization is potentially useful for the structural characterization of biomolecules. The advantages of the FAB–TMS experiment have been discussed in previous works, with the major advantages usually relating to mixture analysis. In this work, FAB–TMS is used to study the dissociation reactions of the $[M + H]^+$ and $[M + Na]^+$ ions of a small peptide, i.e., hippuryl-L-histidyl-L-leucine. This work shows that additional information can be gained by comparing the dissociation reactions of these two different ionic forms. Owing to the specific binding of Na^+ in the organo–alkali metal ion, the collision-induced dissociation spectrum of the $[M + Na]^+$ ion of hippuryl-L-histidyl-L-leucine shows characteristic dissociation reactions not observed for the $[M + H]^+$ ion.

Although fast-atom bombardment (FAB) ionization has proven to be a versatile and sensitive ionization method for many biological compounds (1–10), the FAB–MS spectra are frequently complicated by the presence of impurities and/or adduct ions of the sample and liquid matrix (11–13). For these reasons, several workers have proposed the use of tandem mass spectrometry (TMS) in combination with FAB ionization for structural characterization of large molecules. Several reports dealing with the use of FAB–TMS for structural characterization of biomolecules have appeared in the literature (8, 14–17). Specific examples of such work are the cyclic peptide studies by Gross et al. (16) and the work of Amster and McLafferty on cobalamines (17). We wish to make a specific point regarding the reported collision-induced dissociation (CID) spectra of large biomolecules, viz., the low relative abundances of many of the structurally significant CID product ions, e.g., fragment ions characteristic of the amino acid sequence of peptides (15). The low abundances of the structurally significant ions in the FAB–TMS spectra of peptides are related to the large number of reaction channels available to the collisionally activated ion. During the development of mass spectrometry, several methods for enhancing specific dissociation reactions have been proposed. These methods rely on producing dissociating ions with a narrow range of internal energies, e.g., ion/molecule reactions or chemical ionization (18–22), studies of metastable ions (23, 24), photodissociation methods (25), and angle- and energy-resolved CID (26–28). Alternatively, chemical derivatization reactions of specific functional groups can be employed to enhance specific dissociation reaction channels (29).

A potentially useful and simple method is described in this paper for enhancing the structural information obtained by FAB–TMS. The method relies upon the comparison of the FAB–TMS spectra of $[M + H]^+$ ions and organo–alkali metal ions of the form $[M + A]^+$ or $[M + xA - (x - 1)H]^+$, where A is the alkali metal and $x = 1-3$. Owing to the fact that molecules such as peptides and/or sugars contain highly polar functional groups and that these functional groups have different H^+ and A^+ ion affinities, it follows that the binding sites of H^+ and A^+ may differ. Thus, the site of interaction of the H^+ or A^+ ion with the molecule as well as the nature of the ionic complex may give rise to significant differences in the FAB–TMS spectrum. Although such changes have been observed in secondary ion mass spectrometry (SIMS) spectra (30), the data reported in this paper for the FAB–TMS of the hippuryl-L-histidyl-L-leucine (HHL) $[M + H]^+$ and $[M + A]^+$ ions is a striking example of this behavior.

In this paper, FAB–TMS spectra for the $[M + H]^+$ and $[M + A]^+$ ions (A = Li, Na, K, Rb, and Cs) of hippuryl-L-histidyl-L-leucine (HHL) are reported. First the striking differences in the FAB–TMS spectra for the $[M + H]^+$ and $[M + Na]^+$ ions of HHL are interpreted in terms of structural differences of the ionic complex. Second, the FAB–TMS spectrum of $[M + Na]^+$ ion is compared with the FAB–TMS spectra for the other $[M + A]^+$ ions and these results are discussed in terms of the stability of the ionic complex. Last, the FAB–TMS spectrum of the $[M + 2Cs - H]^+$ ion is discussed and compared with the spectra of the $[M + A]^+$ ions.

EXPERIMENTAL SECTION

The studies reported here were performed with a Kratos MS-50 triple analyzer (31), in the fast atom bombardment (FAB) ionization mode. The FAB ion source used for these studies was the standard Kratos system, equipped with an ION TECH 11-NF saddle field atom gun. Xenon was used for the bombarding fast atom beam; typical operating conditions were beam energies of 6–8 keV and neutral beam currents equivalent to 20–30 μA measured on an ION TECH (Model B 50) current and voltage regulator/meter.

Collision-induced-dissociation (CID) studies were performed in the mass-analyzed ion kinetic energy (MIKE) scan mode, with helium target gas and an incident ion potential of 8 kV (31). All CID spectra were recorded with a collision gas pressure corresponding to a 50% attenuation of the molecular ion beam. A signal-to-noise (S/N) ratio of 5:1 for small peaks and greater than 75:1 for larger peaks in the CID spectra was obtained by signal averaging 16 scans (at a rate of 20 s/scan), using a Nicolet Instrument Corp. 1170 (Model 172/2) signal averager. Spectra were plotted on a standard X-Y recorder.

A solution of HHL was prepared by dissolving ca. 1.5 mg of sample (obtained from Aldrich Chemical Co.; 85,905-2) in 500 μL