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Total ion current of fast atom bombardment mass spectrometry fitted by non-linear curve

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Abstract

Some alkaloids and alkali metal salts were analyzed by fast atom bombardment mass spectrometry (FABMS) with glycerol as matrix, the intensities of their protonated molecules varying with time were investigated. It was found that the curve was decreased exponentially and both alkaloids and salt sample can be fitted by equation $I = a + b\,e^{-kt}$. a, b were constants with a characteristic distribution depending on the ionizability of the sample in the matrix, t was time which was expressed by scan number in this experiment. It was observed that parameter a would be zero when the sample was salt of alkali; when sample was alkaloid, the ratio of initial intensities of protonated molecules was only related to the concentration of the sample, which can be used to analyze sample quantitatively.

Keywords: FABMS; Non-linear curve fitting; Quantitative analysis; Alkaloid; Alkali metal salts

1. Introduction

Fast atom bombardment (FAB) was an ionization technique used in mass spectrometry, it was first reported in 1981 by Barber et al. [1], in which an analyte and liquid matrix mixture was bombarded by a ~8 KeV particle beam such as argon or xenon. Common matrices include glycerol and 3-nitrobenzyl alcohol (3-NBA). The analyte was ionized and it would form protonated molecules denoted as $[M+H]^+$, called quasi molecule ion, or ionized molecules M^+, depending on the polarity of sample, and other fragment ions. FAB has been developed as powerful tool for analyzing structurally significant information from a wide range of materials. This method was well suited to analyze alkaloids [2–4].

There were many reviews discussing the ionization mechanisms and employing FAB technique to analyze kinds of non-volatile and thermal unstable substances [5–14]. Although FAB was used widely for qualitative analysis, it was rarely applied in quantitative analysis [15–20] owing to the factors, which impacts the quantitative results, such as the matrix, pH of the matrix, proton affinity of the analyses and instrument's factor etc.

Ionization events under FAB with a liquid matrix were diverse and complex to understand as noted by Sunner [12]. Two mechanisms, "pre-ionization mechanism"(PIM) and "desorption-ionization mechanism"(DIM) [13,14], were widely discussed. To analyze metal salts by FAB, whatever univalent metal or multivalence metal it would form a charged state ion in the mass spectrometry [21]. It indicated that metal salts existed with metal ions entirely in the liquid solution when they were bombarded by a high-energy atom beam the metal ions would be sputtered from the surface to form current of ions. In this case PIM was good enough to explain it. But for a polar molecule, because the molecules existed in matrix was non-ions or ions partly, the current of ion was dominated by protonated molecules, DIM would be appeared to explain the ionization processes. However, no matter what mechanism was used, it was speculated on that the intensity of both protonated molecule $[M+H]^+$ and metal ions would be decreased exponentially with time. In this work two kinds of substituted hyantoin and alkali metals ions were analyzed to investigate this phenomenon.

2. Experiment

Experiments were performed with a VG70EHF double-focusing mass spectrometry (Micromass instrument). 5,5-

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dimethyl-hyantoin(**1**) and 5-methyl-5-ethyl-hyantoin(**2**) were prepared in our laboratory and were used without further purification; **1** and **2** were dissolved in water and both concentrations were 20 g/L and 5.06 g/L, respectively. The standard solution of K^+ and Li^+ were dissolved in water with concentrations of 1×10^{-6} g/L. Various concentrations of hyantoin were diluted with water. Glycerol was used as the matrix. The water used for preparing the above solutions was doubly distilled in quartz water, which was made in our laboratory

For the analysis, FAB mass spectrometry was performed as follows: Ar atoms were used to bombard the target, the ion gun conditions typically being a 8 KV accelerating voltage and 1 mA emission current, the pressure for the ion source was typically 10^{-4} Pa and 10^{-6} Pa for the analyzer. The mass resolution was approximately 1000. A sample of 1 uL was taken up onto the tip and approximately 2 uL matrix solution was loaded onto the tip too.

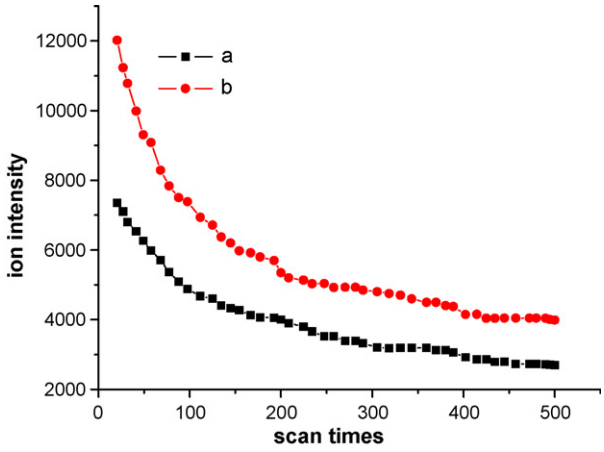

Fig. 1. protoned molecule mass chromatography for sample **1** and sample **2**. (a) 5,5-dimethyl-hyantoin *m/z* 129; (b) 5-methyl-5-ethyl-hyantoin *m/z* 143.

3. Results and discussion

3.1. Total ion current fitted by non-liner curve

According to the current ionization mechanism of FAB, some polar samples such as alkaloids and salts of metal are ionization entirely or partly in matrix before being bombarded by fast atoms. If we let the numbers of ion of sample at some time is N, which will be deceased ΔN when time has passed Δt, amount of ΔN is proportion with Δt, then N at some time can be described by the following equation

$$N(t) = N_0 e^{-kt} \quad (1)$$

N_0 is initial numbers of ion and $N(t)$ can be representative by intensity of peaks

In this experiment, protonated molecules of sample **1** and **2** were monitored with *m/z* 129 and 143, respectively. Fig. 1 was their protonated molecule mass chromatography. Both sample inlet 1 uL with nearly 2 uL glycerol as the matrix; concentration of sample **1** was 20 g/L and sample **2** was 5.06 g/L. Both were scanned 500 times for recording their protonated molecule trace.

It was obvious that intensity of ions was decreased exponentially with time. We took some points by digitizer software from the protonated molecule mass chromatography (Fig. 1) of sample **1** and **2**, then used different exponential equations to fit the curve by Origin 6.0 software. It was found that the equation

$$\acute{\imath} = a + be^{-kt} \quad (2)$$

was better to fit to them rather than Eq. (1) described above.

In this equation $\acute{\imath}$ and t represent the intensity of protonated molecule and scan times, respectively. Constants a and b should be in response to characteristic ionizability of sample in matrix, whose physical meaning will be discussed later. k was the constant of decreasing velocity, which depended on the detection efficiency, response coefficient of the ion and ions yield sputtered by fast atom bombardment etc. $\acute{\imath}^0 = a + b$ was defined as the initial intensity of protonated molecule when scan time (t) was zero. An interesting phenomenon was observed that the constant k for sample **1** and **2** were almost the same in spite of constant a and b was different from each test, the ratios of the initial intensity for sample **1** marked $[\acute{\imath}^0_{129}]$ and sample **2** marked $[\acute{\imath}^0_{143}]$.

$[\acute{\imath}^0_{129}/\acute{\imath}^0_{143} = (a+b)_{129}/(a+b)_{143}]$ close to a constant (as show in Table 1) which meant that ratio was only related to the concentration of sample. According to this, it could be used for quantitative analysis.

3.2. The physical meaning of a and b

The constant a should be decided by the fast atom beam bombardment and the properties of sample, corresponds to desorption-ionization section. The constant b should be the amount of ions of the tested sample in matrix before bombarded by fast atoms, corresponding to section of pre-ionization. If a sample is able to be ionized entirely in matrix, its intensity curve will not appear to be constant a term. In order to investigate this phenomenon, 1 ul 1×10^{-6} g/L KCl and 1 ul 1×10^{-6} g/L LiCl were injected into the mass spectrometry and water as matrix. The reason for using water as matrix was that when using glycerol as matrix the cluster ions such as $(n\text{Gly} + K^+)$ and $(n\text{Gly} + Li^+)$ would be dominant and it would result in the sensitive big fall for detection of K^+ and Li^+. The mass

Table 1
Result of three times test of the sample

	$I_{129} = f(t)$	$I_{143} = f(t)$	k_{129}/k_{143}	$(a+b)_{129}/(a+b)_{143}$
1	$I_{129} = 2734.38 + 4479.04\ e^{-0.00830t}$	$I_{143} = 4097.75 + 8783.35\ e^{-0.00858t}$	0.967	0.56
2	$I_{129} = 112.39 + 1355.38\ e^{-0.00880t}$	$I_{143} = 300.14 + 2295.17\ e^{-0.00891t}$	0.988	0.56
3	$I_{129} = 388.36 + 2989.94\ e^{-0.00856t}$	$I_{143} = 557.69 + 5696.61\ e^{-0.00862t}$	0.993	0.54

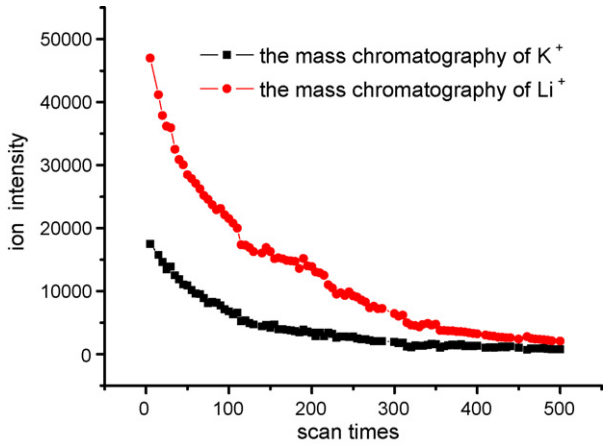

Fig. 2. The mass chromatogram of K^+ and Li^+.

chromatography of K^+ and Li^+ was shown in Fig. 2. We fitted the curve with the Eqs. (1) and (2), the result was shown as Table 2. It could be seen from data in Table 2 that all R^2 can reach 0.99, but the relative error of a was big. It was 49.9% for K^+ and 24.6% for Li^+ when Eq. (2) was used to fit them, So Eq. (1) was more suitable to fit to the curve of the mass chromatography of K^+ and Li^+ rather than Eq. (2). It illuminated from the sputtering theory, when the tested sample, which is purely ionic lattice or existed as ions entirely in the solution, was directly sputtered into the gas phase by the bombardment of fast atom, there would be no parameter a; it coincided with pre-ionization mechanism; while for the sample substitution at C5,5 hyantoin the spectrum also as gradual decease of abundance of protonated molecule appeared the parameter a when its curve fitted by Eq. (2). This process reflected that the sample is the part ionic species in glycerol or the crystals of non-ionic compound, when it was bombarded by fast atom, the protonated sample molecules and the neutral sample molecules were sputtered to form the ion-molecule complexes above the matrix surface at where complex desorption-ionization process would happen. In another word, there were both PIM and DIM during testing polar organic compound by FAB. Therefore the parameter a should be related to desorption-ionization process and b was only related to pre-ionization process. It should also be noted from Table 1 that the value of b was bigger than the value of a, which means that the contribution to intensity of protonated molecules for tested sample is mainly come from its ionized form in matrix, it just accorded with the phenomenon in FAB analysis in which it would enhance the intensity of protonated molecule when the pH of matrix was regulated by adding acid or alkali. If a curve of intensity of protonated molecule can be fitted exactly with Eq. (1), the tested sample will be complete ionic state in matrix, if it fitted exactly with Eq. (2), the tested sample will be partly ionic state in matrix. It can be tentatively suggested that the curve fitted by which of the above equation is sensitive to the state of the tested sample in matrix and ratio of parameter a and b will reveal ionizability of the sample in matrix.

3.3. Quantitative analysis

As having been pointed out above, ratio of the initial intensity was only related to the concentration of samples. Selecting any of them as internal standard, another could be analyzed quantitatively. In the experiment, we selected sample **2** as internal standard to analyze sample **1** quantitatively.

Sample **1** (5,5-dimethy-hyantoin) was prepared with different concentrations (2, 4, 6, 8, 10 g/L) and kept the sample **2** (5-methy-5-ethyl-hyantoin) concentration to be 5.06 g/L. Standard solution (1uL) and the tested sample solution (1uL) were taken up onto the tip, 2 uL glycerol were loaded onto the tip too. Data were obtained before the Ar atoms gun voltage tuned on to ensure the initial point could be scanned. The sample was scanned 500 times for the analysis. It could be seen that the highest point emerged from the protonated molecule mass chromatography of 5-methy-5-ethyl-hyantoin and 5,5-dimethy-hyantoin. We took some points by digitizer software from the highest point of protonated molecule mass chromatography to the bottom and use Origin 6.0 software to fit the curve with Eq. (2). The calibration curve of 5,5-dimethyl-hyantoin was obtained (as shown in Fig. 3). The standard equation is $y = -0.0204 + 0.154 \times (R = 0.99206)$, linear range was 1–25 g/L, and correlation coefficient (r) was 0.99206. The limits of detection for sample **1** and sample **2** are 1 g/L at signal-to-noise ratio of 3. Because decease rate of constant k was lightly when the sample was inlet more than 25 μg, the protonated molecule decrease was nearly invisible in limited testing time.

The relative standard deviations (R.S.D.) was 2.99%, which obtained via six analyses, in which 1 ul of sample **1** (concentration 2 g/L)and 1 ul of sample **2** (concentration 5.06 g/L) and 2 uL glycerol were injected. Some factors influencing on the R.S.D. such as doing experiment in the same day and in other days were also tested, which showed there was little influence on the analysis result. It was demonstrated that the method established in this paper can work with high accuracy, stability and reproducibility.

Table 2
Result of potassium ion and lithium ion′s mass chromatagrphy with different equation fitting

Parameter	Potassium ion		Lithium ion	
	$I = a + b\,e^{-kt}$	$I = b\,e^{-kt}$	$I = a + b\,e^{-kt}$	$I = b\,e^{-kt}$
A	897.2348 ± 447.36863 (49.9%)		1013.53303 ± 250.25847 (24.6%)	
B	41733.97968± 541.33392 (1.3%)	41988.26722 ± 512.37212 (1.2%)	16793.56729 ± 167.68201 (1%)	16561.0059 ± 253.94912 (1.5%)
K	0.00687 ± 0.00026 (3.8%)	0.00641 ± 0.00011 (1.7%)	0.01056 ± 0.00021 (2%)	0.00819 ± 0.00017 (2%)
R^2	0.99585	0.99535	0.99364	0.99509

Values in parenthesis means relative error.

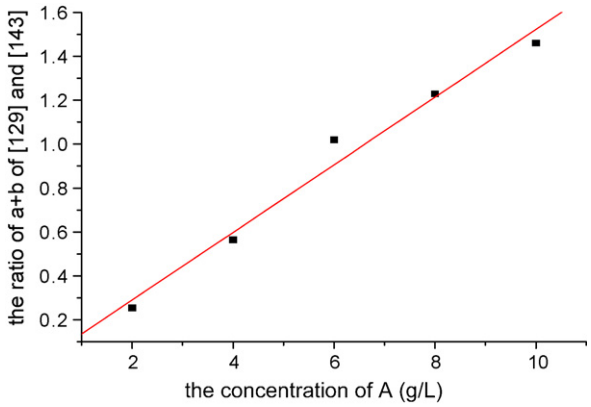

Fig. 3. The calibration of 5,5-dimethyl-hyantoin. Value in square brackets means *m/z*.

4. Conclusion

Substitution at C5,5 hyantoin are able to be analyzed well by FAB, its total ion current of protonated molecule is non-linear attenuation and can be fitted by exponential equation. The parameter *b* could be a candidate for explanation of pre-ionization mechanism and parameter *a* could be a candidate for explanation of desorption-ionization mechanism and their ratio reflect pre-ionizability of the tested sample in matrix. The ratios of the initial intensity of the two similar tested samples can be applied to quantitative analysis.

References

[1] M. Barber, R.S. Bordoli, R.D. Sedgwick, Nature 16 (1981) 325.
[2] M. Bambagiotti-Albertia, S. Pinzautia, G. Monetib, P. Gratteria, S.A. Corana, F.F. Vincieria, J. Pharm. Biomed. Anal. 9 (10–12) (1991) 1083.
[3] H. Ohtaa, Y. Setoa, N. Tsunodaa, Y. Takahashib, K. Matsuurab, K. Ogasawara, J. Chromatogr. B: Biomed. Appl. 714 (2) (1998) 215.
[4] A.F. Casy, J. Pharm. Biomed. Anal. 12 (1) (1994) 41.
[5] M. Takayama, J. Am. Soc. Mass Spectrom. 6 (1995) 114.
[6] M. Takayama, Int. J. Mass Spectrom. Ion Processes 152 (1996) 1.
[7] J. Sunner, Int. J. Mass Spectrom. Ion Processes 86 (1988) 169.
[8] H. Nakata, Org. Mass Spectrom. 29 (1994) 192.
[9] E. De Pauw, Mass Spectrom. Rev. 5 (1996) 191.
[10] W.V. Ligon Jr., S.B. Dorn, Int. J. Mass Spectrom. Ion Processes 78 (1986) 99.
[11] M.L. Gross, Int. J. Mass Spectrom. 200 (2000) 611.
[12] J. Sunner, Org. Mass Spectrom. 28 (1993) 805.
[13] R.J. Day, S.E. Unger, R.G. Cooks, Anal. Chem. 52 (1980) 353.
[14] G. Szekely, J. Allison, J. Am. Soc. Spectrom. 8 (1997) 337.
[15] S. Isomura, K. Ito, M. Haruna, Bioorg. Med. Chem. Lett. 9 (1999) 337.
[16] M.R.M. Domingues, M.G. Santana-Marques, A.J. Ferrer-Correia, Int. J. Mass Spectrom. Ion Processes 165–166 (1997) 551.
[17] R.P. Newton, A.M. Evans, J.I. Langridge, T.J. Walton, F.M. Harris, A.G. Brenton, Anal. Biochem. 224 (1995) 32.
[18] J.C. Paul, T. Rendon, J. Anastassopoulou, T. Theophanides, M.J. Bertrand, Int. J. Mass Spectrom. Ion Processes 142 (1995) 41.
[19] T. Imamura, K. Kudo, A. Nameraa, H. Tokunagab, M. Yashikia, N. Jitsufuchi, T. Kojima, J. Chromatogr. B 731 (1999) 149.
[20] P. Xue, E. Fu, G. Wang, C. Gao, M. Fang, C. Wu, J. Organomet. Chem. 598 (2000) 42.
[21] O.A. Boryak, M.V. Kosevich, V.S. Shelkovsky, V.V. Orlov, Int. J. Mass Spectrom. 194 (1) (2000) 49.