Transactions of the Materials Research Society of Japan 33[4] 1039-1041 (2008)

Quantitative SIMS Analysis of Biological Mixtures with Fast Heavy Ion Irradiation

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We have investigated the secondary ion emission from arginine amino acid thin films under fast heavy ion irradiation using the time-of-flight technique. In a previous study, we reported on the high yield of arginine ions and low relative intensities of fragment ions compared to 10 keV Ar^+ irradiation. These features would be useful for identification of biological mixtures. In this work, we measured secondary ion yields from mixed samples of the drug ipratropium bromide (IB) and cholesterol at various concentrations under 6 MeV Cu^{4+} irradiation. We detected molecular-related ions of IB from mixture films composed of a molar ratio of 0.33% IB to cholesterol at high sensitivity. The yield ratios of IB ions to cholesterol ions showed linear dependence on the molar concentration ratios of IB to cholesterol. These results indicate that secondary ion mass spectrometry (SIMS) using high-energy ions is useful for quantitative microanalysis of drugs in real biological samples.
Keywords: MeV ions, SIMS, biomolecules, quantitative analysis

1. INTRODUCTION

Fast heavy ion impact on a solid surface is followed by electronic excitation around its trajectory, and the excitation energy can be converted to atomic and molecular emission from the surface. This process is referred to as 'electronic sputtering'. The emission mechanism of electronic sputtering is quite different from that of nuclear sputtering, which is the dominant process at projectile energies below several hundreds keV. Desorption of large organic molecules caused by electronic sputtering was first observed in 1974 by Macfarlane and coworkers [1]. The kind of time-of-flight (TOF) mass spectrometry, which uses fission fragments from a ^{252}Cf source, is called plasma desorption mass spectrometry (PDMS) and has been used to analyze masses up to 24,000 amu [2]. We have investigated secondary ion emission from arginine amino acid thin films under 500 keV Au^+ irradiation using the TOF technique, and reported the high secondary ion yield of arginine ions and low relative intensities of fragment ions compared to 10 keV Ar^+ irradiation [3]. These features would be useful for identification of biological mixtures such as cells and tissues. Quantitative analysis of these biological mixtures is important for proteome, metabolome and pharmacokinetic studies. In this work, biological mixtures of cholesterol, which is abundant in cells and tissues, and the drug ipratropium bromide (IB) were analyzed by secondary ion mass spectrometry (SIMS) with 6 MeV Cu^{4+} ion projectiles to investigate the dependence of secondary ion yields from mixed samples on the mixture ratios.

2. EXPERIMENTAL

2.1 Sample Preparation

IB and cholesterol were purchased from Sigma-Aldrich (USA) and Nacalai Tesque (Japan) respectively and were used without further purification. IB is an antimuscarinic agent used in the treatment of obstructive airways disease and allergic rhinitis [4]. The structural diagram of IB is shown in Fig. 1. IB contains quaternary nitrogen and exists as a salt. IB and cholesterol were separately dissolved in ethanol at a concentration of 0.02 mol/dm^3, and the IB solution was mixed with the cholesterol solution in molar ratios of 50%, 25%, 9.1%, 3.2%, 1%, 0.33% to cholesterol. Each mixed solution was deposited on a rotating Si wafer, which was rinsed in water, acetone and 2-propanol using an ultra sonic cleaner. This spin-coating procedure was performed in a clean room under humidity control because the film thickness and smoothness are sensitive to air humidity. The sample thickness was measured using a contact surface profiler (Dektak 3, ULVAC, Japan) and was determined to be about 40 nm, which is sufficiently thin for primary MeV-ion beams to penetrate, avoiding charging-up of the surface.

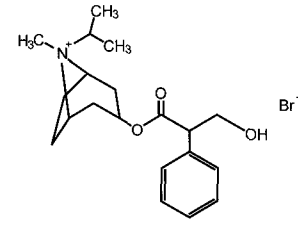

Fig. 1. The structural diagram of ipratropium bromide.

2.2 Secondary Ion Yield Measurement

The experimental setup is schematically shown in Fig. 2. A 6 MeV Cu^{4+} ion beam was provided by Kyoto University's 1.7 MV tandem accelerator. The projectile ion beam was chopped to a width of 50 ns every 100 μs and was incident on the target at an angle of 20° to the surface normal after being collimated to a diameter of 1 mm. Secondary ions were extracted with a kinetic energy of 6.0 keV and detected by a microchannel plate detector after passing through a field-free drift region. The primary ion beam current was measured before and after the TOF measurement with a Faraday cup equipped with an electron suppressor, and was approximately in the range of tens of pA. Mass analysis of secondary ions was performed using a linear TOF technique in a vacuum of 2×10^{-5} Pa. We stored all samples in low humidity before the TOF measurement and assured that there is almost no degradation of samples compared to those measured soon after sample preparation.

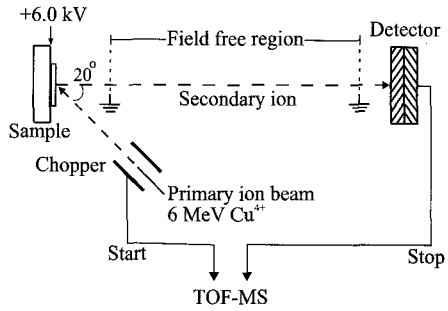

Fig. 2. Experimental setup.

3. RESULTS AND DISCUSSION

An example of a positive-ion mass spectrum for the cholesterol film bombarded with 6 MeV Cu^{4+} ions is shown in Fig. 3. The cholesterol molecules (Ch) were observed in the form of protonated dehydrated ions ($[Ch\text{-}H_2O+H]^+$) and deprotonated ions ($[Ch\text{-}H]^+$). The estimated yields of these ions were 9×10^{-3} and 4×10^{-3} molecular ions/Cu^{4+}. A comparison of $[Ch\text{-}H]^+$ ion yields from the cholesterol samples bombarded with 25 keV Au^+ and 6 MeV Cu^{4+} is shown in Table. 1. The molecular ion yield from the cholesterol sample using SIMS analysis with 6 MeV Cu^{4+} irradiation was found to be more than 700 times higher than conventional SIMS analysis. Because of the high sensitivity of the molecular-related ions, high-energy ion irradiation has an advantage for identifying minute amount of biomolecules from biological mixtures.

A typical mass spectrum of positively charged secondary ions for IB bombarded with 6MeV Cu^{4+} ions is shown in Fig. 4. We observed IB molecular ions in the form of debrominated ions $[IB\text{-}Br]^+$ with intensity about twice as high as the characteristic fragment ions (*m/z* 41). The secondary ion yield of $[IB\text{-}Br]^+$ was about 5×10^{-2} molecular ions/Cu^{4+}. No intense fragment ions were detected between *m/z* 200 and 300. These features of the clear molecular signals with little fragmentation would be useful for identifying IB in mixture samples of biomolecules.

SIMS analysis with 6 MeV Cu^{4+} ion irradiation was

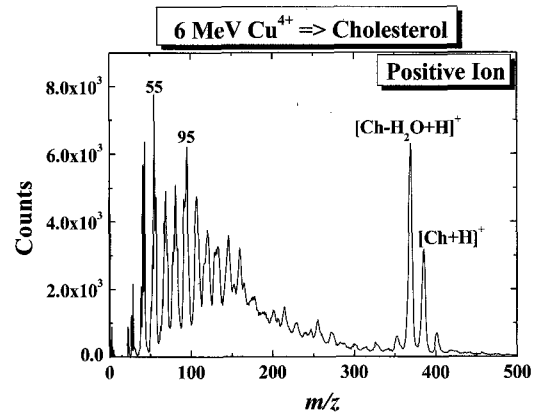

Fig. 3. Positive ion mass spectrum for the cholesterol (Ch) sample bombarded with 6 MeV Cu^{4+}.

Table 1. Comparison of secondary ion yields of the deprotonated ions ($[Ch\text{-}H]^+$) from the cholesterol sample using 25 keV Au^+ [5] and 6 MeV Cu^{4+} primary ion beams.

	Secondary ion yield ($[Ch\text{-}H]^+$)
25 keV Au^+	5.5×10^{-6} [5]
6 MeV Cu^{4+}	4×10^{-3}

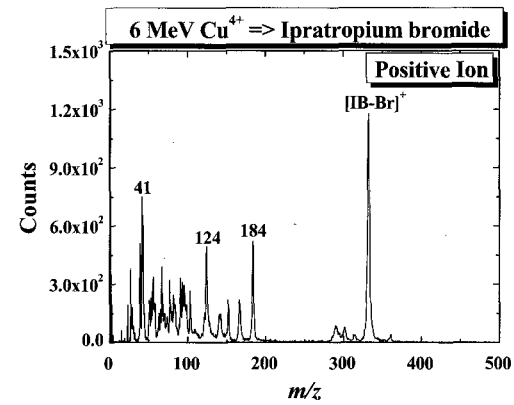

Fig. 4. Positive ion mass spectrum for the ipratropium bromide (IB) sample bombarded with 6 MeV Cu^{4+}.

also employed to mass-analyze mixture samples composed of 50% and 0.33% IB molar concentration ratio to cholesterol (Fig. 5). For the mixture with 50% IB (Fig. 5(a)), the molecular-related ions of IB and cholesterol were detected at high sensitivity in the same form as from the pure constituents. The characteristic fragment ions from IB (*m/z* 124, 184) and from cholesterol (*m/z* 55, 95) were also observed. The estimated yield of IB molecular ions $[IB\text{-}Br]^+$ was 1×10^{-2} molecular ions/Cu^{4+}, which is about five times the yield of cholesterol ions $[Ch\text{-}H_2O+H]^+$. For the mixture with 0.33% IB (Fig. 5(b)), the molecular-related ions of IB were detected at high yield of 4×10^{-3} molecular ions/Cu^{4+}, although the characteristic fragment ions from IB (*m/z* 124, 184) were hardly observed. The signal-to-noise ratio of IB

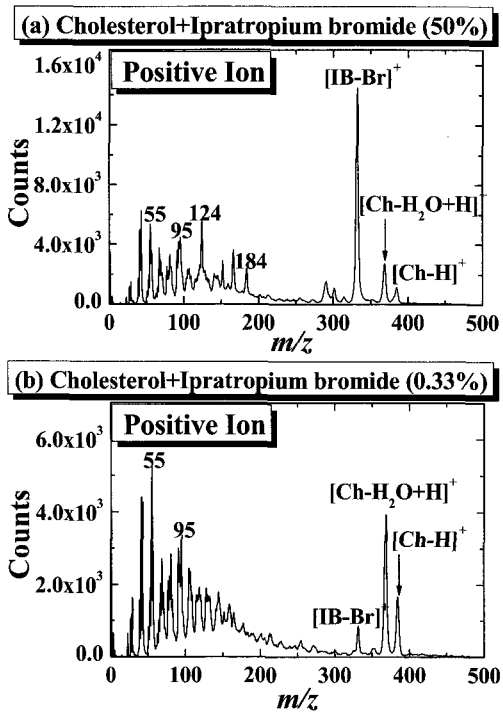

Fig. 5. Positive ion mass spectrum for the mixture samples of ipratropium bromide and cholesterol bombarded with 6 MeV Cu ions. The molar concentration ratios of ipratropium bromide to cholesterol were 50% (a) and 0.33% (b).

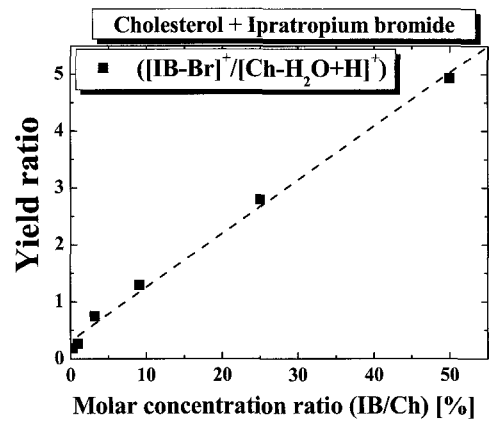

Fig. 6. The yield ratio of ipratropium bromide ions ($[IB\text{-}Br]^+$) to cholesterol ions ($[Ch\text{-}H_2O\text{+}H]^+$) as a function of the molar concentration ratios of Ipratropium bromide (IB) to cholesterol (Ch). The dashed line represents the calibration line of these plots derived by the least-square method.

ions for the mass spectrum in Fig. 5(b) was about 1.5. It would be possible to detect IB ions at several tens ppm by increasing the mass resolution using reflectron TOF instead of linear TOF measurements. Since almost drug concentration in the body is several tens ppm, this method could be used for identifying drugs in real samples.

We measured the yields of the molecular ions emitted from each mixed sample film at IB molar concentration ratios to cholesterol between 0.33-50% (Fig. 6). The yield ratio decreases with the concentration ratio and linear dependence is clearly shown. The present result indicates that it is possible to quantitatively analyze the IB drug in cholesterol samples for SIMS with fast heavy ion irradiation. These features, of high sensitivity and the possibility of quantitative analysis of IB in cholesterol indicate that the SIMS method using high-energy ion irradiation is useful for drug microanalysis in real biological samples. Because the surface chemical environment can be a reason for the suppression or enhancement of the secondary ion yields, which is called matrix effect, it is important to investigate secondary ion yields from drugs and other biomolecules in real samples such as cells and tissues.

4. SUMMARY

We measured the secondary ion yields from mixed sample films at various concentrations of IB and cholesterol under 6 MeV Cu^{4+} irradiation. The molecular-related ions of IB were detected at high sensitivity from the mixture films composed of a molar ratio of 0.33% IB to cholesterol. The yield ratio of IB ions to cholesterol ions showed linear dependence on the molar concentration ratio of IB to cholesterol. These results indicate that SIMS using high-energy heavy ions is useful for quantitative microanalysis of the drug in real biological samples.

References

[1] D. F. Torgerson, R.P. Skowronski and R.D. Macfarlane, *Biochem. Biophys. Res. Commun..*, **60**, 616–621 (1974).

[2] B. Sundqvist, P. Roepstorff, J. Fohlman, A. Hedlin, P. Håkansson, I. Kamensky, M. Lindberg, M. Salehpour and G. Säve, *Science*, **226**, 696–698 (1984).

[3] Y. Nakata, S. Ninomiya, J. Matsuo, *Nucl. Instr. and Meth. in Phys. Res. B*, **256**, 489–492 (2007).

[4] W. Martindale, J. E. F. Reynolds, "The Extra Pharmacopoeia 31st ed.", Ed. by J. E. F. Reynolds, K. Parfitt, A. V. Parsons, S. C. Sweetman, Royal Pharmaceutical Society: London (1996) pp.501–502.

[5] E. A. Jones, N. P. Lockyer and J. C. Vickerman, *Int. J. Mass Spectrom.*, **260**, 146–157 (2007).

(Recieved December 25, 2007 ; Accepted June 30, 2008)