JOURNAL OF MASS SPECTROMETRY, VOL. 33, 480–485 (1998)

SHORT COMMUNICATION

Improvement of Signal Intensities in Static Secondary-Ion Mass Spectrometry Using Halide Additives and Substrate Modification

Arkady I. Gusev, Bernard K. Choi, and David M. Hercules*
Department of Chemistry, Vanderbilt University, Nashville, TN 37235

A new approach is reported for secondary-ion time-of-flight mass spectrometry (TOF-SIMS) sample preparation. The method involves the use of halide additives or halide modification of silver substrate surfaces to promote analyte cationization and protonation. The enhancement of signal intensity has been demonstrated for neutral organic lipophilic and hydrophilic compounds including various small peptides and nucleosides. Improvement factors range from 2–30 for cationized species to 20–2000 for protonated species. However, the new sample preparation does not affect the signal intensities of preformed ionic species. The sample preparation approach is applicable to a wide variety of neutral compounds and should find broad use for organic analysis by TOF-SIMS. © **1998 John Wiley & Sons, Ltd.**

J. Mass Spectrom. **33**, 480–485 (1998)

KEYWORDS: static SIMS; signal enhancement; silver halide; halide additives; small peptides; neutral organic compounds

INTRODUCTION

Secondary-ion time-of-flight mass spectrometry (TOF-SIMS) is a well established analytical technique for the analysis of semiconductors, inorganic compounds, biomolecules, and synthetic polymers.[1–4] The analysis of organic materials typically involves analyte deposition on chemically etched silver substrates. As a result, most lipophilic organic compounds tend to cationize with silver $(M + Ag)^+$. Polar compounds exhibit mostly protonated $(M + H)^+$ or deprotonated $(M\text{-}H)^-$ signals, while preformed (precharged) compounds appear in the TOF-SIMS spectra as $(M + H)^+$, $(M\text{-}H)^-$, or from ion loss, $(M\text{-}Cl)^+$ or $(M\text{-}Na)^-$ species. Low penetration depth and the static nature of TOF-SIMS have a strong influence on secondary ion (SI) yield which determines the method sensitivity and detection limits. Signal intensity and SI yield are affected by several factors including the nature of the analyte, substrate material, substrate surface coverage, presence of contamination, fragmentation, and, most importantly, desorption and ionization efficiencies.[5,6] Various sample preparation approaches have been examined for the purpose of SI yield enhancement and improvement of method sensitivity.[7–14] The use of an ammonium chloride matrix produced considerable signal enhancement for simple sugars, vitamins, small peptides and nucleotides.[7,8] An improvement factor of *ca.* 5 was obtained with excess ammonium chloride.[9] Acidification of the analyte solution resulted in enhancement of $(M + H)^+$ signal intensity for several amino acids whereas the $(M\text{-}H)^-$ signal was maximized when the solution was made basic.[10] Matrix-enhanced SIMS was reported for bimolecular analysis.[12] The method employs a sample preparation procedure similar to that used in matrix-assisted laser desorption ionization (MALDI). The use of the MALDI matrices resulted in substantial signal increase for biomolecules with molecular weights up to 10,000 u. Our group has recently reported the use of a submonolayer of cocaine hydrochloride on a silver substrate deposited prior to analyte deposition to promote analyte cationization.[13] Signal intensity improvement was demonstrated for several lipophilic compounds. The most significant effect was found for samples with large amounts of organic and inorganic contamination *e.g.* crude extracts from blood. It should be noted that a decrease in fragmentation rate was found for the ammonium chloride matrix[9] whereas no significant fragmentation was observed with cocaine modified substrates for the compounds tested.[13] The present communication reports a new approach to TOF-SIMS sample preparation which results in significant enhancement of signal intensities. The sample preparation involves use of halide additives or halide surface modification to promote analyte cationization and/or protonation. The signal intensity improvement has been demonstrated for organic lipophilic and hydrophilic compounds, including various small peptides, nucleosides, and synthetic polymers.

Experimental

The experiments were performed using a TOF-SIMS III mass spectrometer manufactured by Ion-Tof GmbH (Münster, Germany). The instrument has been described in detail elsewhere.[16,17] Briefly, a pulsed sub-

* Correspondence to: D. M. Hercules, Department of Chemistry, Vanderbilt University, Nashville, Tennessee 37235, USA.

CCC 1076–5174/98/050480–06 $17.50

Received 22 August 1997
Accepted 9 January 1998

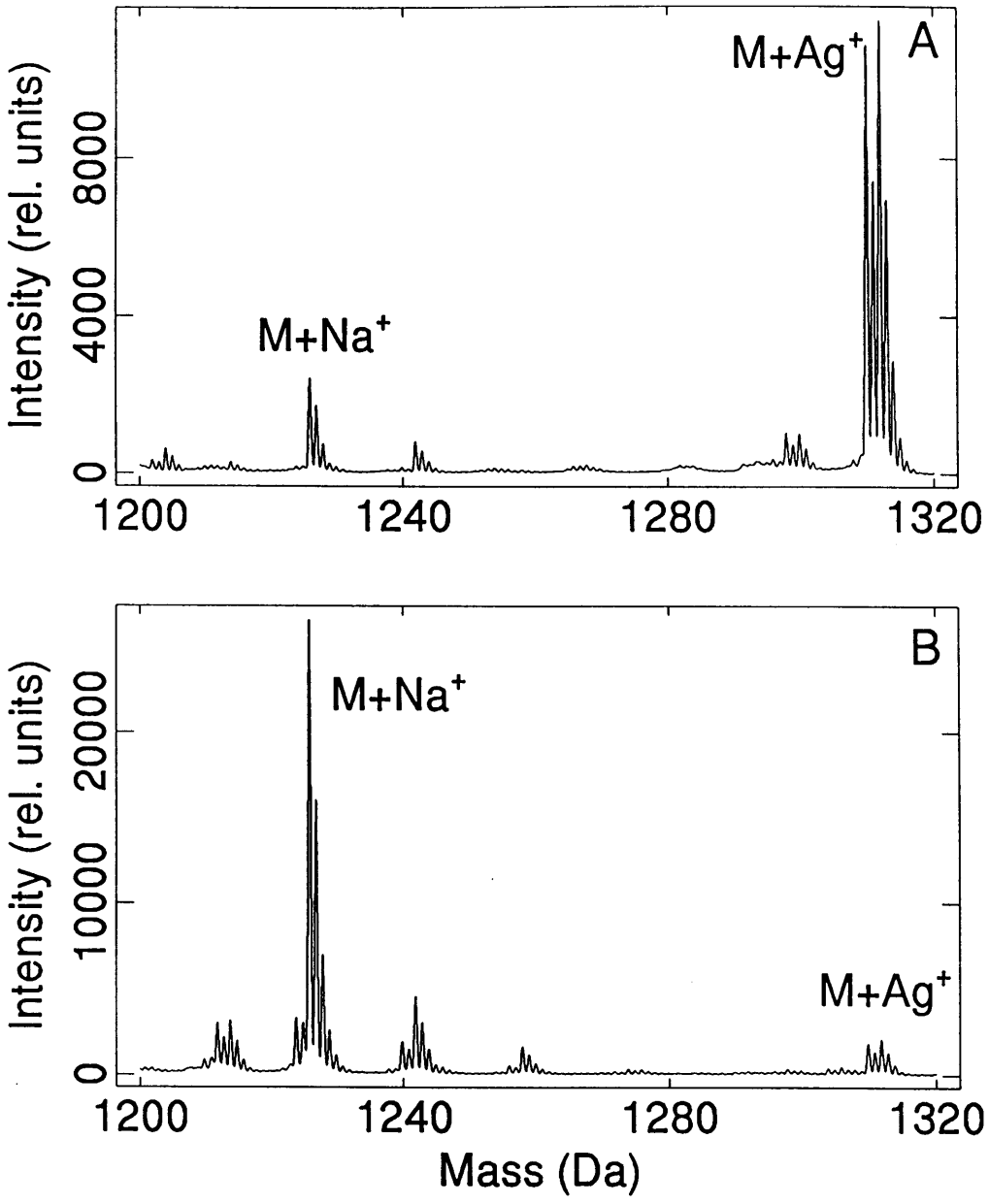

Figure 1. Static SIMS mass spectra of cyclosporin A (10 μg/mL) obtained from (A) an etched silver surface without additives, and (B) with NaBr (1 mg/mL) addition.

nanosecond 10 keV Ar^+ primary ion beam with a diameter of *ca.*50 μm and a pulsed current of 0.1–0.5 pA was used. The secondary ions were accelerated to 3 keV into a reflectron TOF MS and detected by a scintillator-microchannel plate-photomultiplier hybrid detector operating in the single-ion counting mode.

Materials

Nucleotides, angiotensin II, and bradykinin from Sigma (St. Louis, MO). NaBr, NaI, and NaCl were purchased from Fisher Scientific (Pittsburgh, PA). ACS grade HBr, HI, and HCl were purchased from Aldrich Chemical (Milwaukee, WI) and used without further purification. Cyclosporin A (CsA) was obtained from Sandoz Corporation (Basel, Switzerland). All solvents were HPLC grade. Silver foil (0.25 mm thick, 99.9985% purity) was obtained from Alfa Aesar (Ward Hill, MA).

Sample preparation

Three sample preparation methods were studied and compared: (i) the conventional TOF-SIMS sample preparation method on etched silver without additives, (ii) halide additives on etched silver, and (iii) silver substrate modification using halide acids. Standard TOF-SIMS sample preparation for organic analysis involves analyte deposition (1–2 μL) onto a silver substrate (15

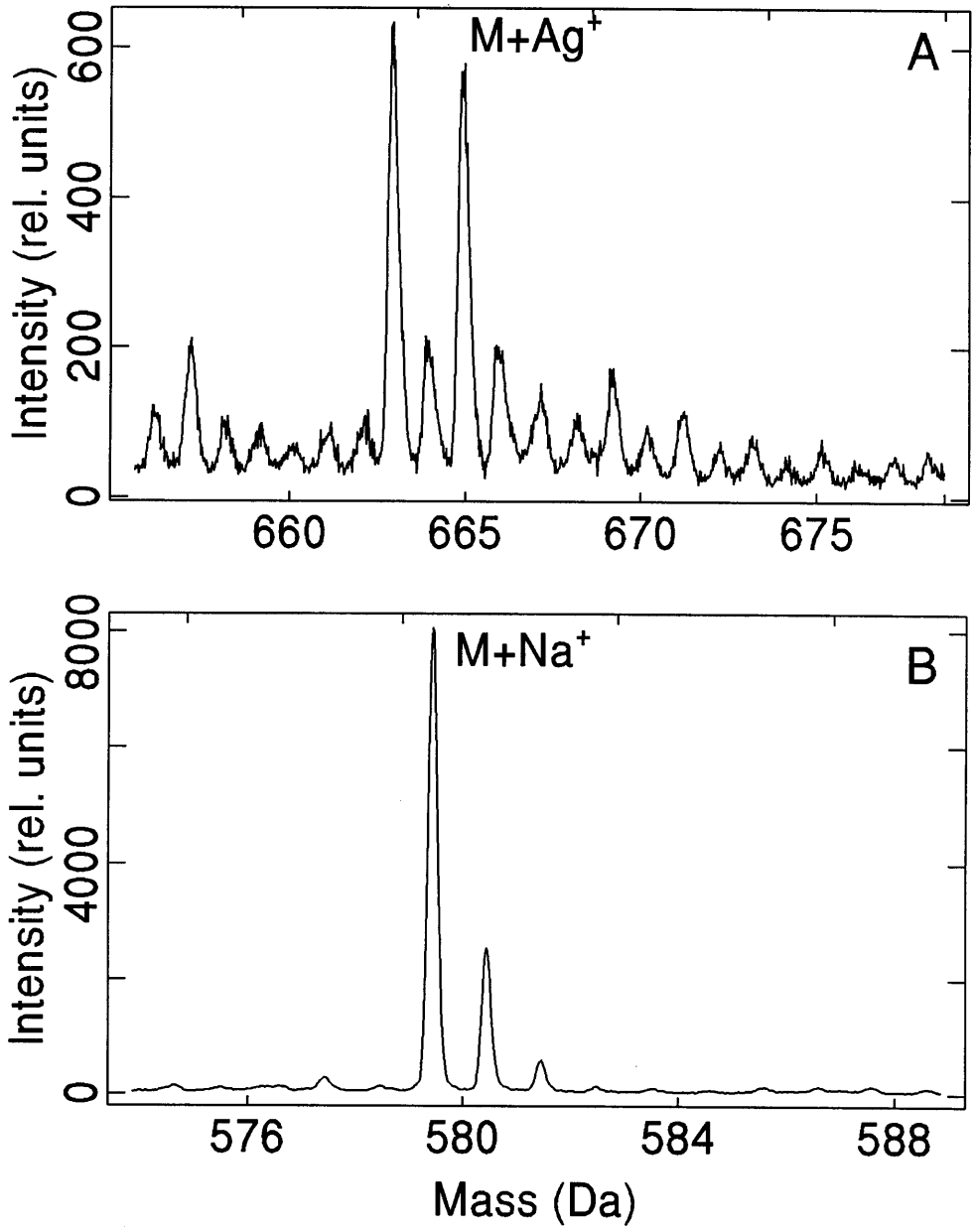

Figure 2. Static SIMS mass spectra of crosslinked adenosine (1 μg/mL) obtained from (A) an etched silver surface without additives, and (B) with NaBr (1 mg/mL) addition.

mm^2) etched in trace metal grade HNO_3 (20% v/v in water). The halide additives (1–2 μL), dissolved in appropriate solvents, were mixed in equal amounts with the analyte directly on the silver substrates. The alternative silver substrate sample preparation involved soaking of previously etched silver substrates in a boiling solution of concentrated HBr, HI or HCl for 5–10 min. The analyte (1–2 μL) was deposited on the halide modified substrate and dried before analysis.

RESULTS AND DISCUSSION

Enhancement of molecular signal intensity and improvement of sensitivity and detection limits can be achieved under the same instrumental conditions by decreasing analyte fragmentation, improving the SI yield, or both. The major focus of this paper will be on the improvement of desorption, cationization and protonation efficiencies of neutral lipophilic and hydrophilic compounds, although analyte molecular fragmentation will be evaluated as well.

Enhancement of cationization efficiency. Most lipophilic compounds tend to cationize well with substrate ions. Etched silver provides an efficient source of cations and has been used for a variety of compounds.[3,4,18–22] Figure 1A shows the mass spectrum of cyclosporin A obtained directly from an etched silver substrate. The major peaks in the spectrum are due to Ag cationization, although Na-and K-cationized species are also observed. The same tendency was reported previously for quantitative analysis of CsA and its major metabolites in blood.[21,22] Although the crude blood extracts

Table 1. Signal improvement factors

	Ion intensity (counts s^{-1})			*Signal*
	$[M+H]^+$	$[M+Na]^+$	$[M+Ag]^+$	*increase*
Cyclosporin A:				
Standard method	5 k	12 k	35 k	Ag vs Na
NaBr addition	1 k	103 k	6 k	~2–5
dA–dA:				
Standard method	8 k	15 k	29 k	Ag vs Na
NaBr addition	15 k	300 k	25 k	~7–9
Brandykinin:				
Standard method	8 k	No signal	2 k	H vs H
HBr substrate	70 k	10 k	7 k	~10
Angiotensin II:				
Standard method	<1 k	No signal	No signal	H vs H
HBr substrate	60 k	No signal	No signal	~100–500

[a] $k = 1 \times 10^3$ counts.

contained a high concentration of alkali metal salts, the Ag cationized peaks were at least an order of magnitude higher than the Na-and K-cationized species. A different effect is observed when alkali bromide (NaBr and KBr), and alkali iodide (NaI and KI) salts are added to the sample, compared to alkali chloride (NaCl and KCl) salts. Figure 1B shows a CsA mass spectrum of an etched silver substrate with NaBr. Equal volumes of NaBr (1 mg/mL in methanol/water 1:1 v/v) and CsA (10 μg/mL in methanol) solutions were mixed directly on the etched silver substrate. In comparison to the standard preparation method, the use of NaBr increased the $(M + Na)^+$ signal by a factor of 12. Thus, the $(M + Na)^+$ signal intensity with NaBr exceeds the $(M + Ag)^+$ signal obtained using standard sample preparation by a factor of *ca.* 2. (see Table 1.) Figure 2 shows mass spectra obtained for crosslinked adenosine

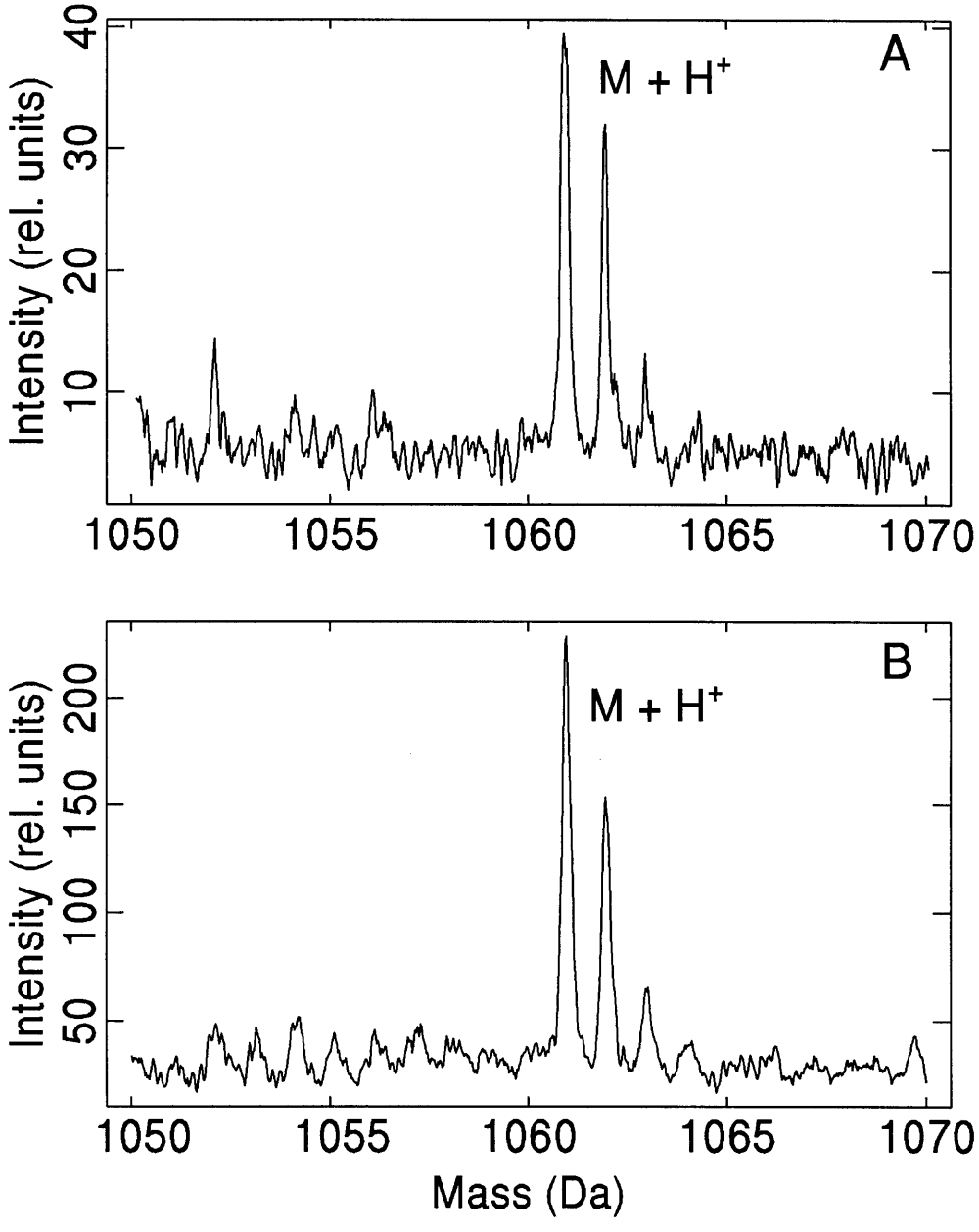

Figure 3. Static SIMS mass spectra of bradykinin (10 μg/mL) obtained from (A) an etched silver surface without additives, and (B) an HBr treated silver substrate.

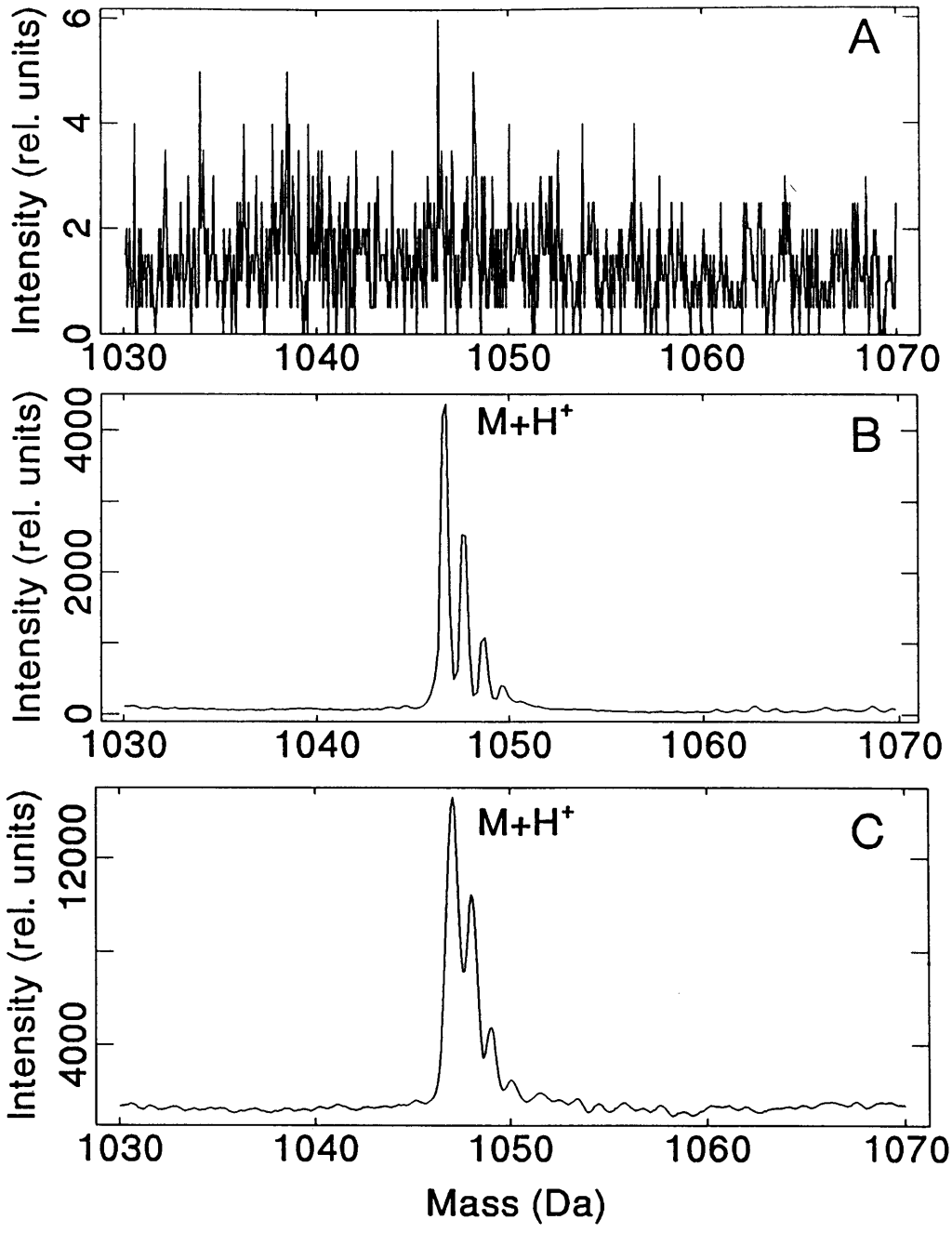

Figure 4. Mass spectra of angiotensin II (10 μg/mL). (A) Static SIMS spectra from an etched silver surface without additives, (B) Static SIMS spectra from an HBr treated silver substrate, and (C) MALDI spectra using the α-cyano-4-hydroxycinnamic acid matrix.

(dA-dA), a new class of potential DNA-carcinogen adduct.[23] Under normal conditions, the dominant analyte signal is $(M + Ag)^+$ while the $(M + Na)^+$ signal is relatively weak. Use of NaBr salt additives improved the signal intensity of the $(M + Na)^+$ peak by a factor of *ca.* 8 over the $(M + Ag)^+$ signal obtained from a standard preparation method (see Table 1). It should be noted that halogen salt additions did not have a significant effect on preformed ions or species which tend to appear most dominatly as protonated molecules.

Enhancement of protonation efficiency. There are two possible mechanisms for forming protonated and deprotonated species in TOF-SIMS spectra. Molecules can be protonated or deprotonated in solution (preformed ions) or ionized in the desorption/ionization process. Preformed ions produce very intense peaks and show high tolerance for substrate surface contamination. For example, analysis of cocaine hydrochloride (preformed ion) has been demonstrated directly from urine with only minimal sample treatment.[24] The detection limits from urine were decreased by a factor of 10 compared to analysis of cocaine from methanol. Neutral compounds can also be protonated in the SIMS ionization process. However, the efficiency of this process in SIMS is generally quite low. For example, angiotensin II, which can easily be ionized in matrix-assisted laser desorption/ionization (MALDI),[25] does not exhibit a measurable signal in SIMS over a broad pH range (pH 1–7) of analyte solution.[26] To improve the efficiency of

proton transfer, the etched silver substrate surfaces were treated with strong halide acids (*e.g.* HCl, HBr and HI) to modify the surface and presumably to change the surface acidity. HCl-treated surfaces exhibited no effect on either protonated or cationized species. However, the use of HBr and HI showed a significant effect on the signal intensities of protonated ions. Figure 3 shows mass spectra of bradykinin obtained from a standard etched silver surface, and an etched silver substrate treated with HBr. Use of the HBr treated substrate resulted in signal intensity enhancement by a factor of *ca.*10, see Table 1. An even more dramatic effect was found for angiotensin II which as stated above does not produce any signal using the standard sample preparation, but which exhibited a strong protonated signal from HBr treated silver. Figure 4 shows TOF-SIMS mass spectra of angiotensin II obtained from (A) an etched silver substrate, and (B) a silver substrate treated with HBr. Figure 4C shows mass spectrum of angiotensin II obtained by MALDI MS using the α-cyano-4-hydroxycinnamic acid matrix. Clearly, use of HBr modified silver enhanced signal intensity by more than 2 orders of magnitude, so that the S/N ratio and signal quality became comparable to that produced by MALDI MS. An increase of signal intensity could be explained by a decrease in fragmentation. However, no significant change in fragmentation was observed for the compounds studied. Thus, the surface must provide favorable conditions (high acidity) for efficient proton transfer. Interestingly, silver surfaces treated with HBr or HI provide efficient means for incorporation of protons and cations, but silver surfaces treated with HCl do not. However, it is clear that the halide-treated surfaces do not just provide protons from adsorbed acid on the surface. In addition, the enhancement of protonated signals is observed only for neutral compounds, no signal improvement was observed for preformed ions. The exact process involved in proton/cation enhancement of SIMS signals by halide modified surfaces is still not clear. Detailed examination of the surface conditions and surface complexes will aid in elucidation of the mechanisms and such studies are now being conducted in our laboratory.

CONCLUSIONS

A new sample preparation method was developed for TOF-SIMS analysis of organics, involving the use of halide additives or halide surface modification to promote analyte cationization and protonation. Significant signal enhancement was achieved for various neutral lipophilic and hydrophilic compounds. Application of NaBr and NaI additives resulted in signal intensity enhancement of cationized peaks by factors 2–30 for lipophilic compounds. Use of silver surface modification by HBr and HI for analysis of neutral hydrophilic compounds led to enhancement of protonated signal intensity by a factor of 20–2000. No signal improvement was observed with NaCl additives or surface modification using HCl. Although the exact mechanism of the effect is still unclear, Br and I atoms on the surface must provide an effective means for incorporation of Na-cations and protons on the silver substrate.

Acknowledgements

This work was supported by the U.S. Environmental Protection Agency (Grant R819809–01), and the National Science Foundation (Grant CHE-9022135). Authors would also like to thank Prof. Marwan C. Houalla and Mr. Anthony J. Nicola for valuable discussion.

REFERENCES

1. J. Bennett, J. A. Dagata, J. Vac. *Sci. Technol. B*., **12**, 214 (1994).
2. F. Lafortune, G. W. Buchko, F. E. Hruska, K. L. Sadana, K. G. Standing, J. B. Westmore. *Nucleosides Nucleotides*, **11**, 1305 (1992).
3. I. V. Bletsos, D. M. Hercules, D. van Leyen, B. Hagenhoff, E. Niehuis, A. Benninghoven *Anal. Chem*., **60**, 938 (1991).
4. I. V. Bletsos, D. M. Hercules, D. van Leyen, A. Benninghoven, C.G. Karakatsanis, J. N. Rieck, *Macromolecules*, **23**, 4157 (1990).
5. D. C. Muddiman, A. H. Brockman, A. Proctor, M. Houalla, D. M. Hercules. *J. Phys Chem*., **98**, 11570 (1994).
6. A. Benninghoven, B. Hagenhoff, E. Niehuis, *Anal. Chem*., **65**, 630A (1993).
7. L. K. Liu, K. L. Busch, R. G. Cooks, *Anal. Chem*., **53**, 109 (1981).
8. S. E. Unger, R. J. Day, R. G.Cooks, *Int.J. Mass Spectrom. Ion Proc*., **39**, 231 (1981).
9. K. L. Busch, B. H. Hsu, Y. X. Xie, R. G. Cooks, *Anal. Chem*., **55**, 1157 (1983A).
10. A. Benninghoven, W. K. Sichtermann, *Anal. Chem*., **50**, 1181 (1978).
11. K. L. Busch, S. E. Unger, A. Vincze, R. G. Cooks, T. Keough, *J. Am. Chem. Soc*., **104**, 1507 (1982).
12. K. J. Wu, R. W. Odom, *Anal. Chem*., **68**, 873 (1996).
13. A. J. Nicola, D. C. Muddiman, D. M. Hercules, *J. Am. Soc. Mass Spectrom*., **7**, 467 (1996).
14. K. L Busch, R. G. Cooks, J., *Science*, **218**, 247, (1982).
15. J. L. Pierce, K. L. Busch, R. G. Cooks, R. A. Walton, *J. Am. Chem. Soc*., **21**, 2597. (1982).
16. E. Niehuis, T. Heller, H. Feld, A.Benninghoven *J. Vac. Sci. Technol. A,* **5**, 1243 (1987).
17. E. Niehuis, P. N. T. van Veltzen, J. Lub, T. Heller, A.Benninghoven, *Surf. Interface Anal*., **14**, 135 (1987).
18. D. C. Muddiman, A. I. Gusev, D. M. Hercules, *Mass Spectrom. Rev*. **14**, 383 (1995).
19. K. Meyer, B. Hagenhoff, M. Deimel, A. Benninghoven, *Org. Mass Spectrom*., **27**, 148, (1992).
20. A. I. Gusev, D. C. Muddiman, A. Proctor, A. G. Sharkey, D. M. Hercules, P. Tata, R. Venkataramanan, W. Diven, *Rapid Com. Mass Spectrom*., **10**, 1215 (1996).
21. D. C. Muddiman, A. I. Gusev, A. Proctor, D. M. Hercules, R. Venkataramanan, W. Diven, *Anal. Chem*. **66**, 2362 (1994).
22. D. C. Muddiman, A. I. Gusev, K. Stoppek-Langner, A. Proctor, D. M. Hercules. P. Tata, R. Venkataramanan, W. Diven, *J. Mass Spectrom*., **30**, 1469 (1995).
23. D. Tsarouhtsis, S. Kuchimanchi, B. L. Decorte, C. M. Harris, T. M. Harris, *J. Am. Chem. Soc*., **117**, 11013 (1995).
24. D. C. Muddiman, A. I. Gusev, L. B. Martin, D. M. Hercules, *Fresenius J. Anal. Chem*. **354**, 103 (1996).
25. A. J. Nicola, A. I. Gusev, A. Proctor, E. K. Jackson, D. M. Hercules, *Rapid Com. Mass Spectrom*., **9**, 1164 (1995).
26. A. J Nicloa, unpublished results (1995).