International Journal of Mass Spectrometry 273 (2008) 84–86

Contents lists available at ScienceDirect

International Journal of Mass Spectrometry

journal homepage: www.elsevier.com/locate/ijms

Short communication

Positive/negative ion ratios and in-plume reaction equilibria in MALDI

Richard Knochenmuss*

Novartis Institutes for Biomedical Research, WSJ 503.1104, 4002 Basel, Switzerland

ARTICLE INFO

Article history:
Received 28 January 2008
Received in revised form 4 March 2008
Accepted 6 March 2008
Available online 16 March 2008

Keywords:
MALDI
Ionization mechanisms
Ion–molecule reactions

ABSTRACT

Recent experimental data on positive vs negative ion ratios in MALDI mass spectra are compared with predictions of the thermodynamic model of secondary ion–molecule reactions. Taking mass balance into account, the ion ratios are predicted to remain close to 1, as long as the forward reactions are favorable. This is remains true for both high and low analyte concentrations, and high or low laser intensities. The data support approach to local thermodynamic equilibrium in the MALDI plume.

1. Introduction

Although a number of models have been proposed for ionization processes in MALDI [1–5], only one has demonstrated quantitative ability to predict and interpret a wide variety of MALDI phenomena and observations [6,7]. This model is based on a two step picture, in which primary matrix ions are first created in a short period of high energy and material density, followed by secondary ion–molecule reactions with analyte neutrals in the expanding plume [8].

Regardless of how the primary ions are formed, secondary reactions in the plume are believed to approach local thermal equilibrium under typical conditions [1,3]. This has the consequence that MALDI mass spectra can be predicted and interpreted using the thermodynamics of these gas-phase reactions. Qualitatively, matrixes can be selected based on physical properties of the analytes, and relative spectral intensities interpreted in terms of the charge transfer processes observed (proton transfer, cation adduction, or electron transfer). The energetically most favorable combination of ions is favored in the mass spectrum, and these are often straightforwardly predictable.

Approach to local thermodynamic equilibrium in the MALDI plume can be sufficiently complete that Boltzmann plots of carefully measured ion ratios can be used to infer gas phase proton affinities [9]. This initially surprising fact becomes very understandable in light of the molecular dynamics simulations which demonstrate that the early plume is a very dense environment in which all but the earliest ablated molecules experience a large number of collisions [10–12].

The model can also be made quantitative, either macroscopically in the form of differential equations [6,7], or microscopically in the form of molecular dynamics simulations [13]. The former method, in particular, is very useful for predicting and interpreting spectra in detail. The relative intensities of matrix and analyte, or of various analytes, can be calculated as a function of readily varied parameters such as analyte concentration(s), laser intensity, wavelength and pulse width, among others. The results are in excellent agreement with experiment, including for unusual phenomena such as the matrix and analyte suppression effects [14,15].

The two step framework and the quantitative models are not restricted to specific ion or reaction types. The concepts have proved consistent with experiment for proton transfer, cationization and electron transfer involving substances ranging from biomolecules to synthetic polymers, and polar or non-polar analytes and matrices. However, MALDI tends to be performed most often in positive ion mode, so there is less data regarding negative ion reactions.

Recently, Dashtiev et al. performed measurements of positive vs negative ion yields of three moderate molecular weight biomolecules, in six MALDI matrixes [16]. Using two detector technologies, and attempting to normalize the instrument response for both polarities, they concluded that the amounts of positive and negative analyte ions produced were about the same, for all combinations of matrix and analyte tested.

While this may not seem surprising in view of the necessary overall charge neutrality of the system, a stronger dependence of the ion ratio was predicted by the authors due to variation of the matrix and analyte proton affinities. As a result, it was concluded that processes other than the gas-phase proton transfer dominate the system, and that the plume equilibrium model is not adequate. This conclusion is troubling, even if it represents only one small data set vs a large body of literature that appears to argue otherwise. The

---

* Tel.: + 41 61 324 4541; fax: + 41 61 324 8868.
E-mail address: rknochenmuss@gmx.net.

1387-3806/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijms.2008.03.004

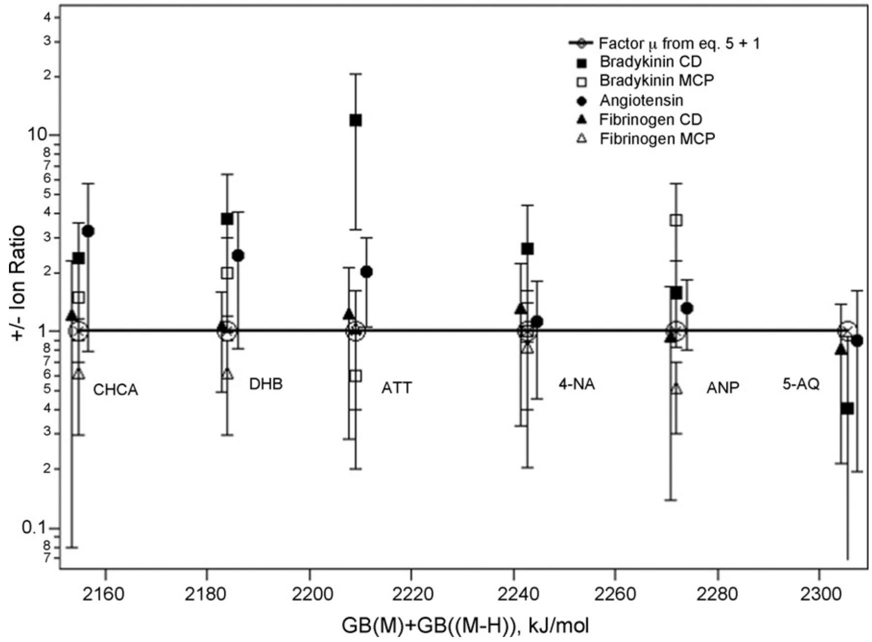

Fig. 1. Positive/negative analyte ion ratios for three analytes and six matrixes. The data and matrix abbreviations are from Ref. [16]. CD stands for charge detector, and MCP for microchannel plate detector. The μ factor from Eq. (5) is plotted as well, vertically offset by 1 for comparison. The temperature was assumed to be 700 K.

present work shows that this conclusion is premature, and that the results of ref. [16] are in fact consistent with the plume equilibrium model.

2. Theory and results

In a dense, hot MALDI plume ion–molecule reactions should approach equilibrium. Assuming protonation and deprotonation reactions to be the major secondary charge transfer processes occurring, reactions 1 and 2 are of interest:

$$MH^+ + A = M + AH^+ \quad (1)$$

$$(M-H)^- + A = M + (A-H)^- \quad (2)$$

where M = matrix and A = analyte.

The associated equilibrium constants are related to the reaction free energies in the usual manner:

$$\begin{aligned} K^+ &= \exp(-\Delta G^+/RT) = [M][AH^+]/[MH^+][A] \\ \Delta G^+ &= GB(M) - GB(A) \end{aligned} \quad (3)$$

$$\begin{aligned} K^- &= \exp(-\Delta G^-/RT) = [M][A-H^-]/[M-H^-][A] \\ \Delta G^- &= GB((A-H)^-) - GB((M-H)^-) \end{aligned} \quad (4)$$

where GB(X) is the gas-phase basicity of species X, defined as $-\Delta G$ for the reaction $X + H^+ = XH^+$.

Solving (3) and (4) for the respective AH^+ and $(A-H)^-$ concentrations, the ion ratio has the following form:

$$\begin{aligned} \frac{[AH^+]}{[(A-H)^-]} &= \left(\frac{[MH^+]}{[(M-H)^-]}\right) \exp\left(\frac{(-\Delta G^+ + \Delta G^-)}{RT}\right) \\ &= \left(\frac{[MH^+]}{[(M-H)^-]}\right) \frac{\mu}{\alpha} \\ \mu &= \exp(-(GB(M) + GB((M-H)^-))/RT) \\ \alpha &= \exp(-(GB(A) + GB((A-H)^-))/RT) \end{aligned} \quad (5)$$

The matrix and analyte dependencies of the ratio are collected in the factors μ and α, respectively. It was argued in ref. [16] that the observed weak dependence of the ion ratio on matrix choice is inconsistent with the plume equilibrium model because of the exponential form of μ. However the sum of the basicities varies by a small relative amount, so μ also changes little. For the matrixes used in ref. [16] the mean is about 2250 kJ/mol, while the range is only about 150 kJ/mol. As seen below in Fig. 1, the expected matrix dependence is in fact small. The $-1/RT$ slope of μ in this semi-log plot is vanishly small.

The slope of μ is consistent with the data, but the absolute ion ratios, and not only the shape of the curve, are of interest. The difference of the reaction free energies in the exponent of Eq. (5), $(\Delta G^- - \Delta G^+)$, would predict that a 10 kJ/mol more favorable reaction 1 yields $[AH^+]/[(A-H)^-] = 5.6$ at 700 K (assuming equal initial matrix ion quantities). Such a ratio could be consistent with a few of

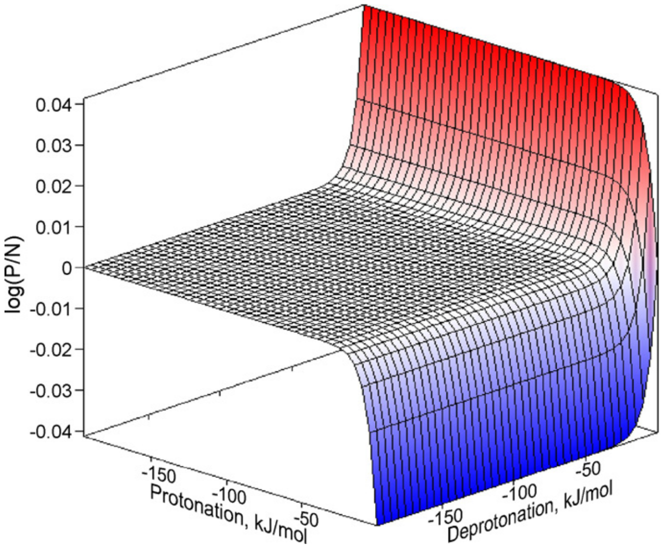

Fig. 2. Positive/negative analyte ion ratios calculated from mass balance Eqs. (6) and (7), with the initial conditions $A^\circ = 10^{-3}$ and $MH^\circ = 10^{-2}$ (excess of primary matrix ions). A logarithmic vertical scale is used to emphasize the symmetry of the ratios vs protonation or deprotonation, ΔG^+ and ΔG^-. Red indicates an excess of positive analyte ions, blue an excess of negative analyte ions. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

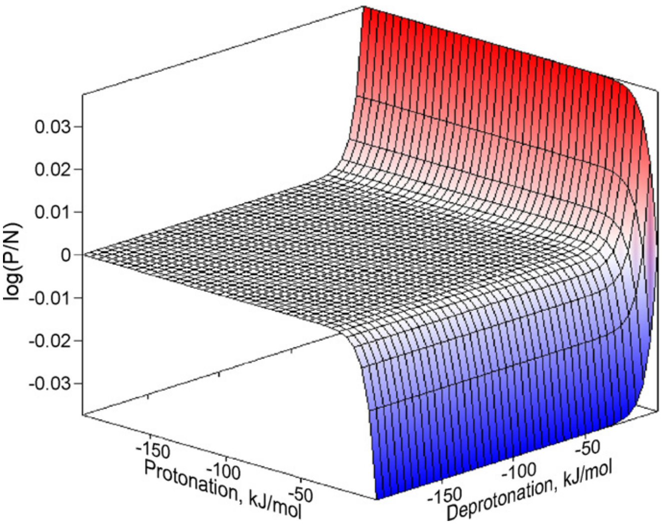

Fig. 3. Positive/negative analyte ion ratios calculated from mass balance Eqs. (6) and (7), with the initial conditions $A^\circ = 10^{-3}$ and $MH^\circ = 10^{-4}$ (excess of analyte). A logarithmic vertical scale is used to emphasize the symmetry of the ratios vs protonation or deprotonation, ΔG^+ and ΔG^-. Red indicates an excess of positive analyte ions, blue an excess of negative analyte ions. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

the data in Fig. 1, but the ΔG difference could easily be much larger, and the predicted ion ratio not at all consistent. This would be true even if both protonation and deprotonation reactions are highly favorable, but with slightly different reaction free energies. This is not a physically reasonable prediction, because if both reactions are favorable, similar quantities of positive and negative analyte ions will be formed, and the ratio will tend to unity.

This apparent problem arises because Eq. (5) does not take into account the limited reactant quantities. The analyte is typically present at 10^{-3} or lower mole ratio vs matrix. The maximally available matrix primary ion concentration during the early plume expansion is in the 10^{-2} range, depending on laser intensity [6,7,13]. A more accurate approach is to solve the mass balance equations, taking into account these initial conditions.

Denote the amount of reacted material in Eq. (3) by x. The sum of reactants and products is normalized to 1. The initial quantities of unreacted positive matrix primary ions and analyte are denoted as MH° and A°:

$$K^+ = \frac{[M][AH^+]}{[MH^+][A]} = \frac{1 - x - (MH^\circ - x) - (A^\circ - x)x}{(MH^\circ - x)(A^\circ - x)} \tag{6}$$

Some algebra yields a quadratic equation:

$$\begin{aligned} 0 &= ax^2 + bx + c \\ a &= 1 - K^+ \\ b &= 1 + A^\circ(K^+ - 1) + MH^\circ(K^+ - 1) \\ c &= -K^+ MH^\circ A^\circ \end{aligned} \tag{7}$$

The smallest root is chosen, corresponding to either MH° or A° as the limiting reagent. The equivalent expression for negative ions replaces K^+ by K^- and MH° by $(M-H)^\circ$. The positive and negative analyte ion quantities can be compared over a range of ΔG values for the protonation and deprotonation reactions. Assuming a nominal analyte/matrix mole ratio of 0.001, as in ref. [16], and a peak primary ion mole fraction of 0.01 (for both MH^+ and $(M-H)^-$), the ion ratios are as shown in Fig. 2. The ΔG values span the range expected for proton transfer reactions between matrix and analyte. Only the region where ΔG^+ and ΔG^- are both negative is plotted, since if either is positive, the corresponding analyte ions will not be formed, and the ion ratio will be zero or infinite.

The positive/negative analyte ratio never deviates significantly from 1, except at the extreme edges where one or the other ΔG is nearly zero. Even at the edges, the ion ratio only increases to 1.09. The nearly uniform value of 1 is consistent with the data of Fig. 1 and with Eq. (5).

This picture does not change for other values of matrix primary ions or analyte concentration. One or the other of these is a limiting reagent in any case. For even moderately favorable thermodynamics, the reaction proceeds far towards this limit. At the limit, there are equal quantities of positive and negative analyte ions. As an example, Fig. 3 shows the case where primary matrix ions rather than analyte are the limiting reagent ($MH^\circ = 10^{-4}$). This corresponds to a weak laser pulse.

Because proton transfer reactions are highly favorable in many MALDI applications (better than −50 kJ/mol) [1], there is seldom a fundamental advantage to one polarity over the other, from an ion formation standpoint. Technical detector issues seem to be more decisive in this respect.

3. Conclusions

Positive/negative analyte ion ratios predicted by the local reaction equilibrium model of MALDI secondary ionization processes are found to be consistent with recent data for three analytes and six matrixes. The calculated ion ratio remains very close to unity over wide ranges of reaction ΔG. The ratio is not predicted to change significantly for widely varying conditions such as analyte concentration, or laser intensity (primary ion quantity).

References

[1] R. Knochenmuss, The Analyst 131 (2006) 966.
[2] H. Ehring, M. Karas, F. Hillenkamp, Org. Mass Spectrom. 27 (1992) 427.
[3] R. Knochenmuss, R. Zenobi, Chem. Rev. 103 (2003) 441.
[4] R. Zenobi, R. Knochenmuss, Mass Spectrom. Rev. 17 (1998) 337.
[5] M. Karas, R. Krüger, Chem. Rev. 103 (2003) 427.
[6] R. Knochenmuss, J. Mass Spectrom. 37 (2002) 867.
[7] R. Knochenmuss, Anal. Chem. 75 (2003) 2199.
[8] R. Knochenmuss, A. Stortelder, K. Breuker, R. Zenobi, J. Mass Spectrom. 35 (2000) 1237.
[9] G.R. Kinsel, D. Yao, F.H. Yassin, D.S. Marynick, Eur. J. Mass Spectrom. 12 (2006) 359.
[10] L.V. Zhigilei, B.J. Garrison, J. Appl. Phys. 88 (2000) 1.
[11] L.V. Zhigilei, P.B.S. Kodali, B.J. Garrison, J. Phys. Chem. B 102 (1998) 2845.
[12] L.V. Zhigilei, E. Leveugle, B.J. Garrison, Y.G. Yingling, M.I. Zeifman, Chem. Rev. 103 (2003) 321.
[13] R. Knochenmuss, L.V. Zhigilei, J. Phys. Chem. B 109 (2005) 22947.
[14] R. Knochenmuss, V. Karbach, U. Wiesli, K. Breuker, R. Zenobi, Rapid Commun. Mass Spectrom. 12 (1998) 529.
[15] R. Knochenmuss, F. Dubois, M.J. Dale, R. Zenobi, Rapid Commun. Mass Spectrom. 10 (1996) 871.
[16] M. Dashtiev, E. Wäfler, U. Röhling, M. Gorshkov, F. Hillenkamp, R. Zenobi, Int. J. Mass Spectrom. 268 (2007) 122.