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APPLICATION NOTE

Quantification of Carbohydrates and Related Materials Using Sodium Ion Adducts Produced by Matrix-Assisted Laser Desorption Ionization

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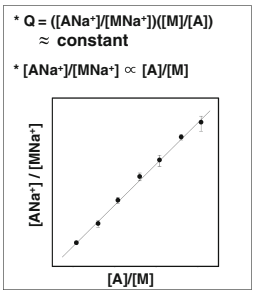

Abstract. The utility of sodium ion adducts produced by matrix-assisted laser desorption ionization for the quantification of analytes with multiple oxygen atoms was evaluated. Uses of homogeneous solid samples and temperature control allowed the acquisition of reproducible spectra. The method resulted in a direct proportionality between the ion abundance ratio $I([A + Na]^+)/I([M + Na]^+)$ and the analyte concentration, which could be used as a calibration curve. This was demonstrated for carbohydrates, glycans, and polyether diols with dynamic range exceeding three orders of magnitude.

Keywords: Quantification of carbohydrate, Quantification of glycan, Quantification by MALDI, Sodium ion adduct

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Introduction

Carbohydrates have important biological and clinical implications [1, 2]. Electrospray ionization (ESI) [3] and matrix-assisted laser desorption ionization (MALDI) [3] are important tools for their analysis. ESI is useful for structural determination and quantification, whereas the fact that carbohydrate-derived ions can be readily produced in the presence of contaminants is the main advantage of MALDI.

In MALDI of many molecules such as peptides, analyte (A)-derived ions appear as a protonated molecule, $[A + H]^+$, or its fragments. In the MALDI spectrum of a carbohydrate acquired using 2,5-dihydroxybenzoic acid (DHB) as the matrix (M), $[A + H]^+$ rarely appears. Instead, the sodium ion adduct, $[A + Na]^+$, more commonly appears. In DHB-MALDI, $[A + Na]^+$ is prominent even when a sodium salt is not added, presumably due to their availability as contaminants. In fact, $[A + Na]^+$ is ubiquitous in the MALDI spectra of compounds containing many oxygen atoms, such as carbohydrates, glycans, and polyether diols.

Although sodium ion adducts are used for structural study of the above oxygen-containing molecules, their use for quantification has been rare. Harvey reported the quantification of oligosaccharides by DHB-MALDI [4] with calibration curves drawn by plotting the abundance of $[A + Na]^+$, or $I([A + Na]^+)$, as a function of the analyte concentration. Such a calibration curve is not useful when contaminants affect $I([A + Na]^+)$.

Harvey [4], Hintze et al. [5], and Sporns et al. [6] used internal standards (IS) to reduce the influence of contaminants. However, the linear dynamic ranges in the calibration curves drawn with $I([A + Na]^+)/I([IS + Na]^+)$ were only 10–100. Furthermore, the curves often became linear only when the data were plotted on a log–log scale. Most recently, Rankin and Mabury [7] reported a linear calibration curve for an acrylate polymer acquired by dithranol-MALDI. Referring to our method for peptide quantification [8], they plotted $I([A + Na]^+)/$

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$I([M+Na]^+)$ as a function of the analyte concentration. The linear dynamic range of the calibration curve thus obtained was only around 10–20, presumably due to the sample inhomogeneity and/or the fluctuation in the early plume temperature.

Our method to quantify peptides without using an internal standard was derived from the observation that two factors mainly determined the reproducibility of the MALDI spectrum, the sample homogeneity and the constancy of the early plume temperature [8]. Methods to control these factors have been reported [9]. We also found that the reaction quotient (Q_H) for the matrix-to-analyte proton transfer, $[M+H]^+ + A \rightarrow M + [A+H]^+$, was nearly constant [8]. This led to a direct proportionality between the analyte-to-matrix ion ratio and the analyte concentration.

$$I([A+H]^+)/I([M+H]^+) \propto I(A)/I(M) \quad (1)$$

Although I(A) and I(M) represent the amounts of A and M in the matrix plume, respectively, we can relate their ratio to the analyte concentration in a sample [8]. Then, Equation 1 becomes a calibration relation. In fact, direct proportionality spanning three orders of magnitude or more was observed for all the peptides and other analytes studied. Moreover, the method was applicable to contaminated samples.

MALDI of molecules containing many oxygen atoms often produce $[A+Na]^+$ preferentially or together with $[A+H]^+$. In this paper, we will show that $[A+Na]^+$ can be used during the quantification of such analytes.

Experimental

A homebuilt MALDI-tandem TOF instrument [10] was used. We fixed the early plume temperature by controlling the total ion count (TIC) in a spectrum that was achieved by feedback-control of the laser pulse energy.

We used DHB as the matrix. It was dissolved in 100% methanol; 100% methanol was the solvent for the analyte also. One μL of a typical sample solution prepared by mixing the matrix and analyte solutions contained 50 nmol of DHB, 100 pmol of NaCl, and variable amounts of an analyte; 0.5 μL of the solution was loaded on a hydrophilic part of a commercial sample plate (ASTA, Suwon, Korea) and vacuum-dried [11]. Although this produced a solid sample that looked homogeneous along the sample plane (yz), their depth profiles, i.e., the analyte distributions along the thickness direction (x), were inhomogeneous (Supplementary Figure S1). A quantification method for analytes in such a sample is described in reference [11]. Addition of 1.0 nmol of NaCl per 1.0 μL of the sample solution did not affect the sample homogeneity.

Peptide Y_5R was purchased from Peptron (Daejeon, Korea). Other materials were purchased from Sigma-Aldrich (St. Louis, MO, USA). These include DHB, maltotriose ($C_{18}H_{32}O_{16}$, 504.2 Da), stachyose ($C_{24}H_{42}O_{21}$, 666.2 Da), Man5GlcNAc2 high-mannose N-linked glycan (Man-5 glycan, $C_{46}H_{78}N_2O_{36}$, 1234.4 Da), fucosylated biantennary complex N-linked glycan (FA2 glycan, $C_{56}H_{94}N_4O_{40}$, 1462.5 Da), polyethylene glycol 1000 (PEG 1000, $H[OCH_2CH_2]_nOH$, n = 8–37), polypropylene glycol 1000 (PPG 1000, $H[OCH(CH_3)CH_2]_nOH$, n = 7–30), and NaCl. Structures of some analytes are shown in Supplementary Figure S2.

Results and Discussion

In the DHB-MALDI spectrum (Supplementary Figure S3) of maltotriose, which is a prototype of carbohydrates, $[A+H]^+$ is absent. $[A+Na]^+$ is essentially the only ion produced from this molecule.

Let us suppose that a carbohydrate is sodiated via a matrix-to-carbohydrate sodium ion transfer occurring in the early plume, reaction 2, which is analogous to the protonation of a peptide.

$$[M+Na]^+ + A \rightarrow M + [A+Na]^+ \quad (2)$$

Its reaction quotient, Q_{Na} (Equation 3), will be nearly constant when it is close to equilibrium.

$$Q_{Na} = \{I([A+Na]^+)/I([M+Na]^+)\}\{I(M)/I(A)\} \approx \text{constant} \quad (3)$$

Equation 3 can be rearranged as follows:

$$I([A+Na]^+)/I([M+Na]^+) \propto I(A)/I(M) \quad (4)$$

To check the validity of the above assumption, we measured Q_{Na} over a maltotriose concentration of 0.3–300 pmol (in 25 nmol of DHB). Two sets of data were acquired, one from samples with 50 pmol and the other with 500 pmol of NaCl added per 0.5 μL of the sample solution. The results in Figure 1a show that Q_{Na} is nearly constant throughout the covered analyte concentration range, suggesting that reaction 2 is nearly in equilibrium, as surmised above. In addition, Q_{Na} is unaffected by the amount of NaCl added. The same data also indicate that the free sodium ions in the matrix plume may not directly participate in the sodium ion transfer.

Regardless of the validity of the above mechanism for the production of $[A+Na]^+$, the fact that Q_{Na} is nearly constant indicates direct proportionality between the sodium ion adduct ratio, $I([A+Na]^+)/I([M+Na]^+)$, and the analyte concentration, I(A)/I(M). This is analogous to the direct proportionality between $I([A+H]^+)/I([M+H]^+)$ and I(A)/I(M) observed for peptides and other basic molecules. The data in Figure 1a are reproduced in Figure 1b in the form of a log–log plot of $I([A+Na]^+)/I([M+Na]^+)$ versus I(A)/I(M), which is a calibration curve. The plots acquired from samples with added NaCl amounts of 50 and 500 pmol look nearly identical. We could not raise the analyte amount beyond 300 pmol because the samples became inhomogeneous. The slopes of the plots are

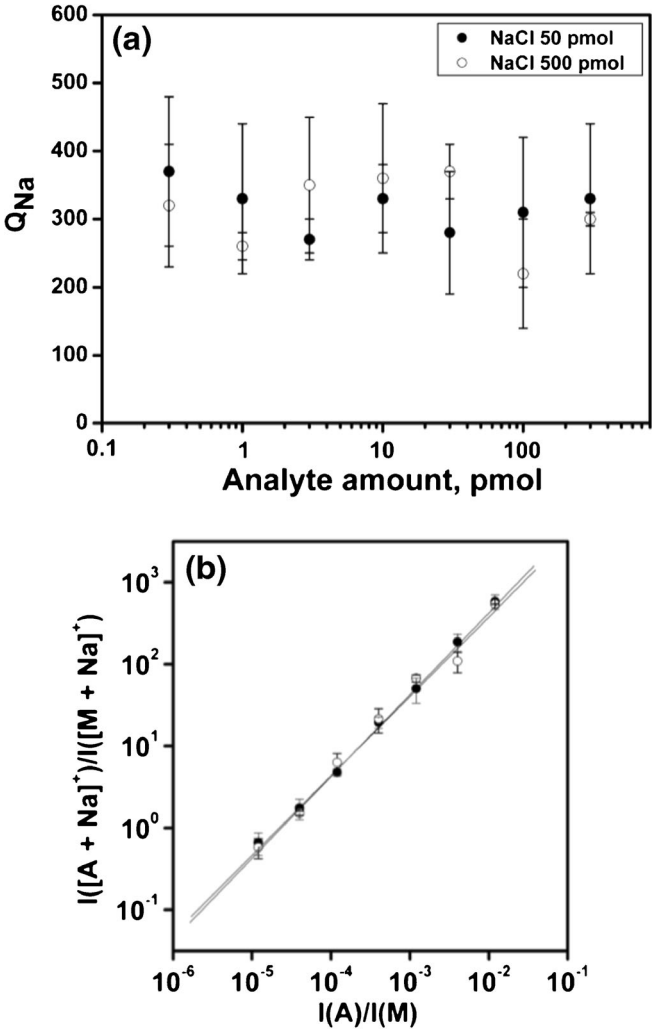

Figure 1. **(a)** The reaction quotient, Q_{Na} (Equation 3), measured with a maltotriose concentration of 0.3–300 pmol in 25 nmol of DHB using a pre-set TIC of 3000. Data from samples with 50 pmol (*filled circles*) and 500 pmol (*open circles*) of NaCl added per 0.5 μL of the sample solution are shown. In **(b)**, the same data have been converted to $I([A + Na]^+)/I([M + Na]^+)$ versus I(A)/I(M) and plotted on the log–log scale. Best fits with the added NaCl of 50 and 500 pmol were y = 0.994x + 4.65 and y = 0.974x + 4.65, respectively. A slope close to 1.0 in the log–log plot means that the ion abundance ratio is directly proportional to the analyte concentration. The same result drawn on a linear scale is shown in Supplementary Figure S3. Error bars indicate one standard deviation from triplicate measurements

close to 1.0, supporting the direct proportionality between the ion ratio and the concentration. As a further demonstration, linear plots of the data are shown in Supplementary Figure S4.

In the peptide quantification based on the protonated ion ratio, we observed that calibration curves deviated from direct proportionality at high analyte concentrations. This was observed when the matrix suppression [12] defined as $1–I([M + H]^+)/I_0([M + H]^+)$ exceeded a critical value, S_c. Here, $I_0([M + H]^+)$ denotes the matrix ion abundance in MALDI of a pure matrix. S_c was found to be matrix-dependent, 50% with DHB

Table 1. Quantification Results for 0.3 and 10 pmol of Maltotriose in 25 nmol of DHB Contaminated with 0.3–30 pmol of Y_5R[a]

Amount of Y_5R, in pmol	Amount of maltotriose loaded, in pmol	Amount of maltotriose determined, in pmol	Matrix suppression,[b] %
0.3	0.3	0.3 ± 0.05	21 ± 6
1.0	0.3	0.3 ± 0.02	25 ± 9
3.0	0.3	0.3 ± 0.04	39 ± 9
10	0.3	0.2 ± 0.04	54 ± 7
30	0.3	0.2 ± 0.03	67 ± 10
0.3	10	11 ± 2	17 ± 8
1.0	10	10 ± 1	24 ± 4
3.0	10	11 ± 1	40 ± 5
10	10	7 ± 1	59 ± 10
30	10	6 ± 1	70 ± 6

[a] 50 pmol of NaCl was also added
[b] $1 – I([M + H]^+)/I_0([M + H]^+)$.

[11]. In the present work, we hoped to establish a similar criterion for quantification based on the sodium ion adduct abundances. However, we could not do this because sodium ions were present in a sample even when we did not add them and because there was no guarantee that the amounts would not change from sample to sample. Fortuitously, however, we observed that the reliability of the quantification results for carbohydrates based on $[A + Na]^+$ was decided by the same factor as for peptides (i.e., the suppression of $[M + H]^+$). As a demonstration, we prepared samples containing 0.3 or 10 pmol of maltotriose, 50 pmol of added NaCl, and 0.3–30 pmol of Y_5R in 25 nmol of DHB. Here, Y_5R was intentionally added, as a contaminant. The quantification results for maltotriose in each sample obtained with the calibration curve in Figure 1b and the measured suppressions of $[M + H]^+$ are listed in Table 1. It is observed that the quantification results are acceptable even when the amount of the contaminant is larger than that of maltotriose by an order of magnitude as long as the matrix suppression is equal to or less than 50%. This is a way of

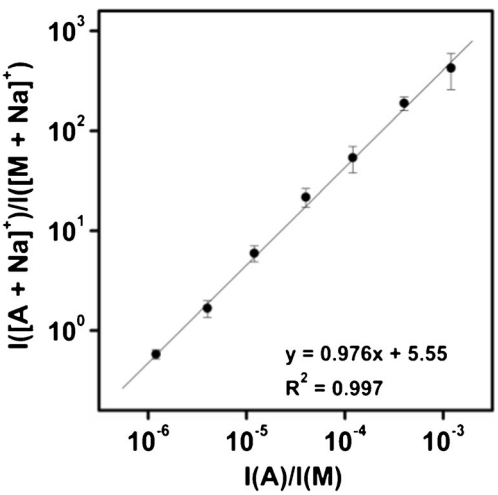

Figure 2. Calibration curve for Man-5 glycan in MALDI with DHB. A pre-set TIC of 3000 was used. A $I([A + Na]^+)/I([M + Na]^+)$ versus I(A)/I(M) plot is drawn on the log–log scale. Error bars indicate one standard deviation from triplicate measurements

checking the influence of the matrix suppression on a quantification result, which is a systematic error. Errors quoted in Table 1 were estimated from the adduct ion abundance ratios for three samples, each averaged over nine different spots. Sample inhomogeneity, less than ideal control of the effective temperature, and other random errors may be responsible for the quoted errors, which were around ±20%. Quantification results for the other analytes are listed in Supplementary Table S1.

We carried out a similar study for stachyose (Gal-Gal-Glc-Fru). A calibration curve acquired for this carbohydrate is shown in Supplementary Figure S5a ($y = 0.980x + 4.84$, $R^2 = 0.997$). S_c in DHB-MALDI of this molecule was 50% also.

Man-5 glycan is important in antibody-dependent cytotoxicity. In its DHB-MALDI spectrum, $[A + Na]^+$ is the most prominent among the analyte-derived ions. The calibration curve in the 0.03–30 pmol range constructed by plotting $I([A + Na]^+)/I([M + Na]^+)$ as a function of I(A)/I(M) is shown in Figure 2. The curve displays direct proportionality over the covered concentration range. We obtained similar results for another glycan, FA2. The calibration curve for FA2 is shown in Supplementary Figure S5b ($y = 0.960x + 5.29$, $R^2 = 0.996$).

Quantification based on the sodiated ion ratio was also investigated for two commercially available polyether diols, PEG 1000 and PPG 1000. Good direct proportionality was observed in their calibration curves, as shown in Supplementary Figures S5c ($y = 0.971x + 4.19$, $R^2 = 0.997$) and S5d ($y = 1.01x + 4.56$, $R^2 = 0.999$).

For the analytes that we studied in this work, we could observe sodium ion adducts down to 0.03–0.3 pmol of each analyte in 25 nmol of DHB. The minimum amounts of sodium ion adducts needed for their detection, or their detection limits, are larger than that of peptides (0.003 pmol, reference [11]) based on protonated ion ratios by an order of magnitude. To use the present method with a commercial instrument, some adaptation of its hardware and software will be needed.

Conclusion

The MALDI-based method to quantify an analyte using the analyte-to-matrix protonated ion abundance ratio that we reported previously cannot be used for molecules that do not produce $[A + H]^+$, such as carbohydrates. When such molecules produce sodium ion adducts, $[A + Na]^+$, we found that the analyte-to-matrix sodiated ion abundance ratio could be used for the quantification.

Acknowledgments

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References

1. Harvey, D.J.: Matrix-assisted laser desorption/ionization mass spectrometry of carbohydrates. Mass Spectrom. Rev. **18**, 349–451 (1999)
2. Harvey, D.J.: Analysis of carbohydrates and glycoconjugates by matrix-assisted laser desorption/ionization mass spectrometry: an update for the period 2005–2006. Mass Spectrom. Rev. **30**, 1–100 (2011)
3. Cole, R.B.: Electrospray and MALDI mass spectrometry. Fundamentals, instrumentation, practicalities, and biological applications, 2nd edn. Wiley, Hoboken (2010)
4. Harvey, D.J.: Quantitative aspects of the matrix-assisted laser desorption mass spectrometry of complex oligosaccharides. Rapid Commun. Mass Spectrom. **7**, 614–619 (1993)
5. Bartsch, H., König, W.A., Straßner, M., Hintze, U.: Quantitative determination of native and methylated cyclodextrins by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. Carbohydr. Res. **286**, 41–53 (1996)
6. Grant, G.A., Frison, S.L., Yeung, J., Vasanthan, T., Sporns, P.: Comparison of MALDI-TOF mass spectrometric to enzyme colorimetric quantification of glucose from enzyme-hydrolyzed starch. J. Agric. Food Chem. **51**, 6137–6144 (2003)
7. Rankin, K., Mabury, S.A.: Matrix normalized MALDI-TOF quantification of a fluorotelomer-based acrylate polymer. Environ. Sci. Technol. **49**, 6093–6101 (2015)
8. Park, K.M., Bae, Y.J., Ahn, S.H., Kim, M.S.: A simple method for quantification of peptides and proteins by matrix-assisted laser desorption ionization mass spectrometry. Anal. Chem. **84**, 10332–10337 (2012)
9. Ahn, S.H., Park, K.M., Bae, Y.J., Kim, M.S.: Efficient methods to generate reproducible mass spectra in matrix-assisted laser desorption ionization of peptides. J. Am. Soc. Mass Spectrom. **24**, 868–876 (2013)
10. Yoon, S.H., Kim, M.S.: Development of a time-resolved method for photodissociation mechanistic study of protonated peptides: use of a voltage-floated cell in a tandem time-of-flight mass spectrometer. J. Am. Soc. Mass Spectrom. **18**, 1729–1739 (2007)
11. Ahn, S.H., Park, K.M., Moon, J.H., Lee, S.H., Kim, M.S.: Preparation of homogeneous solid samples for reproducible and quantitative MALDI. Bull. Kor. Chem. Soc. **37**, 458–462 (2016)
12. Ahn, S.H., Bae, Y.J., Moon, J.H., Kim, M.S.: Matrix suppression as a guideline for reliable quantification of peptides by matrix-assisted laser desorption ionization. Anal. Chem. **85**, 8796–8801 (2013)