

Generation of CsI Cluster Ions for Mass Calibration in Matrix-Assisted Laser Desorption/Ionization Mass Spectrometry

Xianwen Lou, Joost L. J. van Dongen, and E. W. Meijer

Laboratory of Macromolecular and Organic Chemistry, Eindhoven University of Technology, Eindhoven, The Netherlands

A simple method was developed for the generation of cesium iodide (CsI) cluster ions up to m/z over 20,000 in matrix-assisted laser desorption/ionization mass spectrometry (MALDI MS). Calibration ions in both positive and negative ion modes can readily be generated from a single MALDI spot of CsI_3 with 2-[(2E)-3-(4-*tert*-butylphenyl)-2-methylprop-2-enylidene] malononitrile (DCTB) matrix. The major cluster ion series observed in the positive ion mode is $[(\text{CsI})_n\text{Cs}]^+$, and in the negative ion mode is $[(\text{CsI})_n\text{I}]^-$. In both cluster series, ions spread evenly every 259.81 units. The easy method described here for the production of CsI cluster ions should be useful for MALDI MS calibrations. (J Am Soc Mass Spectrom 2010, 21, 1223–1226) © 2010 American Society for Mass Spectrometry

A central issue in mass spectrometry (MS) analysis is to provide molecular weight information of analytes. The degree of mass accuracy largely depends on the instrument type and mass calibration method employed. Nowadays, accurate mass measurements with mass error of only a few parts per million (ppm) can be made using a variety of instrument types [1–5]. High mass accuracy is extremely important in the MS application of molecular formula determination and can significantly improve the level of confidence for compound identification, such as protein/peptide confirmation, through database searching [6–10].

For accurate mass measurements, a MS system must be precisely calibrated. Calibration constants are usually determined by measuring several reference ions covering the mass range of interest. Different calibration methods have been developed in matrix-assisted laser desorption/ionization (MALDI) MS aiming to improve the mass accuracy [11–18]. Nevertheless, a crucial step in calibration remains to be the generation of suitable ions. In principle, any compounds that can yield ions with known masses and do not interfere with the MS analysis might be applied as calibration standards. In MALDI MS, widely used standards include peptides, proteins, and synthetic polymers/oligomers. To establish a precise calibration, a number of standards spreading over the mass range of interest should be included. Because of the analyte suppression effect (ASE) in MALDI [19, 20], some analyte/calibrant ions of interest might substantially or even completely be suppressed. As a consequence, the preparation of suitable

calibrants in MALDI, even for external calibration, is not a simple task and may involve an extensive trial and error procedure. Furthermore, different calibration standards are generally required for different modes of ionization (positive or negative ion modes) and for different mass ranges of interest. In fact, MALDI MS still suffers from the lack of truly convenient mass standard calibrants.

In this contribution, we described a convenient method of generating cesium iodide (CsI) cluster ions for MALDI MS calibration. CsI cluster ions, together with a number of other cluster ions, have frequently been used in electrospray ionization (ESI) MS calibration. Under suitable ESI conditions, cluster ions evenly covering a broad m/z range can be recorded. Calibration curves thus made have many data points and will generally provide a very precise calibration [21–24]. Compared with other types of cluster ions, the CsI cluster ions are quite unique because both Cs and I are monoisotopic. Evidently, the monoisotopic property is desirable in MS calibration where the mass accuracy of peak centroids of the calibrants might otherwise be impaired by the skewed/asymmetric peak shape due to their unresolved isotopic distributions. Another important advantage of CsI cluster ions is that they can be generated in both positive and negative ion modes. These properties of CsI cluster ions also make them very attractive to be used for MALDI MS calibrations. O'Connor and Costello reported the application of CsI clusters for the internal calibration of Fourier transform mass spectrometry (FTMS) with mass accuracy of <1 ppm [13]. However, in their report only small clusters up to Cs_4I_3 were observed. In this study, a simple method is described to generate CsI cluster ions up to

Address reprint requests to Dr. X. W. Lou, Laboratory of Macromolecular and Organic Chemistry, Eindhoven University of Technology, P.O. Box 513, 5600 MB Eindhoven, The Netherlands. E-mail: x.w.lou@tue.nl

mass over 20,000 Da in both positive and negative ion modes in MALDI MS.

Experimental

The MALDI TOF MS measurements were performed with a Voyager-DE Pro (PerSeptive Biosystems, Framingham, MA, USA) instrument equipped with a 337 nm nitrogen laser with 3 ns-duration pulse, capable of executing both linear and reflector modes. In the reflector mode, the accelerating voltage was held at 20 kV and delay time at 350 ns, while in the linear mode the accelerating voltage at 25 kV and the delay time at 400 ns. Mass spectra were acquired by summing spectra from 100 selected laser shots.

CsI₃, CsI, and N(Bu)₄I₃ (tetrabutylammonium triiodide) were purchased from Aldrich (Zwijndrecht, The Netherlands). Three matrices were used in this work. 2-[(2E)-3-(4-*tert*-butylphenyl)-2-methylprop-2-enylidene] malononitrile (DCTB) were synthesized according to Ulmer et al. [25], and α -cyano-4-hydroxycinnamic acid (CHCA) and 2,5-dihydroxybenzoic acid (DHB) were obtained from Fluka (Buchs, Switzerland). Matrix solutions were freshly prepared in tetrahydrofuran (THF) at concentrations of about 100 μ mol/mL. A matrix solution and a sample solution (also in THF) were combined in an Eppendorf tube and vortex-mixed for at least 10 s, and about 0.5 μ L of the mixed solution was pipetted onto a stainless steel MALDI target plate.

Results and Discussion

In ESI MS, CsI cluster ions can readily be generated by electrospraying a CsI solution at an appropriate concentration. When CsI was tried in MALDI TOF MS, however, no such ions were observed. Fortunately, by simply replacing CsI with CsI₃, abundant CsI cluster ions could easily be generated in MALDI MS as well.

Figure 1 shows MALDI TOF MS spectra of CsI₃ with DCTB matrix in the linear mode. CsI cluster species containing singly charged ions every 259.81 Da up to m/z over 20,000 were observed in both positive and negative ion modes. The major cluster ion series observed in the positive ion mode is [(CsI)_nCs]⁺, and in the negative ion mode [(CsI)_nI]⁻. In addition, cluster ion series of [(CsI)_nCs + NaI]⁺ and [(CsI)_nI + NaI]⁻ with much less signal intensity were also observed in the positive and negative ion modes, respectively. In Figure 1, the molar ratio of CsI₃/DCTB is 1/2, and similar results were observed in the molar ratio range of 1/1 to 1/10. It should be noted here that the CsI₃/DCTB ratios applied are quite high compared with the typical analyte/matrix values in normal MALDI TOF MS measurements. Although cluster ions could also be detected at much lower CsI₃/DCTB ratios, the molecular weight of the biggest cluster ions recorded will decrease dramatically with decreased CsI₃/DCTB ratios. The requirement of a high CsI₃/DCTB ratio is probably because the high ratio will favor the formation of large cluster ions.

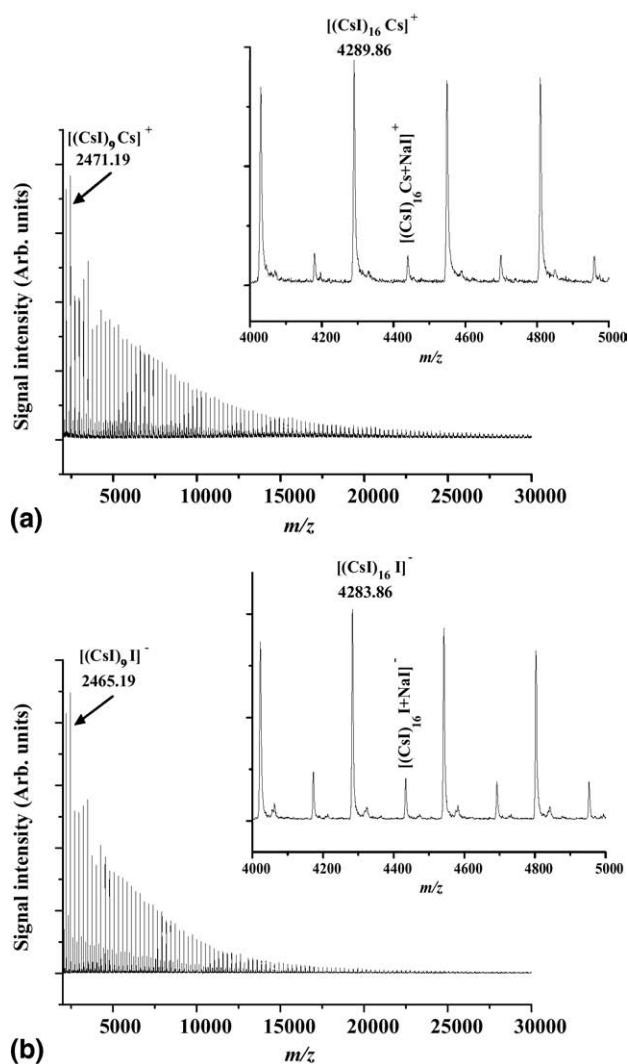


Figure 1. MALDI TOF MS spectra of CsI₃ with DCTB matrix in the linear mode. The molar ratio of CsI₃/DCTB is 1:2. (a) Linear positive ion mode; (b) linear negative ion mode.

On the other hand, it is interesting to see that even direct laser desorption/ionization (LDI) of CsI₃ without matrix could yield cluster ions up to m/z of about 8000. This value is, however, significantly lower than that observed in Figure 1. By comparing the results of LDI and MALDI, it seems that DCTB matrix at a suitable CsI₃/DCTB ratio would facilitate the formation of larger CsI cluster ions.

In addition to being observed in the linear mode, both positive and negative CsI cluster ions could also be recorded in the reflector mode. Figure 2 shows MALDI TOF MS spectra of CsI₃ with DCTB matrix in the reflector mode. Cluster ions of m/z around 3500 can clearly be observed.

Although CsI cluster ions up to m/z over 20,000 could be generated in MALDI using CsI₃ with DCTB as the matrix, it is unclear how these ions were formed. In contrast to the observation of abundant CsI cluster ions, no CsI₃ clusters were observed in the positive ion mode

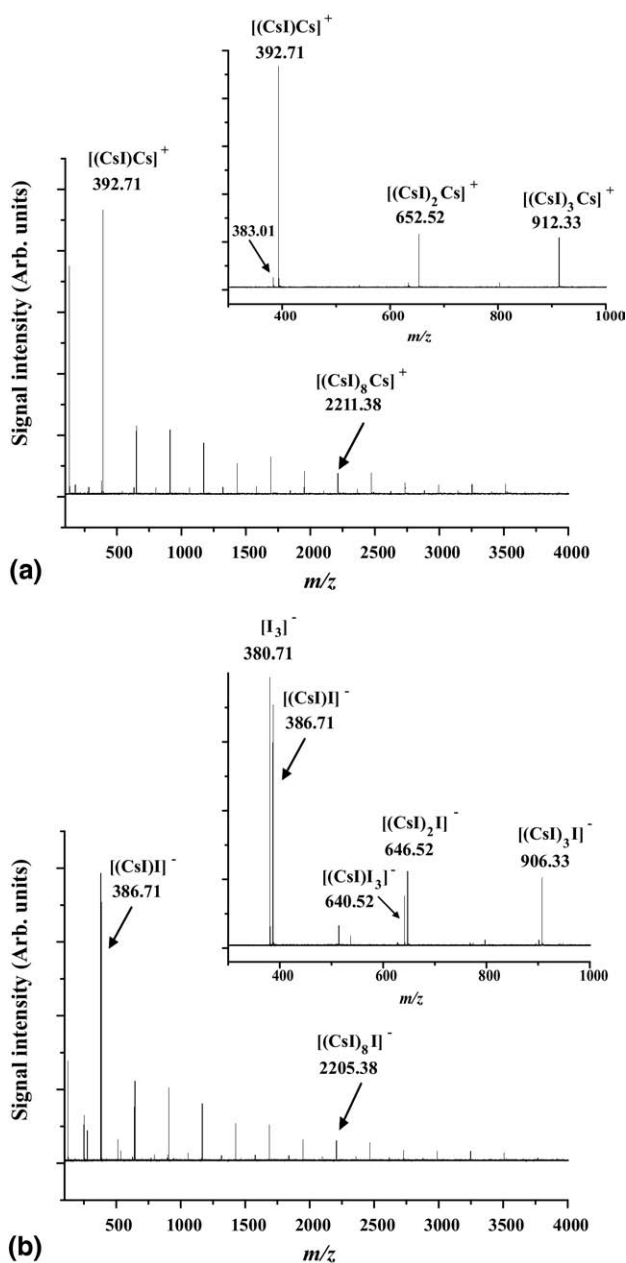


Figure 2. MALDI TOF MS spectra of CsI_3 with DCTB matrix in the reflector mode. The molar ratio of CsI_3 /DCTB is 1:2. (a) Reflector positive ion mode; (b) reflector negative ion mode.

despite of the excessive amount of CsI_3 present in the MALDI sample (see Figure 2a). Similar results were also obtained in the negative ion mode. Concerning the formation of CsI_3 clusters and/or $[I_3]^-$ adducts, only $[I_3]^-$ and a small adduct of $[(CsI)I_3]^-$ were detected with strong signal intensity, and no $[I_3]^-$ adducts with ion mass higher than $[(CsI)_2I_3]^-$ were recorded (see Figure 2b). Furthermore, when CsI was used directly instead of CsI_3 , no CsI cluster ions were detected. This implies that $[I_3]^-$ might play an important role in CsI cluster ion formation. However, it was found that not all triiodides could give analyte cluster ions because no

corresponding cluster ions were observed when another triiodide compound (tetrabutylammonium triiodide ($N(Bu)_4I_3$)) was used instead of CsI_3 .

Besides of DCTB, two other matrices, 2,5-dihydroxybenzoic acid (DHB) and α -cyano-4-hydroxycinnamic acid (CHCA) were also studied. Contrary to what was observed with DCTB matrix, very weak and only low molecular weight CsI cluster ions were observed with DHB while negligible or no such cluster ions at all with CHCA. The results clearly indicate that the formation of CsI cluster ions is strongly matrix-dependent.

Because of the lack of CsI cluster ions with some matrices, an immediate question is whether the CsI cluster ions generated with, for example, DCTB matrix can be applied for the calibration of MALDI MS measurements with other matrices. A major concern of this question is the matrix-dependent behavior of the initial velocity of MALDI ions [26, 27]. In MALDI MS calibrations, the experimental conditions and the instrument design should be calibrated with the reference ions. The influence of ion initial velocity on the accuracy of calibration relies on which type of mass analyzer is coupled to MALDI. For such MALDI MS combinations as MALDI ion-trap, MALDI Fourier transfer ion cyclotron resonance (FT-ICR) and MALDI orthogonal acceleration (oa) time-of-flight (TOF), the variation of ion initial velocity shows no influence on calibration or the influence can be corrected satisfactorily. Under these circumstances, the strong CsI cluster ions produced using DCTB matrix can be employed to calibrate a MS system or MALDI MS measurements without considering the nature of the matrix employed. However, if MALDI is coupled with axial TOF, which is still the most popular type of MALDI MS combination, the influence of ion initial velocity on the calibration accuracy must be considered. The initial velocities of MALDI ions are generally less than 1000 m/s, and mostly in the range of 300–600 m/s [26, 27]. The effects of variation/dispersion of ion initial velocity on ion flight time can largely be corrected in a delayed extraction and by a reflector (in the reflector mode) [28–31]. Therefore, in the delayed reflector mode, the calibration error introduced by using different matrices can significantly be minimized and might be tolerable in many MALDI MS applications. In our laboratory, the CsI cluster ions produced using DCTB matrix were frequently employed for the external calibration of MALDI TOF MS measurements with CHCA and DHB matrices in the delayed reflector mode, and the mass accuracies obtained were well within our instrument specification (0.01% with external calibration in the delayed reflector mode) [32]. Based on the discussions above, it can be concluded that the easy method described here for the generation of both positive and negative CsI cluster ions should be useful for the calibration of MALDI MS measurements.

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