Mass Spectrometry

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Charge-Monitoring Laser-Induced Acoustic Desorption Mass Spectrometry for Cell and Microparticle Mass Distribution Measurement**

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Herein, we report the development of charge-monitoring laser-induced acoustic desorption (CLIAD) mass spectrometry (MS) for rapid mass measurement of cells and microparticles. Different types of mononuclear cells ($CD3^+$ lymphocytes and $CD14^+$ monocytes) were clearly distinguished. Various mass distributions were obtained to distinguish normal T lymphocyte from CEM cancer cells derived from T lymphocytes. This methodology allows different types of cells to be distinguished on the basis of mass measurements. The average mass of polystyrene microparticles with a size of 29.6 μm was measured to be approximately 7×10^{15} Da, which is the largest mass reported so far with mass-spectrometric detection. Furthermore, more than 100000 charges attached to a single 29.6-μm polystyrene particle were observed.

Since the pioneering works on matrix-assisted laser desorption/ionization (MALDI) mass spectrometry (MS) by Karas and Hillenkamp[1] and Tanaka et al.[2] in 1988, the detection of large biomolecules (>10000 Da) by mass spectrometry has become a routine exercise. Recently, viruses were also investigated by mass spectrometry.[3–6] For MALDI measurements, bias can occur as a result of the interaction of matrix compound with cells or viruses during sample preparation. Furthermore, the desorbed matrix particles can interfere with the measurement of analyte microparticles. Thus, it is highly desirable to measure masses of cells and viruses without the need of a matrix.

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Recently, laser-induced acoustic desorption (LIAD)[7–10] was found to allow soft desorption of intact bioparticles, including viruses, bacteria, and whole mammalian cells.[11] In general, these desorbed bioparticles often carry a large number of charges.[11] A quadrupole ion trap is frequently used for light-scattering measurements to determine the mass-to-charge ratio (m/z) for these desorbed bioparticles.[11,12] To determine masses of these bioparticles, the number of charges of the desorbed microparticles needs to be changed by electron bombardment to observe the changes of the light-scattering pattern.[11] This process is quite time-consuming. On average, it takes about 15–30 minutes to determine the mass of one trapped microparticle. As mass distributions of most bioparticles are broad and many microparticles have to be measured to obtain a mass distribution, it becomes impractical to perform routine mass distribution measurements of microparticles. In this work, CLIAD-MS was developed for the rapid measurement of mass distributions of cells and organic microparticles. The number of charges on a cell or a polystyrene microparticle was directly measured by a charge-detection plate without any charge amplification. Time-consuming measurement of microparticle trajectory patterns by light scattering is no longer needed. CLIAD-MS can simultaneously determine both the mass-to-charge ratio (m/z) and total charges (z) on each single cell/microparticle in an ion trap. With CLIAD-MS, the speed of mass measurement for cells can be increased by orders of magnitude relative to light-scattering measurements.[11,12]

In our previous experiments,[11] we found that a single microparticle can have charge numbers in the range of 10–2000 under MALDI or LIAD processes. In this work, samples of cells or microparticles were loaded onto a silicon plate for desorption by LIAD. However, accurate determination of mass by direct measurement of the number of charges on a cell or microparticle is a challenge because of the low number of charges relative to electronic noise. We developed a corona-discharge method to increase the number of charges on a microparticle by more than one order of magnitude so that better signal-to-noise ratios were obtained for an accurate mass determination. Rapid measurement of mass distribution of cells and polystyrene microparticles was demonstrated by using a home-made quadrupole-ion-trap mass spectrometer (QIT-MS) for the determination of m/z and a charge detector for the measurement of z. With CLIAD-MS, we acquired mass spectra over the range of m/z values from 10^9 to 10^{12} using QIT-MS, and signal-to-noise ratios better than 50 for charge measurement were obtained.

CLIAD-MS involves a combination of the following techniques: 1) laser-induced acoustic desorption of microparticles without a matrix,[11] 2) a low-frequency quadrupole ion trap for ultralarge m/z measurement,[13] 3) a pressure-controlled corona discharge to enhance the number of charges on a cell or microparticle, and 4) a compact and low-noise charge detector for total-charge measurement. The experimental setup is shown in Figure 1. Samples of cells or microparticles were loaded directly onto a silicon wafer (thickness 0.5 mm) without a matrix. A frequency-doubled Nd:YAG laser beam ($\lambda = 532$ nm, Laser Technik, Berlin, Germany) with a pulse duration of approximately 6 ns was then shined directly onto the backside of the sample plate to desorb cells or microparticles by LIAD with a power density of around 10^8 W cm^{-2}. The desorbed cells or microparticles were subsequently trapped in the ion-trap device. Each end cap was drilled with a hole. One was for the collection of scattered laser light and the other was for trapped cells or microparticles to exit the trap and subsequently to be detected by the charge-detection plate. A He–Ne laser ($\lambda =$ 632 nm) was used to illuminate the trapped cells or microparticles, and a charge coupled device (CCD) was installed to monitor particles in the ion trap. The microparticles from the laser desorption were trapped with an ac field (ca. 350 Hz, depending on the particle size) in a helium buffer gas (ca. 100 mTorr). A typical image of cells in the ion trap is shown in the inset of Figure 1. Owing to the small light collection angle of the CCD camera, the image size of each particle does not necessarily reflect the true size of each individual cell or microparticle but rather the extent of its stable trajectory. Some cells or microparticles inside the trap might not be observed by the CCD camera because of the small solid angle for light collection.

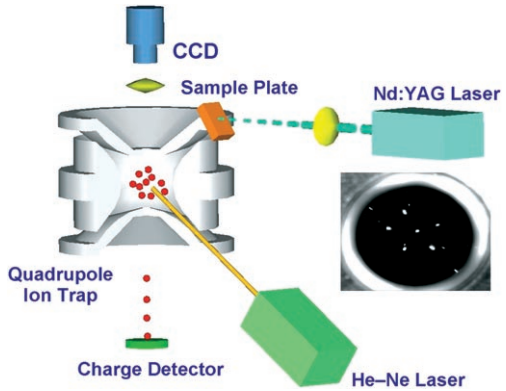

Figure 1. Experimental setup of CLIAD-MS, including a quadrupole ion trap, a pulsed Nd:YAG laser, a He–Ne laser, a charge detector, and a CCD camera. The Nd:YAG laser induces cell desorption. The He–Ne laser illuminates the trapped cells so that they can be detected by the CCD camera. The inset shows a CCD image of the trapped cells.

Ion collectors have been used for detection with a mass spectrometer of large DNA fragments[14,15] and a whole viruses.[16] However, there are always high electronic backgrounds associated with direct charge measurement. We applied a mild corona discharge in the ion trap to reduce the effect of this background and to enhance the number of charges on a microparticle. The pressure often needed to be fine-tuned to give a mild discharge. When a mild corona discharge occurred, blue and white plasma was observed between the ion trap and the desorption plate. An oscillation of plasma as driven by the audio frequency was clearly observed on an oscilloscope. With this mild corona discharge, the number of charges attached to a microparticle or a cell was increased by one to two orders of magnitude depending on the particle size, particle material, and experimental conditions. Both positively and negatively attached microparticles were observed. Mass-to-charge ratios were measured by scanning the frequency to eject charged particles with unstable trajectories.

The ion trap was operated under an axial mass-selective instability mode by scanning the trap driving frequency in the range 350–20 Hz. A voltage of 1520 V was initially applied with a high-voltage transformer driven by an audio-frequency power amplifier and a functional generator. By simply scanning the audio frequency with the functional generator, cells or microparticles could be ejected along an axial direction and the number of charges on each cell or microparticle could be subsequently detected with a charge-detection plate. The mass of each particle was determined according to the measurements of m/z and z for the particle. Methods of mass spectrometry employing charge detection have been demonstrated.[17–19] In this work, the charge detector comprised a conducting plate and a charge-integrator circuit. The charge integrator used a low-noise JFET transistor as the input stage and an operational amplifier (AD8674 Analog Device, USA) and some basic low-pass filtering.[15] The gain of the charge integrators was calibrated by applying a known voltage pulse across a known capacitance to simulate the incoming pulse shape. The charge-to-pulse-height conversion constant of the charge integrator was calibrated to be 52 e mV^{-1}. The rms output voltage noise was measured to be slightly lower than 10 mV, which corresponds to an equivalent noise of about 500 electrons. With a mild corona discharge to increase charge attachment to cells or microparticles, the charge number on each microparticle can be higher than 50000; in such a case, a signal-to-noise ratio higher than 100 can be obtained by LIAD. To determine the mass distribution of cancer cells, we used CLIAD-MS to measure a leukemic cell line, CEM. CEM cells were washed with Dulbecco's phosphate-buffered saline[20,21] (PBS, Gibco BRL) and fixed with 4% paraformaldehyde in PBS for 15 minutes at room temperature. Thereafter, the cells were washed three times in distilled deionized water and were subsequently counted and resuspended before they were placed into the mass spectrometer for analysis.

To check the feasibility of the charge-monitoring mass spectrometer, we first applied this charge detector to measure small molecules such as fullerene (C_{60}). The driving frequency was 200 kHz, and approximately 20 mTorr helium buffer gas was applied. A wide-band power amplifier was used to boost the radio-frequency amplitude to a constant voltage of 150 V, and the ion trap was floated to a dc bias of 2000 V. Since a small molecular ion produced by laser desorption usually has a single charge, the number of ions detected should reflect the

true number of ions produced so that quantitative measurement can be achieved. Our results (Figure 2a) indicate that small ions such as C_{60} ions can be detected by a charge

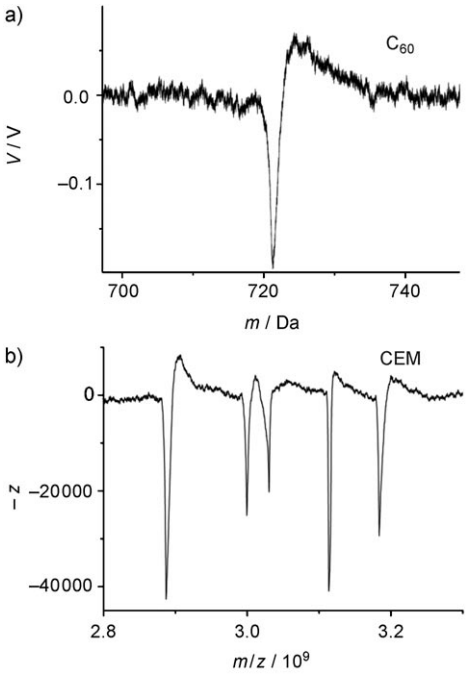

Figure 2. a) Single-scan mass spectrum of C_{60}. The peak height indicates that there were approximately 15000 singly charged C_{60} ions produced during the laser ablation process. The scan time was 1 s. b) Single-scan mass spectrum of CEM cancer cells. Each peak indicates a cell particle and the peak height indicates the number of charges on the particle. The mass of each cell was calculated from simultaneous measurement m/z and z. The scan time was 5 s.

detector with a good mass resolution ($m/\Delta m \approx 500$). Since a Faraday plate used for charge detection has no amplification due to secondary electron emission, a charge-monitoring mass spectrometer can be used to obtain mass spectra without detection efficiency bias. It can be particularly useful for quantitative analysis of organic polymer and biopolymer samples. A typical mass spectrum of CEM cells by CLIAD-MS is shown in Figure 2b. Five peaks are observed. Each peak has a specific m/z value determined by the ejection frequency. The number of charges on each cell was derived from the amplitude at the charge-detection plate. There were about 10 cells on average trapped in each laser pulse. If the scanning rate is fixed at 5 s to cover the entire frequency range, the speed of measurement is estimated to be around 7200 microparticles h^{-1}, which is an improvement of three orders of magnitude over light scattering measurement (2–4 microparticles h^{-1}.[11,12] We noticed that occasionally there were doublets trapped. Since the number of charges on a doublet is about twice that of a single cell, the m/z value should be about the same as that of a single microparticle. Nevertheless, the amplitude corresponding to total charges should be about double. The mass obtained can be determined as a doublet. A conventional mass spectrometer cannot distinguish between M_2^{2+} and M^+, because no charge information can be obtained and m/z is identical for both types of ion.

Mass and charge distributions for polystyrene with a size of 3, 7.2, 10.1, and 29.6 μm are shown in Figure 3.[22] The average masses were measured to be 9.9×10^{12}, 1.3×10^{14}, 3.5×10^{14}, and 7.1×10^{15} Da, respectively, which are in good agreement with the calculated masses of 8.8×10^{12}, 1.2×10^{14}, 3.4×10^{14}, and 8.6×10^{15} Da, respectively. The fwhm values (full width at half maximum) for the masses (Δm) of these particles were measured to be 9.1×10^{11}, 2.3×10^{13}, 6.2×10^{13}, and 1.5×10^{15} Da, respectively. A peak for a polystyrene dimer (2.7×10^{14} Da) was also observed and the population ratio of dimer to monomer is estimated to be around 11% (Figure 3b). Distributions of the number of charges for different sizes of polystyrene are also shown in Figure 3. The number of charges increases as the size increases but is not proportional to the surface area of a microparticle. As many as 250000 charges were observed on a single 29.6-μm polystyrene particle. More studies are needed to resolve the mechanism

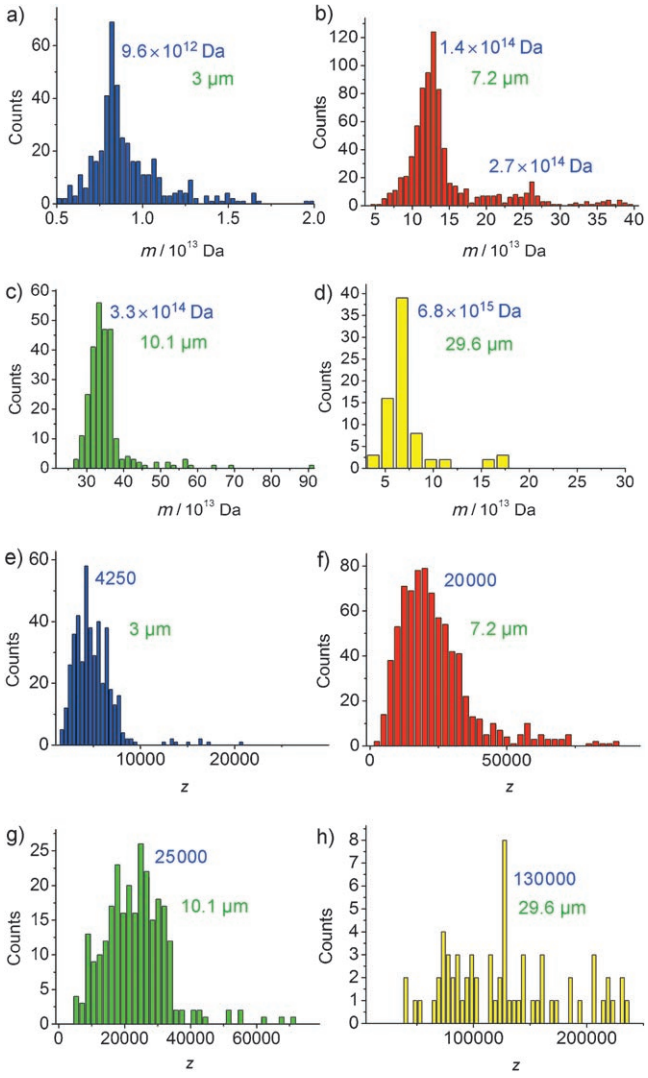

Figure 3. Mass and charge distributions for variously sized polystyrene microparticles. Each count represents a single detected microparticle. Fewer counts were obtained for the 29.6-μm microparticles (d,h) because it is more difficult to trap large particles owing to gravity.

of charge attachment to cells and microparticles under mild corona discharge conditions.

As well as the measurement of polystyrene microparticles, we also applied this method to measure the mass distribution of various types of cells. T lymphocyte ($CD3^+$ cells) and monocyte ($CD14^+$ cells) are major components of peripheral blood mononuclear cells, which play a critical role in the immune system. CLIAD-MS was used to measure mass distributions of lymphocyte and monocyte. The peak positions were assessed to be 2×10^{13} and 4.2×10^{13} Da for lymphocyte and monocyte, respectively (Figure 4). Because of the difference in mass distribution, these two different cells can be clearly distinguished. Since there is some overlap in

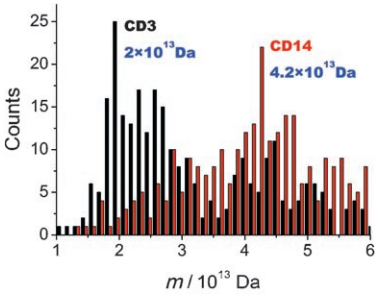

Figure 4. Mass distributions of lymphocyte ($CD3^+$ cells, black) and monocyte ($CD14^+$ cells, red).

mass distribution, it is unlikely that a cell could be identified by measuring the mass of only a few cells. However, CLIAD-MS can be used to distinguish these two types of cells. We also compared CEM leukemia cells with normal lymphocyte ($CD3^+$ cells; Figure 5a). The mass distribution peaks of lymphocyte and CEM cells were determined to be 2.2×10^{13} and 1.1×10^{14} Da, respectively. The average mass of CEM cells is clearly larger than normal lymphocyte. We again can easily distinguish CEM cells from normal lymphocyte. We also mixed an equal number of CEM cells and lymphocyte ($CD3^+$ cells) into the sample. As shown in Figure 5a, the histogram of this mixture sample is almost the same as that obtained by adding the individual spectra of CEM and lymphocyte, which indicates that CLIAD-MS can be used not only to measure a single kind of cell but also mixtures of cells. The size of CEM cells was measured with a particle-sizing device to be 9.8 ± 1.8 μm in diameter, and the average cell weight in air was approximately 3×10^{14} Da.[23] These results suggest some loss of intracellular water in the vacuum chamber of the mass spectrometer. Size distributions may not reflect true mass distributions since the density of a CEM cell can be different from that of a normal lymphocyte owing to its doubled number of chromosomes. The average number of electrons attached to a CEM cell was measured to be approximately 45000, which is about the same as that for a comparably sized polystyrene particle. As the sizes of lymphocyte, monocyte, and Jurkat were measured to be 5.8 ± 1.7, 6.9 ± 1.3, and 8.0 ± 2.2 μm, respectively, we anticipated the average mass of Jurkat to be greater than that of monocyte. Surprisingly, CLIAD-MS showed the mass peak position of Jurkat to be 4.5×10^{13} Da (Figure 5b), which is

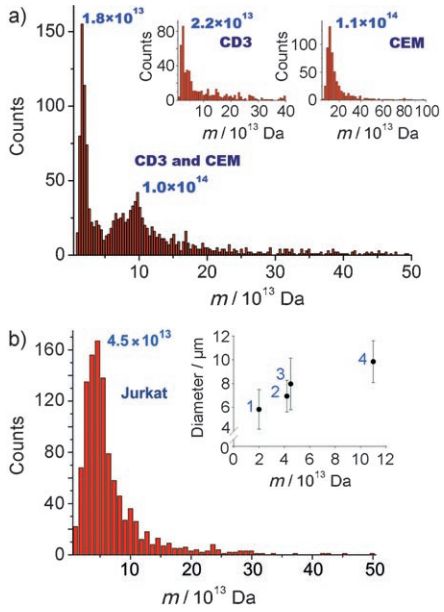

Figure 5. a) Mass distributions of lymphocyte ($CD3^+$ cells), CEM, and an equal-ratio mixture of lymphocyte and CEM. b) Mass distribution of Jurkat cells and a plot of cell size vs. mass. 1: CEM cells, 2: monocyte ($CD14^+$ cells), 3: Jurkat cells, 4: lymphocyte ($CD3^+$ cells).

only 8% heavier than the mass of monocyte (4.2×10^{13} Da), even though Jurkat is 16% larger. The inset in Figure 5b shows the relationship between the size and the mass of the cells. Although there is a general correlation between size and mass, the curve is not exactly linear. Thus, CLIAD-MS is useful for measuring the mass distribution of various types of cells.

In most mass spectrometers, ions are detected by a charge-amplification device such as a microchannel plate (MCP). The detection is based on the ejection of secondary electrons. The efficiency of secondary electron ejection is closely related to the velocity of incoming ions.[24] Therefore, mass spectra of mixtures of large biomolecules usually do not reflect the number of ions detected. With a charge-monitoring mass spectrometer, individual peaks in a mass spectrum should reflect their ion population without detection bias. Since a charge-detection plate has no amplification by secondary electron emission, a charge-monitoring mass spectrometer can be used to obtain mass spectra without detection efficiency bias. The major limitation of the sensitivity with a charge-detection plate is the electronic noise. Recently, Fuerstenau reported the observation an of electronic-noise level equivalent to 100 electrons.[25] With cooling of the detector electronics, the noise could be expected to be reduced by a factor of 5 to reach similar levels with their facility. With this noise level, virus and nanoparticle detection by CLIAD-MS would become feasible. As well as for cancer-cell measurements, CLIAD-MS could be readily used for aerosol measurements.[26]

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[1] M. Karas, F. Hillenkamp, *Anal. Chem.* **1988**, *60*, 2299.
[2] K. Tanaka, H. Waki, Y. Ido, S. Akita, Y. Yoshida, T. Yoshida, *Rapid Commun. Mass Spectrom.* **1988**, *2*, 151.
[3] S. D. Fuerstenau, W. H. Benner, J. J. Thomas, C. Brugidou, B. Bothner, G. Siuzdak, *Angew. Chem.* **2001**, *113*, 559; *Angew. Chem. Int. Ed.* **2001**, *40*, 542; Erratum: S. D. Fuerstenau, W. H. Benner, J. J. Thomas, C. Brugidou, B. Bothner, G. Siuzdak, *Angew. Chem.* **2001**, *113*, 1011; *Angew. Chem. Int. Ed.* **2001**, *40*, 982.
[4] Z. Nie, Y. K. Tzeng, H. C. Chang, Y. Y. Lin, C. Y. Chang, C. M. Chang, M. H. Tao, *Angew. Chem.* **2006**, *118*, 8311; *Angew. Chem. Int. Ed.* **2006**, *45*, 8131.
[5] M. A. Tito, K. Tars, K. Valegard, J. Hajdu, C. V. Robinson, *J. Am. Chem. Soc.* **2000**, *122*, 3550.
[6] S. F. Wong, C. K. Meng, J. B. Fenn, *J. Phys. Chem.* **1988**, *92*, 546.
[7] V. V. Golovlev, S. L. Allman, W. R. Garrett, N. I. Taranenko, C. H. Chen, *Int. J. Mass Spectrom. Ion Processes* **1997**, *169*/170, 69.
[8] V. V. Golovlev, S. L. Allman, W. R. Garrett, C. H. Chen, *Appl. Phys. Lett.* **1997**, *71*, 852.
[9] R. C. Shea, C. J. Petzold, J. L. Campbell, S. Li, D. J. Aserud, H. I. Kenttamaa, *Anal. Chem.* **2006**, *78*, 6133.
[10] J. L. Campbell, M. N. Fiddler, K. E. Crawford, P. P. Gqamana, H. I. Kenttamaa, *Anal. Chem.* **2005**, *77*, 4020.
[11] W.-P. Peng, Y.-C. Yang, M.-W Kang, Y.-K Tzeng, Z. Nie, H.-C. Chang, W. Chang, C.-H Chen, *Angew. Chem.* **2006**, *118*, 1423; *Angew. Chem. Int. Ed.* **2006**, *45*, 1423.
[12] W.-P. Peng, Y.-C. Yang, C.-W Lin, H.-C. Chang, *Anal. Chem.* **2005**, *77*, 7084.
[13] Y. Cai, W.-P. Peng, H.-C. Chang, *Anal. Chem.* **2003**, *75*, 1805.
[14] J. C. Schultz, C. A. Hack, W. H. Benner, *J. Am. Soc. Mass Spectrom.* **1998**, *9*, 305.
[15] S. D. Fuerstenau, W. H. Benner, *Rapid Commun. Mass Spectrom.* **1995**, *9*, 1528.
[16] B. Bothner, G. Siuzdak, *ChemBioChem* **2004**, *5*, 258.
[17] K. M. Lue, A. J. Kelly, Inst. Phys. Conf. Series No. 85, Proceedings of Electrostatics, Oxford, **1987**.
[18] H. Shelton, C. D. Hendricks, R. F. Wuerker, *J. Appl. Phys.* **1960**, *31*, 1243.
[19] R. D. Chen, X. H. Cheng, D. W. Mitchell, S. A. Hofstadler, Q. Y. Wu, A. L. Rockwood, M. G. Sherman, R. D. Smith, *Anal. Chem.* **1995**, *67*, 1159.
[20] The CCRF-CEM cell line (Human T cell lymphoblast-like cell line) was originally derived from a T-cell acute lymphoblastic leukemia. Its chromosome number is 42–47. The line was obtained from the Bioresource Collection and Research Center (Hsinchu, Taiwan). After its inclusion into the present study, the passages were monitored. Cells were cultured at 37 °C in RPMI 1640 medium supplemented with 10% calf serum, 1% L-glutamine, and 1% standard penicillin/streptomycin (10000 IU mL^{-1}) under a 5% CO_2 atmosphere.
[21] G. E. Foley, H. Lazarus, S. Farber, B. G. Uzman, B. A. Boone, R. E. McCarthy, *Cancer* **1965**, *18*, 522.
[22] Microparticles size standards based on polystyrene; monodisperse 7.0-μm and 10.1-μm samples were purchased from Sigma–Aldrich, and 3-μm (SRM 1692) and 29.6-μm (SRM 1961) samples were purchased from the National Institute of Standards and Technology (NIST).
[23] The fixed cells were washed once with PBS and diluted with Isoton 2 (Beckman Coulter). The cell size was determined as particle diameters by using Coulter Counter with a Multisizer 3 instrument (Beckman Coulter, Fullerton, CA).
[24] P. W. Geno, R. D. MacFarlane, *Int. J. Mass Spectrom. Ion Processes* **1989**, *92*, 195.
[25] S. D. Fuerstenau, *J. Mass Spectrom. Soc. Jpn.* **2003**, *51*, 50.
[26] J. L. Jimenez, J. T. Jayne, Q. Shi, C. E. Kolb, D. R. Worsnop, I. Yourshaw, J. H. Seinfeld, R. C. Flagan, X. Zhang, K. A. Smith, J. Morris, P. Davidovits, *J. Geophys. Res. Atmos.* **2003**, *108*, 8425.

