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COMMUNICATION

Characterization of bioparticles using a miniature cylindrical ion trap mass spectrometer operated at rough vacuum

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A miniature cylindrical ion trap mass spectrometer (CIT-MS) equipped with an inexpensive mechanical pump was constructed, and used to measure the masses of cells and microparticles generated by laser induced acoustic desorption ionization. Compared with a previous lab scale quadrupole ion trap mass spectrometer (QIT-MS), the novel miniature CIT-MS had smaller volume (the radius r_0 = 5 mm), simpler ion trap fabrication and overall instrument construction, required a lower trapping voltage, and reduced the weight, power and cost of the instrument. The CIT-MS was calibrated using standard polystyrene beads of 2.982 μm diameter. The CIT-MS was used to measure the total dry weight of human red blood cells (RBCs) from a healthy female adult (2.12×10^{13} Da) and a patient with anemia (1.35×10^{13} Da). The coefficient of variance (CV) for the healthy individual was 21% and that for the patient was 30.4%. The CIT-MS was also applied to guinea pig RBCs and the total dry weight was determined as 1.34×10^{13} Da with a CV of 37.9%. These measurements are consistent with previous QIT-MS measurements. The new miniaturized instrument has potential for applications in field-portable, biological and aerosol analysis.

Introduction

The measurement of ultra-high mass (beyond 1 MDa) bioparticles including viruses, bacteria and cells is of increasing interest.[1–7] Areas of application extend from life sciences, through clinical diagnosis to polymer material analysis. Recently, cantilever transducers have been used as a platform for chemical and biological sensors in the high mass range, and can be used to measure the masses of individual viruses and cells with high precision.[8–11] However, cantilever transducers have to be immersed in liquids to pick up bioparticles, and therefore they are restricted to mass measurements of wet particles. Besides, this method is problematic due to aggregation of bioparticles and contamination of the measured samples. Mass spectrometry offers a novel approach to measure the dry mass of intact bioparticles under vacuum.

Chang and his co-worker successfully measured the dry mass of single viruses and cells with high mass accuracy using their home-made quadrupole ion trap mass spectrometer (QIT-MS) with ion detection by a light scattering method.[12–16] However, the light scattering method requires an electronic gun to alter the particle's charge number (*Ze*), and a charge state assignment program is needed to determine the mass of measured particles.[17] Furthermore, Peng *et al.*[18] adapted the laser induced acoustic desorption (LIAD) ionization method which is able to produce the ions of micron-size particles, although this method has been applied in several ways, such as Kenttämaa's series of work.[19–22] Moreover, Peng *et al.*[23,24] also coupled the LIAD ionization method with a charge detector to achieve fast mass measurement and detection of cells and microparticles using a lab scale QIT-MS. Other efforts[7] on micro-particle analysis have been performed using lab instruments which are costly, non-portable, and high powered. Here we attempt to use cylindrical ion trap (CIT) as a mass analyzer to achieve miniaturization of a QIT-MS mass spectrometer. The advantages of a miniature CIT include low trapping voltage (RF amplitude),[25] low power consumption, and easy mechanical fabrication. Significantly, a CIT-MS can operate under modest vacuum using low applied radio frequency (RF) voltages.

Miniaturization of ion trap mass spectrometers has been implemented in several types of devices, and included miniaturization of toroidal ion traps,[26,27] planar ion traps,[28,29] rectilinear ion traps,[30,31] and CITs.[32] Among these four mass analyzers, CIT is the easiest one for miniaturization because it possesses the simplest geometrical configuration, making it easy to fabricate, miniaturize and multiplex. Therefore, the CIT may be used as the basis for a field-portable analytical instrument. Moreover, CITs can provide unit mass resolution (at low mass) and adequate sensitivity,[32–34] can be constructed easily into arrays of micrometre scale ion traps for high-throughput mass spectrometry,[35–37] and in small size configurations can be operated under rough vacuum conditions using low applied RF voltages.

The *m*/*Ze* range of CIT analyses is usually no more than 2000, but Nie *et al.* measured single viral particles of 100 MDa with tens of charges by a light scattering method using a CIT analyzer under high

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vacuum.[12] The mass analyzer internal radius was 5 mm, and the experiments operated at low q values ($q \leq 0.4$) where the pseudo-potential model can accurately describe the zero-order secular frequency.[38–40]

$$\omega = \frac{V}{\sqrt{2}\left(\frac{m}{Ze}\right)r_0^2\Omega} \quad (1)$$

where, ω is the secular frequency, V is the zero-to-peak voltage of the applied RF with angular frequency Ω, m is the mass of the ion, Z represents the charge number, e is the elementary charge, r_0 is the radius of the trap.

In order to expand the applications of mass spectrometry in the field of *in situ* analysis, we constructed a CIT-based miniature mass spectrometer without a turbo pump. This miniature CIT-MS was applied in rapid measurements of microparticles and cell masses. The masses of individual microparticles were derived from the m/Ze, which was measured by the CIT-MS in the mass-selective instability RF-scan mode, and the Ze value obtained from the charge detector. Standard polystyrene microspheres (diameter 2.982 μm) were used for mass calibration and red blood cells (RBCs) of human (including a healthy female adult and a patient with anemia) and a guinea pig were measured. Our results show that CIT-MS provides fast analysis, appropriate mass accuracy, easy fabrication and compactness of the experimental setup. Therefore, the CIT-MS shows potential for use as a field-portable mass spectrometer, especially in the ultra-high mass range.

Experimental section

Materials

Polystyrene size standard (2.982 μm diameter) was purchased from the US National Institute of Standards and Technology (NIST). The polystyrene sample was washed with distilled water and diluted to about 1×10^6 particles per mL before use. The guinea pig and human's red blood cells (both healthy and patient) were obtained from the Key Laboratory of Analytical Chemistry for Living Biosystems, Chinese Academy of Science. Prior to mass measurement, the cells were rinsed with phosphate buffer saline (PBS) solution to remove the serum proteins. The cells were recovered by centrifugation and then fixed in 0.25% glutaraldehyde in PBS (RBCs: 0.25% glutaraldehyde = 1 : 10, v/v) for 1 h. The fixed RBCs were washed in distilled water and the washings removed by centrifugation. Finally, the fixed RBCs were re-suspended in distilled water to give a concentration of about 1×10^6 cells per mL.

Experimental setup

Fig. 1 shows the homemade miniature CIT-MS instrument equipped with a LIAD ion source and charge detector. The CIT mass analyzer ($r_0 = z_0 = 5$ mm) was machined as described in previous reports.[12] A pulsed Nd:YAG laser (Dawa200, Beamtech Co. Ltd.) operating at its second harmonic (532 nm, pulse width 7 ns, energy 30 mJ per pulse) was focused with a diameter of about 300 μm on the back of the silicon plate (0.5 mm thick) to produce ions using LIAD ionization.[18–22] The pulse power density was estimated to be greater than 6×10^9 w cm^{-2}. Ions were introduced to the CIT through the gap (2 mm) between the cylindrical ring electrode and the flat end-cap electrode.

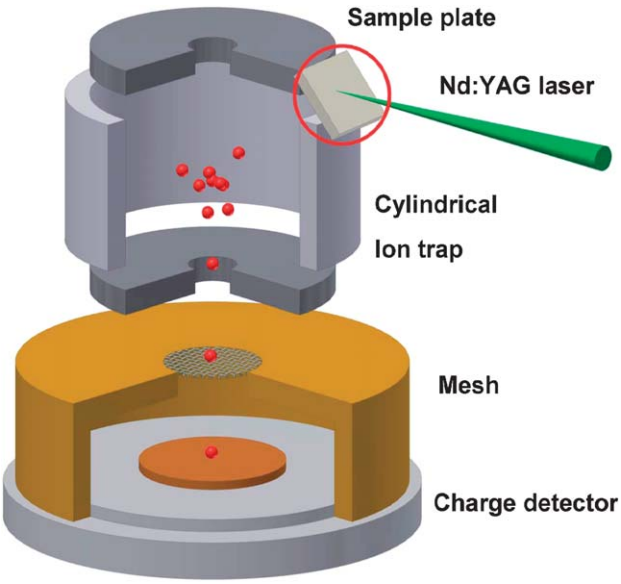

Fig. 1 Schematic illustration of the experimental setup. The CIT-MS consists of a LIAD ion source, a CIT mass analyzer, a charge-sensitive detector, and a mechanical pump.

Particle trapping conditions

The RF trapping voltage was provided by a synthesized function generator (DS345, Stanford Research) and amplified by a power amplifier (Model 5/80, TREK), and then connected to the ring electrode. For the mass selective instability scan, the RF was scanned linearly from 900–300 Hz for 5 seconds at a constant amplitude (1000 V, zero-to-peak). The particles were ejected through a 1 mm diameter hole into the end-cap which was grounded during the RF scan. A home-made charge detector was employed to detect the ejected ions while the m/Ze value was obtained from the RF voltage and frequency at the time of ejection. To eliminate the influence of the RF voltage on the charge detector, a shielding plate with a mesh (85% transmission) was placed between the end-cap and charge detector about 10 mm from the exit of the end-cap.

CIT-MS operating pressure

Appropriate vacuum conditions are critical for the trapping of ions. To reduce both the weight and volume of the CIT-MS instrument, compact pumps must be used. Ouyang *et al.* characterized ion trap mass spectrometers operated at a relatively high pressure (50 mTorr).[41,42] The CIT employed in this study was only half the size of that previously used in ultra-high mass QIT-MS (internal radius 10 mm), and this reduced the trapping voltage (RF amplitude) by four-fold. Therefore, high vacuum pumping was no longer critical for the trapping of microparticles, and our setup could operate under rough vacuum (about 50 mTorr). The fact that the turbo molecular pump was no longer needed in this experiment simplified the whole vacuum system and thus reduced the weight of the device and made miniaturization possible.

CIT-MS operation in instability mode with frequency-scan

Cooks *et al.* have shown that the mass spectrometer using CIT as the mass analyzer could work in the mass-selectively instability scan

mode, but there was a mass shift due to the non-ideal electrode geometry which introduced extra multipole fields.[32,36,37] There are still other factors which also impact the mass determination, such as the effective Mathieu q value under the vacuum conditions and a larger error in the determination of Ze.[43] Hence, in order to calibrate the experiment setup, we introduced a correction factor, η, in the normal mass analysis equation of QIT-MS:

$$\frac{m}{Ze} = \eta \frac{8V}{q_{\text{eject}}\Omega^2(r_0^2 + 2z_0^2)} \quad (2)$$

where, q_{eject} denotes the ejection point of Mathieu parameter (0.908 was adopted here for the ideal case), z_0 is the distance from the center to the end-cap, and V, r_0, m, Z, Ω have been mentioned in eqn (1). The value of η is constant over the whole mass range as it is dependent only on the geometry of the electrodes. To determine the value of η, the CIT-MS was calibrated with the NIST polystyrene size standard (2.982 μm diameter, 8.81×10^{12} Da mass). Peng *et al.*[17] and Trevitt *et al.*[44] also proposed using the NIST polystyrene size standard as a mass standard for particle MS.

Results and discussion

We detected directly the number of charges carried by the ejected charged micro-particles using a charge detector as the charged micro-particles were ejected out from the ion trap. Both the mass-to-charge ratio (m/Ze) and the charge number (Ze) were obtained and thus the mass of the ions could be calculated. Fig. 2 shows a typical mass spectrum obtained from the CIT-MS. The horizontal axis represents m/Ze value, which is directly obtained from the ejected RF frequency when the system is operated in the RF-frequency scanning mode (from 900–300 Hz). The vertical axis is the charge numbers of the ejected ions, which is proportional to the induced electric current signal intensity (in voltage) measured by the charge detector when the ejected ions hit the Faraday disc. This representation is different from the conventional mass spectrum, which shows the intensity of the ion signal (or the abundance of ions) on the y-axis. Here, the transformation ratio between z and voltage (mV) was determined as about 61 charges per mV. As shown in Fig. 2, the background noise of the charge detector was about 10 mV (about 600 charges). This background noise is similar to that observed in a lab scale QIT-MS. Typically, a mass spectrum consists of 3–8 peaks (here, it's just 5 peaks in Fig.2) and each peak represents a single ejected ion. In general, more than two hundred particles are required to obtain a mass histogram distribution. The peaks will be used as the signal-to-noise ratios were bigger than 6.

First, we measured the NIST standard polystyrene microparticles and recorded their mass distribution. Using Gauss-plot fitting (blue curve in Fig. 3), the observed mean mass was 7.606×10^{12} Da. The standard deviation (SD) of the mass distribution was known as 1.4×10^{12} Da and the coefficient of variance (CV) was 18%. However, the known mean mass and CV of NIST standard polystyrene microparticles are 8.8×10^{12} Da and 1.6%, respectively. As discussed above, this mass difference was corrected by the correction factor, η, which was determined as 1.16 in this experiment. Fig. 3 shows the mass distribution of the polystyrene microparticles after the mass correction. The CV value is a little larger than that obtained in earlier QIT-MS (16% therein),[43] which may be due to the small volume of CIT and increased Coulomb effects. These results obtained by CIT-MS show no distinct difference in mass resolution in high mass range from those of QIT-MS, which means that CIT-MS can be also used as a mass analyzer and can be used in ultra-high mass measurement. Table 1 is the detailed comparisons of CIT-MS and QIT-MS.

In an application of the methodology, we detected the red blood cells (RBCs) of human (including a healthy female adult and a patient with anemia) and a guinea pig. The human' RBCs was obtained from hospital. The mass histogram of the healthy human' RBCs is shown in Fig. 4a. The mass distribution exhibits a fairly symmetry profile with respect to its mean mass. The measured mean mass was 2.12×10^{13} Da, which is equivalent to a mean corpuscular weight (MCW) of 35.2 pg, and the SD was 0.44×10^{13} Da (CV 21%). These results are similar to those of a previous study where a MCW of 34.8 pg (SD = 0.46×10^{13} Da) was obtained by QIT-MS for a healthy male adult, and the mean corpuscular hemoglobin (MCH) value of 31.0 pg was determined using the automated hematology analyzer.[43]

Fig. 4b shows the mass histogram of RBCs of the patient with anemia. The measured mean mass is 1.35×10^{13} Da, corresponding

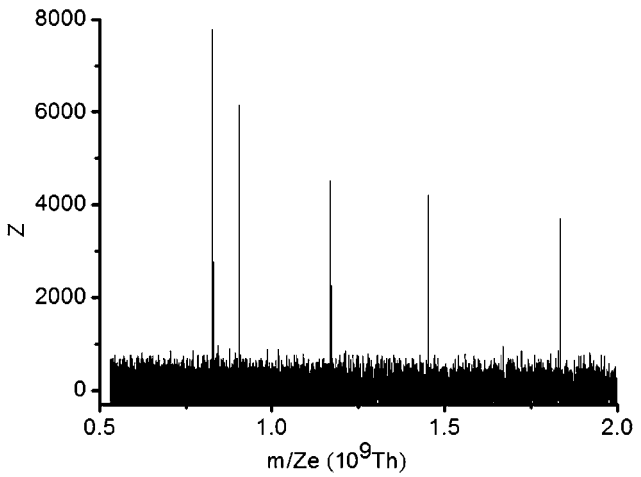

Fig. 2 Typical mass-to-charge *vs.* charge spectrum of polystyrene microparticles. The peak intensity indicates the measured charge number of the microparticles.

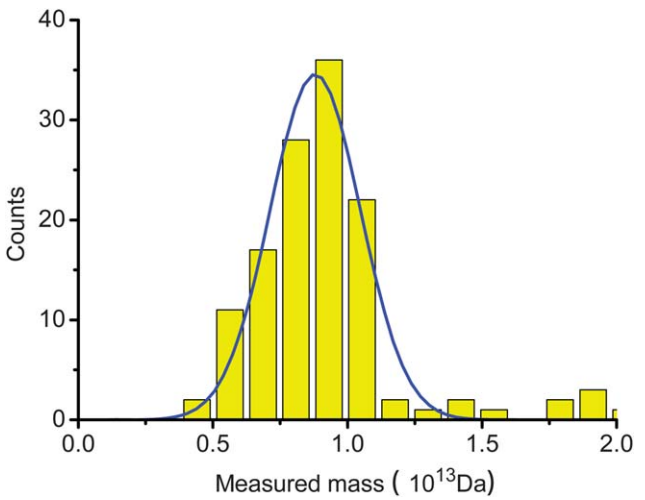

Fig. 3 Mass histogram of polystyrene size standard (diameter 2.982 μm). The blue line is a Gauss plot.

Table 1 Experimental comparisons of CIT-MS and QIT-MS using NIST 3 μm polystyrene mass standard

	Radius of ring electrode (r_0)	RF voltage ($V_{0\text{-}p}$)	Frequency scan range/Hz	Averaged peaks per scanning	CV	Vacuum
CIT-MS[a]	5 mm	1000	900–300	5	18%	Without turbo pump
QIT-MS[b]	10 mm	1500	450–150	8	16%	With turbo pump

[a] This work. [b] Our pervious work in ref. 43.

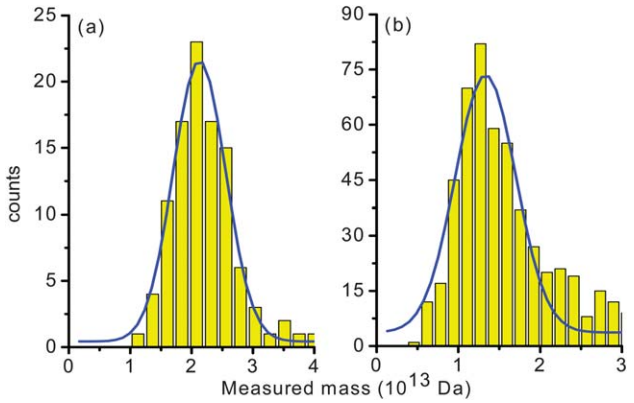

Fig. 4 (a) Mass distribution of healthy human RBCs. The blue line is the Gaussian fit. (b) Mass distribution of RBCs from a patient with anemia. The blue line is the Gauss plot.

to 22.4 pg, which is obviously smaller than the value of healthy human's RBCs. Compared with earlier measurements on anemic patients, including those with iron-deficiency anemia (28.8 pg) and thalassemia (20.6 pg),[43] this result is closer to that of thalassemia than iron-deficiency anemia. Although we cannot deduce from this fact that the anemic patient in this study has thalassemia, the possibility is high. The CV value for the anemic patient (30.4%) was much larger than that of the healthy individual (21%), which maybe exhibits the degree of pathological changes.

CIT-MS was also applied to RBCs from a guinea pig (Fig. 5). The mean mass was 1.34×10^{13} Da, which corresponds to a MCW of 22.2 pg, and the SD value in this measurement was 5.08×10^{12} Da (CV value 37.9%). This MCW valve was very similar to that of the anemic

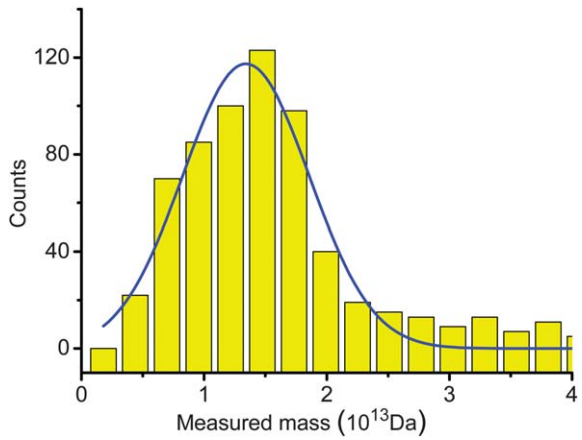

Fig. 5 Mass distribution of guinea pig RBCs. The blue line is the fitted Gaussian curve.

patient. We previously measured the MCH value of guinea pig RBCs at about 18.0 pg using QIT-MS,[43] and in comparison the MCW value measured by CIT-MS is 19% smaller. The 19% difference would probably be attributed to membrane, membrane-associated proteins, enzymes, other intracellular components attached with RBCs. Besides, the MCH/MCW ratio for our current and earlier results is 0.81, which is very close to that obtained in our previous measurements of animal RBCs.[43]

The speed of the mass analysis is also a critical factor for the development of the miniaturization of mass spectrometer, especially for the field-portable mass spectrometer. Peng *et al.*[23,24] demonstrated that rapid analysis of microparticles by QIT-MS is possible. In fact, Nie *et al.*[43] accomplished the mass measurement of several types of RBCs, including humans and animals, and expended merely 30 min for each sample. Here, using the new combination technique of CIT with a charge detector, we can rapid analyze micro-particles, with each mass histogram comprising more than 200 individual samplings. It can be estimated that the analysis time of the current CIT-MS is up to 20 min for each sample. By optimizing the data acquisition program, the analysis speed of CIT-MS can be shorted further.

Conclusion

We constructed and characterized a miniature CIT-MS for measurements in the ultra high mass region. The CIT-MS was calibrated with polystyrene microparticles and the mass spectra of human and guinea pig RBCs were measured. The results were consistent with those from a laboratory QIT-MS instrument. Therefore, the miniaturized CIT-MS is a good alternative to lab scale ion trap systems because it is portable, reduces the size of the instrument and enables easy fabrication. In the future, we can use ambient mass spectrometry techniques to analyze samples without pretreatment in CIT-MS.

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