Cover Feature:
Quantitative Analysis of Gold Nanoparticles in Single Cells with Time-resolved ICP-MS
Jinhui Liu, Lingna Zheng, Junwen Shi, Xing Wei, Xue Li, Mingli Chen,
Meng Wang, Jianhua Wang, and Weiyue Feng
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# Front Cover Article

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Quantitative Analysis of Gold Nanoparticles in Single Cells with Time-resolved ICP-MS

Jinhui Liu, Lingna Zheng, Junwen Shi, Xing Wei, Xue Li, Mingli Chen, Meng Wang, Jianhua Wang, and Weiyue Feng

ABSTRACT: Single-cell inductively coupled plasma mass spectrometry (SC-ICP-MS) has been introduced for the analysis of intracellular essential elements and nanoparticles (NPs) at the single cell level. However, it is still quite challenging for accurate and reliable determination. In this work, a high-efficiency sample introduction system was used for single cell analysis with ICP-MS. The system includes a microconcentric nebulizer, a low-volume single pass spray chamber, and a syringe pump. The transport efficiency of single cells was greatly improved to ~12%. In addition, \(^{197}\text{Au}\) signals in individual HepG2 cells, after incubation with gold nanoparticles (AuNPs) at the concentrations of 0.1, 0.5, and 1 \(\mu\)M for 12 h, were analyzed by time-resolved ICP-MS with dwell times of 100 \(\mu\)s and 5 ms, respectively. The \(^{197}\text{Au}\) signal-to-background ratio (S/B) at 100 \(\mu\)s dwell time was much higher than at 5 ms. For quantitative analysis, AuNP standard reference materials were used for calibration. The SC-ICP-MS data using NP calibration were in good agreement with those using solution ICP-MS analysis, validating the developed SC-ICP-MS method.

INTRODUCTION

Cells are the basic structural and functional units of living organisms. Traditional biomedical research is mainly based on cell population, which masks the differences between individual cells. Rapid advances in analytical techniques enable scientists to conduct biomedical research at a single cell level.\(^{1}\)\(^{2}\) Single cell analysis cannot only obtain the heterogeneity and diversity of the individual cell, but also offers profound insight into the various biological processes of the living organisms, which has become of great interest to the scientific community.

Nanoparticles (NPs) show unique characteristics and are significantly different from their large-size counterparts.\(^{3}\)\(^{4}\) After exposure to the living organism, NPs will be internalized into the cells, which is similar in process to the large macromolecular substances and viruses.\(^{5}\) NPs can promote cellular proliferation or differentiation\(^{6}\)\(^{7}\) and induce cytotoxicity, oxidative stress, and even apoptosis.\(^{8}\)\(^{9}\) For estimating cellular uptake of NPs, conventional methods are based on the analysis of the cell populations, which can only provide average information of the large numbers of cells and ignore the differences of individual cells.\(^{10}\) However, single cells behave differently after environmental stimuli, such as exposure to NPs, even the cells with the same genotype may show great heterogeneity.\(^{11}\)\(^{12}\) Therefore, the study of the uptake of NPs by single cells is crucial, and reliable methods for single cell analysis are urgently needed.

Several analytical techniques have been established for the analysis of elements in biological samples: for example, atomic absorption spectrometry (AAS),\(^{13}\) atomic fluorescence spectrometry (AFS),\(^{14}\) and inductively coupled plasma optical emission spectroscopy (ICP-OES).\(^{15}\) The above methods, however, are still difficult for the analysis of NPs in single cells mainly due to their high detection limits. Inductively coupled
plasma mass spectrometry (ICP-MS), using a time-resolved mode, has already been developed for the analysis of intracellular elements, named single cell (SC)-ICP-MS. \textsuperscript{16,17} Li et al. studied the signals of \textsuperscript{238}U in single Bacillus subtilis cells by time-resolved ICP-MS and found that the individual cells behave in an ICP more like solid particles rather than wet droplets. \textsuperscript{18} Ho et al. determined the Mg content in individual algae cells by time-resolved ICP-MS. \textsuperscript{19} Zheng et al. determined quantum dots, Gd\textsubscript{2}O\textsubscript{3}(OH)\textsubscript{22}, and cisplatin with time-resolved ICP-MS and studied their uptake behavior at a single cell level. \textsuperscript{20,21}

Traditional sample introduction systems are not suitable for SC-ICP-MS because of the low detection efficiency of single cells (typically < 1\%). Many advances in instrument development have been made to increase the efficiency. Single cell pre-treatment devices of the microdroplet generation were coupled with time-resolved ICP-MS for the measurement of cellular uptake of NPs, \textsuperscript{22,23} which improved throughput and precision of SC-ICP-MS analysis. Wei et al. developed high-throughput droplet-free single cell sampling devices based on inertial force in spiral pipes, which removed the oil phase and therefore avoided carbon deposition. \textsuperscript{24,25} More efforts need to be made to further improve the transport efficiency of single cells.

Data acquisition modes have a significant effect on the results of SC-ICP-MS. The duration of transient signals from a single particle / cell was reported to be ~0.5 ms in plasma. \textsuperscript{26,27} In single cell analysis, most quadrupole ICP-MS analyses suffer from a settling time, which may miss single cell events, and cause cycle loss. In addition, many commercial quadrupole ICP-MS instruments can only operate with dwell times at the millisecond range, and thus the dwell times are reported as being longer than the duration of a single cell, reported as 1 ms, \textsuperscript{28} 4 ms, \textsuperscript{29,29} 5 ms, \textsuperscript{20,22} and 10 ms. \textsuperscript{23,30} Under these circumstances, the number of NPs introduced into the ICP-MS should be properly controlled to avoid overlapping signals from more than one NP. In addition, the signal-to-background ratios (S/B) deteriorate by using long dwell times. Data processing methods, such as iterative algorithms as proposed by Pace et al. \textsuperscript{31} are needed to distinguish cellular events from background noises, which is quite challenging, especially for the weak signals of single cells.

In this work, the uptake of AuNPs by individual HepG2 cells was determined by SC-ICP-MS. A high-efficiency sample introduction system was employed to improve the transport efficiency of the single cells. A new generation of quadrupole ICP-MS with zero settling time and 100 μs dwell time was used to increase the signal-to-background ratio (S/B). For quantitative analysis, AuNP standard materials were used as the calibration standards. The quantitative results were compared with the results by cell digestion and solution analysis with ICP-MS.

**EXPERIMENTAL**

**Chemicals.** Ultrapure water (18.2 MΩ cm, Milli-Q water purification system, Millipore Corporation, USA) was used throughout this work. The AuNP certified reference material GBW(E)120127 (diameter: 43.7 ± 1.5 nm) was purchased from the National Central Nanoscience and Technology (Beijing, P.R. China). Dubbecco's Modified Eagle Medium (DMEM) high glucose was purchased from Thermo Scientific (USA). Fetal bovine serum (FBS) and Penicillin Streptomycin were purchased from Gibco BRL Co. Ltd. (USA). Trypsin-EDTA solution was bought from the Beyotime Institute of Biotechnology (Beijing, P.R. China). ICP-MS multi-element standards, containing 10 mg L\textsuperscript{-1} Be, Ce, Fe, In, Li, Mg, Pb, U, were purchased from PerkinElmer, Inc. (USA). Nitric acid (MOS grade) was bought from Beijing Chemical Reagent Company (Beijing, P.R. China). The gold standard solution was bought from the National Institute of Metrology (Beijing, P.R. China).

**Cell culture and treatments.** A human liver cancer cell line (HepG2) was used in this work. The HepG2 cells were cultured at 37 °C in 5% CO\textsubscript{2} in high glucose (2 g L\textsuperscript{-1}) DMEM medium, which contained 10% FBS, 100 μg mL\textsuperscript{-1} streptomycin, and 100 IU mL\textsuperscript{-1} penicillin. After the AuNPs solution was sonicated, the HepG2 cells were incubated for 12 h with AuNPs at 0.1, 0.5, and 1 μmol L\textsuperscript{-1}. The cells were collected after trypsin digestion, centrifugation, and thorough washing with 0.9% NaCl solution. For single cell analysis, one part of the collected cells was immobilized with 70% (v/v) pre-cooling ethanol at 4 °C for 12 h, centrifuged, counted, and resuspended in ultrapure water with a cell density of 10\textsuperscript{5} mL\textsuperscript{-1}. The cells' morphology was examined with a bright field microscope (EVOSFL Auto, Life Technologies Corporation, USA). The remaining cells were counted with a hemocytometer, digested with nitric acid in closed vessels at 150 °C for 12 h, and analyzed with solution nebulization ICP-MS.

**Time-resolved ICP-MS measurement.** A quadrupole ICP-MS (NexION 300D, PerkinElmer, Inc., USA) was used in this work. The sample introduction system for SC-ICP-MS was composed of a HEN microconcentric nebulizer (Meinhard, USA), a low-volume, single pass spray chamber (Viktor Beijing Technology Co. LTD, P.R. China), and a SP120PZ syringe pump (World Precision Instruments, UK). The typical instrumental parameters are shown in Table 1. The single cell suspension was measured in a time-resolved mode at the dwell time of 100 μs or 5 ms. The transport efficiency of the system was calculated by the ratios of single-cell events determined by ICP-MS to single cells in suspensions introduced into the ICP-MS, which had been counted in advance with a hemocytometer. The data collection was carried out through the Syngistix Single Cell Application Module. All data were processed using Origin 8 software (OriginLab Corporation, USA) and Excel 2018 software (Microsoft, USA).
Table 1. Typical Parameters for SC-ICP–MS

<table>
<thead>
<tr>
<th>Parameter</th>
<th>PerkinElmer, NexION 300D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nebulizer</td>
<td>Microconcentric nebulizer</td>
</tr>
<tr>
<td>(Meinhard HEN)</td>
<td></td>
</tr>
<tr>
<td>Spray Chamber</td>
<td>Low-volume, single pass spray chamber (Viktor Beijing)</td>
</tr>
<tr>
<td>Carrier gas</td>
<td>Argon</td>
</tr>
<tr>
<td>Nebulizer gas flow (L min⁻¹)</td>
<td>0.62</td>
</tr>
<tr>
<td>Makeup gas flow (L min⁻¹)</td>
<td>0.55 (Total flow)</td>
</tr>
<tr>
<td>Auxiliary gas flow (L min⁻¹)</td>
<td>1.2</td>
</tr>
<tr>
<td>Plasma gas flow (L min⁻¹)</td>
<td>18</td>
</tr>
<tr>
<td>RF Power (W)</td>
<td>1600</td>
</tr>
<tr>
<td>Sample flow rate (μL min⁻¹)</td>
<td>40</td>
</tr>
<tr>
<td>Dwell time</td>
<td>100 μs, 5 ms</td>
</tr>
<tr>
<td>Data acquisition (s)</td>
<td>100</td>
</tr>
<tr>
<td>Isotope determined</td>
<td>¹⁹⁷Au</td>
</tr>
</tbody>
</table>

RESULTS AND DISCUSSION

Preparing single cell suspensions. The preparation of single cell suspensions is crucial for SC-ICP-MS analysis. In addition, the integrity of the cellular structure needs to be maintained after cell fixation.³² It can be seen from Fig. 1 that the HepG2 cells are monodisperse and the integrity is preserved after fixation. In order to reduce the interference and improve the S/B ratios, the cells are re-suspended in ultrapure water. In SC-ICP-MS, the number of cells in suspension must be carefully controlled, based on the calculation of transport efficiency, to ensure that only one cell enters the plasma during each dwell time.

Transport efficiency of the high-efficiency sample introduction system. In this work, a new high-efficiency sample introduction system is designed and produced. Fig. 2 shows the schematic diagram of the system, which mainly consists of a microconcentric nebulizer and a single pass spray chamber. The microconcentric nebulizer produce finer droplets that can transport more efficiently through the single pass spray chamber. Two make-up gas flows are introduced tangentially through the chamber walls, thus preventing single cells from sticking to the walls and improving the transport efficiency of the cells. The new sample introduction system, which is designed by us and built by Viktor Beijing Technology, is easily installed on the ICP-MS. Fig. 3 shows internal and external views of the system. The nebulizer and spray chamber in the system are easily aligned with the injector and are prevented from accidental damage.

According to previous reports, the single cell events detected by ICP-MS in each dwell time can be calculated by the Equation (1):

\[ n = \varepsilon \cdot Q_S \cdot C \cdot t_{dwell} \]  

(Eq. 1)

where \( n \) is the number of detected cells in a dwell time (s); \( \varepsilon \) is the transport efficiency (TE) of single cells; \( Q_S \) is uptake rate of single cell suspensions (mL s⁻¹); \( C \) is the number concentration of single cells (mL⁻¹); \( t_{dwell} \) is the dwell time (s).

In SC-ICP-MS analysis, \( n \) should be less than 1. The key parameter, transport efficiency (\( \varepsilon \)), which is dependent on the sample introduction system used, is determined by introduction of a known number of cells. In this work, a known number of single cells...
cells in suspension were counted with a hemocytometer, introduced into the ICP-MS, and analyzed in a time-resolved mode. The ratios of the single-cell events to the single cells introduced were calculated as the transport efficiency. Our results showed that the transport efficiency of the system for single cells was ~12%, which is better than many reports in the literature. Under these circumstances, the optimal number of cells in suspension was \( \sim 10^5 \text{mL}^{-1} \) for SC-ICP-MS.

**Quantitative analysis of Au NPs in single cells.** The quadrupole ICP-MS used in this work can continuously monitor a single isotope with zero settling time and a dwell time as low as 10 μs.

**Fig. 4** shows the SC-ICP-MS spectra of \(^{197}\text{Au} \) in the HepG2 cells at the dwell time of 100 μs and 5 ms, respectively. The Au signals of 5 ms dwell time were higher than those of 100 μs dwell time. However, the \(^{197}\text{Au} \) signal-to-background ratio (S/B) at 100 μs dwell time was much higher than at 5 ms. For example, **Fig. 5** is the enlargement of two spike signals labeled by red circles in Fig 4, showing that the S/B ratio of 100 μs dwell time was 27-fold higher than that of 5 ms. Therefore, the 100 μs dwell time was chosen for subsequent experiments. Our results showed that in comparison to a 5 ms dwell time, the dwell time of 100 μs can improve the S/B ratios in SC-ICP-MS, which agrees with the results in the literature.
Table 2 Au Mass in the Cells Exposed to AuNPs

<table>
<thead>
<tr>
<th>Exposure concentrations of AuNPs (μM)</th>
<th>SC-ICP-MS</th>
<th>Solution analysis by ICP-MS</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1 (n=491)</td>
<td>23 ± 8</td>
<td>24.2</td>
</tr>
<tr>
<td>0.5 (n=1618)</td>
<td>25 ± 16</td>
<td>26.9</td>
</tr>
<tr>
<td>1.0 (n=1504)</td>
<td>26 ± 17</td>
<td>35.4</td>
</tr>
</tbody>
</table>

n: the number of single cells determined.

Both the NPs and the trace element standard solution can be used as calibration for the quantification of NPs in single cells. In this work, AuNP standard materials were used for the quantitative analysis by SC-ICP-MS. The calibration method depends on the assumption that the intracellular AuNPs exhibit the same ionization behaviors to the AuNPs in suspension. In order to validate the SC-ICP-MS results, the average masses of the AuNPs in the cell populations were also determined by acid digestion and solution analysis with ICP-MS. No detectable Au signals were found in the control cells, thus the Au signals in the single cells come from the intracellular AuNPs. Fig. 6 shows the histogram of distribution of the AuNPs in single cells after exposure of 0.1, 0.5, and 1.0 μM for 12 h. The uptake of the AuNPs by single HepG2 cells follow the Gaussian distribution. According to the specification from the manufacturer, one AuNP is 43.7 nm in diameter and contains ~0.843 fg Au on average. When the dwell time is set at 100 μs, one AuNP produces a peak area of 3.13 ± 0.11 count ms in SC-ICP-MS. The quantitative results (Table 2) show that the cellular uptake of AuNPs increases with an increase in AuNP exposure dose. The SC-ICP-MS data using NP calibration are in good agreement with those using solution ICP-MS analysis, validating the developed SC-ICP-MS method. The data obtained provide clear evidence that single cells behave differently after AuNP exposure.

CONCLUSIONS

In this work, a high-efficiency sample introduction system was used for SC-ICP-MS analysis which improves the transport efficiency of single cells up to ~12%. In comparison with a 5 ms dwell time, the S/B ratios can be greatly improved by collecting data at a dwell time of 100 μs. In SC-ICP-MS, calibration by standard NPs can provide accurate results. The SC-ICP-MS has the capability to evaluate cell-to-cell variation and is expected to be widely applied in the future in biomedical research.

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New Possibilities for the Determination of Volatile Organic Compounds by Their Molecular Ions in Air Using μs-Pulsed GD TOFMS

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ABSTRACT: A recent trend in glow discharge analysis, previously considered as a ‘purely inorganic’ technique, is related to the effective ionisation of volatile organic compounds (VOCs). This approach was demonstrated to be capable of analysing VOCs in both model gas mixtures and ambient air. In the current study, the possibility of the direct determination of VOCs of different classes of organic compounds (including toluene, p-xylene, chlorobenzene and 1,2,4-trimethylbenzene) in ambient air using microsecond pulsed glow discharge time-of-flight mass spectrometry (μs-Pulsed GD TOFMS) with copper hollow cathode was demonstrated. The ionisation processes with the formation of molecular ions M+, which can be used for quantification, were discussed. The fragmentation of detected molecular ions of VOCs was found to be quite low, which benefits both qualitative and quantitative determination. The ease of identification and relative simplicity of the mass spectrum is promising for the analysis of VOC mixtures. One of the possible applications of the designed method is the direct determination of VOCs in human exhaled breath for the diagnosis of lung diseases, including lung cancer. However, revealing its potential applicability for this purpose requires further research.

INTRODUCTION

A recent trend in glow discharge analysis, previously considered a ‘purely inorganic’ technique, is related to the effective ionisation of the volatile organic compounds (VOCs). This approach was demonstrated to be capable of analysing VOCs in both model gas mixtures and ambient air.1,2 Several studies addressed the application of glow discharge mass spectrometry (GDMS) in molecular analysis.1,2 Fandino et al. investigated two variants of GDMS application: as a detector in gas chromatography and under a continuous flow of VOCs (benzene, toluene, ethylbenzene, and xylene) in an artificial air mixture.3 The afterglow of the discharge was used for the ionisation, employing mainly the Penning process. The limits of detection (LODs) of the VOCs in artificial air were ca. 1 ppb. The authors pointed out the similarity of acquired GDMS spectra to electron impact spectra. However, these similarities were not sufficient for simple mass spectra library-based identification, especially for the multicomponent systems. Additionally, the determination of VOCs in real ambient air or human exhalation is a more challenging task than for the artificial air mixture, mainly due to the presence of moisture with high variability. Moisture affects all ionisation processed in the glow discharge.

Nunome et al. used a novel coaxial discharge cell consisting of a cylindrical anode with a meshy cylindrical cathode inside.2 The
The aim of the current study is to develop a µs-Pulsed GD TOFMS method for the direct, fast, and highly sensitive determination of VOCs in the air using molecular ions under the minimised level of fragmentation of the analytes.
(Sigma-Aldrich, Merck, Darmstadt, Germany). After the introduction of VOCs, the Tedlar® bag was heated with a fan for 2-3 minutes to ensure full evaporation. Since the concentrations of the VOCs in the bag were below the saturated vapour concentration for the analytes, total evaporation of the VOC matter enabled its quantification in the bag. The capillary was introduced through a septum into the Tedlar® bags and the laboratory air, or air containing the VOCs, entered the discharge cell at a constant rate through the capillary due to the drop in pressure between the discharge cell and the ambient atmosphere. The switch between the Tedlar® bags was undertaken through repositioning the capillary’s edge. There were no valves in the sample introduction system. Before the analysis of every VOC containing sample, the background spectra of the bag filled with ambient air were acquired.

**Discharge parameter optimization and calibration.** The following parameters were optimised: discharge pulse duration, discharge cell pressure, discharge pulse frequency and repelling pulse delay relative to the discharge pulse. Constant air pressure of 61.3 Pa was maintained in the cell, which was determined by the air introduction capillary geometry (inner diameter of 75 µm and length 8 cm). The argon partial pressure in the discharge cell was regulated using a piezoelectric device (measured under switched off air-flow). A five-component VOC mixture was used for the optimisation (Table 2, C4). Three four-component VOC mixtures were used for calibration (Table 2, C1-C3).

**Statistics.** All measurements, including the determination of the LODs and the precision of the method (as relative standard deviation – RSD), were undertaken in 6 replicates (n = 6). All results are expressed as the mean ± confidence interval (n = 6, P = 0.95).

The LODs were estimated using the following equation (Eq. 5) based on refs.12,13:

\[
\text{LOD} = 3 \cdot C \cdot \Delta I_{bg}/(I - I_{bg}) \quad \text{(Eq. 5)}
\]

where \(I\) = integral intensity of the compound under study, \(I_{bg}\) = background intensity in the range ±0.15 Da from the peak centre, \(\Delta I_{bg}\) = standard deviation of background intensity, \(C\) = concentration of the compound in the sample.

### RESULTS AND DISCUSSION

Contrary to a previous study,3 we introduced a supplementary argon flow into the discharge cell since the discharge could not be properly initiated without it. The increase of air pressure reduced the sensitivities of the VOCs and increased the intensity of the copper associates, as observed in our previous study.5 The optimised pressure provided signal maximisation of the analytes and led to minimisation of the background intensities. VOC ionisation was undertaken via short discharge pulses (2.0-3.5 µs).

Such short pulses effectively excited the metastable level of the molecular nitrogen (N2*). The excited N2* molecules induced the ionisation of VOCs via the Penning process (Eq. 1).

At the same time, short pulses ensured a low degree of compound dissociation and low background intensities. This indicates high selectivity and improves the LOD values. The efficiency of the Penning process in the ambient air atmosphere (Eq. 1) is related to the fact that the excitation energy of the metastable N2* (11.1 eV) exceeds that of most of the VOCs. The ionisation energy of some VOCs and inorganic gases presents in the atmosphere and/or the exhalation are indicated in Table 3. As shown in Table 3, only acetonitrile has a higher ionisation energy than that for metastable N2* and metastable argon (Ar*).14 The ionisation energies of other VOCs suffice to exploit metastable levels of N2* and Ar* for an effective Penning process.

The increase of discharge pulse frequency, in principle, increases the sensitivity of VOC determination (cps/ppm). Thus, the use of the highest frequency possible seems to be reasonable to employ. At the same time, a frequency increase, corresponding to the decrease of the pulse period Ti, causes some change in the integral intensities for the mass spectra acquired. Fig. 2 shows the corresponding dependence for 5 VOCs and 60Cu on the pulse period in the range of 200 to 400 µs. The use of a discharge pulse period shorter than 200 µs was not possible, due to the specific requirements of the detecting system; for Ti > 400 µs, discharge instability or failed ignition was observed. The data in Fig. 2 indicate that the longer Ti increases the signal intensities for the VOCs, especially for 1,2,4-trimethylbenzene and p-xylene. The

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**Table 2. Concentrations of VOCs in Mixtures used. Mixtures C1-C3 Were Used for Calibration; Mixture C4 Was Used for Optimisation**

<table>
<thead>
<tr>
<th>VOC</th>
<th>C1</th>
<th>C2</th>
<th>C3</th>
<th>C4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Propanol-1, ppm</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>87</td>
</tr>
<tr>
<td>Toluene, ppm</td>
<td>12</td>
<td>35</td>
<td>94</td>
<td>94</td>
</tr>
<tr>
<td>Chlorobenzene, ppm</td>
<td>15</td>
<td>45</td>
<td>120</td>
<td>120</td>
</tr>
<tr>
<td>p-Xylene, ppm</td>
<td>12</td>
<td>35</td>
<td>93</td>
<td>93</td>
</tr>
<tr>
<td>1,2,4-Trimethylbenzene, ppm</td>
<td>12</td>
<td>36</td>
<td>95</td>
<td>95</td>
</tr>
</tbody>
</table>

**Table 3. Ionisation Energies (IE) of VOCs and Inorganic Gases**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Molecular weight</th>
<th>IE, eV</th>
<th>Compound</th>
<th>Molecular weight</th>
<th>IE, eV</th>
</tr>
</thead>
<tbody>
<tr>
<td>CO</td>
<td>28</td>
<td>14.0</td>
<td>Benzene</td>
<td>78</td>
<td>9.2</td>
</tr>
<tr>
<td>N2</td>
<td>28</td>
<td>15.6 (11.1*)</td>
<td>Toluene</td>
<td>92</td>
<td>8.8</td>
</tr>
<tr>
<td>NO</td>
<td>30</td>
<td>9.3</td>
<td>n-Heptane</td>
<td>100</td>
<td>9.9</td>
</tr>
<tr>
<td>O2</td>
<td>32</td>
<td>12.1</td>
<td>o-Xylene</td>
<td>106</td>
<td>8.6</td>
</tr>
<tr>
<td>Ar</td>
<td>40</td>
<td>11.6* (11.6*, 11.7*)</td>
<td>p-Xylene</td>
<td>106</td>
<td>8.4</td>
</tr>
<tr>
<td>CO2</td>
<td>44</td>
<td>14.3</td>
<td>m-Xylene</td>
<td>106</td>
<td>8.6</td>
</tr>
<tr>
<td>SO2</td>
<td>64</td>
<td>12.3</td>
<td>n-Octane</td>
<td>114</td>
<td>9.8</td>
</tr>
<tr>
<td>Acetonitrile</td>
<td>41</td>
<td>12.2</td>
<td>Chlorobenzene</td>
<td>113</td>
<td>9.1</td>
</tr>
<tr>
<td>Propanol-1</td>
<td>60</td>
<td>10.2</td>
<td>1,2,4-trimethylbenzene</td>
<td>120</td>
<td>8.3</td>
</tr>
</tbody>
</table>

*Excitation energy of metastable level is shown in parentheses.

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www.at-spectrosc.com/as/article/pdf/2021031 122 At. Spectrosc. 2021, 42(3), 120-127
The dependencies of mass spectrum signal intensities for 5 VOCs on the discharge pulse period. Air partial pressure 61.3 Pa, argon pressure 25.0 Pa, pulse duration 2.5 µs, repelling pulse delay 110 µs. C4 VOC mixture was used for the optimisation (Table 2).

The dependencies of mass spectrum signal intensities for 5 VOCs on the discharge pulse duration. Air partial pressure 61.3 Pa, argon pressure 25.0 Pa, discharge pulse period 250 µs, repelling pulse delay 110 µs. C4 VOC mixture was used for the optimisation (Table 2).

The optimal T-value was found to be in the range of 250-350 µs. Finally, the period of 250 µs was selected since this value also provided increased sensitivities in cps/ppm.

Figure 3 shows the dependence of signal intensities for 5 VOCs on the discharge pulse duration. The increase of pulse duration caused some increase of VOC molecular ion intensities, possibly related to the improved excitation efficiency of the discharge gas and, thus, higher concentration of N₂*. The optimal discharge pulse duration was found to be in the range of 2.5-3.5 µs. Longer pulses accelerate the sputtering of the cathode material, increasing the concentration of the copper atoms in the plasma, reducing the concentration of N₂* and the output of the Penning process (Eq. 1), due to the non-elastic collisions of the electrons with the copper atoms.

The increase of discharge cell pressure considerably reduced the mass spectral intensities of the molecular ions (argon pressure above 28.7 Pa) and increased the intensities of the background components (Fig. 4). This effect is probably related to the accelerated sputtering of the Cu cathode and, consequently, the elevated electron temperature and the reduced concentration of the metastable N₂*. The increase of the pressure from 24.0 to 28.7 Pa led to a considerable increase in the mass spectrum signal intensities of chlorobenzene and toluene. The increase was also observed for the NO⁺, Cu⁺ and m/z 43 component ([M-OH]+) for propanol-1. At the same time, the intensities of p-xylene and 1,2,4-trimethylbenzene were slightly affected. The intensity maxima for chlorobenzene, toluene, and component m/z 43 for propanol-1 are shifted towards higher pressure, compared to p-xylene and 1,2,4-trimethylbenzene. A possible explanation may be related to the asymmetric charge transfer with NO⁺ (see Eq. 6). The intensity of this reaction increases up to 30 times with the increase of pressure from 24.0 to 28.7 Pa.

\[
\text{NO}^+ + M \rightarrow \text{NO} + M^+ \quad \text{(Eq. 6)}
\]

The ionisation energies of toluene and chlorobenzene (Table 3) are comparable to that of NO (corresponding ionisation energy differences are 0.37 and 0.20 eV, respectively). However, for p-xylene and 1,2,4-trimethylbenzene, the ionisation energies differ more considerably (0.83 and 1.0 eV, respectively). Notably, the...
The probability of the dissociation processes (Eq. 7 and Eq. 8) appears to be considerably higher than that of the Penning ionisation of propanol:

$$\text{C}_3\text{H}_7\text{OH} + \text{N}_2^*(\text{Ar}^*) \rightarrow \text{C}_3\text{H}_6\text{OH}^+ + e^- + \text{N}_2 (\text{Ar})$$  \hspace{1cm} (Eq. 9)

which may explain our observations for the propanol-1 mass spectrum.

Additionally, the intensity of $\text{C}_3\text{H}_7^+$ is considerably lower than that of $\text{C}_3\text{H}_6\text{OH}^+$, which corresponds to the higher difference between the OH-group bond energy and the metastable $\text{N}_2^*$ level energy (0.5 eV) compared to that of the CH$_3$-group (0-0.25 eV).

An analogous process was observed for $n$-octane, for which the difference between the bond energy of the methyl and excitation energy of $\text{N}_2^*$ is also low (-0.2 eV). Fig. 6a demonstrates the $\mu$s-pulsed GD TOFMS mass spectrum of $n$-octane. The appearance energy values for $n$-octane fragmentation are shown in Table 4. The intensity of the fragment components of $[\text{M}-2\text{CH}_3]^+$ and $[\text{M}-3\text{CH}_3]^+$ is considerably higher than that for $\text{M}^+$. On the other hand, for $p$-xylene, the methyl bond energy is higher than the excitation energy for $\text{N}_2^*$ and $\text{Ar}^*$ resulting in a relatively low degree of fragmentation (Fig. 6b, Table 5).

**Figure 5** shows a mass spectrum range of 4 VOCs (a) and 5 VOCs (b) under optimised discharge parameters. Notably, primarily the molecular ions of the VOCs can be observed in the mass spectrum, followed by protonated and deprotonated ions with considerably lower intensity. However, for propanol-1, the molecular ion is virtually absent (Fig. 5b). Instead, two components of $m/z$ of 43 ($[\text{M}-\text{OH}]^+$) and 45 ($[\text{M}-\text{CH}_3]^+$) were present. We suppose that such behaviour of propanol-1 may be related to its resonance dissociation in the discharge. The excitation energies of molecular nitrogen and argon (Table 3) are quite close to the bond energy for the methyl group in the $\text{C}_3\text{H}_7\text{OH}$ molecule (11.1 eV) and the bond energy of the OH group in the molecule (11.6 eV). Thus, highly effective resonance dissociation of $\text{C}_3\text{H}_7\text{OH}$ may take place:

$$\text{C}_3\text{H}_7\text{OH} + \text{N}_2^* (\text{Ar}^*) \rightarrow \text{C}_3\text{H}_6\text{OH}^+ + \text{CH}_3^- + \text{N}_2 (\text{Ar})$$  \hspace{1cm} (Eq. 7)

$$\text{C}_3\text{H}_7\text{OH} + \text{N}_2^* (\text{Ar}^*) \rightarrow \text{C}_3\text{H}_6^+ + \text{OH}^- + \text{N}_2 (\text{Ar})$$  \hspace{1cm} (Eq. 8)

The use of reaction (Eq. 6) to increase the intensities of some VOCs is accompanied by a considerable elevation of the background components. Thus, the high NO$^+$ concentration mode was not used in further studies.
Table 4. Appearance Energy for n-octane

<table>
<thead>
<tr>
<th>Fragment ion</th>
<th>Appearance energy, eV</th>
</tr>
</thead>
<tbody>
<tr>
<td>C(_6)H(_4)(^+)</td>
<td>11.2</td>
</tr>
<tr>
<td>C(_6)H(_6)(^+)</td>
<td>11.1</td>
</tr>
<tr>
<td>C(_6)H(_7)(^+)</td>
<td>11.4</td>
</tr>
<tr>
<td>C(_7)H(_8)(^+)</td>
<td>11.1</td>
</tr>
<tr>
<td>C(_8)H(_9)(^+)</td>
<td>11.0</td>
</tr>
<tr>
<td>C(_9)H(_10)(^+)</td>
<td>11.1</td>
</tr>
<tr>
<td>C(<em>{10})H(</em>{11})(^+)</td>
<td>11.2</td>
</tr>
<tr>
<td>C(<em>{11})H(</em>{12})(^+)</td>
<td>10.3</td>
</tr>
<tr>
<td>C(<em>{12})H(</em>{13})(^+)</td>
<td>10.6</td>
</tr>
<tr>
<td>C(<em>{13})H(</em>{14})(^+)</td>
<td>10.9</td>
</tr>
</tbody>
</table>

Table 5. Bond Energies for Some VOCs

<table>
<thead>
<tr>
<th>Compound</th>
<th>Ion</th>
<th>Appearance energy, eV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetonitrile</td>
<td>C(_2)H(_3)N(^+)</td>
<td>13.9</td>
</tr>
<tr>
<td>Benzene</td>
<td>C(_6)H(_5)(^+)</td>
<td>14.4</td>
</tr>
<tr>
<td>Chlorobenzene</td>
<td>C(_6)H(_5)(^+)</td>
<td>14.0</td>
</tr>
<tr>
<td>Propanol-1</td>
<td>C(_3)H(_5)(^+)</td>
<td>15.7</td>
</tr>
<tr>
<td>Toluene</td>
<td>C(_6)H(_5)(^+)</td>
<td>11.7</td>
</tr>
<tr>
<td>m-Xylene</td>
<td>C(_6)H(_5)(^+)</td>
<td>11.8</td>
</tr>
<tr>
<td>n-Octane</td>
<td>C(_8)H(_17)(^+)</td>
<td>10.9</td>
</tr>
</tbody>
</table>

Table 6. LODs for VOCs in Air Mixture Assessed for 10 Min Mass Spectrum Acquisition Using 3σ Criterion

<table>
<thead>
<tr>
<th>VOC</th>
<th>LOD, ppb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Toluene</td>
<td>2</td>
</tr>
<tr>
<td>Chlorobenzene</td>
<td>3</td>
</tr>
<tr>
<td>p-Xylene</td>
<td>0.5</td>
</tr>
<tr>
<td>1,2,4-Trimethylbenzene</td>
<td>5</td>
</tr>
</tbody>
</table>

Notably, since the ionisation takes place in pulsed discharge afterglow under low atomic temperature (average discharge power of 3 W), the processes (6) and (7) may be accomplished only under the comparable energies of metastable N\(_2\)\(^*\) and Ar\(^*\) and the bond energy of the dissociating group. Indeed, the bond energy for CH\(_2\) in p-xylene and toluene, as well as the Cl atom in chlorobenzene, are 11.9, 13.7 and 11.8 eV, respectively. Although the difference with excitation energy of molecular nitrogen appears to be not too large (0.8, 2.6 and 0.7 eV, respectively), the [M-CH\(_2\)]\(^+\) components are virtually absent in the mass spectrum. The intensities of protonated and deprotonated toluene ([M+H]\(^+\) and [M-H]\(^+\)) are also relatively low.

Another notable fact, as can be seen in Fig. 5, under selected discharge conditions, the intensities of the copper components (\(^{64}\)Cu\(^+\) and \(^{68}\)Cu\(^+\)) are low. This indicates that all copper-based cluster components (such as CuOH\(^+\), CuAr\(^+\), CuO\(^+\), Cu\(^2+\), etc.) are extremely low, not affecting the determination of the VOCs. The optimised discharge parameters providing the maximised VOC intensities under minimised intensities of the background components are as follows: air pressure 61.3 Pa, argon pressure 25.0 Pa, discharge pulse duration 2.5 µs, discharge pulse frequency 4 kHz, repelling pulse delay 110 µs. Lower values of pressure or shorter pulse duration resulted in unstable discharge.

Under the optimised discharge conditions, calibrations were established for 4 VOCs (Fig. 7). The relative uncertainty of the calibration points was within 10%. The LODs, evaluated using the 3σ criterion for the registration time of 10 min, are presented in Table 6. The LOD values in the range of 0.5-5 ppb were achieved, which is perspective for the real application of the new approach for a wide range of analytical tasks, including the determination of VOCs in human exhalation.

When comparing our results with previous studies\(^{1,3,5}\), the following aspects may be identified. The combination of several factors related to the nature of the pulsed glow discharge resulted in the combination of two beneficial outcomes for the online detection mode, which are often hard to achieve together. These are high ionisation efficiency and low degree of fragmentation of the VOCs. The reason for the low degree of fragmentation is related to the primary ionisation mechanism of the VOCs in the current study – the Penning process with metastable molecular nitrogen. Bouza et al. also used the Penning mechanism for VOC ionisation.\(^1\) However, since Ar\(^*\) rather than N\(_2\)\(^*\) was used for the ionisation, which has a higher excitation energy (the difference of 0.66 eV), it resulted in a significantly higher fragmentation level for the VOCs.\(^1\) Besides, we used a relatively low discharge power of 3 W, additionally reducing the degree of fragmentation. In our previous study,\(^3\) we used copper-associates CuM\(^+\) for the detection of VOCs and observed a low degree of fragmentation under the discharge parameters favouring their formation; however, it resulted in more than one order of magnitude reduced sensitivity compared to the use of molecular ions as in the current study.

When analysing the VOC mass spectra, we noticed the presence of rather an intensive component of O\(_2\)\(^+\), related to the ionisation of inorganic gases present in the air. O\(_2\) has a relatively low difference between its ionisation energy and excitation energy of the metastable level of argon (0.36 eV, Table 3). The ionisation of other inorganic gases, such as N\(_2\), SO\(_2\), CO, CO\(_2\), etc., by the Penning process is not possible since their ionisation energies
considerably exceed the excitation energy of the metastable Ar* and N₂*. However, since the analytical system used is capable of effective ionisation of the components with high-energy electron impact, we explored the prospect of determining inorganic gases in the air. Fig. 8 represents a mass spectrum of the air sample obtained under a short repelling pulse delay of 8 µs. Under such repelling pulse delay, the ions formed by the high-energy electron packet are detected. The flow of the high-energy electron packet is formed at the front of the discharge pulse and is capable to ionise the components with the highest ionisation energies. The obtained mass spectrum (Fig. 8) indicates that the proposed analytical system may combine the determination of organic (VOCs) and inorganic components in the gaseous phase. This is an additional potential benefit of the designed analytical system, which requires further research.

CONCLUSIONS

The possibility of direct determination of VOCs of different classes of organic compounds (i.e. toluene, p-xylene, chlorobenzene, and 1,2,4-trimethylbenzene) in ambient air using TOF-MS with a µs-DC pulsed glow discharge with copper HC was considered. Ionisation processes with the formation of the molecular ions M⁺, which can be used for quantification, were discussed. Effective Penning ionisation of different VOCs was demonstrated. At the same time, another ionisation process (electron ionisation) was shown to be feasible by variation of the repelling pulse delay. This process can be used for the determination of inorganic compounds (N₂, O₂, CO₂, H₂O, etc.)

The fragmentation of detected molecular ions of VOCs was found to be rather low, which benefits both the qualitative and the quantitative determination. The ease of identification and the relative simplicity of the mass spectrum is promising for the analysis of VOC mixtures, which was also demonstrated. One of the possible applications of the designed method is the direct determination of VOCs in human exhaled breath for the diagnosis of lung diseases, including lung cancer. However, revealing its potential applicability for this purpose requires further research.

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Notes
The authors declare no competing financial interest.

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REFERENCES


Accurate Measurement of Chromium Isotopic Compositions in Geological Reference Materials by Double-Spike MC-ICP-MS

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ABSTRACT: The δ53/52Cr values of twenty-five geological reference materials (GRMs) were determined including igneous rocks, coal, shale, stream/ocean sediments and soils, with high-precision double spike MC-ICP-MS (Neptune Plus). Previously measured GRMs, including MUH-1, OKUM, DTS-2B, and JB-3, were used to monitor the long-term analytical precision and accuracy. The resulting method yielded a long-term precision of ≤ 0.06‰ based on these GRMs, and the δ53/52Cr values were excellently consistent with their previously reported values. Most of the seventeen new GRMs reported here yielded δ53/52Cr values within a narrow range from -0.20‰ to 0.01‰, with an average of -0.14 ± 0.03‰ (2SD), which was the same as the unfractionated Bulk Silicate Earth (BSE). However, SCO-2 (shale) yielded a δ53/52Cr value of 0.19 ± 0.03‰ (2SD) and GBW07334 (ocean sediment) yielded a δ53/52Cr value of 0.01 ± 0.05‰ (2SD), which were different from the BSE. Thus, SCO-2 and JH-1 (-0.20 ± 0.02‰) can serve as good candidate GRMs for interlaboratory comparisons.

INTRODUCTION

Chromium (Cr) exists in the natural environment mainly as two valence states (Cr³⁺ and Cr⁶⁺), and it has four stable isotopes (⁵⁰Cr, ⁵²Cr, ⁵³Cr and ⁵⁴Cr, Laeter et al., 2003).¹ Because of the short half-life of ⁵³Mn decaying to ⁵³Cr (3.7±0.4Ma), radiogenic ⁵³Cr was used as an astronomical chronometer to identify the evolutionary processes of the early solar system (Götz and Heumann, 1988).² With the development and improvement of multiple-collector inductively couple plasma mass spectrometry (MC-ICP-MS), the measured precision of Cr isotope compositions has been improved to ~0.02‰ (2SD)²-⁶ and stable Cr isotopic fractionation in terrestrial sample can be identified. Since Cr is a redox-sensitive element, and the redox transformation between Cr (III) and Cr (VI) can result in 7.6‰ Cr isotopic variation.⁷,⁸ Therefore, Cr isotopes have been used to reconstruct the redox conditions of the paleo-ocean and atmosphere.⁹,¹₀ In addition, Cr (VI) is a soluble, carcinogenic, and common toxic contaminant in modern environments due to its extensive use in many industries such as electroplating and leather tanning.¹²-¹⁴ In contrast, Cr (III) is less soluble and toxic. Thus, an effective way of remediating Cr (VI) pollution is to reduce Cr (VI) to Cr (III). Because heavier Cr isotopes preferentially stay in the remaining Cr (VI)⁷, ⁵³Cr/⁵²Cr ratio can be used to indicate and quantify the extent of Cr (VI) reduction in contaminated groundwater systems.¹⁴-¹⁸ High precision and accuracy data are a prerequisite for applying Cr isotopes to solve the abovementioned problems.

Geological reference materials (GRMs) play an important role in calibrating analytical methods for quality assurance and interlaboratory comparisons.¹⁹,²⁰ For Cr isotopes, commonly used GRMs include JP-1 (Peridotite),³,²¹-²³ BHVO-2 (Basalt),²⁴,²⁵ JDo-1 (Carbonatite),²⁶,²⁷ and SRG-1b (Shale).²³ SDO-1 was a shale standard for Cr isotopes but it has been discontinued. In addition, very few GRMs for environmental and ultramafic samples have been reported.⁵,²³ Recent studies suggested that the stable Cr isotope system has a great potential in identifying the pollution sources of stream sediments, and it could be utilized as a proxy for...
oxygen fugacity to study planetary differentiation and magmatic evolution.\textsuperscript{28,29} Hence, the Cr isotopic ratios of more GRMs with different lithologies need to be further determined.

In this study, the Cr isotopic ratios of twenty-five, including ultramafic rocks, coal, shale, soil, stream and ocean sediment, were measured by double-spike MC-ICP-MS. Among them, eight GRMs have been published previously and were used to validate our methods, and eighteen samples were reported for the first time. The aim for this work is to expand the data set (data set is 2 words) of Cr isotopic values in GRMs for interlaboratory comparisons when measuring a wide range of geological and environmental samples.

**EXPERIMENTAL**

**Reagents and materials.** The HNO\textsubscript{3}, HCl and HF (optima-grade) were purchased from Beijing Institute of Chemical Reagents and distilled with Savillex\textsuperscript{TM} (USA) DST-1500 stills before using in sample chemical purification. Ultrapure water (18.2 M$\Omega$·cm\textsuperscript{-1}) was obtained from a Milli-Q Element system (Millipore, USA). High-purity reagents, such as (NH\textsubscript{4})\textsubscript{2}S\textsubscript{2}O\textsubscript{8} (99.8%), NH\textsubscript{3}•H\textsubscript{2}O (99.999%), NH\textsubscript{4}OH•HCl (99.995%) and 35\% H\textsubscript{2}O\textsubscript{2} (guarantee reagent), were purchased from Alfa Aesar (China). Cation and anion exchange resins (AG50W-X8, 200-400m) resin. Chromium was separated from residual matrices. Briefly, the samples were successively passed through 2 ml AG50W-X8 (200-400m) resin and 2 ml AG1-X8 (100-200 m) resin to remove Ca, Fe and some other matrix elements in step I. Step II was used to remove Ti and V by passing thought 1ml AG1-X8 (200-400m) resin. Chromium was separated from residual matrices during step III, where Cr (III) was oxidized to Cr (VI) by a strong oxidant Ammonium persulfate ((NH\textsubscript{4})\textsubscript{2}S\textsubscript{2}O\textsubscript{8}) at circumneutral pH (pH=5.9, adjusted with NH\textsubscript{3}•H\textsubscript{2}O). After oxidation, samples were centrifuged, and the supernatants were passed through columns charged with 2ml AG1-X8 (100-200m) resin. High-purity Cr was eluted from the column by 8 ml 2 M H\textsubscript{2}SO\textsubscript{4} solution. The double-spike ($^{50}$Cr-$^{54}$Cr) used in this work was the same as that in Zhu et al.\textsuperscript{23}

Twenty-six GRMs were measured in this study. Peridotite (JP-1, PCC-1 and DTS-2b), basalt (BRP-1), shale (SCO-2), and coal (CLB-1) were obtained from the United States Geological Survey (USGS). Gabbro (JGB-1,2), basalt (JB-3), and hornblendite (JH-1) were purchased from the Geological Survey of Japan (GSJ) (City). Ultramafic rock (MUH-1) and Komatiite (OKUM) were bought from International Association of Geoanalysts (IAG) (UK). Ocean and stream sediments (GBW07325, -31, -34, -35, GSD-1, 4, 8, 14, 16, 19) and soils (GSS-19, 20, 21) were obtained from the Institute of Geophysical and Geochemical Exploration (IGGGE), China.

**Sample digestion.** The digestion method for various samples were described in detail in Zhu et al.\textsuperscript{23} Briefly, igneous rock samples, including peridotite, basalt, gabbro and rhyolite, were digested in 15 ml PFA beakers. Approximately 50 mg of powdered samples were mixed with 3 ml HNO\textsubscript{3}•H\textsubscript{2}O•HF (1:2) and decomposing for 8 - 12 h at 150 °C degrees. Dried samples were then treated with 4 ml Aqua regia (1:3) (HNO\textsubscript{3}/HCl for 6 - 12 h at 130 °C and then dried; this process was repeated until no solid residue was observed in the dissolved solution. Other samples, including soils, coal, ocean and stream sediment, were digested with customized high-pressure bombs: ~100 mg of powdered samples was mixed with 3ml HNO\textsubscript{3}•HF (4:1) and heated for 36 h at 185 ± 5 °C. The digestion procedures were repeated again for completely dissolving samples. All the digested samples were dissolved in 2M HNO\textsubscript{3} mixed with 0.5\% (V/V) H\textsubscript{2}O\textsubscript{2} for storage.

**Chromatographic separation.** The chemical purification of all samples was conducted in a class 100 hood at the Isotope Geochemistry Laboratory of China University of Geosciences, Beijing. Following previous procedures.\textsuperscript{23} Briefly, $^{50}$Cr-$^{54}$Cr double spike was mixed with sample solution before separation in order to correct for potential isotope fractionations during chemical purification and isotope measurement. According to the optimized $^{50}$Cr\textsubscript{spike}/$^{50}$Cr\textsubscript{sample} ratio of 0.4, sample solutions containing 600 ng Cr were spiked with suitable amount of double-spike. The sample-spike mixtures were sealed in 15 ml PFA beaker and heated for over 6 h at 100 °C to achieve isotopic equilibrium between sample and spike. The chemical purification scheme described in Zhu et al.\textsuperscript{23} was used to separate Cr from the sample matrices. Briefly, the samples were successively passed through 2 ml AG50W-X8 (200-400m) resin and 2 ml AG1-X8 (100-200 m) resin to remove Ca, Fe and some other matrix elements in step I. Step II was used to remove Ti and V by passing thought 1ml AG1-X8 (200-400m) resin. Chromium was separated from residual matrices during step III, where Cr (III) was oxidized to Cr (VI) by a strong oxidant Ammonium persulfate ((NH\textsubscript{4})\textsubscript{2}S\textsubscript{2}O\textsubscript{8}) at circumneutral pH (pH=5.9, adjusted with NH\textsubscript{3}•H\textsubscript{2}O). After oxidation, samples were centrifuged, and the supernatants were passed through columns charged with 2ml AG1-X8 (100-200m) resin. High-purity Cr was eluted from the column by 8 ml 2 M HNO\textsubscript{3} with 0.5\% H\textsubscript{2}O\textsubscript{2}. Cr eluents were evaporated at 130 °C and redissolved in 2\% HNO\textsubscript{3} and diluted to 25 μg L\textsuperscript{-1} for Cr isotope measurement.

**Table 1. Instrument Parameters**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cup configuration</strong></td>
<td>L\textsubscript{3} (Ti), L\textsubscript{2} (V,Cr), L\textsubscript{1} (V), C\textsubscript{60} (Cr), H\textsubscript{1} (Cr), H\textsubscript{2} (Fe,Cr), H\textsubscript{4} (Fe)</td>
</tr>
<tr>
<td><strong>Inlet system</strong></td>
<td>L\textsubscript{1} 15.1–15.7 L/min</td>
</tr>
<tr>
<td></td>
<td>L\textsubscript{2} 1.3 L/min</td>
</tr>
<tr>
<td></td>
<td>L\textsubscript{3} 0.91–1.10 L/min</td>
</tr>
<tr>
<td></td>
<td>L\textsubscript{4} 1250 W</td>
</tr>
<tr>
<td></td>
<td>H type (sample), X type (skimmer)</td>
</tr>
<tr>
<td><strong>Spay chamber temperatures</strong></td>
<td>Medium M/ΔM ≥ 6500</td>
</tr>
<tr>
<td></td>
<td>110 °C</td>
</tr>
<tr>
<td></td>
<td>160 °C</td>
</tr>
<tr>
<td><strong>Desolvator temperatures</strong></td>
<td>Ar sweep gas 5.1–6.3 L/min</td>
</tr>
<tr>
<td></td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>Sample uptake 110 μL/min</td>
</tr>
<tr>
<td></td>
<td>Sensitivity ($^{50}$Cr) ≥300 V/μg mL\textsuperscript{-1}</td>
</tr>
</tbody>
</table>
Mass spectrometry. The measurement of Cr isotope ratios was conducted on a Thermo Fisher Scientific (USA) Neptune Plus MC-ICP-MS at the Isotope Geochemistry Laboratory, China University of Geosciences, Beijing. The instrument was equipped with 9 Faraday cups connected to 1011 Ω amplifiers. Among them, seven Faraday cups L3, L2, L1, C, H1, H2 and H4 were used to detect the signal intensities of 49Ti, 50Cr, 51V, 52Cr, 53Cr, 54Cr and 56Fe in the static mode. A customized Aridus II (Teledyne CETAC, USA) with a 110 μL min−1 micro-concentric PFA nebulizer (ESI, USA) was used to introduce sample solutions into the instrument.23,26 Other operating parameters for measuring Cr isotope are listed in Table 1.

As presented in our previously referenced work,23 all isotopes were measured in the medium resolution mode to avoid polyatomic interferences, such as 40Ar21Cr and 40Ar18N on 52Cr and 54Cr, respectively. Each analysis consists of three blocks of 60 cycles, each cycle with 4.19 s integration time and 3 s idle time. Before each sample/standard analysis, the sample introduction system was washed with 2% HNO3 (V/V) until the signal intensity of 52Cr and 54Cr dropped to less than 3 mV. On-Peak-Zero (OPZ) was used to subtract the contribution of blank signal and background noise from sample signals. All data were reported relative to SRM 979: δ53Cr = ([53Cr/52Cr]sample/(53Cr/52Cr)SRM 979 - 1) × 1000. The analysis of every three or four samples were bracketed by spiked SRM 979 with similar Cr concentrations to monitor the instrument stability. The δ53Cr values of SRM 979 were used to normalize the sample values: δ53Cr = δ53Crsample − δ53Cr SRM 979.23

RESULTS AND DISCUSSION
Blank contributions
The total procedural Cr blank, including sample digesting and chemical purification, ranged from 0.7 to 1.2 ng with an average value of ~1.0 ng, which was within the range of previous studies (0.12~17 ng).4,26,31,32 Our previous study demonstrated that the blank has negligible effects on measured sample δ53Cr values when the procedural blank is less than 0.4% of load Cr size.26 In this study, Cr blank was less than 0.2% of the load Cr mass.

Precision and Accuracy
The SRM 979 and several GRMs were repeatedly measured to assess the precision and accuracy of the reported method. Spiked SRM 979s were measured 57 times during two analytical sessions separated by 6 months. The two standard deviation (2SD) of these results was 0.03‰ (Fig. 1), which was similar to previous studies.25,26,31,33 GRMs including GSD-1, JB-3, JGB-1, JP-1, DTS-2b, MUH-1, and OKUM were repeatedly digested, purified and measured in different analytical procedures, with 2SDs (n=4-7) of 0.03‰, 0.02‰, 0.03‰, 0.03‰, 0.03‰, 0.02‰, and 0.04‰, respectively, which are consistent with values reported,6,21,22,28,34 demonstrating that the long-term precision of δ53Cr measurement for actual samples was better than 0.06‰.

As show in Fig. 2, the mean δ53Cr values of SRM 979, GSD-1, JB-3, JGB-1, DTS-2b, MUH-1 and OKUM were 0.00 ± 0.03‰
Concentrations of chromium, such as PCC-1 (-0.05 ± 0.04 ‰)
processes is small.

yielded δ
GBW07334 and GBW07335 collected in South China ocean
GBW07334 and GBW07335 fall within the range of oxic sediments, consistent with the fact that they were likely deposited in oxic environment.

(iii) Soil reference materials. The δ
Cr of GSS-19, 20, 21
were -0.11 ± 0.06 ‰ (2SD, n=4), -0.09 ± 0.02 ‰ (2SD, n=4) and
-0.12 ± 0.06 ‰ (2SD, n=4), respectively. Taking previously reported soil samples into consideration, the average δ
Cr of soils is estimated to be -0.10 ± 0.33 ‰ (2SD, n=17). Soil is the residual product of chemical weathering of parent rocks, many processes can lead to Cr isotope fractionation during weathering, such as oxidation of Cr(III) to Cr(VI) and/or adsorption of Cr
VI by biomaterials. Published δ
Cr values for soil profile samples range from -0.57 ‰ to 0.23 ‰, and our soil δ
Cr data are within this range.

(iv) Shale and coal reference materials. The δ
Cr of SCO-2 was 0.19 ± 0.03 ‰ (2SD, n=4), which is within the range of other previously published shale reference materials, such as SGR-1b (0.30 ± 0.06 ‰) and SDO-1 (-0.08 ± 0.05 ‰), and GSR-5 (-0.15 ± 0.03 ‰). The total range of δ
Cr in published shale GRMs is relatively small with 0.45‰, which may be due to varying redox states for their deposition environments. The δ
Cr of CLB-1 was -0.09 ± 0.04 ‰ (2SD, n=8), which was the first coal reference material published so far for Cr isotopes.

CONCLUSIONS

The δ
Cr of twenty-five GRMs were measured using double-spike MC-ICP-MS. Repetitive digestion, purification and measurements yielded a precision of less than 0.06‰ (2SD), similar to previous studies. Eight of these GRMs have been measured previously, and the measured values are consistent with reported values, confirming the high-precision and accuracy of the generated data. The δ
Cr of seventeen new GRMs were reported for the first time, δ
Cr values ranging between -0.20 ‰ and 0.19 ‰. Among the investigated GRMs, GBW07334 (0.01 ± 0.05‰), JH-1 (-0.20 ± 0.02‰), and SCO-2 (0.19 ± 0.03‰) are slightly different from the unfractionated BSE, and thus are good candidate reference materials for interlaboratory comparisons.

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Notes

The authors declare no competing financial interest.
Table 2 Cr Isotopic Composition of Geological Standards in this Study and in the Literature

<table>
<thead>
<tr>
<th>Sample</th>
<th>Sample type</th>
<th>Reference</th>
<th>Cr (μg/g)</th>
<th>δ⁵³Cr (‰)</th>
<th>2SD</th>
<th>n</th>
<th>U</th>
</tr>
</thead>
<tbody>
<tr>
<td>OKUM (IAG)</td>
<td>Komatite</td>
<td>This study</td>
<td>2301</td>
<td>-0.10</td>
<td>0.03</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Average</td>
<td></td>
<td>-0.10</td>
<td>0.04</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>MUH-1 (IAG)</td>
<td>Ultramafic rock</td>
<td>This study</td>
<td>2385</td>
<td>-0.07</td>
<td>0.025</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Average</td>
<td></td>
<td>-0.07</td>
<td>0.025</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>DTS-2b (USGS)</td>
<td>Peridotite</td>
<td>This study</td>
<td>21250</td>
<td>-0.10</td>
<td>0.03</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Average</td>
<td></td>
<td>-0.10</td>
<td>0.03</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>PCC-1 (USGS)</td>
<td>Peridotite</td>
<td>This study</td>
<td>3769</td>
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<td>0.04</td>
<td>4</td>
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<tr>
<td></td>
<td></td>
<td>Average</td>
<td></td>
<td>-0.07</td>
<td>0.04</td>
<td>4</td>
<td></td>
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<tr>
<td>JP-1 (GSJ)</td>
<td>Peridotite</td>
<td>This study</td>
<td>2894</td>
<td>-0.10</td>
<td>0.03</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Average</td>
<td></td>
<td>-0.10</td>
<td>0.03</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>CLB-1 (IGGE)</td>
<td>Coal</td>
<td>This study</td>
<td>99.8</td>
<td>-0.19</td>
<td>0.03</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Average</td>
<td></td>
<td>-0.19</td>
<td>0.03</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>GSS-19 (IGGE)</td>
<td>Soil</td>
<td>This study</td>
<td>51.2</td>
<td>-0.11</td>
<td>0.06</td>
<td>4</td>
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<tr>
<td></td>
<td></td>
<td>Average</td>
<td></td>
<td>-0.11</td>
<td>0.06</td>
<td>4</td>
<td></td>
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<tr>
<td>GSS-20 (IGGE)</td>
<td>Soil</td>
<td>This study</td>
<td>45.2</td>
<td>-0.09</td>
<td>0.02</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Average</td>
<td></td>
<td>-0.09</td>
<td>0.02</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>GSS-21 (IGGE)</td>
<td>Soil</td>
<td>This study</td>
<td>57.2</td>
<td>-0.12</td>
<td>0.06</td>
<td>4</td>
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<tr>
<td></td>
<td></td>
<td>Average</td>
<td></td>
<td>-0.12</td>
<td>0.06</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>CB-1 (IGGE)</td>
<td>Coal</td>
<td>This study</td>
<td>10.1</td>
<td>-0.09</td>
<td>0.05</td>
<td></td>
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</tr>
<tr>
<td></td>
<td></td>
<td>Average</td>
<td></td>
<td>-0.09</td>
<td>0.05</td>
<td>4</td>
<td></td>
</tr>
</tbody>
</table>

* The Cr concentrations were calculated using double-spike method, Lu et al., (in press) presented the method in detail.
* The Number of analyses.
* The value was calculated from 95% confidence interval of independent replicate analyses that take the student-t distribution into account.

ACKNOWLEDGMENTS

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4. C.-F. Li, L.-J. Feng, X.-C. Wang, S.-A. Wilde, Z.-Y. Chu, and
Temperature Difference Between Gas Species in Absorption Measurements Using Diode Laser Absorption Spectroscopy and Its Effect on Temperature Reduction

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ABSTRACT: The observation of isotope shifts due to a difference in mass number by diode laser absorption spectroscopy (DLAS) is a powerful approach for the isotope analysis of radionuclides. The spectral resolution for the detection of slight shifts can also be enhanced by a temperature reduction using adiabatic expansion. In our previous studies, we reported that the translational temperature was successfully decreased to approximately 180 K in xenon isotope analysis using a supersonic plasma jet. However, there remains a considerable uncertainty regarding the significant temperature reduction compared with the temperature of argon atoms at the edge of the supersonic plasma jet, which is at 790 K. In this study, temperature differences between two species of three mixed gas patterns (neon/argon, argon/strontium, and argon/xenon) were investigated using low-pressure glow discharge plasma. The temperature differences for the mixed gas patterns were clearly observed and are sufficient evidence to support our previous results. The relationship between temperature differences and energy levels of lower states used as absorption transitions is also discussed.

INTRODUCTION

Radioisotope abundance due to radioactive decay provides clues to its origin and age; thus, isotope analysis techniques are essential tools for obtaining accurate information.1-4 In fact, radioactive elements (e.g. 90Sr, 134Cs, and 137Cs) were released into the environment as a result of the accident at the Fukushima Daiichi Nuclear Power Plants in 2011,5,6 and the quantification of their abundance is essential for the safe treatment and disposal of the contaminated radioactive waste.7 Among them, 90Sr (half-life of 30 years) is one of the most hazardous nuclides because it accumulates in biological bone and emits beta radiation (energy maximum of 0.54 MeV) to surrounding tissues over a long period of time. Strontium isotope analysis has been widely conducted by conventional measurement methods such as inductively coupled plasma mass spectrometry (ICP-MS) and liquid scintillation counting.5,8

ICP-MS, which is the most common isotope analysis method, characterizes isotopes by the mass difference of the elements. Thus, there is difficulty in measuring isotopes with the same mass, such as uranium (238U) and plutonium (238Pu), which are the major elements in nuclear waste, including in spent fuel.9-11 In strontium analysis, 89Y and 90Zr, which are daughter and grandchild nuclides, respectively, considerably interfere with 90Sr detection. To prevent technical issues during measurement, it is necessary to separate each element using chemical pretreatment, which usually is very time-consuming (approximately 2–4 weeks).7 Therefore, the development of a measurement method with an easy sample pretreatment and achieves high accuracy essential for rapid analysis. Recently, highly sensitive optical spectroscopic methods for isotope analysis, such as absorption spectroscopy (diode laser
absorption spectroscopy (DLAS) and fluorescence spectroscopy (laser-induced fluorescence (LIF)), have been developed.\textsuperscript{12,13}

DLAS, which uses plasma as an atomization source, can characterize isotopes by a difference in the energy level of each element and the isotope shifts using diode lasers with high-wavelength selectivity; therefore, this approach has gained attention as a useful technique for isotope analysis. Because high resolution allows the detection of slight wavelength shifts, DLAS can distinguish isotopes with the same mass without any chemical pretreatment of the samples. However, the high-temperature condition of plasma observed with conventional instrumentation, which usually reaches 5000–10,000 K during laser ablation, decreases the spectral resolution as a result of the Doppler broadening because of thermal atomic motions.\textsuperscript{14-20} For example, a 100-times increase in temperature usually causes a 1/10 decrease in resolution.\textsuperscript{21}

In a previous study,\textsuperscript{22} we proposed a rapid isotope analysis method without sample preparation by using a supersonic plasma jet. The arc discharge plasma in the high-temperature section upstream of a supersonic nozzle leads to a vacuum chamber; then, the translational temperature considerably decreases with an increase in the flow velocity because of adiabatic expansion. In a different study,\textsuperscript{23} we used xenon gas as a target where a temperature reduction was successfully achieved and was estimated at approximately 180 K. However, in the above-mentioned study,\textsuperscript{22} the temperature of the argon atoms at the edge of the plasma jet was determined to be 790 K. Thus, the results obtained with DLAS measurements suggested the possibility that the translational temperature of the xenon atoms differed from that of the argon atoms.

The present study aimed to investigate the temperature differences between two species by conducting temperature measurements using DLAS in a mixed gas plasma containing noble gases (neon, argon, and xenon) and strontium. As a plasma source, a low-pressure glow discharge plasma was used to generate a temporal and spatial steady-state plasma. A liquid phase sample, which contained a strontium compound, was supplied to an electrode before plasma generation. On the basis of the temperature differences obtained between the two species in glow discharge plasmas of the three mixed gas patterns, we discuss the relationship between the temperature differences and the energy levels of each atomic electron transition as well as the effect on the temperature reduction for the strontium atoms.

**EXPERIMENTAL**

**Glow discharge plasma.** A glow discharge plasma was produced between two cylindrical 20 mm diameter copper electrodes that were approximately 50 mm apart. The supply voltage can be adjusted between 0 and 3 kV with a high-voltage power supply (METRONIX: HSV2K-30). In the temperature measurements of the two species, two pinholes were positioned in front of each window because laser beams must pass through the same optical path in the glow discharge plasma. The operating conditions of the gas mixture were optimized to obtain temperatures for the two species simultaneously. An optical chopper (Thorlabs: MC200B), combined with a lock-in amplifier (Stanford Research Systems: SR830), was used to improve the signal-to-noise ratio.

**Diode laser absorption spectroscopic system.** Fig. 1 shows the schematic diagram of the diode laser absorption spectroscopic system that uses a glow discharge plasma. DLAS analysis was performed using the single longitudinal mode diode lasers (Hitachi: HL6322G and HL8325G) which were tuned to the neon, argon, and xenon absorption lines. These laser line widths (approximately 100 MHz) were one order of magnitude narrower than the absorption line width (a few GHz) in the low-pressure glow discharge plasma. To detect strontium, an external cavity diode laser (Toptica: DL100) was used with the laser line width at approximately 100 kHz. Each laser wavelength was scanned over an absorption spectrum by modulating the laser operating current with a ramp function controlled with a low-cost function generator (A&D Company: AD-8624A). The repetition frequency was approximately 1 Hz and the scanning width approximately 10 GHz. The relative laser wavelength was observed using solid etalons (EKSMA: UVFS flat etalon finesse 30) with a free spectral range of 1.15 GHz for the 630 and 830 nm bands. An optical cavity consisting of two high-reflective mirrors (SigmaKoki Co., LTD.: PSCM95-25.4C6.35-1000-460) was installed in the free space for the 460 nm band. The transmitted laser beams through the plasma and the etalon were detected using a photodetector (Thorlabs: DET10/M). Their voltage signals were recorded using an oscilloscope (Yokogawa: DL850E) with a 12-bit resolution at the maximum sampling rate of 10 MS s\textsuperscript{-1}.
RESULTS AND DISCUSSION

To investigate the temperature differences in DLAS measurements, we generated glow discharge plasmas for the three mixed gas patterns (neon/argon, argon/strontium, and argon/xenon) and conducted the temperature measurements for two gases using each atomic absorption line. The wavelengths of neon, argon, strontium, and xenon in air were 638.30 nm (Ne I, $3s^{1/2} - 3p^{1/2}$), 826.45 nm (Ar I, $4s^{1/2} - 4p^{1/2}$), 460.73 nm (Sr I, $5s^{1/2} - 5p^{3}$), and 823.16 nm (XeI, $6s^{3/2} - 6p^{3}$), respectively. The incident laser beam intensities that the plasmas were exposed to were set to sufficiently low power (<10 μW) to prevent absorption saturation. To detect strontium, a SrCl$_2$ reagent sample (Fujifilm Wako Pure Chemical Co.: Strontium standard solution Sr 1000) was introduced into the electrode. Notably, only the strontium atoms were generated by the sputtering argon ions.

The absorption spectra of the neon, strontium, and xenon atoms comprised plural absorption lines because of the existence of isotopes, as shown in Fig. 2. In nature, neon, argon, strontium, and xenon have the following isotopes: three ($^{20}$Ne, $^{21}$Ne, and $^{22}$Ne), three ($^{36}$Ar, $^{38}$Ar, and $^{40}$Ar), four ($^{84}$Sr, $^{86}$Sr, $^{87}$Sr, and $^{88}$Sr), and nine ($^{124}$Xe, $^{126}$Xe, $^{128}$Xe, $^{129}$Xe, $^{130}$Xe, $^{131}$Xe, $^{132}$Xe, $^{134}$Xe, and $^{136}$Xe), respectively. Under low-pressure conditions, the absorption profiles have an inhomogeneous broadening of the Doppler width, $\Delta \nu_D$, and these features can be expressed in Equations (1) and (2) as:

$$f(\nu) = \frac{1}{\sqrt{\pi} \Delta \nu_D} \exp \left(-\left(\frac{\nu - \nu_0}{\Delta \nu_D}\right)^2\right) \quad \text{Eq. (1)}$$

$$\Delta \nu_D = \frac{\nu_0}{c} \sqrt{\frac{2k_B T}{M_A}} \quad \text{Eq. (2)}$$

where $\nu_0$, $M_A$, and $k_B$ are the center frequency, atomic weight, and Boltzmann constant, respectively. The neon, strontium, and xenon absorption spectra were treated with simplified convolutions of the Gaussian functions by considering each mass number, natural abundance, and oscillator strength. Considering that the isotope abundance of $^{40}$Ar is two orders of magnitude higher compared to other atoms, the absorption spectra of the argon atoms were fitted using a Gaussian function of the Igor Pro 6.2 software. The obtained absorption spectra agreed well with the fitting curves.

Fig. 3a-c shows the translational temperature measured at some discharge conditions of the three mixed gas patterns: (a) neon and argon, (b) argon and xenon, (c) argon and strontium. Remarkably, the temperature differences between the two species were clearly observed in the low-pressure glow discharge plasmas of these three patterns. The number densities of the strontium generated by ion bombardment increased approximately 46 times with an increase in the discharge current (Fig. 3c).

---

Fig. 2 Absorption profiles of (a) neon, (b) strontium, and (c) xenon atoms.
Fig. 3 Translational temperatures in mixed gas plasmas of three patterns. (a) Neon and argon atoms, (b) Argon and xenon atoms, (c) Argon and strontium atoms, and number densities of strontium atoms. Error bars represent standard deviations.

Fig. 4 Typical temperature differences in mixed gas plasmas of three patterns.

Fig. 4 shows the temperature differences between argon and the other atoms at each typical discharge condition. Indeed, similar to noble gases, these results showed that the temperature of the neon atoms was 84 K higher than for the argon atoms. Conversely, the temperature of the xenon atoms was 76 K lower than for the argon atoms. In the case of strontium detection, the temperature of the strontium atoms was 119 K lower than for the argon atoms.

This study attempts to identify the contributing factors for the temperature differences in DLAS measurements. Here, we discuss the relationship between the temperature measurements and the energy levels of the absorption transitions. DLAS has a feature where the obtained absorption spectrum is given by the line-integrated value over the optical path in the plasma because of tomography measurements. Considering that the absorption coefficient, \( k(\lambda) \), has a spatial distribution, the absorbance can be expressed in Equation (3) as:

\[
-\ln \left( \frac{I}{I_0} \right) = \int_0^L k(\lambda) \, d\lambda \quad \text{(Eq. 3)}
\]

where \( I, I_0, \) and \( L \) are the transmitted beam intensity, incident beam intensity, and optical path length, respectively. Therefore, the temperature can be estimated as the average temperature of the optical path. If there is a difference in either the absorption coefficients or the optical paths, the temperatures obtained by fitting the integrated spectrum to the Gaussian function can also vary.

Figure 5 shows the relationship between the temperature differences and the energy levels of the absorption transitions, where the vertical axis indicates the energy level of a lower state in the absorption transition. In the present study, it was found that
the temperature differences were closely related to the energy levels, and the temperature decreased with a decrease in the energy level of the lower state. Most importantly, the energy levels of the excited states of the noble gases depend on the mass number.

Figure 6 shows the conceptual images of temperature difference. Fig. 6a assumes that the glow discharge plasma has a spatial temperature distribution. Because atoms at a lower energy level are likely to be excited compared with those at a higher energy level, the temperature distribution due to the difference of normalized population distributions depends on the energy level.

It also can be seen that the population changes of the two energy states are negligible near the center of the temperature distribution (Fig. 6, area I), whereas the changes increase with a decrease in temperature (Fig. 6, area II). Hence, the temperature distribution is due to the difference in the population distributions of the two species. The contribution of atoms at the base (Fig. 6, area III) of the absorption spectra are negligible. In Fig. 6, area II, the gradient of the argon atoms is smaller than for the neon atoms, i.e., the argon atoms are widely distributed in the plasma in comparison to the neon atoms.

The difference in population distribution can affect the absorption spectra in the temperature measurements by DLAS. Fig. 6b illustrates the absorption process by a probe laser beam in an optical path. Because the spatially detectable range of the argon atoms in an excited state extends to a lower temperature, the absorption spectrum (which is obtained as the line integral value over an optical path) sharpens; thus, the temperature can be estimated to be lower than for neon. Notably, there is a difference in the atomic behavior of ground and excited states because the atomic number density in the ground state is inversely proportional to the translational temperature according to the equation of the state. However, because the strontium atomization process depends on the sputtering of the argon ions in the glow discharge plasma, strontium atoms in the ground state are mostly distributed in the high-temperature area near the electrode.

In summary, the effect of temperature difference supports previous results regarding the temperature reduction for xenon atoms which is due to the lower energy levels of the argon and the xenon atoms. For strontium isotope analysis using the supersonic plasma jet system developed in our previous studies, it will be more practical to sharpen an absorption spectrum of the strontium atoms, in addition to temperature reduction. Because of adiabatic expansion using a supersonic nozzle, the atoms in the ground state are expected to be distributed more near the edge of the supersonic plasma jet rather than near the center. Additionally, because
absorption transitions from the ground states in the isotope analysis of metallic elements can be used (e.g. Cs I, 852.1 nm; U I, 394.4 nm; and Pu I, 420.6 nm), significant temperature reductions can be expected for xenon atoms, in comparison to argon atoms.

CONCLUSIONS

The temperature measurements for two atomic electron transitions of different species revealed that there was an apparent temperature difference between the two species (list species). This phenomenon was attributed to the energy level differences in the lower states of absorption transitions. Thus, the present findings support our earlier study which showed that the translational temperature of the xenon atoms decreased considerably during xenon isotope analysis. The temperature measurements of the argon and strontium atoms indicate that the effect of temperature difference sharpened spectral broadening of the strontium atoms.

This study also showed that for strontium isotope analysis in a supersonic plasma jet, the temperature measurements for the argon and strontium atoms are still required. For temperature evaluations by DLAS without spatially resolved measurements, the effect of temperature difference should be analyzed by carefully considering the spatial population distribution in the case of an absorption transition from a relatively lower energy state, such as the ground state. Otherwise, the translational temperature will be underestimated.

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Concentrations of Total As and As Speciation in Chinese Rice Wine and Associated Risk Assessment in Main Producing Provinces

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ABSTRACT: Rice and rice products have been identified as significant sources of As. Concerns have been raised about the presence of As in rice wine. This study collected 79 rice wine samples from China. High-performance liquid chromatography-inductively coupled plasma mass spectrometry was used to determine total As and As species concentrations. The average concentration of total As was 14.6 μg L⁻¹, and the concentration of As (III) (arsenite), As (V) (arsenate), dimethylarsinic acid (DMA), and arsenobetaine (AsB) were 2.86 μg L⁻¹ (0.970–6.08 μg L⁻¹), 7.22 μg L⁻¹ (2.24–22.9 μg L⁻¹), 3.92 μg L⁻¹ (1.58–7.82 μg L⁻¹) and 0.620 μg L⁻¹ (ND-0.950 μg L⁻¹), respectively. MMA (monomethylarsonic acid) and AsC (arsenocholine) were not detected. The THQs (target hazard quotients) for chronic noncarcinogenic risks (skin lesions as the point of departure) were below 1, suggesting that the Chinese population did not encounter a significant noncarcinogenic risk. However, the mean values of MOE (margin of exposure) for lung cancer were below 100 (62.1 to 75.1) for male drinkers, indicating a potential carcinogenic risk.

By comparing the As species of rice wines and the main raw material, it was found that the methylation increased DMA during fermentation.

INTRODUCTION

Arsenic (As) is a carcinogenic and highly toxic metalloid found diffusely in the earth’s crust, water, soil, plants, and food.¹² As can accumulate in the human body through food intake, which is a serious health risk to consumers. Inorganic arsenic (As), mainly arsenite and arsenate, is listed as a Group I carcinogenic by the International Agency for Research on Cancer (IARC) and presents potential damage to the skin, lungs, kidneys, nervous system, respiratory system, and urinary system.³ Minimizing the intake of As is essential to a healthy life. However, As contamination has become a severe environmental threat in some parts of the world. Apart from drinking water, food is the primary source of As for humans due to the exceedingly high ability of As to accumulate in organic tissue. Plants become a source of As because herbicides and insecticides are used heavily on crops.⁴,⁵ In particular, rice is the staple food in East Asia and is affected by the As in soil and water. Rice products, such as rice wine, are also affected.¹,⁶

The toxicity of As depends strongly on the compounds it forms with specific chemical structures, rather than merely the total content. As has proved to be more poisonous than the organic As species, including monomethylarsonic acid, dimethylarsinic acid (DMA), arsenobetaine (AsB), arsenocholine (AsC), and arsenosugars.⁷,⁸ Hence, a determination of the total As concentration cannot provide full, accurate information about As toxicity.

Research has shown that the concentrations of total As and As species change during grape and wine processing.⁹–¹¹ especially
during fermentation, due to the microbes that metabolize As. Bertoldi et al. assessed the risks associated with the ingestion of grape wines from 10 vineyards with soils rich in As. They found no difference in As content in 7 red wines before or after malolactic fermentation. As was higher in 7 white wines, suggesting that the biological As absorption by bacteria is negligible. However, Aguilar et al. observed that As content decreased during fermentation and maceration in the wine-making process. They speculated that the physical and chemical changes (i.e. the existence of volatile compounds and the formation of colloidal substances as sediment) were predominant and caused As loss—the chemical transformations of As species may occur during fermentation and influence the As species concentrations. Aguilar et al. noted that the As(V) and DMA concentrations dropped in the red wine, whereas the As(III) concentration rose. In addition, they testified that 40% of the spiked As(V) was converted to As(III), and no more than 1% of the added organic As species were demethylated into inorganic forms. However, 7% of the DMA was converted to MMA. The authors proved that DMA and MMA are the main As species that originated from inorganic As due to biometylation of As. MMA(V) could be formed from As(III) combined with CH₃⁺ undergoing oxidation. The reaction continued until the formation of DMA(V) and DMA(III). Therefore, the biometylation of As was regarded as an important way to enhance the As tolerance attributed to the lower toxicity of MMA(V) and DMA(V) when compared to As.

Rice wine is a traditional fermented wine in China and is known as the world’s three ancient wines along with beer and grape wine. It is produced from cereals (mainly rice) fermented with yeast and several bacteria. In the production of rice wine, starch is hydrolyzed and saccharified in the presence of microorganisms. They are also the substrate for ethanol fermentation, which is the source of the flavor of the wine. Rice wine contains many nutrients, such as proteins, amino acids, bioactive peptides, phenols, and oligosaccharides, which have antioxidant, hypotensive, and immunoregulatory qualities; they also help in lowering cholesterol. Presently, customers and researchers are concerned about the nutritional value and safety of rice wine. According to the International Office of Vine and Wine (OIV), the maximum As concentration allowed in wines is 200 μg L⁻¹. However, there is a risk associated with rice wine due to the accumulation of As in rice. Investigations have focused on the presence of As in beer, red wine grapes, and white wine grapes; however, there is no risk assessment report on the presence of As in rice wine. This research aims to collect and analyze comprehensive data about As in rice wine to assess dietary exposure by determining the total As and As species concentrations using high-performance liquid chromatography-inductively coupled plasma mass spectrometry (HPLC-ICP-MS).

Margin of exposure (MOE) and target hazard quotient (THQ) were used to assess the risks associated with As ingestion. MOE evaluates the carcinogenic risks of certain contaminants listed by the Expert Committee on Food Additives (JECFA); THQ is used to assess noncarcinogenic risks. Furthermore, possible As species alterations in the wine processing were speculated to correspond with rice materials, which is aimed at providing support for understanding the risk level of rice wine.

**EXPERIMENTAL**

**Sample collection and pretreatment.** Seventy-nine bottles of rice wine were randomly collected as samples from the Zhejiang and Jiangsu provinces, considered the leading producers of rice wine. All wine samples were stored at 4 °C in a refrigerator before analysis, and aliquots of the samples were directly taken from the bottles. In addition, 203 rice samples were collected from the Zhejiang and Jiangsu provinces. Every rice sample (with hulls) was collected from five random, well-distributed points of one paddy field (>100 m²). After harvesting, a quarter of every rice sample (at least 2 kg) was randomly separated for analysis. Then, all samples were transported to the laboratory as soon as possible to air-dry and to obtain a constant weight. To remain consistent with the rice wine-making process, hulls and bran layers were removed. The rice samples were then ground and sieved with a 0.45 mm mesh sieve and stored in bags at 4 °C before analysis.

**Reagents and solutions.** As standard (GBW08611) with 1000 μg mL⁻¹ of As was purchased from the National Standard Material Center (Beijing, China) and used for calibration in total As determination. Other reagents were purchased from Sinopharm Chemical Reagent Co., Ltd, China. For As speciation, 5 μg mL⁻¹ As (V), As (III), MMA, DMA, AsB, AsC standards were purchased from the National Standard Material Center (Beijing, China). These standard solutions were mixed with ultrapure water and diluted to 0.2, 0.4, 1.0, 4.0, and 10.0 μg mL⁻¹. Ultrapure water (18 M Ω cm) was obtained from a Milli-Q water purification system (Millipore, Paris, France). The injection volume was 20 μL. Nitric acid (65–68%, GR) and other reagents used were purchased from the National Standard Material Center (Beijing, China).

**Instrumentation.** The total As concentration was determined using inductively coupled plasma mass spectrometry (ICP-MS, iCAP RQ, Thermo Fisher scientific company, USA). The parameters used were according to Caruso et al. with some modifications: incident RF power, 1550 W; plasma gas flow rate, 14 L min⁻¹; spray chamber temperature, 3 °C; intermediate Ar gas flow rate, 0.8 L min⁻¹; carrier Ar gas flow rate, 1.04 L min⁻¹. The collision mode with He gas (4.8 mL min⁻¹) was used to reduce interference by polyatomic ions. The As isotope signal was measured.

For As speciation, a chromatograph (HPLC, Dionex UltiMate
3000, Thermo Fisher Scientific company, USA) was coupled with the ICP-MS instrument. A Dionex IonPac AS7 column (250 mm × i.d. 4 mm) was employed for As species separation. The mobile phase flow rate was 1.0 mL min\(^{-1}\) with solution A, (NH\(_4\))\(_2\)CO\(_3\) (100 mM) and solution B, (NH\(_4\))\(_2\)CO\(_3\) (5 mM). The elution gradient was: 0–3.5 min, 100% solution B; 3.5–7.5 min, 20% solution B; 7.5–10 min, 100% solution B.\(^{28}\)

The total As and As species concentrations in rice samples were determined using high performance liquid chromatography–hydride generation–atomic fluorescence spectrometry (HPLC-HG-AFS) (AFS 8220, Beijing Titan Instruments Co., Ltd., China). The mobile phase flow rate was 1.0 mL min\(^{-1}\) with 15 M (NH\(_4\))\(_2\)HPO\(_4\) (pH 6.0). 7% HCl solution and 20% KBH\(_4\) dissolved in 5% KOH solution as the reducing reagent. The operational parameters were set according to Huang et al.\(^{29}\)

**Total As determination in rice wines by ICP-MS.** A mixture of 2 mL rice wine, 8 mL HNO\(_3\), and 2 mL H\(_2\)O\(_2\) (30%, v/v) was prepared in 50 mL polypropylene tubes. The mixture was heated at 120 °C for 2 h on a heating block and then diluted with 2% HNO\(_3\) (v/v) to 50 mL.\(^{30}\)

**As speciation in rice samples by HPLC-HG-AFS.** A 1.0 g rice sample was blended with 10 mL of 0.02 M TFA, 50% (v/v) methanol, and 0.02 M HNO\(_3\). After uniform mixing, the mixture was heated at 90 °C for 1 h and centrifuged at 5000 rpm for 10 min. Then, 4 mL supernatant was concentrated to less than 1.5 mL with a controlled nitrogen flow, and 120 μL H\(_2\)O\(_2\) was added to the concentrated solution heated at 70 °C for 0.5 h to oxidize As(III) to As(V). Then, the solution was filtered through a 0.45 μm polypropylene filter before analysis.\(^{31}\)

**As speciation in rice wine samples by HPLC-ICP-MS.** The rice wine samples were prepared as described above, and then the extracts were diluted ten-fold by adding ultrapure water and filtered through 0.45 μm polypropylene filters before As determination.\(^{31}\) A 100 μL filtrated sample was injected into the chromatographic column. Chromatograms for a standard (1.0 μg L\(^{-1}\)) with the investigated As species and a rice wine sample are shown in Fig. 1. The limit of detection (LOD) for As species by HPLC-HG-AFS and HPLC-ICP-MS were calculated based on 3 times the signal-to-noise ratios (Table 1)

**Risk assessment of As through rice wine consumption.** The estimated daily intake (EDI)\(^{32}\) of As depended on its concentration and wine consumption. The EDI was calculated by equation (1):

\[
\text{EDI} = (E_F \times E_D \times F_{IR} \times C) / (W_{AB} \times T_A)
\]

\(^{(1)}\)

Table 1. Limits of Detection (LODs) of As Species for HPLC-HG-AFS and HPLC-ICP-MS

<table>
<thead>
<tr>
<th>Methods</th>
<th>Unit</th>
<th>AsB</th>
<th>AsC</th>
<th>MMA</th>
<th>DMA</th>
<th>As(III)</th>
<th>As(V)</th>
<th>As(_i)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPLC-HG-AFS</td>
<td>μg kg(^{-1})</td>
<td>—</td>
<td>—</td>
<td>8.00</td>
<td>6.00</td>
<td>—</td>
<td>—</td>
<td>6.00</td>
</tr>
<tr>
<td>HPLC-ICP-MS</td>
<td>μg L(^{-1})</td>
<td>0.230</td>
<td>0.440</td>
<td>0.230</td>
<td>0.310</td>
<td>0.540</td>
<td>0.340</td>
<td>—</td>
</tr>
</tbody>
</table>

\(E_F\) is the exposure frequency (365 days/year), \(E_D\) is the exposure duration (70 years), \(F_{IR}\) is the ingestion rate of rice wines (g/person/d), \(C\) is the Asi concentration in rice wines (μg/kg), \(W_{AB}\) the average body weight (kg), and \(T_A\) is the average exposure time \((E_F \times E_D)\). The average body weights of males and females are 60 kg and 55 kg, respectively.

**Target hazard quotient.** THQ was used to access noncarcinogenic risks for residents (pods are skin lesions) through the consumption of rice wines contaminated with As. THQ value >1 indicates risks for humans.\(^{33}\) Otherwise, it is the opposite. The THQ was calculated using equation (2):

\[
\text{THQ} = \text{EDI} / \text{BMDL}_{0.5} \quad \text{(skin lesions)} \quad \text{(2)}
\]

MOE is an index applied to evaluate carcinogenic risk. BMDL\(_{0.5}\) was primarily used instead of the provisional tolerance week intake (PTWI, 15 μg/kg bw/week) in the calculation due to the revocation of PTWI by JECFA in 2010.\(^{34}\) BMDL\(_{0.5}\), the benchmark dose levels (BMDL) for 0.5%, increased risk of lung cancer. According to JECFA, the lower limit of BMDL\(_{0.5}\) induced by As was 3–5 μg/kg bw, whereas 3 μg/kg bw was adopted here. MOE \(\geq 100\) indicates no carcinogenic risk for humans and MOE \(\leq 100\) implies a lower exposure risk. The MOE was calculated following equation (3):

\[
\text{MOE} = \text{BMDL}_{0.5} / \text{EDI} \quad \text{(lung cancer)} \quad \text{(3)}
\]

**Exposure assessment model.** The dietary exposure to As\(_i\) was calculated depending on model construction theories and the Monte Carlo method and bootstrap values. The mean values and percentiles (P97.5) for individual samples were obtained in this study.
Statistical analysis. Final experimental results were calculated using the statistical software SAS 9.2 (SAS, USA).

RESULTS AND DISCUSSION

Concentrations of As species in Rice and Rice Wine Samples.

Rice is the main material in rice wine production and contains starch, protein, fat, and cellulose. These basic components are responsible for the aroma and taste of rice wine. To better investigate the alteration of As concentration during the fermentation, 203 rice samples were analyzed from the same Chinese province from where the rice wine samples were collected. The total As concentration was 65.8 μg kg⁻¹ (Table 2). MMA was not detected in any rice sample. The average concentrations of As⁰ and DMA were 52.6 and 13.5 μg kg⁻¹, respectively. The As⁰ and DMA contributed to 79.9% and 20.5% of total As concentration, respectively.

The average total As concentration in rice wine samples was 14.6 μg L⁻¹ and MMA and AsC were not detected in any sample. The As (sum of As (III) and As(V)) contributed to 69.1% of total As concentration, and the average As in the rice wine was 10.1 μg L⁻¹ (4.43–24.0 μg L⁻¹), reflecting the presence of As in rice grain. The average concentrations of As (III), As(V), DMA and AsB were 2.86 μg L⁻¹ (0.970–6.08 μg L⁻¹), 7.22 μg L⁻¹ (2.24–22.9 μg L⁻¹), 3.92 μg L⁻¹ (1.58–7.82 μg L⁻¹), and 0.620 μg L⁻¹ (ND-0.950 μg L⁻¹), respectively (Table 2). As(V) was the largest contributor to the total As concentration (49.5% of total As), followed by DMA (27.4% of total As), As (III) (19.6% of total As), and AsB (4.51% of total As). In addition, DMA was the predominant organic As species and accounted for 86.3% of organic As.

As shown in Table 2, the average concentrations of total As and As in rice wine samples were lower than in rice samples, which may be due to the dilution during wine production. Aguilar et al. found that the total As concentration in rosé and red wines declined during the fermentation and maceration stages. However, the proportion of organic As increased after fermentation. In particular, AsB was detected in rice wines, which accounted for 4.51% of the total As concentration. Although the DMA concentration in rice wine was less than that in rice, the proportion was the opposite. This suggests that As methylation occurred during fermentation, which might be due to As metabolism by microorganisms (e.g., yeasts, fungi, and bacteria) present in the medium. Lu et al. found 10 bacterial genera in Shaoxing rice wine; Bacillus and Lactobacillus accounted for the largest proportion. Bacteria and fungi (28 genera of bacteria and 13 of fungi) were detected in Hong Qu glutinous rice wine, including Bacillus ginsengiihi, Pantoecia sp., Monascus purpureus, and Aspergillus niger.

Besides, yeasts used to produce alcohol exists in rice wine diffusely, like Saccharomyces cerevisiae, Pichia sp. and Trichosporon sp. Yeast was found to be able to methylate inorganic As into organic As in soil environment. Zeng et al. found that As (III) might be oxidated and methylated by some fungi (e.g., Trichoderma asperellum SM-12F1, Penicillium janthinellum SM-12F4, and Fusarium oxysporum CZ-8F1) and generate As(V), MMA, and DMA. The conversion of As species is also related to temperature and pH during the fermentation process. Vriens et al. testified that the methylation of As depends on the temperature. The production of rice wine is carried out in an open environment. As the temperature of surface water and air increases, the rate of methylation increases. At high pH, the organic mass, moderate moisture, and temperature contribute to biotransformation mediated by As. Meanwhile, the pH and redox potential are affected by the quantity of heat released from the microbial metabolism, which influences As speciation.

Exposure estimate

The study population was divided according to their habits of drinking rice wine: general population (all consumers ≥18 years old) and drinking population. Each of these groups was further divided by gender (male/female) and further by age (18–44, 45–59, and ≥60). As seen from Fig. 2 and Table S1, the average

| Table 2. Mean Concentration and Proportion of As species in 79 Rice Wine and 203 Rice Samples |
|-------------------------------------------------|-----------------|-----------------|----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Sample  | Concentration and Proportion | AsB  | AsC  | MMA  | DMA  | As(III) | As(V) | As  | Total  |
| Rice wine | Concentration (μg L⁻¹) | 0.620 | ND* | ND  | 3.92 | 2.86 | 7.22 | 10.1 | 14.6 |
|          | Proportion (%)         | 4.51  | 0.00 | 0.00 | 27.4 | 19.6 | 49.5 | 69.1 | —   |
| Rice    | Concentration (μg kg⁻¹) | —     | —    | ND  | 13.5 | —    | —    | 52.6 | 65.8 |
|          | Proportion (%)         | —     | —    | 0.00 | 20.5 | —    | —    | 79.9 | —   |

* ND: not detected.
consumption of rice wine by all consumers and drinkers in five provinces were 7.40 and 250 mL day\(^{-1}\) (mL d\(^{-1}\)), respectively. Among the general population, the mean consumption by males was higher than that for females, which accounted for 14.4 and 1.10 mL d\(^{-1}\), respectively. The group with the highest rice wine consumption was that of adults between 45 and 59 years of age, and the group with the lowest consumption consisted of adults between 18 and 44 years. The total average consumption by drinkers was 255 mL d\(^{-1}\); an average of 273 mL d\(^{-1}\) for males and 142 mL d\(^{-1}\) for females. The highest consumption was by people between 45 and 59 years of age, and the minimum consumption was by the 60+ age group.

The average concentration of As\(_i\) was taken as the mean intake for dietary exposure assessment, and the estimated daily intakes per body weight of the different groups were obtained. The percentile (P97.5 in this study) was drawn to determine the intake for dietary exposure assessment, and the estimated daily intakes for any group did not exceed P97.5 indicated that the estimated highest exposure intake dose by male and female consumers was 5.12 \(\times 10^{-3}\) and 3.91 \(\times 10^{-4}\) μg/kg bw/d by males and females, respectively. Among drinkers, the indices for males and females were 0.0970 and 0.0550 μg/kg bw/d, noticeably greater than those for all consumers.

### Evaluation of noncarcinogenic and carcinogenic risk

THQ and MOE have been considered the main indices to assess the noncarcinogenic and carcinogenic risks of ingested As\(_i\) in daily rice wine consumption. For THQ calculation, it was assumed that the absorbed dose of As\(_i\) was equal to the intake dose. PTWI (15 μg/kg bw/week) was the denominator in the formula. In some studies\(^{38}\) it has been found that skin lesions, lung cancer and urinary bladder cancer may rise when humans are exposed to As\(_i\) 15 μg/kg bw/week or less. These findings resulted in the withdraw of PTWI by JECFA in 2010\(^{39}\). Instead, BMDL\(_{0.5}\) (5.4 μg/kg bw/d) for skin lesions was used to estimate the As\(_i\) toxicity. For MOE, the BMDL\(_{0.5}\) of for lung cancer was lower than for urinary bladder cancer, so the BMDL\(_{0.5}\) lung cancer (3.0-5.0 μg/kg bw/d) was considered to calculate MOE according to U.S. Environmental Protection Agency (USEPA)\(^{40}\). Thus, the lowest of daily dose (3.0 μg/kg bw/d) of As\(_i\) was adopted in this study.

As shown in Table 3, the THQs for the general population were less than 1, indicating no noncarcinogenic risk (skin lesions in this case) to people due to rice wine consumption.\(^{41}\) However, because of the higher rice wine consumption, the THQ values for males were higher than those females in each gender group; P97.5 for any group did not exceed 1. These results indicated that the hazard of As\(_i\) exposure was permissible. The average MOE values for the general population, both males and females, were 1.24 \(\times 10^{-3}\) (1.19 \(\times 10^{-3}\)-1.53 \(\times 10^{-3}\)) and 1.49 \(\times 10^{-4}\) (1.06 \(\times 10^{-4}\)-2.41 \(\times 10^{-4}\)), respectively; they were 65.5 (62.1-75.1) and 115 (97.1-143) for male and female drinkers, respectively (Table 4). For the general population, all MOE values were > 100, demonstrating no carcinogenic risk for people in general. Nevertheless, among drinkers, the MOE values for males and those for females in the 45–59 age group were < 100, suggesting a potential carcinogenic risk (lung cancer) due to As\(_i\) intake. P97.5 for all age groups of female drinkers was lower than 100 (45.9-67.4), indicating a considerable proportion of females were exposed to the risk of lung cancer. From Table 4, it can be deduced that the exposure risk is related to the consumption of rice wine by drinkers; the higher the consumption of rice wine, the greater the carcinogenic risk. For the 45–59 age group, a higher carcinogenic risk was observed than for any other age group; this is related to Chinese drinking habits and features. The MOE values indicated that a large intake of rice wine might increase the carcinogenic risk; hence it should be noted by the people.

### Table 3. Dietary Exposures of As\(_i\) in Rice Wine for Different Age and Gender Groups (THQ for Noncarcinogenic Effects)

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>All people</th>
<th>Drinker</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>18-44</td>
<td>6.55 (\times 10^{-4})</td>
<td>1.39 (\times 10^{-4})</td>
</tr>
<tr>
<td>45-59</td>
<td>6.66 (\times 10^{-4})</td>
<td>1.41 (\times 10^{-4})</td>
</tr>
<tr>
<td>60-</td>
<td>8.40 (\times 10^{-4})</td>
<td>1.78 (\times 10^{-4})</td>
</tr>
<tr>
<td>Sum</td>
<td>8.06 (\times 10^{-4})</td>
<td>1.71 (\times 10^{-4})</td>
</tr>
</tbody>
</table>

### Table 4. Dietary Exposures to As\(_i\) in Rice Wine for Different Age and Gender Groups (MOE for Lung Cancer)

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>General population</th>
<th>Drinker</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>18-44</td>
<td>1.53 (\times 10^{-1})</td>
<td>721</td>
</tr>
<tr>
<td>45-59</td>
<td>1.50 (\times 10^{-1})</td>
<td>709</td>
</tr>
<tr>
<td>60-</td>
<td>1.19 (\times 10^{-1})</td>
<td>563</td>
</tr>
<tr>
<td>Sum</td>
<td>1.24 (\times 10^{-1})</td>
<td>586</td>
</tr>
</tbody>
</table>
compare the risks due to As exposure, the total As and As species concentration in wines from different countries are summarized in Table 5, which shows that As$_i$ was detected in wine. The As concentration in wines procured from the United States was higher than those from China and central Europe, indicating that the consumption of these wines could cause cancer. However, the total As concentration ($\leq 56.0$ μg L$^{-1}$) in all wines was lower than the regulated limit of 100 and 200 μg L$^{-1}$ in Canada and OIV, respectively. The total As concentration and As species in different wine types varied from one country to another, and As$_i$ was in general higher than organic As (Table 5).

### CONCLUSIONS

The total As and As species concentration found in rice wine and rice samples varied. The concentration of As$_i$ decreased after fermentation; AsB was detected in rice wine, while As$_i$ was the predominant As species in all samples. The results of THQ showed no significant noncarcinogenic risk (skin lesions) to local rice wine-consumers. The MOE values < 100 demonstrated that local people might be exposed to carcinogenic risk (lung cancer) due to the consumption of rice wine. However, the amount of As$_i$ ingested did not exceed 200 μg L$^{-1}$, as permitted by OIV. In addition, this study emphasized the risks of excessive rice wine consumption; the whole intake of As$_i$ should be considered.

### ASSOCIATED CONTENT

Please contact the corresponding author for the Supporting Information (Table S1).

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### ACKNOWLEDGMENTS

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### REFERENCES


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Table 5. Reported Concentrations of Total As and As Species in Wines from Different Countries

<table>
<thead>
<tr>
<th>Country</th>
<th>Type</th>
<th>n</th>
<th>Total As, μg L$^{-1}$</th>
<th>Inorganic As (μg L$^{-1}$)</th>
<th>DMA (μg L$^{-1}$)</th>
<th>MMA (μg L$^{-1}$)</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Mean (range)</td>
<td>Mean (range)</td>
<td>Mean (range)</td>
<td>Mean (range)</td>
<td></td>
</tr>
<tr>
<td>United States</td>
<td>Red wine</td>
<td>46</td>
<td>6.76 (0.32-23.2)</td>
<td>6.12 (0.40-20.5)</td>
<td>0.55 (0.02-2.66)</td>
<td>0.09 (0.08-0.17)</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>White wine</td>
<td>26</td>
<td>22.6 (1.52-42.5)</td>
<td>9.50 (0.570-30.4)</td>
<td>0.82 (0.42-1.87)</td>
<td>0.17 (0.08-0.47)</td>
<td></td>
</tr>
<tr>
<td>United States</td>
<td>Rosé</td>
<td>NA</td>
<td>16.7</td>
<td>16.0</td>
<td>0.72</td>
<td>ND</td>
<td>43</td>
</tr>
<tr>
<td></td>
<td>White wine</td>
<td>NA</td>
<td>12.1</td>
<td>11.4</td>
<td>0.72</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Red wine</td>
<td>NA</td>
<td>2.20</td>
<td>1.70</td>
<td>0.47</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Spain</td>
<td>Grape juice wine</td>
<td>15</td>
<td>7.07 (4.00-11.3)</td>
<td>1.80 (0.60-8.80)</td>
<td>3.64 (1.3-8.3)</td>
<td>1.63 (1.50-2.50)</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>White wine</td>
<td>15</td>
<td>9.71 (4.60-14.0)</td>
<td>1.81 (0.60-4.59)</td>
<td>4.82 (0.7-10.4)</td>
<td>3.08 (1.50-8.10)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sherry wines</td>
<td>15</td>
<td>11.0 (2.00-15.1)</td>
<td>1.71 (0.60-4.00)</td>
<td>4.35 (0.70-15.1)</td>
<td>4.92 (1.50-9.60)</td>
<td></td>
</tr>
<tr>
<td>China</td>
<td>Rice wine</td>
<td>79</td>
<td>14.6 (11.7-24.7)</td>
<td>10.1 (4.43-24.0)</td>
<td>3.92 (1.58-7.82)</td>
<td>ND</td>
<td>This study</td>
</tr>
<tr>
<td>Argentina/Brazil</td>
<td>White wine</td>
<td>14</td>
<td>25.7 (ND)</td>
<td>ND (2.90-28.1)</td>
<td>NA$^a$ (0.456-1.07)</td>
<td>ND</td>
<td>11</td>
</tr>
<tr>
<td>Chile</td>
<td>Central Europe $^+$</td>
<td>7</td>
<td>5.92 (3.74-7.32)</td>
<td>4.54 (1.10-6.76)</td>
<td>0.57 (2.08-3.90)</td>
<td>ND (ND-0.44)</td>
<td>44</td>
</tr>
<tr>
<td></td>
<td>Grape juice wine</td>
<td>39</td>
<td>4.99 (0.83-21.0)</td>
<td>4.82 (ND-22.0)</td>
<td>0.16 (ND-0.600)</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td></td>
<td>White wine</td>
<td>29</td>
<td>3.95 (0.46-15.7)</td>
<td>3.79 (ND-16.1)</td>
<td>ND (ND-3.95)</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Red wine</td>
<td>6</td>
<td>9.59 (7.28-12.5)</td>
<td>8.99 (0.740-11.3)</td>
<td>0.17 (ND-1.20)</td>
<td>ND (ND-0.72)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Late harvest wine</td>
<td>6</td>
<td>11.1 (7.94-18.8)</td>
<td>10.22 (0.50-16.5)</td>
<td>0.14 (ND-0.55)</td>
<td>0.640 (ND-1.41)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ice wine</td>
<td>9</td>
<td>1.88 (0.63-6.07)</td>
<td>1.10 (ND-6.21)</td>
<td>0.07 (ND-1.25)</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>13 countries $^+$</td>
<td>Red wine</td>
<td>147</td>
<td>4.00 (&lt; 0.1-56.0)</td>
<td>NA ($&lt; 0.200-10.7$)</td>
<td>NA$^a$ (0.456-1.07)</td>
<td>NA (0.30-1.01)</td>
<td>32</td>
</tr>
</tbody>
</table>

* ND: not detected; $^+$ NA: not analysis; $^+$ German, Austria, Switzerland; $^+$ Wine collected from France, Spain, Slovenia, Italy, Greece, Morocco, South Africa, Tunisia, China, Australia, Chile, Argentina, and the United States.
Protected Geographical Identification of Honey by Spark Discharge-assisted Laser-induced Breakdown Spectroscopy

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ABSTRACT: Honey is a natural food that is valued worldwide for its nutritional and therapeutic values. Therefore, authentication of honey according to the geographical origin is a guarantee of the genuine properties. In this article, an evaluation of spark discharge-assisted laser-induced breakdown spectroscopy (SD-LIBS) for certification of the geographical origin of honey is reported. Forty-nine samples of multifloral honey produced in four Argentine provinces were considered. The results showed the best classification performance was obtained using smoothing, generalized least squares weighting (GLSW) and mean centering for spectral preprocessing, added to the k-nearest neighbor (k-NN) or Support Vector Machine (SVM) classification algorithms, which provided 100% of correct classification. More importantly, the results of Partial Least Squares – Discriminant Analysis (PLS-DA) pointed to N, Ca, K, Cu, Fe and Mn as key elements for the certification of geographical origin. In addition, the greatest potential of N stands out for the discrimination of the origin of honey. These findings confirm SD-LIBS as a promising tool for authentication of honey quality, providing a simple, fast and environmentally friendly solution. The method can be useful for industry, the market and others related to food authenticity.

INTRODUCTION

Consumers’ lifestyle inquires certificates to prove genuine characteristics of food. Some differentiated foods are labeled by protected geographical identification (PGI) and protected designation of origin (PGO) that requires a premium price for products. Food fraud covers cases where there is a violation of food law, which is intentionally committed to obtain a financial gain through consumer deception. 1

Honey is the third most adulterated food in the world. 2 Produced by bees from the nectar of plants, honey is a naturally sweet food valued worldwide for its nutritional and therapeutic values. The properties mentioned are intrinsically related to the geographical origin of honey. 3,4 Therefore, authentication of honey according to its origin is an important requirement that demands reliable, fast and reproducible analytical methods.

The method traditionally used to determine botanical and geographical origin of honey is the pollen analysis which reflects the type of vegetation from which the nectar was collected by the bees. 5 The method mentioned is time-consuming and requires great skill and technical experience in pollen morphology. 6 In addition, if honey undergoes a filtration process this type of
In order to simplify the determination of the origin of the honey, methods based on the elemental analysis of the honey composition have been proposed. These methods use analytical techniques, such as atomic absorption spectrometry (AAS), inductively coupled plasma optical emission spectroscopy (ICP-OES) and inductively coupled plasma mass spectrometry (ICP-MS) combined with multivariate data analysis. The disadvantages of the aforementioned techniques are the high cost per analysis, since they require gases and high-purity reagents, in addition to the time for the sample pretreatment. Particularly for honey samples, analytical difficulties may arise due to its high carbohydrates content, which influence the performance of the mentioned analytical techniques. Sample pretreatment by wet digestion using concentrated acid and heating or dry ashing followed by ash dissolution in concentrated nitric acid are time-consuming and costly procedures besides not being environmentally friendly. In contrast, the direct analysis of diluted samples using techniques that depend on the nebulization of the sample, such as flame atomic absorption spectrometry (FAAS), ICP-OES and ICP-MS is critically affected by the effects of transport. Considering these disadvantages, direct analysis techniques such as Raman, infrared and some sensors have been explored for classification of honey. However, the elemental composition of the samples cannot be accessed by these techniques.

Laser-induced breakdown spectroscopy (LIBS) is an analytical technique capable of performing direct and fast multielement analysis with minimal or no sample preparation, without the use of chemical consumables, such as solvents and gases. LIBS is based on the measurements of atomic and ionic emission of elemental sample constituents excited in a plasma. A single LIBS analysis takes a few seconds to perform. In addition to the elemental analysis, the correlation between spectral fingerprint and other samples properties is also possible. Due to its potential, LIBS has been successfully applied to detect food fraud. Despite the attractive analytical characteristics, LIBS shows low detectability, which makes some types of application difficult. For this reason, some strategies have been developed to improve the sensitivity of the LIBS, such as the spark discharge-assisted LIBS (SD-LIBS), which increases emission intensities by reheating the plasma.

Recently, Zhao et al. (2020) achieved good results for the classification of honey according to geographical origin. The authors used a LIBS system composed by a high-energy laser operating at 532 nm, a high-resolution spectrometer and an intensified detector. In contrast, low-cost LIBS systems have received great attention and expanding the applicability of the technique. The low sensitivity and spectral resolution of these instruments have been circumvented using some simple devices to increase sensitivity and applying different spectral processing to extract the appropriate analytical information.

In this work, a low-cost LIBS system coupled with a spark-discharge (SD) for authentication of geographical origin of honey was evaluated, taking advantage of the speed and reliability of the LIBS and aiming to provide an accessible device for authentication and traceability of honey.

**EXPERIMENTAL**

**Samples.** For this study, forty-nine samples of multifloral honey collected in harvesting season between 2015 and 2016, from four provinces of the Northeast region of Argentina (Fig. 1), were used. The extraction and mixing of honey were carried out in an extraction room authorized by the National Service for Agri-food Health and Quality. Each sample corresponded to a composite sample, prepared from the mixture of samples extracted from ten beehives. The use of composite sample was chosen because it provides an unbiased estimation of the population average. Thus, eleven composite samples were obtained from the province of Formosa (F), ten from Chaco (CH), fourteen from Corrientes (C), and fourteen from Misiones (M). The honey samples were stored in polypropylene flasks at room temperature until analysis.

**LIBS analysis.** The spectra were acquired using a LIBS system designed for direct analysis of solids, which is equipped with a Q-switched laser Nd:YAG 1064 nm Big Sky Ultra 50 (Quantel, Co., Bozeman, MT, USA), an optical fiber bundle, and four spectrometers HR2000+ (Ocean Optics Co., Dunedin, FL, USA), featuring an optical resolution of 0.1 nm (full width at half maximum) and a spectral range from 200 nm to 630 nm. The laser...
the sample holder of LIBS system, which can be moved in the x-y directions. The sampling chamber is equipped with video camera to monitor the analyses. A spark discharge device was coupled with LIBS to increase the detectability of emission lines. The spark discharge was obtained using two cylindrical pure tungsten electrodes fixing 4 mm between them and 2 mm above the sample surface. The DC voltage signal was 4300 V. More details on the electric discharge system can be found in Vieira et al. (2018). Twenty spectra were measured for each sample by spreading lasers pulses on the surface of the sample. 

Chemometric analysis. All chemometric analysis were performed using MATLAB 2013a (MathWorks Inc., Natick, MA, USA) with PLS toolbox 7.3.1. (Eigenvector Research Inc., WA, USA). The spectral profile of all samples was first evaluated to detect outliers. Spectra showing an anomalous profile, evidenced by the absence of emission signals in any wavelength range, were discarded. Thereafter, each individual spectrum was processed by Whittaker filter for baseline fitting and multiplicative scatter correction (MSC) to normalize the effects of fluctuations, which is common in the LIBS analysis. A principal component analysis (PCA) was performed using each preprocessed spectrum. The spectrum that showed scores values beyond the confidence limits (95% level) in the first principal component was excluded. Finally, the spectra corresponding to each sample were averaged. Afterward, the spectrum set was divided into subsets for calibration and validation: two-thirds of the samples were considered for calibration (9 C, 7 CH, 7 F, and 9 M), and one-third for external validation (5 C, 3 CH, 4 F, and 5 M).

Three methods of classification were evaluated, Partial Least Squares Discriminant Analysis (PLS-DA), k-nearest neighbor (k-NN), and Support Vector Machine (SVM). The number of latent variables (LV) used in the PLS-DA model and the number of nearest neighbors (k) were chosen according to the number of correct classifications of the calibration samples during cross-validation. The SVM model was developed using the radial basis kernel type. The values of the SVM parameters (ν and γ) were automatically optimized during cross-validation. The SVMs used for binary classification within a multiclass strategy were based on 0 and 1, with a threshold of 0.5.

The classification methods described were evaluated in combination with the following preprocessing techniques: baseline correction by Whittaker filter, first and second derivatives (15 variables per window), Savitzky-Golay smoothing, MSC by mean, MSC by median, normalization by area, Standard Normal Variate (SNV), Pareto scaling, Poisson scaling, generalized least squares weighting (GLSW), and external parameter orthogonalization (EPO). After the application of each preprocessing, the data were mean centered or autoscaled.

RESULTS AND DISCUSSION

The experimental data were first submitted to the removal of outliers. Thereafter, the remaining spectra were evaluated by PCA, and the scores plot (PC1 versus samples) was used as a control chart. Spectra showing scores values out of the confidence limits (95% level) were excluded. The observed outliers may be due to the honey meniscus formed in the sampling flask, which provided different laser focal distance in relation to sample surface, considering the center and the edges of the flask. These differences led to the exclusion of 19% of the spectra. Therefore, the replicates for each sample ranged from 5 to 20. The useful spectra from each sample were adjusted to the baseline, normalized, and averaged.

The classification models were developed using the average spectra of each sample. The first step of the modeling was to determine the most suitable spectral preprocessing for each classification method (PLS-DA, k-NN and SVM). The results of correct classifications for thirteen preprocessing strategies and 30 combinations are shown in Table 1. The mean centering does not influence k-NN and SVM modeling, so this preprocessing was only maintained by convention. Consequently, autoscaling had the same effect as variance scaling using these methods. The combination of smoothing, GLSW, and mean centering provided the highest number of correct classifications for the three methods performed. Since the GLSW attenuates spectral variables that vary in the same class, variables correlated to the classes present greater weight in the modeling. Therefore, this preprocessing was the most important to provide correct classifications. In addition, the Savitzky-Golay smoothing reduced the spectral noise, made emission peaks more defined, and increased model fit. The three methods evaluated for honey classification were able to separate the four classes: the k-NN (k=3) and SVM (ν=0.5, γ=10^-6) models provided 100% correct classification for the external validation set, and PLS-DA (9 LV) model provided 94% of correct classification (Table 1).

SVM and k-NN methods do not allow a visualization of the relationship between spectral variables and the class clusters. Thus, the PLS-DA method was used for such visualization and interpretation. The scores plot (Fig. 2) shows the separation of the four honey classes in the first three LVs. The PLS-DA model classified correctly all validation samples, except for a F sample, which was classified as region C honey.

The correlation between the spectral variables and the honey classes was assessed using loading and scores values. This analysis reveals that M samples were separated by the 1st LV (positive values), the CH samples by the 2nd LV (negative values), and the C and F samples were separated by the 3rd LV (C with negative
Table 1. Evaluation of Preprocessing Strategy for Each Classification Method

<table>
<thead>
<tr>
<th>Preprocessing</th>
<th>PLS-DA Cal</th>
<th>PLS-DA Val</th>
<th>k-NN Cal</th>
<th>k-NN Val</th>
<th>SVM Cal</th>
<th>SVM Val</th>
</tr>
</thead>
<tbody>
<tr>
<td>MC</td>
<td>87%</td>
<td>46%</td>
<td>33%</td>
<td>57%</td>
<td>46%</td>
<td>45%</td>
</tr>
<tr>
<td>BC+MC</td>
<td>84%</td>
<td>46%</td>
<td>33%</td>
<td>57%</td>
<td>64%</td>
<td>53%</td>
</tr>
<tr>
<td>1st der+MC</td>
<td>91%</td>
<td>60%</td>
<td>32%</td>
<td>41%</td>
<td>28%</td>
<td>40%</td>
</tr>
<tr>
<td>2nd der+MC</td>
<td>87%</td>
<td>60%</td>
<td>29%</td>
<td>26%</td>
<td>36%</td>
<td>58%</td>
</tr>
<tr>
<td>Sm+MC</td>
<td>97%</td>
<td>78%</td>
<td>37%</td>
<td>46%</td>
<td>71%</td>
<td>64%</td>
</tr>
<tr>
<td>Sm+1st der+MC</td>
<td>94%</td>
<td>75%</td>
<td>42%</td>
<td>46%</td>
<td>50%</td>
<td>50%</td>
</tr>
<tr>
<td>Sm+2nd der+MC</td>
<td>91%</td>
<td>55%</td>
<td>37%</td>
<td>52%</td>
<td>36%</td>
<td>40%</td>
</tr>
<tr>
<td>MSC(mean)+MC</td>
<td>88%</td>
<td>28%</td>
<td>17%</td>
<td>31%</td>
<td>0%</td>
<td>25%</td>
</tr>
<tr>
<td>MSC(median)+MC</td>
<td>88%</td>
<td>52%</td>
<td>31%</td>
<td>33%</td>
<td>53%</td>
<td>36%</td>
</tr>
<tr>
<td>Norm.+MC</td>
<td>94%</td>
<td>47%</td>
<td>21%</td>
<td>37%</td>
<td>25%</td>
<td>25%</td>
</tr>
<tr>
<td>SNV+MC</td>
<td>88%</td>
<td>23%</td>
<td>17%</td>
<td>31%</td>
<td>17%</td>
<td>20%</td>
</tr>
<tr>
<td>GLSW+MC</td>
<td>100%</td>
<td>89%</td>
<td>100%</td>
<td>95%</td>
<td>100%</td>
<td>89%</td>
</tr>
<tr>
<td>Sm+GLSW+MC</td>
<td>100%</td>
<td>94%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>EPO+MC</td>
<td>100%</td>
<td>59%</td>
<td>42%</td>
<td>56%</td>
<td>33%</td>
<td>35%</td>
</tr>
<tr>
<td>Pareto Scaling+MC</td>
<td>100%</td>
<td>88%</td>
<td>24%</td>
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<td>61%</td>
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<tr>
<td>Poisson Scaling+MC</td>
<td>100%</td>
<td>69%</td>
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<td>40%</td>
</tr>
<tr>
<td>AS</td>
<td>100%</td>
<td>71%</td>
<td>37%</td>
<td>40%</td>
<td>64%</td>
<td>62%</td>
</tr>
<tr>
<td>BC+AS</td>
<td>100%</td>
<td>74%</td>
<td>32%</td>
<td>23%</td>
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<td>20%</td>
</tr>
<tr>
<td>1st der+AS</td>
<td>100%</td>
<td>69%</td>
<td>26%</td>
<td>41%</td>
<td>25%</td>
<td>45%</td>
</tr>
<tr>
<td>2nd der+AS</td>
<td>100%</td>
<td>74%</td>
<td>32%</td>
<td>51%</td>
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<td>15%</td>
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<tr>
<td>Sm+AS</td>
<td>100%</td>
<td>88%</td>
<td>29%</td>
<td>30%</td>
<td>54%</td>
<td>58%</td>
</tr>
<tr>
<td>Sm+1st der+AS</td>
<td>100%</td>
<td>69%</td>
<td>33%</td>
<td>51%</td>
<td>25%</td>
<td>50%</td>
</tr>
<tr>
<td>Sm+2nd der+AS</td>
<td>100%</td>
<td>69%</td>
<td>33%</td>
<td>46%</td>
<td>36%</td>
<td>45%</td>
</tr>
<tr>
<td>MSC(mean)+MC</td>
<td>100%</td>
<td>68%</td>
<td>14%</td>
<td>15%</td>
<td>0%</td>
<td>11%</td>
</tr>
<tr>
<td>MSC(median)+MC</td>
<td>100%</td>
<td>46%</td>
<td>17%</td>
<td>45%</td>
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<tr>
<td>Norm+AS</td>
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<tr>
<td>SNV+AS</td>
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<td>17%</td>
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<td>48%</td>
<td>100%</td>
<td>66%</td>
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<tr>
<td>Sm+GLSW+AS</td>
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<td>51%</td>
<td>100%</td>
<td>88%</td>
<td>100%</td>
<td>94%</td>
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<tr>
<td>EPO+AS</td>
<td>100%</td>
<td>74%</td>
<td>41%</td>
<td>50%</td>
<td>53%</td>
<td>43%</td>
</tr>
</tbody>
</table>

Note: Calibration set (Cal), external validation set (Val), mean centering (MC), baseline correction by Whittaker filter (BC), first derivative (1st der), second derivative (2nd der), smoothing (Sm), multiplicative scatter correction (MSC), normalization by area (Norm.), standard normal variate (SNV), generalized least squares weighting (GLSW), external parameter orthogonalization (EPO), and autoscaling (AS).

Fig. 2 Score plot of the PLS-DA model (only validation samples).

and F with positive values). The score plot also shows a greater separation of class M from the others, while classes C and F were more similar. These results corroborate the elemental composition of samples (Table 2), obtained by assigning spectral emission lines according to the NIST LIBS database. A typical LIBS spectrum of a honey sample showing the emission lines that influenced class discrimination is shown in Fig. 3.

Class M samples had a relatively lower N content, while C and F samples showed the relatively higher content of this element, suggesting that C and F honeys have a high protein content in contrast to M honey. In addition, the C samples showed the relatively higher Ca content, resulting in the separation of this class by the 3rd LV of the model, and the medium K and Fe content provided the separation of the F samples. The CH samples were
Table 2. Emission Lines Related to Each Class

<table>
<thead>
<tr>
<th>Class</th>
<th>Peak (nm)</th>
<th>NIST assignments</th>
<th>Intensity among classes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Misiones</td>
<td>499.5</td>
<td>N II (499.6 nm)</td>
<td>lowest</td>
</tr>
<tr>
<td></td>
<td>552.9</td>
<td>N I (553.0 nm)</td>
<td>lowest</td>
</tr>
<tr>
<td></td>
<td>553.5</td>
<td>N II (553.5 nm)</td>
<td>lowest</td>
</tr>
<tr>
<td></td>
<td>567.6</td>
<td>N II (567.6 nm)</td>
<td>lowest</td>
</tr>
<tr>
<td></td>
<td>568.5</td>
<td>N II (568.6 nm)</td>
<td>lowest</td>
</tr>
<tr>
<td></td>
<td>324.7</td>
<td>Cu I (324.7 nm)</td>
<td>highest</td>
</tr>
<tr>
<td>Chaco</td>
<td>404.1</td>
<td>Mn I (404.1 nm)</td>
<td>medium</td>
</tr>
<tr>
<td></td>
<td>434.7</td>
<td>Mn II (434.6 nm)</td>
<td>medium</td>
</tr>
<tr>
<td></td>
<td>396.9</td>
<td>Ca II (396.9 nm)</td>
<td>highest</td>
</tr>
<tr>
<td>Corrientes</td>
<td>422.8</td>
<td>Ca I (422.7 nm)</td>
<td>highest</td>
</tr>
<tr>
<td></td>
<td>466.2</td>
<td>N I (466.2 nm)</td>
<td>highest</td>
</tr>
<tr>
<td></td>
<td>404.2</td>
<td>K I (404.4 nm)</td>
<td>medium</td>
</tr>
<tr>
<td>Formosa</td>
<td>439.7</td>
<td>Fe II (439.8 nm)</td>
<td>medium</td>
</tr>
<tr>
<td></td>
<td>464.3</td>
<td>N II (464.3 nm)</td>
<td>highest</td>
</tr>
</tbody>
</table>

Fig. 3 Typical LIBS spectrum of a honey sample with emission line assignments related to the studied classes.

distinguished from the others by their relative higher levels of Cu and medium levels of Mn, and N did not show significant influence on segregation.

Three class of samples were separated according to the N content, suggesting that N plays a fundamental role in discriminating the geographical origin of honey. According to Imdorf et al., bees feed mainly on nectar and pollen, the latter being the main source of protein for the entire colony. Thus, the supply and quality of pollen, which directly influence the amount of N, could be related to the geographical production of honey. Furthermore, Fehner et al. using ICP-MS data from the same samples used here, obtained a classification method for geographical origin reaching 76% of prediction accuracy. These results suggest that the absence of N in the data set may decrease the accuracy of the predictions. SD-LIBS presented attractive features for authentication of honey in terms of cost and performance, in addition to the ability to measure N, which is not feasible with ICP-MS. Thus, the methods developed provide new horizons for honey quality analysis.

CONCLUSIONS

Authentication of geographical origin of Argentine honey was assessed using a low-cost SD-LIBS and chemometric tools. Adequate spectral preprocessing provided k-NN and SVM models capable of accurately classifying honey according to their production regions. The results suggested N as the most important element to discriminate the studied classes. Complementary contributions from Ca, K, Cu, Fe, and Mn were also important for geographical discrimination of honey. Considering the possibility of measuring N and the importance of this element for geographical discrimination of honey samples, the proposed methods place LIBS in a prominent position in comparison with the conventional atomic techniques generally used for geographical analysis of honey (e.g. FAAS, ICP-OES and ICP-MS). Furthermore, the use of SD-LIBS provides fast, clean and direct analysis, providing a low-cost device for the control of food quality.

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Notes
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Elemental Imaging of Alumina Ceramic Tube Using Laser Ablation-Inductively Coupled Plasma-Mass Spectrometry (LA-ICP-MS)

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ABSTRACT: Laser ablation-inductively coupled plasma-mass spectrometry (LA-ICP-MS) is an attractive analytical technique for the direct analysis of samples found in the geology, biology, and the environmental and material sciences. However, few reports have discussed the difficulty found in the analysis of curved surface samples by LA-ICP-MS where the main focus is on the curved surface. In this work, LA-ICP-MS was used to map the elemental images of curved surface samples by segmentation and recombination. In addition, the influence of parameters, such as laser spot size, laser fluence, repetition rate, scan speed, dwell time and washout time on lateral resolution were investigated. The developed method was applied to the imaging analysis of lanthanum in an alumina ceramic tube, and the results showed that lanthanum was not uniformly distributed in the tube.

INTRODUCTION

Alumina ceramics are widely used in the energy, aerospace, machinery, automobile, metallurgy, chemical industries, as well as in the electronics and other fields because of their excellent properties of high temperature resistance, wear resistance, corrosion resistance, erosion resistance, and oxidation resistance. In order to improve the mechanical, electrical, optical or thermal properties of alumina ceramics, additives are usually added to improve sintering, densification, microstructure and crystal phase composition of the materials, such as rare earth oxides. Fang et al. reduced the densification rate by doping Y$_2$O$_3$ and La$_2$O$_3$ in alumina. Yang et al. found that La$_2$O$_3$ could improve the densification degree and transmittance of transparent alumina ceramics. Wu et al. reported that the wear resistance of alumina ceramics containing La$_2$O$_3$ was 43% higher than without it. Therefore, the distribution of rare earth oxides in alumina has become a topic of concern. The electron probe microanalysis (EPMA) results showed that the rare earth oxides distribution at the grain boundary of alumina transparent ceramics was a non-equilibrium thermodynamic composition distribution. Thompson et al. studied the distribution of La$_2$O$_3$ and Y$_2$O$_3$ in polycrystalline alumina ceramics by secondary ion mass spectrometry (SIMS), and found that La$^{3+}$ and Y$^{3+}$ were mainly distributed at the grain boundary and on the surface of the pores. The above results showed that the elemental distribution had an important influence on the structure uniformity, which was also helpful to obtain better ceramics. Therefore, the uniformity of the elemental distribution is an important index to evaluate the properties of ceramics.

So far, many techniques for elemental distribution analysis were developed, mainly SIMS$^{4,8}$, laser ionization mass spectrometry (LIMS)$^{9}$, glow discharge mass spectrometry (GDMS)$^{10}$ and laser ablation inductively coupled plasma mass spectrometry (LA-ICPMS)$^{11,12}$. Compared with conventional solution-based analytical methods, direct solid analysis by mass spectrometry can provide more efficient analysis without a complicated sample pretreatment.

LA-ICP-MS has become an attractive analytical technique for the direct analysis of solid samples due to its direct analysis of solid samples, in-situ micro area and micro loss analysis, and high sensitivity and low detection limits of MS. It has been used for the determination of micro- and trace elements and the measurement of isotope ratios, as well as in microanalyses, in-depth profiling,
and elemental imaging. And imaging has been widely applied in many fields, including geology, biology, and the environmental and material sciences. However, most of the LA-ICP-MS imaging samples should be provided with a planar surface, and there were few reports on curved surface samples. This was mainly the “focus” required in LA-ICP-MS analysis, and the difference of laser focusing would cause laser energy fluctuation resulting in wrong imaging data.

In this work, LA-ICP-MS was used for elemental imaging of the curved surface samples by segmentation and recombination. In addition, the influence of parameters on the lateral resolution, an important index of imaging analysis, were investigated in detail. The developed method was applied to the imaging analysis of lanthanum in alumina ceramic tubes.

**EXPERIMENTAL**

A quadrupole-based X Series II ICP-MS system (Thermo Fisher, USA), combined with an NWR-213 laser ablation system (NewWave, USA), was used for imaging analysis. Table 1 summarized the typical experimental parameters. Helium, used as the carrier gas for cell purging, was mixed with argon when introduced into the plasma. The data were drawn into two-dimensional images by Iolite software (Iolite 4.0). The alumina ceramic tubes were supplied by Shengnuo Optoeletronic Technology (Qing Hai) Co., Ltd. The tube surface was cleaned with 2% HNO₃ and deionized water, dried, and then fixed in the sample cell with double-sided tape. When rotation of the sample was required, the sample cell needed to be opened, the position marked, and then rotated to the next section for ablation.

**RESULTS AND DISCUSSION**

**Optimization of LA-ICP-MS parameters**

The optimization process of the LA-ICP-MS imaging analysis conditions mainly includes: (i) minimum limits of detection; (ii) maximum spatial resolution; and (iii) minimum time of analysis. Spatial resolution is divided into horizontal resolution and vertical resolution, which refers to the size of two adjacent points that can be distinguished. In this work, the optimized spatial resolution refers to the lateral resolution.

**Laser ablation mode.** Elemental imaging can be completed by spot ablation mode or line ablation mode. For spot ablation, the X-Y stage moves to a location, ablates a few shots, then repeats in a new location until it covers the whole sample. Image blurring can be avoided by leaving enough waiting times between two spots, which are from microseconds to a few seconds (0.1–10 s, depending on the cell volume) to ensure total particulate washout from the cell. Günther et al. developed a low dispersion sample chamber (tube cell), which made an improvement in the imaging capabilities by reduction of the single LA shot duration to 30 ms (full width at 1% maximum). Vanhaecke et al. designed a low-dispersion small sample cell embedded in the cobalt sample chamber integrated into LA-ICP-MS and achieved a sub-micron spatial resolution. Under optimum conditions, single pulse responses with a full width at 10% of the maximum peak intensity (FW0.1M) of ~ 1 ms can be achieved. These low dispersion sample chambers made spot ablation a good choice. For line ablation, the X-Y stage moves continuously in a straight line. Compared with spot ablation, the analysis speed of line ablation is faster and the data processing is simpler, but the disadvantage is that the data are easily affected by the washout time of the sampling cell. Considering the influence of analysis speed and spatial resolution, the line ablation mode was chosen for imaging analysis in this experiment.

**Spot size.** Generally, spatial resolution is used to describe the number of pixels used to make up an image. In the same physical size, the more pixels, the higher spatial resolution of the image. Thus, the spot size had a big impact on spatial resolution. Typically, the imaging lateral resolution range for minerals, ceramics and other sample types were from 20 to 100 μm. Recent achievements in bio-imaging have led to improved lateral resolutions down to 1 μm. Small spatial resolution for biological samples is essential to obtain effective information on elemental distribution because the diameter of a single mammalian cell is about or less than 20 μm. However, low lateral resolution of micron scale is not extremely necessary for surface imaging of large bulk materials in centimeter scale to study the uniformity of elemental distribution, such as doped ceramics or crystal materials. Considering the character of the alumina ceramic tube, analysis time and spatial resolution, the spot size was set to 100 μm.

In addition, the laser fluence and the repetition rate affect the lateral resolution by increasing or decreasing the signal intensity. Laser fluence should be estimated to obtain enough signals based on the statistically different signal-noise ratio and the energy threshold of the samples. It is usually necessary to control excessive laser fluence to avoid abating the underlying glass slide in tissue imaging, which was of no concern in the ablation of the
Fig. 1 Al images were obtained at different dwell times (a) 1 ms; (b) 5 ms; (c) 10 ms; (d) 50 ms; (e) 100 ms. Laser spot size: 100 μm, repetition rate: 10 Hz, scan speed: 100 μm/s, and laser energy: 10%.

ceramic samples. The estimation results showed that the laser energy was greater than approximately 2 J/cm² (5% laser energy and 100 μm spot size), the signal and background of lanthanum were significantly different, and the degree of plasma ionization was high, which was obtained by determining the U/Th ratio in NIST 610 under the same conditions. The laser fluence of approximately 2.5 J/cm² (10% laser energy and 100 μm spot size) was chosen in this work. The laser repetition rate was also one of the important influencing factors. An increase in laser repetition rate resulting in denser ablation does not improve the spatial resolution of the images because there was not enough time interval to wash out the adjacent laser shot. At higher repetition rates, the signal overlap between adjacent shots will lead to image blurring. A low repetition rate may cause the laser shot not go through the sample completely, so the laser repetition rate will be determined according to the scan speed and washout time in this work.

Consequently, the trade-off among spatial resolution and sampling time of the elemental image of the alumina ceramic were investigated, including the parameters of the laser scan speed, dwell time and washout time.

**Washout time.** The aerosol generated by each laser shot takes time to traverse the cell, the tubing, the torch before being detected in the detector, known as washout time. The washout time was usually calculated from the transient signals as the time taken by the signal to fall to 1% of its peak value. Besides the sampling time and scan speed, a washout time of the ablation cell had great influence on the lateral resolution resulting in signal broadening. Doble’s group’s research showed that fast scan speeds were applicable for imaging experiments, making the measurements more time-efficient. However, the washout behavior of the used cell plays a significant role for the maximum obtainable scan speed and thus, image quality might suffer from blurring effects.

In this work, washout time as a function of different parameters (He flow rate, dwell time) was assessed by analyzing the Al signal in a commercial sampling cell. A steady decrease in washout times was observed with an increase in He flow rate from 600 mL/min (2.5 s) to 1000 mL/min (1.5 s). For the following experiments, the He flow rate was set at 1000 mL/min. Considering the relationship between dwell time and signal acquisition, dwell time was also investigated. The results (not given here) showed that dwell time has little effect on washout time, which fluctuated between 1.8 s and 2.0 s. Therefore, the washout time of a commercial sampling cell used in this experiment was 1.5 s - 2.5 s, which was comparable to routinely applied ablation cells. Despite the many efforts made, most of the routinely applied ablation cells have an experimental washout time in the order of a few seconds for a 99% intensity drop from the peak maxima. For only a few examples it was reported that the washout time was as short as a few milliseconds.

**Dwell time.** Quadrupole ICP-MS acquires data for an allocated mass-to-charge ratio (m/z) for a specified time period, or dwell time, before moving to the next m/z. This is continued until the data have been acquired for all designated m/z. This is continued until the data have been acquired for all designated m/z. Exactly, the quadrupole mass analyzer is not a simultaneous data collection technique, though it is able to rapidly acquire information for each
m/z. In addition, the dead time will produce when the m/z is switched, which is the reason that the acquisition time is not equal to the sum of all dwell time of m/z. Limiting signal experiments were performed using 5 dwell times for each measured mass (1, 5, 10, 50 and 100 ms) using 100 μm spot size, 100 μm/s scan speed, 10 Hz repetition rate and 10% laser energy. The results from Fig. 1 showed that the number of lateral pixels obtained at the dwell times of 1, 5, 10, 50 and 100 ms was about 60, 40, 30, 10 and 5 under the same laser ablation conditions, which was obtained by taking the dead time into account. Obviously, more element signal data were detected at a smaller dwell time for each laser spot, resulting in higher lateral resolution. The image with a dwell time of 1 ms has the image missing in Fig. 1e, which was due to the long dwell time resulting in data loss. The outline of images with dwell time of 5, 10 and 50 ms were similar.

Dwell time should be as short as possible but ensure good differentiation between signal and noise to avoid biased intensity. To meet the requirement of statistical difference, the minimum difference between the low error bar of the samples and the high error bar of the background was 0. The minimum dwell time can be calculated by the following formula:

\[
(I_{\text{sam}} - 3 \times \sigma \times I_{\text{sam}}) - (I_{\text{bkg}} + 3 \times \sigma \times I_{\text{bkg}}) \geq 0
\]

where \( I_{\text{sam}} \) and \( I_{\text{bkg}} \) were the intensity of the sample and the background, respectively. \( \sigma \) was the standard deviation of the signal counts not CPS. The minimum intensity of La was more than 500 and the background signal was less than 100 from test ablation. According to the above formula, the shortest dwell time was about 12 ms. Therefore, combined with the above experimental results, the dwell time chosen was 10 ms.

**Scan speed.** Shortening the analysis time is mainly determined by the laser scan speed. Kanicky found that the relative broadening of the images was related to enlarging the laser spot and increasing the scan speed as well, while the scan speed affects the broadening more strongly than the laser spot diameter. The scan speed was investigated in this work and the results are shown in Fig. 2. To make sure the laser hits at least once everywhere in the region of interest, the value of spot size multiplied by repetition rate should be greater than or equal to scan speed, that is, the scan speed should be less than 1000 μm/s in this work. Fig. 2d shows obvious color differences between the middle and the sides, which was due to the inconsistent focus. In addition, there was no obvious broadening at the scan speed of 50 – 200 μm/s as shown in Fig. 2a-c. It can be seen that the scan speed was higher than 400 μm/s, since the laser stage moved too fast to focus properly. Therefore, considering to shortened analysis time, the scan speed was chosen as 200 μm/s.

**Lanthanum imaging on alumina ceramic tube.**

The difficulty of elemental imaging on an alumina tube lies in the
multi-line focusing of non-planar surfaces. In this work, the La imaging of the alumina ceramic tube was established by segmentation and recombination under the optimized experimental parameters. The focusing range of the tube placed in a flat was investigated. It was found that the optimal range of the tube with an outer diameter of 0.68 cm used in this experiment was less than 1 mm. According to the formula of arc length and central angle, the semicircular tube to be imaged was segmented into 10 equal parts. After one-part is scanned, slowly rotate to the next until these 10 scans are completed in turn. Finally, the 10 images were recombined in order to form the La map of the aluminum oxide tube by Photoshop software (Fig. 3). The imaging results showed that lanthanum was not uniformly distributed in the alumina ceramic tube.

CONCLUSIONS

In this study, the influence of the parameters for laser spot size, laser fluence, repetition rate, scan speed, dwell time and washout time on the lateral resolution were investigated in detail. The elemental imaging method for the alumina ceramic tube by LA-ICP-MS was established by segmentation and recombination to obtain the optimal laser focus. The optimal focusing range of the tube with an outer diameter of 0.68 cm was determined to be 1 mm. Laser ablating of the tube was achieved by establishing 10 optimal focusing ranges of the tube, and then recombining every image to form the complete elemental maps of the tube. The lanthanum images of the alumina ceramic tube showed that lanthanum was not uniformly distributed in the tube. The results of this study show that LA-ICP-MS is a powerful imaging technology and will be widely used in archaeology and materials science. Future work will focus on rapid elemental imaging of irregularly shaped samples without going through segmentation and recombination.

REFERENCES


Determination of Trace Cd and Pb in Edible Salt and Soy Sauce by ETAAS Using Fluorescent Carbon Nanoparticles (FCNs) as Matrix Modifier

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ABSTRACT: Lead (Pb) and cadmium (Cd) are non-essential but extremely noxious metallic elements, and the study of their impact on environmental pollution is of utmost importance. In this report, an economical and environmentally friendly matrix modifier, fluorescent carbon nanoparticles (FCNs), is utilized for the electrothermal atomic absorption spectrometry (ETAAS) determination of trace Pb and Cd in edible salt and soy sauce. FCNs have been characterized for use with UV-Vis spectroscopy, fluorescence (FL), Fourier transform infrared spectrum (FT-IR) and transmission electronic microscopy (TEM). In comparison to traditional matrix modifiers, FCNs can effectively eliminate matrix interference. Using the proposed FCNs, the ETAAS method achieved a linearity of 10–50.0 μg L⁻¹ for Pb and 0.4–4.0 μg L⁻¹ for Cd; a limit of detection (LOD) for Pb in edible salt of 0.0140 mg kg⁻¹ and in soy sauce of 0.0470 mg kg⁻¹, and for Cd in edible salt of 0.0015 mg kg⁻¹ and in soy sauce of 0.0005 mg kg⁻¹. The method of additions chemical matrix modifier was used for Pb and Cd detection in this study. Excellent accuracy (93.0–101.0% recovery) and precision (0.19–0.85 %) of this procedure were obtained for soy sauce and edible salt. This work provides a new and economical strategy for the determination of trace Pb and Cd and is expected to facilitate future studies in the use of FCNs as a matrix modifier.

INTRODUCTION

Salt and high-salt foods with a relatively complicated production process, such as soy sauce and edible salt, may contain high levels of Pb and Cd when produced near Pb and Cd sources. Therefore, the accurate and rapid detection of trace Pb and Cd in high-salt foods is considered to be an important area for contamination studies. Establishing whether edible salt and high-salt foods are contaminated with Pb or Cd is important to properly assess their risk to humans. In China, the guideline values for Pb is set at 1.0 mg kg⁻¹ in condiments, in addition, the limit value of Pb are 2.0 mg kg⁻¹ and 3.0 mg kg⁻¹ in edible salt and fish sauce, and for Cd are set at 0.5 mg kg⁻¹ and 0.1 mg kg⁻¹ in salt and fish seasonings (GB 2762-2017), respectively. These levels of Pb or Cd are difficult to determine accurately by available analytical methods due to their low content and the NaCl interference effects in mass and/or spectral superposition.

A new, sensitive and reliable method for determining trace Pb and Cd levels in high-salt food samples is an important tool to identify and treat poisoning cases. At present, electrothermal atomic absorption spectrometry (ETAAS) and inductively coupled plasma mass spectrometry (ICP-MS) are the two main methods for trace elements determination in food testing and risk assessment laboratories. Although ICP-MS has high sensitivity for multi-elemental analysis, it has many disadvantages, such as high cost, risk of sample contamination, requires sophisticated operation and results in mass interferences. ETAAS may be more attractive for real sample analysis.

In general, the use of a matrix modifier allows high pyrolysis temperatures, thereby reducing or eliminating evaporation and vapor phase interferences and minimizing background signals. As
early as 1975, Ediger et al. reported Ni(NO₃): as a matrix modifier. At present, there are more than 60 conventional matrix modifiers reported for ETAAS analysis which can roughly be divided into organic reagents, inorganic reagents and reactive gases. Traditional matrix modifiers, a matrix technique eliminating interference, are used for routine analysis of trace Pb and Cd because of their simplicity and adaptability. However, some shortcomings, such as the use of Pd, make matrix modifiers expensive, non-environmentally friendly and not good for use with high-salt samples. With the development of matrix modifiers, some carbon-based matrix modifiers have emerged, including activated carbon, palladium-bearing activated carbon and multiwalled carbon nanotubes. FCNs are generally small oxygenous carbon-supported with good water solubility, low toxicity, high chemical stability and of low environmental hazard. In addition, FCNs are easy to prepare without heating, simply mix acetic acid, water with di-phosphorus pentoxide, and dissolve the mixture to obtain FCNs in minutes. Their use has been widely studied, but less as matrix modifiers.

In this study, FCNs are used as a new matrix modifier to improve the accuracy and precision of ETAAS determination of Pb and Cd in high-salt samples. The proposed FCNs assisted ETAAS method was evaluated for the analysis of soy sauce and edible salt samples.

### EXPERIMENTAL

**Instrumentation.** The UV-Vis absorption spectra were studied using a Cary 5000 spectrophotometer (Varian, USA). The fluorescence (FL) spectra were measured with a Cary Eclipse fluorescence spectrophotometer (Agilent, Australia). A FT-IR spectrum was obtained using the Varian 660-IR spectrometer in the range of 500–4000 cm⁻¹ (Varian, USA). Transmission Electron Microscopy (TEM) of the FCNs was obtained on a JEM-2100 microscope (Japan Electron Optics, Japan). The PnAAcłe 900Z atomic absorption spectrometer (AAS) (PerkinElmer, USA), equipped with furnace and coated graphite tube with integrated platform, including an AS-900 autosampler furnace autosampler, were used for the determination of Cd and Pb in high-salt food products (purchased at local supermarket). The system consists of an eight-lamp mount with a Pb hollow cathode lamp operated at 10.0 mA and a spectral bandwidth of 0.7 nm. The analytical wavelength of Pb was set at 283.31 nm and the analytical wavelength of Cd was set at 228.80 nm. Syngistix software was used for data integration and processing. Absorbance was calculated by the area of the atomic absorption peak and the peak position. The absorbance peak was adjusted to obtain maximum absorbance signal and excellent peak. The samples were digested using the Titan MPS microwave sample preparation system (PerkinElmer, USA).

**Reagents and sample procedures.** Deionized water used for the preparation of all blank, standard and sample solutions were obtained from a Milli-Q system (Millipore, USA). Standard solutions containing 4.0 µg L⁻¹ Cd and 50.0 µg L⁻¹ Pb were freshly prepared by appropriate dilution of the stock solutions containing 1000 mg L⁻¹ of each element (National Center for Analysis and Testing of Organic Metals and Electronic Materials, P.R. China) in 0.2% (v/v) nitric acid (Suzhou Crystal Clear Chemical Co., China). The reagents (CH₂COOH and P₂O₅) were purchased from Tianjin Kernel Chemical Reagent Co. Ltd. (China). All solutions were prepared with deionized water. All glassware and plastic containers were washed with tap water, soaked in 20% nitric acid overnight, then filled with volume with tap water and deionized water, the containers emptied and dried at room temperature. Salt was used in the studies of optimization, and validation and application of the methodology was carried out. After optimization, the validated methods were applied to the analysis of both the edible salt and soy sauce samples.

The determination of Pb and Cd by ETAAS was carried out using an injection solution of 0.2% nitric acid, FCNs, salt sample (1%) and standard solution (Fig. 1). In order to determine the concentration of Cd and Pb in the salt sample, 0.500 g salt was dissolved in 50.00 mL solution with HNO₃ 0.2% (v/v). In the optimization of the graphite furnace heating procedure, 50.0 µg L⁻¹ Pb solution and 4.0 µg L⁻¹ Cd solution were added to the salt sample. 16.0 µL of the salt solution and 5.0 µL FCNs were injected into the graphite tube for the analysis of Pb and Cd.

**Synthesis of FCNs.** The FCNs were synthesized according to the literature with some modifications. A homogeneous mixture consisting of 1 mL glacial acetic acid and 80 µL water was quickly added to 2.5 g P₂O₅ in a 50 mL beaker without stirring. In this system, the upper temperature was mainly controlled by vaporizing the glacial acetic acid at its boiling point (117°C). The nanobubbles of glacial acetic acid vapor then served as the templates for the nano-sized structures. Finally, the dark brown FCNs were collected by dispersing them in deionized water.
MΩ cm), followed by adjustment of the pH to 7.0 with NaOH, and dilution to 100 mL by deionized water in a brown volumetric flask. The obtained FCNs solution is stable for at least one month in the refrigerator. The concentration of the FCNs was 4.2 g L⁻¹ as calculated by the C element in the glacial acetic acid.

RESULTS AND DISCUSSION

Spectral characteristics of FCNs. Fig. 2A shows the typical TEM images of as-prepared FCNs at low magnification. It can be seen that most of the FCN particles are spherical in shape and uniformly distributed. The sizes of the FCNs are less than 25 nm, which clearly shows that a nano-sized morphology is achieved. The particle sizes and shapes are similar to those reported in the literature.⁷³,²⁹ Fig. 2B shows the UV-Vis and FL spectra of the FCNs. A broad absorption around 297 nm and a sharp absorption at 247 nm were observed. The peak at 247 nm was ascribed to a π–π* transition of the aromatic C=C bonds,²⁹ while the shoulder at 297 nm is attributed to a n–π* transition of the C=O bonds.³⁷ When the excitation wavelength was between 350 and 420 nm, the FL emission peak was located at 500 nm. Thus, there was essentially no change in the emission wavelength and agrees with information from the literature.³⁷ The FL spectra show that the FCNs have fluorescence properties.

Optimization of experimental conditions. The effect of FCNs on the Pb and Cd analysis in edible salt and soy sauce was investigated since without a modifier, there is low thermal stability of the analyte.

(a) For the analysis of Pb, the highest pyrolysis temperature was 600 °C without addition of FCNs. After addition of the modifier, the pyrolysis temperature increased to 900 °C without loss of element due to volatilization. This demonstrates that the modifier adopted not only improved the sensitivity of the measurement since there was an increase in the value of the analytical sign, but also improved the thermal stability of Pb in the sample.²⁵ In the optimization study of the atomization temperature, it was observed that the lowest temperature (1600 °C) provided the highest analytical sign. Such result was already expected due to the volatile character of Pb.³⁸ In the pyrolysis study, it was observed that the background (BG) values are higher when a modifier is added. However, under the optimized conditions of pyrolysis (900 °C) and atomization (1700 °C), the BG value of the FCNs as modifier was lower. Thus, the pyrolysis and atomization temperatures adopted for Pb were 900 °C and 1700 °C for the FCNs, respectively (Table 1).

(b) For the analysis of Cd, the integrated absorbance without modifier was lower than with the FCNs. It should be noted that in the absence of a modifier, Cd started to volatilize at 400°C and after adding the modifier, the volatilization temperature increased to 600 °C. This suggested that the thermal stability of the element in the sample was improved when FCNs were used as the modifier. In the atomization study, it was verified again that the chemical sensitivity was enhanced by using FCNs as the modifier. Thus, the optimum pyrolysis condition and atomization temperature of Cd was 600 °C and 1600 °C, respectively. These results are attributed to the good solubility, stability and large active surface area of the FCNs and were, therefore, used as the modifier in subsequent measurements.

Analytical performance. Validation of the methods developed was verified by analyzing the parameters of system suitability, linearity, LOD, quantification limits (LOQ), characteristic
calculating the RSD of five determinations of Pb (10.0 µg L\(^{-1}\)) and Cd (0.8 µg L\(^{-1}\)) at an interval of one hour on the same day under the same experimental and laboratory conditions. The experimental data showed that the RSD for Pb (10 µg L\(^{-1}\)) and Cd (0.8 µg L\(^{-1}\)) were 0.72% and 0.21%, respectively, indicating that the method has high precision.

The analytical curves were obtained by different methods of standard addition to verify whether there was a matrix effect in the determination. For Pb and Cd analysis of the salt solution (1%), the analytical curves ranging from 10.0 to 50.0 µg L\(^{-1}\) and 0.4 to 4.0 µg L\(^{-1}\) were obtained, respectively. For each metal, each point of the analytical curves was analyzed in triplicate. The linearity of the analytical curves was checked at the 95% confidence level. Fig. 3 shows the regression line in the prediction interval.

Verification of the linearity of Pb and Cd was obtained by ETAAS. The salt and soy sauce samples were analyzed in the same way, and the results are listed in Table 2. The accuracy of the proposed method for the determination of Cd and Pb in these samples was evaluated by addition and recovery tests using two levels (Table 2). The recoveries of Cd and Pb in the two salt food samples ranged from 93.0% to 94.0% and from 93.4% to 101.0%, respectively. These values are in the acceptable range as recommended by GB/T27417-2017, which is 80–110% for 0.1–1.0 mg kg\(^{-1}\). In summary, the obtained results show satisfactory agreement with good precision and verify that FCNs are a suitable matrix modifier for the determination of Pb and Cd in high-salt food samples.

Possible mechanism of modification. In order to understand why the addition of FCNs can increase the pyrolysis temperature of Pb and Cd, FT-IR was used to analyze the structure of the FCNs. Fig. 2C shows the FT-IR spectrum of the FCNs. An apparent absorption peak of the −OH group at about 3449 cm\(^{-1}\) and an absorption peak of the C=O group appeared, conjugated with the oxygen-containing functional groups like −OH and C=O have the ability to bind to the metal elements, which may be the reason why the Pb and Cd. Stability of the Pb and Cd by FCNs is mainly owed to the hydroxyl and carboxyl. It is broken down and produced methane, hydrogen, carbon monoxide and other deoxygenizing substances. The complex matrix is deoxygenized by deoxygenizing substances in the pyrolysis steps. Pyrolysis temperature increased after the formation of these structures, the oxygen-containing functional groups like −OH and C=O group appeared, conjugated with aromatic carbons at 1658 cm\(^{-1}\). These data show that the FCNs are rich in carboxylic groups. A peak at 1542 cm\(^{-1}\) from a conjugated C=C stretching vibration was observed, indicating that unsaturated carbon bonds formed during the carbonization process. During the formation of these structures, the oxygen-containing functional groups like −OH and C=O have the ability to bind to the metal elements, which may be the reason why the Pb and Cd.
FCNs were added as the modifier in the ETAAS test process, thereby reducing an amount of loss and increasing the elemental sensitivity in the pyrolysis step. In all, in the absence of a modifier, the Pb and Cd values decrease when the pyrolysis temperature is 600 °C and 400 °C, respectively, and Pb and Cd are lost proportionally with an increase in temperature. Thus, the addition of FCNs can increase the pyrolysis temperature of Pb and Cd and improve the measurement accuracy and sensitivity.

CONCLUSIONS

The FCNs were prepared without external heat treatment. The results of this study show them to be an excellent modifier that improves the detection sensitivity of Pb and Cd in ETAAS analysis. The oxygen-containing functional groups on the surface of the FCNs enable both Pb and Cd to form structures at a higher pyrolysis temperature, thereby reducing elemental loss in the heat treatment process. This work provides a new direction for the application of FCNs and a new idea for high-precision and low-cost determination of trace Pb and Cd by ETAAS.

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