Determination of lead in fish samples by slurry sampling electrothermal atomic absorption spectrometry

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Ultrasonic slurry sampling electrothermal atomic absorption spectrometry (USS-ETAAS) was applied to the determination of lead in several fish samples. The influences of instrument operating conditions and slurry preparation on the signal were examined. Palladium and ammonium nitrate were used as the modifier to improve the signal. Since the sensitivity to lead in various fish slurries and aqueous solutions was different, the standard additions method was used for the determination of lead in these fish samples. The method was applied to the determination of lead in dogfish muscle reference material (DORM-2) and a swordfish muscle sample purchased from the local market. The analysis results agreed with the reference value. The accuracy was better than 6%. The precision between sample replicates was better than 16% with the USS-ETAAS method. The detection limit of lead estimated from standard additions curve was about 0.053–0.058 μg g⁻¹ in different samples.

Introduction

Electrothermal atomic absorption spectrometry (ETAAS) is a useful technique for the determination of lead in various samples and numerous studies have been published.¹–¹⁴ Most of the methods involve prior dissolution of the samples. A variety of digestion methods for measuring total lead in biological samples, and both wet and dry ashing, or a combined procedure, have been reported. All these methods involve the risk of contamination or loss of lead and it is clear that methods involving minimal sample handling are required.

In recent years, the direct analysis of solids and slurries by ETAAS has received much attention in an attempt to eliminate problems associated with conventional wet oxidation and dry-ashing sample preparation procedures. Ultrasonic slurry sampling is one of the methods for direct solid sample introduction that has been successfully used in ETAAS.¹⁵–¹⁷ Compared with traditional sample preparation methods such as acid digestion and dry ashing, slurry sampling offers the benefit of reducing the possibility of analyte loss before analysis. Furthermore, slurry sampling combines the benefits of solid and liquid sampling and permits the use of conventional liquid sample handling apparatus such as an autosampler. Very few studies using the slurry-based approach to determining lead in food samples have been published.¹¹

Most of the methods which exist for determining the concentrations of elements in fish samples require digestion of the sample with acid before analysis.⁴,¹⁸ In this study, ultrasonic slurry sampling (USS) ETAAS was used to determine the concentrations of lead in several fish samples directly. The influences of instrument operating conditions and slurry preparation on the signal were investigated. The method was used for the determination of lead in dogfish muscle reference material (DORM-2) and a swordfish muscle sample purchased from the market.

Experimental

Apparatus and conditions

AAS measurements were made on a Perkin-Elmer (Norwalk, CT, USA) Zeeman/S100 PC atomic absorption spectrometer equipped with an HGA-6100 graphite furnace atomizer. A lead hollow cathode lamp (HCL) and pyrolytic graphite-coated graphite tubes with integrated platforms (Perkin-Elmer, Überlingen, Germany) were used throughout. The sample introduction system included a Model AS-60 autosampler equipped with a USS-100 ultrasonic slurry sampler. The USS-100 was set at 25 W (10% power) and a 10 s mix time was used to mix slurries before injection of 20 μl sample aliquots for analysis. The optimized instrumental parameters and heating programme are given in Tables 1 and 2, respectively. The peak area of the transient signal was used for data handling.

Reagents and slurry preparation

H₂O₂, NH₄NO₃ and EDTA were obtained from Merck (Darmstadt, Germany), trace metal grade HNO₃ (70% m/m)

Table 1  Equipment and operating conditions

<table>
<thead>
<tr>
<th>Step</th>
<th>Temperature/°C</th>
<th>Ramp/s</th>
<th>Hold/s</th>
<th>Gas flow rate/ ml min⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drying 1</td>
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<td>5</td>
<td>10</td>
<td>250</td>
</tr>
<tr>
<td>Drying 2</td>
<td>120</td>
<td>5</td>
<td>20</td>
<td>250</td>
</tr>
<tr>
<td>Pre-treatment</td>
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<td>5</td>
<td>10</td>
<td>250</td>
</tr>
<tr>
<td>Drying 1</td>
<td>150</td>
<td>10</td>
<td>20</td>
<td>250</td>
</tr>
<tr>
<td>Drying 2</td>
<td>200</td>
<td>10</td>
<td>35</td>
<td>250</td>
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<td>Ash</td>
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<td>5</td>
<td>30</td>
<td>250</td>
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<td>Atomization</td>
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<td>250</td>
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<tr>
<td>Cooling</td>
<td>20</td>
<td>5</td>
<td>5</td>
<td>250</td>
</tr>
</tbody>
</table>

* Used for selection of modifier and slurry preparation parameters.
Results and discussion

Selection of modifier

Modifiers are commonly used in ETAAS analysis. The use of a modifier would change the chemical or physical characteristics of the sample and/or the atomizer surface in order to improve quantification. In this study, several modifiers, including EDTA, Pd, NH₄NO₃ and a mixture of Pd and NH₄NO₃, were tested for the best signal of lead. The drying temperature was set at 150 °C for 20 s and 200 °C for 35 s; the ash temperature was set at 500 °C; and the atomization temperature was set at 1700 °C. We found that the absorbance of lead increased and the peak shape improved significantly when 500 °C. We found that the absorbance of lead increased and the peak shape improved significantly when 500 °C. We found that the absorbance of lead increased and the peak shape improved significantly when 500

Effect of slurry preparation on ion signal

ETAAS has been successfully applied to the analysis of slurries.15–17 Certain factors such as particle size, analyte partitioning, maximum slurry concentration and slurry homogeneity were important for the success of the analysis of slurries by ETAAS.15,16 The effects of several parameters of the slurry preparation on the ion signals were investigated in this work as described below.

An important factor in the slurry technique is the slurry concentration. However, dilution of the slurry can only be carried out within a limited range. The effect of dilution factor on lead signal was studied and the result is shown in Fig. 3. As shown, the sensitivity (absorbance concentration) of lead

Fig. 1 shows the effect of the amount of Pd modifiers on the integrated peak area of lead. As shown, the signal increased with increase in Pd concentration and reached a maximum when the concentration was 700 µg ml⁻¹. In subsequent experiments, a Pd concentration of 700 µg ml⁻¹ was selected. Fig. 2 shows the effect of the concentration of NH₄NO₃ in the prepared slurry on the integrated peak area of lead. As shown, the signal increased with increase in NH₄NO₃ concentration and reached a maximum when the NH₄NO₃ concentration was about 1% m/v. In subsequent experiments, an NH₄NO₃ concentration of 1% m/v was selected.

Fig. 1 Effect of Pd concentration on Pb signal. The slurry solution contained 2% m/v swordfish sample and 1% m/v NH₄NO₃. Each data point represents the mean of five measurements. All data are relative to the first point.

Fig. 2 Effect of NH₄NO₃ concentration on Pb signal. The slurry solution contained 2% m/v swordfish sample and was spiked with various amounts of NH₄NO₃. The platform was thermally pre-treated with 20 µl of 700 µg ml⁻¹ Pd. Each data point represents the mean of five measurements. All data are relative to the first point.

from Fisher (Fair Lawn, NJ, USA), Triton X-100 from Sigma (St. Louis, MO, USA), palladium powder from Kojundo Chemical Laboratory (Saitama, Japan) and lead element standard solution (1000 µg ml⁻¹) from SPEX (Metuchen, NJ, USA).

The applicability of the method to real samples was demonstrated by the analysis of dogfish muscle reference material DORM-2 (National Research Council of Canada, Ottawa, Canada) and a swordfish muscle sample purchased from the local market. The latter sample was cut into small pieces and dried in an oven at 60 °C. The dried fish samples were then ground at room temperature for 30 min with a Retsch MM2000 mixer mill and sieved using a Retsch (Haan, Germany) VE1000 sieving machine. The powder with particle size < 100 µm were collected for subsequent experiments. In order to avoid cross-contamination, the mixer mill and sieving machine were cleaned after each usage. Before weighing for analysis, the fish samples were dried to constant mass by drying at reduced pressure at room temperature in a vacuum desiccator over Mg(ClO₄)₂ for 24 h.

The slurry was prepared as the following procedure. A 0.2 g portion of the powder material was transferred into a 10 ml calibrated flask. Suitable amounts of NH₄NO₃, H₂O₂ and Triton X-100 were added to give a final solution containing 1% m/v NH₄NO₃, 1.5% v/v H₂O₂ and 0.1% v/v Triton X-100. After various amounts of element standard solutions had been added, these slurries were diluted to volume with pure water. For standard addition analysis, the slurries were spiked with various amounts of lead (0, 1, 2, 5, 10, 20 and 30 ng ml⁻²) in the final solutions) standard. The slurry was then sonicated for 10 min in an ultrasonic bath and 1 ml aliquots were removed as needed for analysis with the use of a pipette while the slurry was being mixed with a vortex mixer. These aliquots were then deposited in the autosampler cups for analysis. A reagent blank was carried through the procedure, as outlined above, to correct for any analyte in the reagents used for slurry preparation. The concentration of lead was then determined from the standard addition calibration curve. For the studies of the effect of electrothermal atomization conditions and slurry preparation on the signal, a swordfish slurry sample was prepared according to the procedure described above.

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An important factor in the slurry technique is the slurry concentration. However, dilution of the slurry can only be carried out within a limited range. The effect of dilution factor on lead signal was studied and the result is shown in Fig. 3. As shown, the sensitivity (absorbance concentration) of lead

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Effect of slurry preparation on ion signal

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Fig. 1 Effect of Pd concentration on Pb signal. The slurry solution contained 2% m/v swordfish sample and 1% m/v NH₄NO₃. Each data point represents the mean of five measurements. All data are relative to the first point.

Fig. 2 Effect of NH₄NO₃ concentration on Pb signal. The slurry solution contained 2% m/v swordfish sample and was spiked with various amounts of NH₄NO₃. The platform was thermally pre-treated with 20 µl of 700 µg ml⁻¹ Pd. Each data point represents the mean of five measurements. All data are relative to the first point.

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increased with increase in dilution factor. This could be due to the alleviation of non-spectroscopic effects when the dilution factor was increased. In order to balance sample homogeneity, analyte signal and the complete vaporization of the introduced sample, a dilution factor of 50 was used in subsequent experiments.

The concentration of acid in the slurry solution could affect the rate of the extraction of the metal ions and the precision of signal measurement. Table 3 shows the effect of various additives on the absorbance of lead. As shown, the addition of HCl and HNO₃ reduced the signal of lead. In subsequent experiments, no acid was used in slurry preparation. However, it is interesting to see that the addition of 1% v/v H₂O₂ increased the lead absorbance slightly. Further, the precision was improved when 1% m/v H₂O₂ was added. In USS-ETAAS analysis, the direct introduction of suspensions could be problematic because of high background values or the build-up of carbonaceous residue, both of which reduce sensitivity and precision. The addition of H₂O₂ to biological samples has proved very effective in preventing carbonaceous residues building up inside the tube. The effect of H₂O₂ concentration in the slurry sample on the lead signal was also studied in this work, and the result is shown in Fig. 4. As shown, the lead signal increased with increase in H₂O₂ concentration and reached a maximum when the H₂O₂ concentration was 1.5% v/v. Furthermore, as shown in Fig. 5, a more symmetrical and sharp peak could be obtained when 1.5% v/v H₂O₂ was added. In USS-ETAAS analysis, the direct introduction of suspensions could be problematic because of high background values or the build-up of carbonaceous residue, both of which reduce sensitivity and precision. The addition of H₂O₂ to biological samples has proved very effective in preventing carbonaceous residues building up inside the tube. The addition of H₂O₂ to biological samples has proved very effective in preventing carbonaceous residues building up inside the tube. The addition of H₂O₂ to biological samples has proved very effective in preventing carbonaceous residues building up inside the tube.

In another experiment, we found that the concentration of surfactant did not affect the lead signal significantly. However, a more symmetrical and sharp peak could be obtained when a small amount of Triton X-100 was added. Since the sample homogeneity could affect the repeatability of the signal determined, for better precision in subsequent experiments 0.1% v/v Triton X-100 was used in all slurry preparations.

Selection of ash and atomization temperatures

Fig. 6 shows the effect of ash temperature and atomization temperature on the lead signal. As shown, the signal of lead increased slightly with increase in ash temperature and reached a maximum when the temperature was about 1100 °C. This could be due to the removal of more volatile matrix during the ash stage and alleviation of the non-spectroscopic interference in the atomization stage. However, the signal of lead decreased rapidly when the ash temperature was > 1100 °C. This could be due to the vaporization of lead. In subsequent experiments, the ash temperature was set at 1100 °C.

As shown in Fig. 6, the signal of lead did not change significantly until the atomization temperature was > 2100 °C. A more symmetrical and sharper peak could be obtained when a higher atomization temperature was used. In subsequent experiments, 2100 °C was selected as the atomization temperature. A summary of the electrothermal atomization temperature programme is given in Table 2.

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Table 3

<table>
<thead>
<tr>
<th>Additive</th>
<th>Absorbance</th>
<th>RSD(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No other additive</td>
<td>0.036 ± 0.002</td>
<td>5.55</td>
</tr>
<tr>
<td>1% v/v HNO₃</td>
<td>0.038 ± 0.002</td>
<td>5.26</td>
</tr>
<tr>
<td>1% v/v HCl</td>
<td>0.040 ± 0.002</td>
<td>5.00</td>
</tr>
<tr>
<td>1% v/v H₂O₂</td>
<td>0.048 ± 0.001</td>
<td>2.08</td>
</tr>
</tbody>
</table>

Values are means of seven measurements ± standard deviation. The slurry solution contained 2% m/v swordfish sample and 1% m/v NH₄NO₃ and was spiked with various additives.

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**Fig. 3** Effect of dilution factor on Pb signal. The slurry solution contained various amount of fish sample and 1% m/v NH₄NO₃. Each data point represents the mean of five measurements.

**Fig. 4** Effect of H₂O₂ concentration on Pb signal. The slurry solution contained 2% m/v swordfish sample and 1% m/v NH₄NO₃ and was spiked with various amounts of H₂O₂. Each data point represents the mean of five measurements. All data are relative to the first point.

**Fig. 5** Effect of H₂O₂ additive on Pb peak shape: (a) without H₂O₂ and (b) with 1.5% v/v H₂O₂. The slurry solution contained 2% m/v swordfish sample, 1% m/v NH₄NO₃ and various amounts of H₂O₂.
The detection limit of lead in different samples was determined from the standard additions curve for lead. It was based on the usual definition of the concentration of the analyte yielding a signal equivalent to three times the standard deviation of the reagent blank signal \((n = 7)\). The detection limit estimated from the standard addition curve was in the range 0.053–0.058 \(\mu g \cdot g^{-1}\) for lead in different samples. A better detection limit is to be expected with more purified reagents.

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**References**