Determination of Lead in Milk by Graphite Furnace Atomic Absorption Spectrometry

Introduction
Milk is one of the basic food groups in the human diet, both in its original form and as various dairy products. It is well-known that lead (Pb) is toxic and causes damage to the nervous system; it has a particularly detrimental effect on young children and it has become a cause of major concern since the 1970s. As per World Health Organization (WHO) standards, the permissible limit of lead in drinking water is $10 \, \mu g/kg$ (parts per billion, ppb). However, the Chinese guidelines for maximum levels of lead content is set at $20 \, \mu g/kg$ (ppb wet weight) in infant formula (use of milk as a raw material measured by fluid milk diluted from powder, referring to the product ready-to-use) and at $50 \, \mu g/kg$ (ppb) in fresh milk, respectively. Lead analysis has traditionally been one of the major applications of graphite furnace atomic absorption spectrometry (GFAAS) worldwide.

In order to ensure protection of consumers, analysis should be sensitive, efficient, and cost-effective so that more effective monitoring can be accomplished. Because GFAAS is a mature technique, it is well-understood and routinely used and is certainly suitable for this determination. Sample preparation is an important part of an analysis, yet can be time consuming.

Generally, milk is an emulsion or colloid of butterfat globules within a water-based fluid. The exact components of raw milk vary by different animal species, but it contains significant amounts of lactose, fat, protein and minerals as well as vitamins. Due to the relative interference resulting from such a complex matrix, complete decomposition of milk samples prior to instrumental measurement by microwave or heating block acid digestion is generally recommended. This approach, however, is more time-consuming and poses a more rigorous requirement on quality assurance than simple dilution when concentrations of lead are to be determined at $\mu g/kg$ level in the final solution, which is extremely sensitive to reagent blank contribution and environmental contamination. To overcome these issues, you will use a simple and direct dilution method for sample preparation, followed by automated analysis using GFAAS. This method minimizes sample preparation, and also reduces potential contamination, while still maintaining the speed of analysis.

Experimental Conditions
Instrumentation
A PerkinElmer® GFAAS system will be used for measurements of lead (Pb) in different milk samples. The spectrometer's transversely heated graphite atomizer (THGA) with Zeeman background correction provides a constant uniform temperature distribution across the entire length of the graphite tube. This allows a full implementation of the Stabilized Temperature in graphite furnace to obtain a stable analysis where we can analyze complex sample matrices using aqueous
standard solutions as calibration for suspended sample solutions to get accurate and precise results. Maximum atomic signals can be obtained with minimum memory effect and potential interference.

The GFAAS spectrometer is equipped with an autosampler and a PerkinElmer Lumina™ single-element Pb hollow cathode lamp is used as the light source. A standard THGA tube and polypropylene autosampler cups will be used throughout all measurements. The instrument is controlled by AA WinLab32™ software running under windows.

A summary of the GFAAS instrument settings is listed below.

**Parameter Value**
- Wavelength: 283.3 nm
- Slit Width: 0.7 nm
- Lamp Current: 10 mA
- Signal Measurement: Peak Area
- Measurement Type: AA-BG
- Integration Time: 5 s
- Replicates: 3
- Sample Volume: 20 μL

**Sampling**
One milk sample per student from a different source will be analyzed for its lead content. These may include milk powder, skimmed milk powder, whole milk, low-fat milk, and children or infant milk. All the samples collected should be clearly labeled and taken to the laboratory then kept refrigerated until analysis.

**Sample Preparation**
For the preparation of all solutions, ultrapure deionized (DI) water should be used throughout. Metal-free polypropylene vials and pipette tips should be pre-cleaned with diluted nitric acid (~1% HNO₃) and rinsed thoroughly with DI water before use. For the subsequent GFAAS analysis, deionized water is used as a blank. About 50 g sample of liquid milk or 6 g of solid milk powder is accurately weighed (to 0.1 mg) and transferred into a 250 mL measuring flask which is subsequently diluted to about 150 mL, and shaken vigorously for a few minutes to ensure homogeneity, then completed to mark and also further shaken to homogenize. The obtained suspension solution is ready for GFAAS measurement using the autosampler. These suspensions should be stable for the lab period. Even the more challenging total fat milk powder prepared by this rapid dilute-and-shoot procedure can be stable for this duration, which is sufficient for the inter-day variability check. The same procedure is used to prepare the blank.

*** All your glassware should be cleaned using lead free 1% HNO₃ and rinsed with ultrapure deionized water ***

**Preparation of Standard Solutions**
As the concentration of Pb in milk samples is generally very low, all the reagents used must be of ultra-pure grade. Thus, reagents of best purity should be used.
Calibration curves will be constructed using the **method of standard** addition as follows:

1. Transfer **1.00 mL** of the **100 ppm Pb$$^{2+}$$** stock solution (provided) into a precleaned **1.00 L** measuring flask and complete to mark using deionized water. This results in a **100 ppb Pb$$^{2+}$$** standard solution.

2. Into each of five **50 mL** measuring flasks transfer **20 mL** of the previously prepared milk sample. Then add **0.00, 2.00, 5.00, 10.00, and 20.00 mL** of the **100 ppb Pb$$^{2+}$$** solution, **shake well**, and complete to mark with deionized water. These are your solutions which contain **0.00, 4.00, 10.00, 20.00, and 40.00 ppb** of the added standard Pb$$^{2+}$$ solution.

3. Your blank is ultrapure deionized water.

4. A summary of volumes and solution composition is shown in the following Table:

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>mL Sample</th>
<th>mL Standard</th>
<th>mL water to Mark</th>
<th>Absorbance Signal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank</td>
<td>0.00</td>
<td>0.00</td>
<td>50.00</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>20</td>
<td>0.00</td>
<td>30.00</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>20</td>
<td>2.00</td>
<td>28.00</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>20</td>
<td>5.00</td>
<td>24.50</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>20</td>
<td>10.00</td>
<td>20.00</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>20</td>
<td>20.00</td>
<td>10.00</td>
<td></td>
</tr>
</tbody>
</table>

5. Load the autosampler with your blank and samples according to the method you have built using the AA Winlab32 software. Choose Linear, with Intercept calibration curve.

The temperature program for the analysis of lead is optimized to provide maximum matrix decomposition without loss of analyte. A suitable furnace temperature program used by PE personnel is given below:

**Furnace temperature program for the direct measurement of lead in milk samples**

<table>
<thead>
<tr>
<th>Step</th>
<th>Temp. (°C)</th>
<th>Ramp Time (sec)</th>
<th>Hold Time (sec)</th>
<th>Internal Flow</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Drying</td>
<td>130</td>
<td>5</td>
<td>30</td>
</tr>
<tr>
<td>2</td>
<td>Drying</td>
<td>150</td>
<td>15</td>
<td>30</td>
</tr>
<tr>
<td>3</td>
<td>Drying</td>
<td>450</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>4</td>
<td>Pyrolysis</td>
<td>600</td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td>5</td>
<td>Atomization</td>
<td>1600</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>6</td>
<td>Clean-out</td>
<td>2500</td>
<td>1</td>
<td>5</td>
</tr>
</tbody>
</table>

For Pb determination, complete mineralization of the milk components is not necessary. All data should be calculated from 3 replicate readings for each solution using peak-area (integrated absorbance) integration.
Results and Discussion

Report your results as µg lead/kg milk (for liquid milk) or µg Pb/kg reconstituted milk (for milk powder, assume 120 g of whole milk powder are needed to reconstitute 1 kg of milk). Do not forget to specify milk state, type, as well as Production Company.