Determination of Aluminum in Biological Samples by Atomic Absorption Spectrophotometry with a Graphite Furnace

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Aluminum, generally considered non-essential and non-toxic, may accumulate in toxic amounts in the brain in cases of chronic renal failure. We describe a procedure for its analysis in biological fluids by atomic absorption spectrophotometry with a graphite furnace. No sample preparation is required and the procedure is sensitive at the appropriate concentrations. A sample of serum or urine is pipetted into the interior of the graphite tube, where it is sequentially dried, charred, and atomized. Precautions for sample handling are discussed and instrument settings are defined. Precision and accuracy of the method are evaluated, as are the effects of salts, protein content of serum, and specific gravity of urine. Serum of 23 persons who were not consuming aluminum-containing antacids contained 28 ± 9 (SD) μg of Al per liter (1.02 ± 0.33 μmol/liter).

Additional Keyphrases: trace elements • chronic renal failure • normal values • urine • sample-handling precautions

Because of its ubiquitous distribution, aluminum is encountered daily in many forms and from a variety of sources. This is usually adventitious, but in a few instances its ingestion is purposeful, most commonly in the form of antacids.

In the special case of chronic renal failure, large amounts of aluminum-containing compounds are given to prevent the accumulation of phosphate (1). As this has produced evidence of possible toxic effects of aluminum (2), it becomes important to measure this element in these patients. This paper describes a method for doing so by graphite-furnace atomic-absorption spectrophotometry.

Values reported for aluminum concentration in serum or plasma as measured by various methods vary greatly (3-10, Table 1). The procedure we describe is adequately sensitive, requires no sample preparation when serum and urine are used, and is relatively simple and rapidly performed. Here we report this technique, describing the necessary precautions needed for accuracy and precision.

Materials and Methods

Equipment

We used a Model 306 atomic absorption spectrophotometer, equipped with a Model HGA-2000 graphite furnace, a deuterium arc background correction system, a Model 56 chart recorder, a Model PRS-7A printer interface and sequencer, an Intensitron aluminum hollow-cathode lamp, and standard graphite tubes and cones, all from Perkin-Elmer Corp., Norwalk, Conn. 06856. A voltage stabilizer was used between the current source and the spectrophotometer, background corrector, and chart recorder. Filtered air was used to purge the deuterium arc background corrector.

Standards and Reagents

Standard aluminum stock solutions were prepared in several ways and compared. They were stored in polyethylene bottles at 4 °C.

1. From salts. Solutions of Al₂(SO₄)₃•18 H₂O were found more stable than those of K₂Al₂(SO₄)₃•24 H₂O. For a concentration of 1 mg of Al per liter, 12.26 mg Al₂(SO₄)₃•18 H₂O was dissolved to make 1 liter of solution. This concentration sufficed to make acidification for stability unnecessary, eliminating one source of contamination. Dilutions for use were prepared daily from the stock.

2. Aluminum metal. Aluminum foil of 99.997% purity (Alfa Division, Ventron Corp., Danvers, Mass. 01923) was weighed on a Cahn electrobalance and 1.921 mg was dissolved in 5.6 ml of concentrated H₂SO₄. Solution was facilitated by adding 6 drops of a 300 g/liter solution of H₂O₂. The final concentration was 960.5 μg of aluminum and 90 mmol of H₂SO₄ per liter. To check that there was no contamination from the glassware or reagents, we first prepared a blank acid solution and assayed it and the standard was prepared in the same 2-liter volumetric flask.

A standard was also prepared from the foil with HCl, which required gentle heating to effect solution. The final concentration was 1.0155 mg of Al and 40 mmol of HCl per liter.

3. Commercial standard. A solution of Al₂(SO₄)₃ was obtained from Alfa Division.

The water was purified by reverse osmosis and then passed through a mixed-bed resin exchanger (Continental Water, Melrose Park, Ill. 60160), run for a few minutes before collection. No Al could be detected in it.

Procedures for Obtaining Specimens

Many systems for obtaining specimens by venipuncture were tested for their possible contribution to aluminum contamination. Our results were best with a “Monoject” (Sher-
Table 1. Serum or Plasma Aluminum—Reported Values

<table>
<thead>
<tr>
<th>Al concn., µg/liter</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spectrographic</td>
<td></td>
</tr>
<tr>
<td>240 ± 120 (30)*</td>
<td>3</td>
</tr>
<tr>
<td>450 ± 20 (122)</td>
<td>4</td>
</tr>
<tr>
<td>551 ± 172 (63)</td>
<td>5</td>
</tr>
<tr>
<td>Atomic absorption</td>
<td></td>
</tr>
<tr>
<td>37 ± 25 (29)</td>
<td>6</td>
</tr>
<tr>
<td>24 ± 5 (20)</td>
<td>7</td>
</tr>
<tr>
<td>340 ± 190 (21)</td>
<td>8</td>
</tr>
<tr>
<td>240 ± 55 (5)</td>
<td>9</td>
</tr>
<tr>
<td>28 ± 9 (23)</td>
<td>This paper</td>
</tr>
<tr>
<td>Neutron activation</td>
<td></td>
</tr>
<tr>
<td>1460 ± 261 (5)</td>
<td>9</td>
</tr>
<tr>
<td>72 ± 70 (10)</td>
<td>10</td>
</tr>
</tbody>
</table>

* Numbers in parentheses indicate number of people tested.

Wood Medical Co., St. Louis, Mo. 63105) 3-ml disposable syringe (cat. no. 158150) and a “Monject” 20-gauge 1½” disposable needle (cat. no. 250), as described elsewhere (11). This system proved to be free of detectable aluminum. The serum from the centrifuged blood was pipetted directly into the furnace or decanted into a previously prepared test tube. Plasma was obtained by transferring the whole blood to previously prepared tubes containing 18 µl of sodium citrate solution (300 g/liter) per milliliter of whole blood and centrifuging.

Urine specimens were obtained as 24-h collections in previously prepared containers, with no preservative. Volume and specific gravity were recorded for each.

Except for the preparation of standards, all laboratory ware was plastic, chiefly polypropylene, and all was tested for contamination. Once free of contamination, the same tubes were rinsed well and reused. Regular washing with nitric acid showed no advantage because of its aluminum content (12). Unnecessary addition of reagents and transferring to other containers was avoided.

Grettie et al. (13) reported Parafilm as a source of contamination, but we found it not to release aluminum. Therefore, all sample tubes were covered with Parafilm until analyzed.

Another source of contamination was sweat from the hands, which is rich in trace metals (14); thus handling of all associated materials was kept to a strict minimum.

Instrumental Adjustments

Set the voltage to 115 V with a voltage stabilizer to smooth out fluctuations in line voltage, which interfere with the absorption signals. Make certain that all connections are grounded.

Purge the furnace with 99.995% argon with the flow adjusted to 1.1 liter/min. The expected increase in sensitivity with argon as compared to nitrogen was observed (15).

Activate the automatic gas-flow interrupt to maintain the atom cloud in the light beam longer.

Adjust the flow of water for cooling the furnace to about 2.7 liter/min.

Balance the hollow cathode beam and the deuterium arc beam at a gain setting of midline on the energy meter. Because of the much greater intensity of the aluminum lamp as compared to the deuterium lamp, reduce the current to the aluminum lamp to accomplish the matching.

Set the signal peak mode at slit width setting No. 4, to provide a spectral band width of 0.7 nm.

Tune the monochromator to the peak of the emission line of the aluminum hollow-cathode lamp at 309.3 nm.

Insert a graphite tube into the furnace, aligning it with a glass rod with well-fired ends, to prevent damage, instead of the aluminum rod furnished with the instrument. Align the tube so that the sample hole is centered in the injection port, facilitating introduction of samples. The graphite tubes are asymmetric with respect to the placement of the gas inlet holes around the sample hole, but tube insertion either way produced the same absorption signal. The tubes can be placed so that the gas inlet holes are either in a horizontal or a vertical plane. Carbon residue from serum and plasma samples is more prone to accumulate when the tubes are placed with the holes in the vertical position; consequently, the tubes are always placed with the front holes in the horizontal position. The sample hole of the standard tubes is too small to allow reproducible placement of samples, resulting in sample loss when the micropipetor tip is withdrawn. To prevent this, increase
1% absorption) was 30 pg (1.11 pmol). With a 25-μl sample this requires a concentration of aluminum of 1.2 μg/liter (44.5 nmol/liter). The within-day precision for 10 consecutive analyses of serum gave a coefficient of variation of 2.9%. Results obtained for 14 serum samples by the method of additions minus those from a standard curve did not differ significantly (mean difference = -2.61 ± 4.47 (SD) μg of Al per liter). The concentration of Al in the sera ranged from 13 to 137 μg/liter. The analytical recovery of aluminum added to 10 samples of serum with Al concentrations ranging from 4 to 136 μg/liter, was 101.3 ± 7.2 (SD) %. The interday variation of the method was determined by analyzing 15 sera on separate days. These samples were stored at -7 °C and values for them ranged from 12 to 131 μg of Al per liter (0.45-4.86 μmol/liter). The mean difference between the two groups of data was 2.56 ± 5.46 μg of Al per liter (95.0 ± 202.5 nmol/liter). No significant statistical difference between these two groups of data was present, as the t-test for paired observations produced a P > 0.05.

To test for effects of matrix upon the aluminum analyses, standards in serum, water, and urine, covering a wide range of concentration, were analyzed (Figure 7). The method of additions established the concentration of the original serum and urine samples, after which aqueous aluminum sulfate standards were added to increase the concentrations. The difference between the serum and urine lines were not of statistical significance, and pooling the results produced a line with slope = 2.37 × 10⁻³ and y-intercept = 11.70 pg of Al per liter. This line differed significantly from the water line (P < 0.01). Comparing the pooled serum and urine line with the regression forced through the origin, which had a slope = 2.9 × 10⁻³. The correlation coefficients of all lines were significant at the 0.1% level. From this it was determined that standards should be prepared in a matrix corresponding to that of the samples to be analyzed.

We investigated possible causes of the lower absorbance values for standards in serum and urine from those in aqueous solutions of aluminum sulfate. We found, as have others, that aluminum chloride solutions of the same aluminum concentration give lower absorbance values than those prepared from the sulfate. Because AlCl₃ sublimes at 178 °C, and because chloride is the predominant anion in serum and urine, low absorbance for these matrices would be suspected. We found that addition of NaCl to any of the samples decreased the observed absorbance, while addition of sulfate enhanced it. Initial experiments indicated that adding 13 mg of Na₂SO₄ per milliliter of serum gave maximal enhancement. Other salts had similar effect, but we found Na₂SO₄ most convenient. Addition of sodium sulfate had no effect on standards prepared as aluminum sulfate, but the lower values observed with aluminum chloride standards were increased to those observed for aluminum sulfate standards by the addition of sodium sulfate. A small correction was necessary for the aluminum content of the sodium sulfate.

At this point, it was necessary to decide if it is really necessary to add sulfate to all samples. We determined the concentration of aluminum in a serum pool by the method of
Fig. 1. Curve obtained on using pipettors of various volumes. A standard aluminum sulfate solution, 100 μg of Al per liter, was used.

Fig. 2. Chart-recorder tracing, demonstrating the need for removal of the residue from the graphite tube between analyses of serum and plasma. During five consecutive analyses of a sample, the residue was not removed until after the fourth determination. This was especially necessary if drying was at 100 °C.

The diameter of the sample hole from 2.08 mm to 2.78 mm with a drill bit. Condition new tubes by heating them at 2600 °C until the absorbance returns to the baseline. Use the deuterium arc background corrector for all measurements, purging with air at a 4-unit setting on the power supply flowmeter. Operate the chart recorder in the automatic mode with the pen actuator leads shorted, so that the pen responds at all times but the chart starts to move only during the final seconds of the charring stage. Zero the recorder to the chart baseline with both the recorder zero and the spectrophotometer auto-zero plus peak-start combination. Operate the recorder in the servo position at a speed of 10 mm/min and usually at 10 mV. If the printer-sequencer is used, connect it to its own power source to eliminate drain when the unit is activated.

These instrument settings are summarized in Table 2.

Sample Delivery

We used Oxford pipettors to deliver samples into the interior of the graphite tube. Some of the attendant tips were irregularly contaminated with aluminum. This contamination could not be removed by washing with nitric acid, and Karin et al. (16) reported that aluminum could not be leached from the plastic matrix by acid washing. We washed the tips for 75 min, with continuous stirring, in a 6 g/liter solution of Na₂EDTA. They were rinsed with several changes of water and stirred for 60 min in a large volume of water. The tips were air dried in the container in which they had been washed. Aluminum could not be detected when the tips were used to add pure water to the furnace. The pipettor was used in the delivery mode and a new tip used for each sample. The sample was delivered while the tip touched the bottom of the graphite tube opposite the opening. A linear response was found with sample volumes up to 25 μl (Figure 1). This volume was used for most analyses.

Drying conditions. The drying settings were determined while viewing the sample within the graphite tube. With a 25-μl volume, 100 °C for 60 s was sufficient to dry aqueous and urine samples. Higher temperatures caused loss of samples due to spattering. However, this temperature was found to be too low for serum. The residue formed on drying at 100 °C creates a small mound during the charring phase which interferes with subsequent analyses (Figure 2). The gradual increase in absorbance could be avoided by loosening the residue with a quartz rod and blowing it out with a rubber bulb. To avoid the need for this, serum samples were dried at 350 °C for 60 s, but occasionally some carbon remained and had to be removed as above. Drying serum at either temperature gave the same absorbance values when proper care was used to see that the graphite tube was clear after each firing and no losses had occurred when using the lower temperature.

Atomizing. With 25 μl of aqueous aluminum standard, the absorbance increased linearly with increasing atomizing temperature to 2600 °C (Figure 3). At higher temperatures, the rate of absorbance increase was less, so that 2600 °C was chosen as optimal.

The minimum atomizing time at 2600 °C needed for the absorbance to return to the baseline and avoid carryover was 12 s. This time also was adequate for serum and urine samples.

Charring. To optimize the charring conditions, we determined the minimum temperature and time to eliminate nonspecific background absorption at 307 nm, a non-absorbing wavelength for aluminum. As shown in Figure 4, when charring 25-μl samples for 60 s, the minimum charring temperature is 1300 °C and is the same for both serum and urine. The minimum charring time for 25-μl samples of serum and urine at 1300 °C is 60 s, as illustrated in Figure 5.

The optimal charring temperature for 25-μl samples of serum and urine as determined at the primary wavelength for aluminum, 309.3 nm, is 1500 °C (Figure 6).

Results

A comparison of the three aluminum sulfate standards gave identical response. When using 25 μl of standard, the sensitivity (defined as the quantity of aluminum needed to give a
additions, using either aluminum chloride or aluminum sulfate standard, and in the presence and absence of added sodium sulfate. The results are given in Table 3, where the slopes of the lines and the calculated aluminum concentrations are given. When aluminum chloride was used in the method of additions, the value for the calculated aluminum content of the serum in the presence of Na₂SO₄ was significantly higher, but not so high as that obtained with the aluminum sulfate standard. When sodium sulfate was added to the set prepared with added aluminum sulfate, higher absorbances were indeed obtained, but the slope of the method of additions line was also increased so that the calculated aluminum concentration of the serum was the same. This suggests that the same analytical values can be obtained with and without the addition of sodium sulfate if the correct calibration line is used, and if the aluminum sulfate standards are used.

To further determine the need for the addition of sodium sulfate, we analyzed 15 serum and 20 urine specimens with and without the addition of 13 mg of Na₂SO₄ per milliliter (92 mmol/liter). Calculations were made from the corresponding standard curves. In both cases the difference in concentration due to the sodium sulfate was insignificant (P > 0.05). The function of the sulfate is probably to lower the molar ratio Cl⁻/SO₄²⁻ and so prevent sublimation of AlCl₃. It may also furnish oxygen for the formation of aluminum oxide, as suggested by Campbell and Ottaway (17).

To study the effect of serum and urine components on the analytical results, we assayed a pool of each for aluminum by the method of additions, an aluminum sulfate standard of the same concentration being used to dilute each. The protein concentration, as determined by the biuret method, had no significant effect on the aluminum concentration (Figure 8). The coefficient of variation of the absorbance values was 3.4%.

When the spectrophotometer response was compared with the specific gravity of the urine dilution (Figure 9), the coefficient of variation of the absorbance was 3.2%. Thus the concentration is not considered to have had a significant effect on the results.

The reference value for 23 sera by the method described is 28 ± 9 (SD) μg of Al per liter (range 12–46) or 1.02 ± 0.33 μmol/liter (range 0.045–1.71). The only criterion we used for including samples was that the donor was not taking aluminum-containing antacids. The range of values is lower than found by many investigators (Table 1), and we think this to be due to the elimination of many manipulations and reagents that act as sources of contamination. For this sample size, sex and age did not contribute significantly.

In the analysis of urine from 12 normal men who were not taking antacids, the Al excretion was 45 ± 32 (SD) μg/24 h (range 6–92), or 1.67 ± 1.19 μmol/24 h (range 0.22–3.41).

**Discussion**

Campbell and Ottaway (17) point out the importance of the anion component of the standard solution. Many papers dealing with atomic absorption spectrophotometry with a graphite tube do not indicate the anion composition. Although AlCl₃ sublimes at 180 °C, some workers (18) have used the chloride in the standard. Independent of the anion, the furnace conditions are such that all aluminum compounds are converted to Al₂O₃ before the atomizing temperature is reached (15, 17). The question then is whether any AlCl₃ vaporizes before being converted to the oxide. That this happens is suggested by the lower absorbance values obtained with standards in hydrochloric acid compared to those in sulfuric acid. This is of special concern for biological materials that have a high chloride content. The atomic absorption of such materials can be increased by adding sodium sulfate to change the proportion of anion that is present as the chloride. We found that this is not necessary in practice when we analyzed serum and urine in the presence and absence of added sodium sulfate. Because the results were the same in either case, we elected not to add the sulfate and thereby avoided a source of contamination.

Various programs have been used for the graphite furnace. Agreement as to the charring and atomizing temperatures and times is uniform. Drying of aqueous standards and urine requires a temperature near 100 °C, but if serum is dried at this temperature, the charring leaves a mound of carbon at the sample site which interferes with subsequent measurements unless it is removed. Fuchs et al. (6) avoided this by first drying for 60 s at 100 °C, then for 60 s at 300 °C. By observing the sample during the drying and charring stages, we found that drying serum at 350 °C gave the desired results. Early in the drying, the sample bubbled a little; it then formed a small mound and started to decompose. By the time the charring step was reached, the residue was flat in the graphite tube and the carbon combusted completely. An occasional sample of serum would leave non-combustible carbon in the tube, so that it is advisable for the operator to observe the inside of the tube before adding a new sample of serum.

The method described offers advantages over existing techniques for the quantitation of aluminum in serum and urine. It is adequately sensitive and, as no reagents are added it is less prone to contamination. The sensitivity is greater than that reported by Blotcky et al. (12) for the analysis of aluminum in urine by neutron activation.

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**Table 3. Effect of Na₂SO₄ on Aluminum Assay of Serum. Method of Additions in the Absence and Presence of Na₂SO₄**

<table>
<thead>
<tr>
<th>Na₂SO₄ addition, (18 g/liter of serum)</th>
<th>Standard, as chloride</th>
<th>Standard, as sulfate</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>Slope 2.85 Al, μg/l</td>
<td>Slope 2.76 Al, μg/l</td>
</tr>
<tr>
<td>Yes</td>
<td>80.4</td>
<td>86.3</td>
</tr>
</tbody>
</table>

**Fig. 8. Effect of protein content on serum aluminum determinations**

Total protein determined by the biuret method.

**Fig. 9. Effect of relative density (specific gravity) on urinary aluminum determinations**

Specific gravity determined from refractive index.
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References