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Russell D. Brauer
University of Kentucky

J. David Robertson
University of Kentucky

Pankaj Sharma
Purdue University

Robert A. Yokel
University of Kentucky, ryokel@email.uky.edu

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Aluminum and phosphorus separation: Application to preparation of target from brain tissue for ^{26}Al determination by accelerator mass spectrometry

Russell D. Brauer[a,b], J. David Robertson[c], Pankaj Sharma[d], and Robert A. Yokel[a,b]

[a]College of Pharmacy, [b]Graduate Center for Toxicology and [c]Department of Chemistry, University of Kentucky, Lexington, Kentucky 40536-0082, and [d]PRIME Lab, Department of Physics, Purdue University, W. Lafayette, IN, 47907-1396.

Corresponding author:

Robert A. Yokel, Ph.D.

Pharmacy Building

University of Kentucky Medical Center

Rose Street

Lexington, KY 40536-0082

Phone: 606-257-4855

Fax: 606-257-7564

email: ryokel1@pop.uky.edu

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Abstract

Acid digested brain containing 4 mg added ^{27}Al was ashed at 1000°C to prepare an Al_2O_3 target for accelerator mass spectrometry analysis of ^{26}Al. A glassy-like material usually resulted which was thought to be aluminum oxyphosphate. The separation of aluminum and phosphate was investigated. Aluminum, but not phosphate, was bound by a cation exchange resin (AG 50-X8). Hydrofluoric acid eluted the aluminum from the resin. Removal of phosphate from acid digested brain by this method produced an amorphous material after ashing that was easier to recover from the porcelain crucible and had a higher AMS beam current. This procedure to separate aluminum from phosphate may have utility in other applications.

1. Introduction

Aluminum (Al) plays a major role in the generation of encephalopathy in renally-impaired subjects [1]. It has been implicated in the etiology of Alzheimer's disease and other neurodegenerative disorders [2]. There is concern about occupational Al exposure [3]. The only radioisotope of Al available for research, ^{26}Al, has a half-life of ≈ 720,000 years [4]. Toxicokinetic studies utilizing ^{26}Al and scintillation counting are not practical due to the very slow decay and high cost of this isotope. The application of accelerator mass spectrometry (AMS) to quantitate ^{26}Al [5] enables the study of Al toxicokinetics at physiologically relevant Al concentrations. The brain is of primary interest in such studies. AMS analysis of ^{26}Al requires separation and conversion of the Al to Al_2O_3. The literature does not contain a rigorously developed, or described, procedure. Some reports provide minimal description of the methods [6]. Walton et al [7] digested brain by repeated oxidation in nitric and then a nitric/perchloric acid mixture, precipitation of Al with 8-hydroxyquinoline (oxine) and then ashing at 1000°C for 4 hours. Kobayashi et al [8,9] digested freeze dried brain in nitric acid and then added perchloric acid. Al was purified by cation exchange, treated with ammonia water and the product ashed at 1000°C. The details of the cation exchange were not reported nor shared with us in response to a

request.

Similar methods have been reported to prepare non-brain samples for AMS. They include acid digestion followed by ashing at 800°C [10], precipitation of Al with oxine before ashing [11], and acid digestion followed by $Al(OH)_3$ precipitation [12,13]. A detailed procedure for separating Al from geological samples, using a cation exchange resin, has been described [14]. A 1N HCl solution was used to adsorb the Al onto the column, and a 6N HCl solution eluted the Al. The fate of P was not followed in this method.

2. Experimental procedures, results and discussion

2.1. Preparation of the AMS target by conventional methods

We initially prepared brain for AMS by acid digesting the brain and then ashing the residue. Carrier Al (4 mg of ^{27}Al as the HCl; Aluminum ICP/DCP standard solution, Aldrich) was added to the brain sample prior to acid digestion to maximize production of a sample homogenous in ^{27}Al distribution. The brain was dried at 110°C overnight and acid digested in a teflon screw-cap container in 3 ml of a 2:1 HNO_3:$HClO_4$ mixture on a hot plate, set at its lowest heat. This procedure has been extensively used to prepare homogenous solutions for electrothermal atomic absorption spectroscopy (ETAAS) [15,16]. After acid evaporation in a semi-closed acid trapping desiccator [15], 0.2 ml of 35% HNO_3 was added to dissolve the resultant material and enable its transfer to a

porcelain crucible. This transfer step was repeated, the liquid was evaporated, and the sample ashed at 1000°C. This produced an amorphous sample on occasion, but usually a glass-like material (glaze) which was very difficult to impossible to remove from the porcelain crucible. Neither the ramp time to 1000°C nor the time at 1000°C seemed to influence the appearance of the product. Similar inconsistent results were obtained by Richard Flarend (personal communication), which were not adequately pursued to ascertain the cause of the inconsistency. Although this glaze product could be utilized in AMS analysis, it was not ideal. Scraping it from the crucible introduced potential contamination with Si from the crucible and metal from the scraper. Target Al_2O_3 samples are typically mixed with Ag powder as a binder to facilitate production of steady negative ion beam current. Mixing was difficult with the glacial product. There was the risk of fracturing the material during its preparation for loading into the sample holder for AMS analysis and resultant ^{26}Al contamination of the sample loading area. The Al beam current of brain prepared in this manner was relatively low (Table 1). This could be due to poor Al_2O_3 formation or contaminants. It would lower the precision and detection limit of the AMS analysis. In contrast, similar processing of serum consistently resulted in amorphous material with a beam current averaging 94 nA.

2.2 Attempts to prepare a better target for AMS analysis suggested phosphorus interfered with Al_2O_3 formation.

We attempted to produce a sample that was easy to remove from the porcelain crucible, that had less beam current suppression and was composed of higher purity Al_2O_3.

Ashing at 500°C produced an amorphous material. However, it's low beam current, 34 nA, was thought to be due to incomplete Al_2O_3 formation. The beam current of an acid digested brain that was not ashed, 10 nA, supported this interpretation. Ashing samples at 1000°C, that had originally been ashed at 500°C, resulted in a glaze.

It was suspected that the glaze was an inorganic material because organic materials should thermally decompose under these conditions. An Al oxyphosphate was suspected because phosphorus interferes with Al_2O_3 formation, forming an $AlPO_4$ when Al and P are co-ignited [17]. High temperature reactions on graphite surfaces with Rutherford backscattering spectrometry demonstrated that lead and cadmium, when heated with excess phosphate, form metal-oxyphosphates that were stable in vacuum up to 900°C [18]. Because Al is also modified by the addition of excess phosphate, it supported the hypothesis that the glaze was an Al oxyphosphate. Thin sample PIXE analysis of a glaze sample of brain showed the presence of ≈ equimolar Al and P; considerable Na, K, Ca, Fe and Si; but no detectable Cl or S. The Al source was the 4 mg of ^{27}Al

added to the ≈ 1.8 gm of wet brain. The P can be attributed to the ≈ 3 mg P/gm wet mammalian brain [19,20,21,22]. Of the elements present in brain at concentrations > 0.1 mg/gm (Cl, K, Mg, N, Na, P, and S; [21,22,23,24]), only P (with O) would be expected to react with Al. The much lower P concentration in serum (0.04 mg/ml) and lack of glaze formation when serum was similarly acid digested and ashed, was consistent with a role of P in glaze formation. This hypothesis, and the alternate hypothesis that the glaze was Al silicate, were tested by preparing a brain sample by the identical procedure but ashing in a platinum crucible. The ashed product was a glaze. When scraped from the crucible its color suggested the presence of platinum. PIXE analysis showed large, and approximately equal, Al and P peaks, a large Pt peak (attributed to the Pt crucible as no measurable Pt was observed in the sample scraped from the porcelain crucible), but no measurable Si. Very low Si is consistent with the low ppm Si concentrations in all living organisms [25]. Two grams of wet brain would contain ≈ 4 μg Si, 3 orders of magnitude less than the 4 mg of ^{27}Al added to these samples. As serum averages 21 μM Si, a serum sample would have even less Si. Therefore, we concluded that the glaze formed from the acid digested brain was not an Al silicate.

When 0, 4 or 8 mg P was added to 4 mg Al and ashed at 1000°C, an amorphous material, an intermediate product and a glaze

resulted, respectively. These results further supported the hypothesis that considerable P produces an Al oxyphosphate glaze. Al phosphate is stable at 1000°C. Ashing at a higher temperature in the presence of oxygen or in a vacuum is required to convert the Al to Al_2O_3. These were not practical.

To determine if increasing oxygen would facilitate Al_2O_3 formation, an acid digested brain and a serum sample were ashed in a quartz tube furnace in a 95/5% oxygen/carbon dioxide at 1000°C. A glaze resulted from the brain and an amorphous material from the serum.

2.3 Attempts to separate Al from P by selective Al precipitation, solubilization of Al, and precipitation of P.

To selectively precipitate Al, rat brains to which 4 mg ^{27}Al were added were acid digested, and the product redissolved in acid. This was adjusted to pH 6.2, the nadir of the aqueous solubility of Al [26]. The supernatant and one wash were analyzed for Al by ETAAS (Perkin Elmer 4100ZL) and for P using the Sigma Kit #670 for inorganic phosphate. Although ≈ all of the Al was in the precipitate, > 50% of the P also precipitated. In a second attempt to selectively precipitate Al, 5 moles of oxine were added per mole of Al, to precipitate Al [27]. Oxine forms an insoluble $Al(oxinate)_3$ at pH 5 with a stability product $\beta_3 = 10^{34}$. The

solution was adjusted to pH 5, 8 or 11. Again, the precipitate contained nearly all of the Al and most of the P.

An attempt was made to selectively precipitate P and solubilize Al as $Al(OH)_4^-$. The AOAC gravimetric method [28]) was used, at pH 8.3, 9.3, 10.4, 11.3 and 11.9. No condition selectively precipitated either Al or P, as determined by the Al and P in solution.

To determine if alkalinization of the Al prior to ashing is beneficial, as conducted in [12,13], 4 mg Al and 8 mg P were prepared for ashing under three conditions. When ashed at 1000°C a glaze formed. When adjusted to pH 13 with NaOH a non-glaze material resulted that was difficult to remove from the crucible and to break apart. Similarly, precipitation at pH 6.0 and washing the precipitate, then dissolution and pH adjustment to 13 produced a similar product, although slightly easier to remove from the crucible. Alkalinization slightly improved the product but these procedures did not produce a material that could be easily removed from the crucible and disintegrated.

2.4 Separation of Al from P by ion exchange.

Given the inability to selectively precipitate Al or P, separation by ion exchange was investigated. A cation exchange resin containing sulfonic acid functional groups (AG 50-X8, 100-200 mesh; Bio-Rad) was identified as the only available resin expected to bind Al at a low pH, where $Al(OH)_3$ would not form. It

avidly complexed Al, but not P, within 5 minutes. Washing the resin with 0.2% HNO_3 eluted P, but not Al. Attempts to elute the Al with HNO_3 were not completely successful, even after addition of 50 ml of 70% HNO_3 to 4 mg Al bound to 2 gm resin. Based on HF elution of Al from this resin [29], we assessed the ability of HF (Fisher Scientific) and HF plus HNO_3 to elute 4 mg of Al from 2 gm of resin. Fifteen ml of 0.1N HF did not totally elute the Al, but 1N HF did (Figure 1), resulting in 104% recovery. Recovery was not improved by addition of HNO_3 to the HF nor greater contact time between the acid and resin. After eluting the P with 5 ml of 0.2% HNO_3 thrice, no measurable P was found in the HF eluant, providing a method to separate Al from P.

To determine if the resin contained inherent Al that could be mobilized by 1N HF to significantly contribute to added Al, 2 gm of resin was washed thrice with 5 ml of 1N HF. This mobilized ≈ 6 μg Al, equivalent to 0.15% of the 4 mg of ^{27}Al added as carrier to samples processed for AMS. Rinsing the resin with 1N HF to remove inherent Al prior to its use impaired the resin's ability to bind Al, so was not possible.

Ashing 2 mg Al and 2 mg P at 1000°C produced a glaze. When the resin was used to separate the P from the Al, the ashed product of the HF elutions was an amorphous solid.

This separation method was applied to rat brains. Four mg ^{27}Al was added to 4 rat brains which were acid digested as

described in 2.1 above. After acid evaporation the residue was solubilized in 0.2 ml 35% HNO_3 and 2-3 ml H_2O_2. For two of the samples, this was diluted to 0.2% HNO_3 by addition of 35 ml H_2O, to which 2 gm resin was added. Analysis of the supernatant showed ≈ 99% of the Al bound to the resin and the presence of 2.3 and 3.0 mg of P. Washing the resin with 5 ml 0.2% HNO_3 thrice removed ≈ 0.2% of the Al and 0.06 & 0.13 mg P. All of the P was in the first rinse and was probably derived from the solution originally surrounding the resin. Washing the resin with 5 ml 1N HF thrice recovered 64 and 88% of the Al from the 2 samples, compared to the Al in the dissolved residue of the two brains that did not undergo solid phase Al extraction. No measurable P was recovered. Because AMS analysis utilizes only part of the sample, and determines the $^{26}Al/^{27}Al$ ratio, it is not necessary to obtain quantitative Al recovery. The liquid in the 1N HF eluants was evaporated in the acid-trapping desiccator (above) and the residue ashed at 1000°C. The result was an white amorphous material that was easily removed from the crucible and had a higher AMS beam current than traditionally processed samples (Table 1). In contrast, ashing the residue of the two acid digested brains which did not undergo P removal, and which contained 2.4 mg P, resulted in a glaze from one, and a glassy-amorphous material from the other that was more difficult to remove from the crucible than the amorphous material from the HF eluants.

Therefore, the procedure which we developed, shown in Figure 2, effectively separates Al from P and improves AMS analysis of ^{26}Al.

3. Conclusions

Aluminum and P can be separated using a sulfonic acid cation exchange resin, using the batch method. This enables preparation of Al_2O_3 with minimal P interference. This separation method may be applicable to other problems. For example, P is a concern for neutron activation analysis (NAA) because it undergoes a nuclear reaction to form ^{28}Al, as does ^{27}Al when it absorbs a thermal neutron. The technique proposed herein may overcome the interference of P in Al assay by NAA, if quantitative recovery can be accomplished or a suitable internal standard identified.

One limitation to the application of the present method might be the Al inherent in the resin. This could become important if ultratrace level separation was attempted. Due to the ability of this resin to avidly complex Al, scrupulously clean labware should be used to avoid Al complexation from the labware, as was conducted in the present work.

This method was developed to separate Al from the P in brain. Bone has over 10 times the brain Al concentration, ≈ 50 mg P/gm [19]. Heart, kidney, liver, muscle, pancreas, spleen and thyroid have P concentrations at least 50% of those in brain [19,20]. This method should avoid formation of a glaze when these tissues

are ashed, if this problem occurs.

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Figure Legends:

Figure 1. Elution of Al from 2 gm of AG 50-X8. Four mg of Al was introduced with 4 mg of P in 0.2% HNO_3. Elution with 5 ml of 0.2% HNO_3 thrice prior to elutions shown in the Figure resulted in removal of most of the P. No measurable P was observed during the elutions shown in the figure.

Figure 2. Procedure developed to prepare brain tissue for AMS analysis of ^{26}Al.

Table 1. The low energy negative ion beam current for Al analyzed from 9 targets prepared from brains ashed at 1000°C without P removal, from 2 targets prepared from brains from which P was removed prior to ashing at 1000°C and from commercial grade Al_2O_3.

sample	^{27}Al beam current (nA)
brains ashed at 1000°C without P removal	52, 29, 50, 47, 45, 33, 33, 52, 64 mean = 45, range = 29-64
brains from which P was removed prior to ashing at 1000°C	94 & 117
commercial grade Al_2O_3	200