REVIEW/COMMENTARY

Aluminum in Parenteral Products: Analysis, Reduction, and Implications for Pediatric TPN

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ABSTRACT: Performance and cost of the best available analytical methodology for the measurement of aluminum in parenteral products are presented. Typical levels of aluminum in representative solutions are summarized. A methodical approach to the minimization of aluminum contamination in the manufacturing process is considered in light of aqueous aluminum chemical considerations. Results of long-term clinical follow-up studies of infants maintained on currently manufactured TPN solutions indicate no adverse pathology arising from aluminum.

This paper will attempt to present an integrated understanding of a number of seemingly unrelated facets of the issue concerning levels of aluminum in parenteral solutions. The strides made in the analytical methodology, responsible for making routine measurement possible, will be shown as will the cost to implement this technology in a quality control setting. The aqueous chemistry of the metal will be examined to understand the complexity of the reactions involved. This is necessary to effectively troubleshoot a manufacturing process for the purpose of minimizing the aluminum levels in the product. A survey of typical values of aluminum to be expected in some representative solutions will be shown. Finally, the results of a long-term follow-up study involving over a hundred pediatric patients maintained on TPN over a ten-year period will be presented in an attempt to evaluate the likelihood of demonstrable pathology accruing from TPN admixtures, as currently compounded.

I. Quantitative Methods

Until relatively recently, the acquisition of accurate and precise determinations of aluminum was considered something of an art. Versieck and Cornelis (1) have reviewed the literature on the estimation of aluminum in human plasma or serum. The 17 reviewed papers had been published between 1960 and 1979 and showed mean aluminum concentrations ranging from 3.72 to 1460 ppb. Since electrolyte levels in humans are regulated within narrow limits by the kidneys and other systems, most of the variation among the means was attributed to analytical problems.

The analytical methodology has evolved extensively since that time. Because of the essential role that the analysis plays in implementation of an enforceable standard, it is well to briefly review the improvements made to overcome the wide variability shown by previous investigators during the last decade. The technique that most commends itself for our purposes is graphite furnace atomic absorption spectrometry. It is specific, can be made sufficiently precise, and is automatable. In this methodology, Figure 1, a liquid drop of sample is heated at various stages, to successively dry the sample, char off the organic interferences, and char off the low boiling inorganic salts. Finally, the temperature is sharply raised to volatilize the aluminum in the sample and atomize it, so that it intercepts a beam of light at the specific wavelength of aluminum. This results in the atomic absorption process that leads to estimation of mass by reduction of light intensity (2).

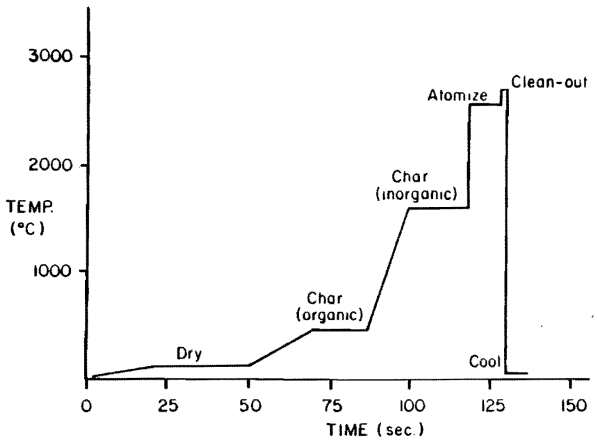

Figure 1—Typical graphite furnace atomic absorption heating program.

[*EDITOR'S NOTE:* This is the fourth in a series of four presentations on "Aluminum in Parenteral Products" from the PDA Annual Meeting, Chicago, IL, October 1988. They are being published as a group in this issue.]

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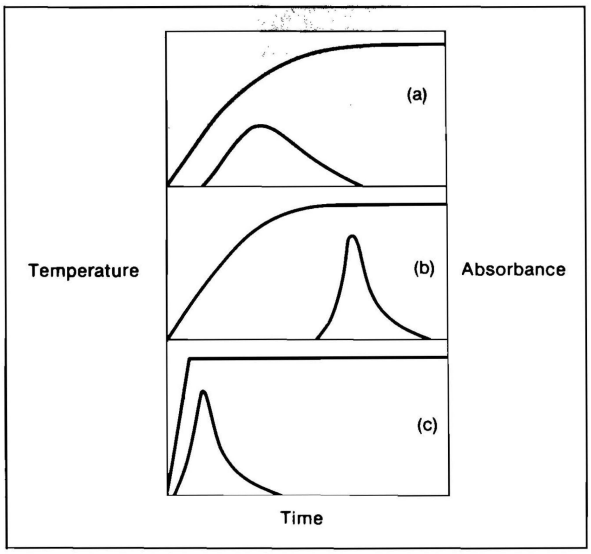

Figure 2—Furnace temperature (left) and absorbance (right) vs. time profiles, characterizing several modes of instrumental performance: (a), slowly heated furnace tube; (b), slowly heated furnace tube with L'vov platform; (c), rapidly heated furnace tube.

The problems suffered by earlier investigators occurred because the heating profiles shown in Figure 2 for the furnace temperature were too slow in attaining a plateau of stable instrumental performance. The sample aluminum atomic cloud, formed during the rapidly changing furnace temperature, resulted in large variability of the results. The problem was solved in two ways. The heating rate of later model furnaces was increased so as to move the onset of stable operating temperature earlier in the run cycle. Additionally, the atomization of the sample was delayed as shown in Figure 2. The sample is deposited on top of a L'vov platform, Figure 3, rather than on the bottom of the furnace tube which is what is directly heated. This delays onset of volatilization until the establishment of equilibrium instrumental conditions (3).

But the major improvement occurred in background correction capability. As depicted in Figure 4, despite attempts to remove the matrix earlier in the heating program, nonspecific absorption will occur during atomization due to residual interferences. Earlier optical designs permitted too broad a wavelength range around the absorbance peak to enter the monochromator. Also, they certainly could not differentiate between absorption due to aluminum and that due to extraneous background absorption occurring at the apex of the aluminum peak. This capability was accomplished with the introduction of the polarized Zeeman technique (2).

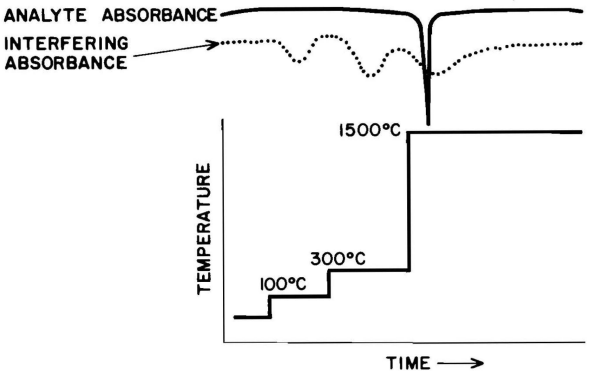

Figure 4—Interfering absorbance superimposed upon heating profile.

As shown schematically in Figure 5, a magnetic field is placed around the furnace tube. This causes the absorption line of aluminum to split into several component lines, separated by wavelength. At the same time, the absorption lines are now polarized; they will absorb light only if (in addition to a wavelength match) the light is similarly polarized in the same direction with respect to the direction of the magnetic field. For example, the central line in Figure 5 will absorb only light which has its electric vector polarized parallel with respect to the magnetic field. To take advantage of this, the unpolarized light from the analyzing lamp is split into components polarized parallel and perpendicular to the magnetic field, by interposing a rotating polarizer in the optical path. Only the parallel component is absorbed by the sample aluminum, while the nonspecific background will absorb both components

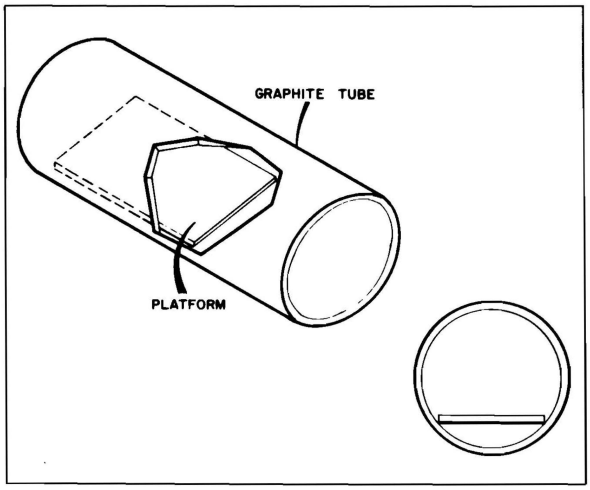

Figure 3—L'vov platform.

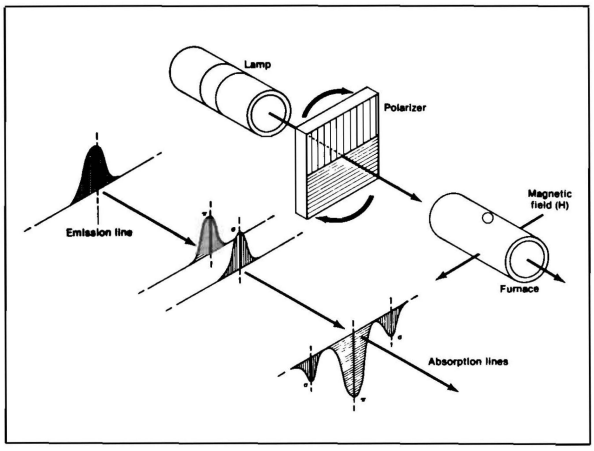

Figure 5—Schematic diagram of polarized inverse Zeeman graphite furnace atomic absorption spectrometer.

TABLE I. GFAAS—Aluminum Assay Validation Composite Average for Days 1–3

Instrument	Sample	Conc. ppb Al	Precision CV(%)	% Recovery 9.9 ppb Al
Zeeman 5000	1	4.7	3.66	100.6
	2	2.5	6.07	102.7
Model 603/2100	1	5.3*	11.7	63.3
	2	2.8*	39.3	62.9

* Spike recoveries used to correct sample concentrations.

equally. This is the principle behind this enhanced background correction technique (2).

The improved performance, attendant with polarized Zeeman capability, is apparent in Table I (4). (Full experimental details are found in the reference cited.) Levels of aluminum in Dianeal® CAPD solution are analyzed by two graphite furnace atomic absorption instruments, one with and one without Zeeman (but with deuterium) background correction. Without Zeeman, a recovery of only 60% is found for a 10 ppb aluminum spike intentionally added to the sample. With Zeeman capability, the recovery is increased to 100%. Techniques that have low recovery usually have variable recovery as well, which leads to high coefficients of variation. This is the case here, as a C.V. of almost 40% is found for the conventional instrument, but a respectable 6% is found for the Zeeman instrument.

The method developed in our laboratory, Table II, features acidification of the sample in nitric acid, and utilization of magnesium nitrate, a matrix modifier. This makes for a more forgiving method in terms of tolerating widely varying sample types (5). The performance of the method when applied to the analysis of four types of parenteral

TABLE II. Polarized Zeeman Graphite Furnace Atomic Absorption Spectrophotometric Procedure and Program

Sample preparation: Dilution with 4% HNO_3/0.4% $Mg(NO_3)_2$
Instrument = Perkin-Elmer Zeeman 5000AAS with HGA-500 Graphite Furnace and AS-40 Autosampler
External Standards = 0, 5, 10, and 20 ng/mL in 4% HNO_3/0.4% $Mg(NO_3)_2$

Sample Size = 20 μL	Tube Type = Carbon pyro with L'vov Platform
Wavelength = 309.3 nm	Slit = 0.7 nm
Mode = Abs	Current = 25 mA
Int. Time = 4.0 sec	Signal = Peak Area

Step	Temperature (°C)	Ramp Time (s)	Hold Time (s)
1	90	10	10
2	100	10	25
3	500	30	10
4	1500	20	10
5 (Read)	2550	0	5
6	2600	1	4
7	20	1	5

Ar Int. Gas Flow = 300 cc/min except for Steps 1 and 5.

TABLE III. Comparison of Baxter and Alfrey Methods for Determination of Aluminum in Parenteral Solutions

	Baxter			Alfrey	
	Mean (μg/L)	C.V. (%)	Within-Unit C.V (%)	Mean (μg/L)	C.V. (%)
10% Travamulsion	14.5	5.4	0.4	13.3	19.4
25% Albumin	280.0	14.5	2.5	317.0	12.2
Heparin sodium (1000 U/mL)	47.8	6.0	1.3	50.7	2.8
5% Dextrose	<1			<2	

solution is shown in Table III. Also shown for comparison are the results of Dr. Allen Alfrey, to whom we sent a number of samples from the same lot of each of the four solutions. The mean values found by both methods agree. The coefficient of variation, representing container to container variability is shown in the first set of parentheses for the Baxter data, and is compared to that for the Alfrey method. The second set of parentheses for the Baxter data indicates C.V. for repeated determinations from a single container, and is indicative of intrinsic variability in the instrumental and sample preparation.

These data are comparable to those reported by Koo, Table IV (6), who found that LVP's typically contain less than 50 ppb and that most of the problem occurs for phosphate salts, both sodium and potassium, as well as for gluconate. It is clear that in order for any method to be useful in determining aluminum levels in clinically important solutions, validation work should be designed with these matrices in mind. As part of work performed on behalf of a PDA task force to evaluate analytical methodology for aluminum in parenteral solutions, we compared the performance of the method developed in our laboratory to that of one submitted by Dr. Ted Rains of the National Institute of Standards and Technology, Table V. Phosphate containing solution, as well as amino acids, with and without electrolytes, a simple electrolyte solution, and heparin were subjected to the inter-method study. As it was originally submitted, the NIST procedure

TABLE IV. Sources of Aluminum Contamination in Parenteral Nutrition Solutions

Product	Number Tested Sample/Lot/Mfg.	Al (ug/L)
Sterile water	7/7/3	<5
Dextrose water (5–50%)	13/13/3	<5
Cryst. amino acids (5–10%)	17/17/3	<5–47
Soybean oil emulsion	4/4/1	<5
Sodium chloride	3/3/1	<5–5
Sodium acetate	1/1/1	<5
Sodium phosphate	7/5/2	<5–2370
Sodium lactate	2/2/1	184
Potassium chloride	9/9/5	<5–17
Potassium phosphate	4/4/2	90–2300
Calcium gluconate	11/11/5	1100–5600
Calcium chloride	5/4/3	5–19
Magnesium sulfate	5/5/3	<5–5

TABLE V. Comparison of Baxter and NIST Methods for Determination of Aluminum in Parenteral Solutions

Solution	Baxter (μg/L) Mean	SD	NIST Method of Addition (μg/L) Mean	SD	NIST External Standards (μg/L) Mean	SD
8.5% Travasol® inj. with electrolytes	16.0	1.5	21.0	0.2	18.6	1.2
8.5% Travasol® inj. without electrolytes	10.5	1.7	9.7	4.5	9.5	4.2
Plasma-Lyte® solution	1.5	1.4	2.4	4.2	2.3	4.0
Heparin lock	<0.4		<3.6		<3.6	
Sodium phosphate	250.0	1.7	302.0	60*	193.0	14*

* Dilution factor increased to improve reproducibility.

featured a method of addition technique. This is fine for imparting a robustness to the method in terms of matrix compensation for a wide variety of different solution types, but it makes for lower sample throughput. One must assay not only each sample, but each sample intentionally spiked with a known addition of aluminum at several different levels. To evaluate the capability of the NIST method to be run faster, we compared its performance utilizing external standards. Comparable results were found for the mean values of all three methods for the first four solutions, although the NIST methods have a somewhat high CV at 4% for repeated injections.

Irreproducible results were found with the NIST methods when applied to the phosphate solution. We suspected that the acceptable performance from the Baxter method arose from the incorporation of the matrix modifier $MgNO_3$ in the sample preparation. This reduces sensitivity of the method to phosphate in the sample matrix (5). As stated by Slavin et al., the mechanism of reduction of interference effects is attributable to imbedding of the aluminum in a matrix of magnesium oxide. This delays vaporization of the analyte until the magnesium oxide is vaporized. In any event, lacking this modifier in the sample dilution step, the NIST procedures incurred wide variability. We attempted to mitigate this problem by increasing the sample dilution factor, in an attempt to dilute out the phosphate interference. This was somewhate successful, in that the means now found by the NIST methods did not differ statistically from that of the Baxter method, although a standard deviation of 60 ppb resulted. The lesson here is that even though a method appears to work well for the determination of aluminum in four sample matrices, there is no guarantee that it will work on the fifth.

Assay variability is particularly a problem with methods operating at the trace level. Sample contamination, adsorbtion to the walls of the sample container, and limitations in sensitivity of the analytical method become more onerous as the analyte concentration is decreased. To underscore this are the results of an analysis of fifty interlaboratory round robin studies conducted by the Association of Official Analytical Chemists (7), shown in Figure 6. Each study involved at least twenty laboratories. All different kinds of analytical methods as applied to many types of analytes were studied. The major factor leading to assay variability was identified as the concentration level of the analyte sought. Precision for the major ingredient of a dosage form poses no problem. But as the concentration of the substance sought decreases, the interlaboratory coefficient of variation increases, following a power law. At the ppb level, a C.V. of 40% was found. It is clear that especially at trace levels, a careful validation of the analytical method is essential.

The large interlaboratory variability found by the AOAC at the ppb level would seem to be at odds with the rather close agreement among aluminum levels reported in Tables III and V of this work. The minimal interlaboratory variability found here is attributable to the select nature of the three laboratories whose work is presented. Far from representing a random sample of average analytical outfits, these three laboratories have spent many years dedicated especially to the particular analytical concerns associated with the determination of trace levels of aluminum. Our laboratory, for example, is continually involved with resolution of erroneous results for aluminum reported by contract laboratories who analyze parenteral solutions infrequently.

An estimate of the cost to validate a method is summa-

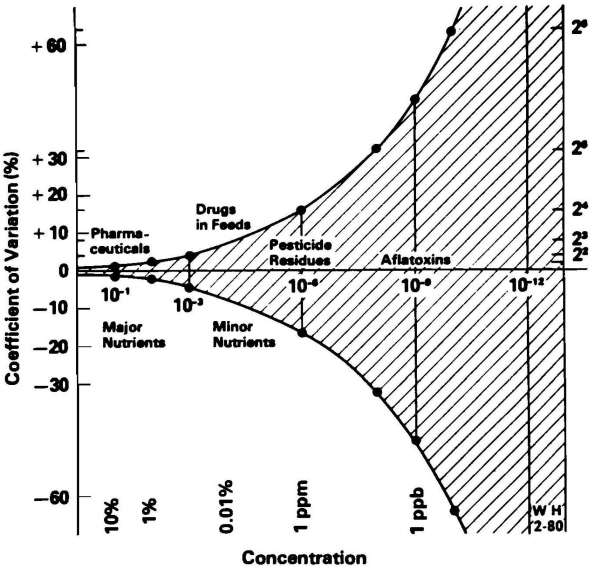

Figure 6—Interlaboratory coefficient of variation as a function of concentration. [Reprinted with permission from *J. Assoc. Off. Anal. Chem.*, Vol. 63, No. 6, pp. 1344–1354 (1980).]

TABLE VI. Aluminum Determination by Atomic Absorption Spectrophotometry

Cost Analysis	
Method Development and Validation for 20 Solutions	
Manpower: 20 man-months	
Cost: $160,000	
Operating Costs per Manufacturing Site	
Instrumentation	
2 Polarized Zeeman graphite furnace atomic absorption spectrometers with autosamplers	$136,000
Preventative maintenance contracts	6,500/yr
Personnel	
2 Chemists	80,000/yr
Supplies	
Graphite tubes and platforms	16,800/yr
Graphite cones, reagents, labware	1,350/yr
Argon	1,200/yr
Total	$105,850/yr

TABLE VII. Component Contributions to Aluminum Level in TPN Mixture

Component	Component Al Conc. (μg/L)	Contribution to TPN (μg/L)	Percent of Contribution
10% Amino acids	47	17.6	7.0
50% Dextrose	3	1.5	0.6
Sterile water	<DL*	0	0
Calcium gluconate	5200	223	88.7
Magnesium sulfate	4	0	0
Potassium chloride	<DL*	0	0
Sodium acetate	50	0.7	0.3
Sodium iodide	<DL*	0	0
Selenium	1170	3.5	1.4
Chromium chloride	960	0.5	0.2
Copper sulfate	462	1.2	0.5
Manganese sulfate	471	3.3	1.3
Zinc sulfate	60	0.2	0.1
Total		251.5	100.1

* <DL means less than a detection limit of 0.4 μg Al/L.

rized in Table VI. Approximately one man-month per solution type is estimated. Using typical overhead expense figures, twenty solutions would cost $160,000 for validation. Capital expenditure would cost almost $140,000, assuming one purchased a polarized Zeeman graphite furnace atomic absorption spectrometer, as well as a backup, for those occassions when the first failed to operate. This would become necessary if mandatory testing were imposed, to prevent shutting down production simply because the primary analytical instrument failed. Typical operating costs, including maintenance contracts, supplies, and the chemists, skilled in trace metal analysis, would run in excess of $100,000 per year.

II. Minimizing Aluminum Levels in the Product

Assuming that one were required to implement testing, what are the sample matrices for which validation should be performed? To answer this, we analyzed a typical TPN admixture to assess the contribution to the total aluminum level from each of the components shown in Table VII. It was found that the amino acids contributed about 3% of the total aluminum. While some of the trace metal additives had quite high aluminum concentrations, their overall contribution was quite low, a few percent, by virtue of the small volumes employed in the admixture. Calcium gluconate, on the other hand, contributed about 89% of the total aluminum found. These figures are in substantial agreement with the data reported by Koo (6), who also found that less than 3% of the aluminum arose from the amino acids, and about 89% resulted from the calcium gluconate. Clearly, the largest reduction of aluminum levels in TPN admixtures would accrue from addressing the most heavily contaminated components.

We have seen that phosphate salts, gluconate salts, and citrate salts as well, carry the highest aluminum burden. To understand why this is the case we must understand the structure of aluminum in water (8), Figure 7. In highly acidic aqueous solutions, aluminum exists as a triply positively charged ion, coordinated to six neutral water molecules. As such, it has a very high charge density. It forms quite strong complexes with negatively charged oxyanions by electrostatic attraction, especially if they are capable of binding at multiple ligand sites about this octahedron. Such is the case for phosphate, citrate, and gluconate (9). In the process of preparation of raw materials made from these anions, complexation with aluminum is inevitable, and this is carried through to the final dosage form. Precipitation of aluminum salts in pharmaceutical preparations, buffered with phosphate, have been discussed previously (10, 11, 12).

As the pH is raised, protons are hydrolyzed off the coordinating water molecules, leaving negatively charged hydroxide ions in their place. This reduces the overall charge on the complexed ion, and hence the coulombic repulsion between such species. Mutual approach is facilitated, and in fact dimers are formed (8), as two octahedra share an edge. As the pH rises still further the positive charge decreases, permitting ring structures to form (13) as each octahedron shares two edges with neighboring complexes. With pH in the neutral region, the charge decreases to zero. There is no longer repulsion among the species, and extended networks develop (13), attaining colloidal dimensions, eventually precipitating out of solution as aluminum oxide, Figure 8. With further rise in the

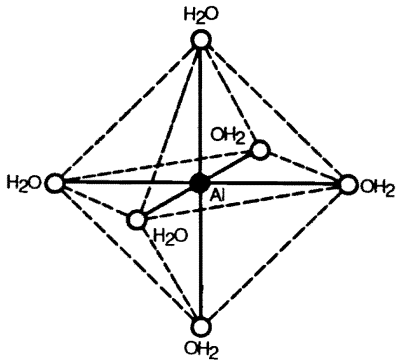

Figure 7—Schematic representation of aquo-aluminum ion $Al(H_2O)_6^{3+}$.

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