

Reduction of Aluminum Levels in Dialysis Fluids Through the Development and Use of Accurate and Sensitive Analytical Methodology

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ABSTRACT: Various analytical methods of determining the aluminum content of CAPD (continuous ambulatory peritoneal dialysis) solutions and associated raw materials were developed and compared. The methods include two graphite furnace atomic absorption procedures and an aluminum-lumogallion fluorescence technique that can quantitate aluminum in CAPD solution at, and in some cases below, the 1- $\mu\text{g/liter}$ level with good accuracy and precision. These methods were then used to examine the possible contributions to aluminum content from all aspects of the production of CAPD solutions at Travenol Laboratories, Inc. The major source of aluminum to the low, but measurable, levels in CAPD solutions was lactic acid. With the use of USP grade or equivalent ingredients, especially a low-aluminum-content lactic acid, these solutions can be prepared with aluminum levels below 10 $\mu\text{g/liter}$.

Introduction

Concern about trace levels of aluminum in parenteral products has arisen in connection with contamination incurred from the container (1, 2) closure (3) or the drug (4, 5) itself. Excessive intake of aluminum from dialyzing fluids (6–17) and aluminum containing phosphate binders (18–26) has been associated with dialysis dementia and bone disease in patients with chronic renal failure. The maximum aluminum levels in dialysis fluids which have been considered as “safe” by various researchers have ranged from 10 to 50 $\mu\text{g/liter}$ (10, 15–17, 21). The part per billion accuracy required of analytical methods used to study these aluminum levels is available to only a few laboratories willing to expend the effort to achieve reliable results. In contrast, the prevalence of expensive atomic spectroscopic instrumentation would suggest trace metal analysis to be a routine undertaking. The numerous contradictory reports within the literature imply that while such determinations may be widespread, inaccuracy is pervasive (27). The erroneous values adversely affect our understanding of the clinical problem, thereby preventing rational determination of what constitutes safe levels of aluminum.

The present studies describe reliable analytical methodology necessary to assess the concentration of aluminum in dialysis fluid products. In the absence of a recognized reference standard for aluminum in this matrix, the reliability of the methods was established through interlaboratory and intermethod comparisons. This required corroboration of results by redundant methods based upon dissimilar physical principles.

Utilizing these validated methods, aluminum levels in DIANEAL[®], peritoneal dialysis fluid manufactured by Travenol Laboratories, Inc., were found to be far lower than the 50- to 100- $\mu\text{g/liter}$ levels implicated in patient cases involving excessive aluminum loading. To assure continued low levels, a program was undertaken to evaluate sources contributing to the small, though measurable, aluminum levels in DIANEAL[®] solutions. This process was simplified by the numerous worldwide manufacturing facilities, that lent themselves to comparative analysis. During this study, several plants were found with exceedingly low levels in their product. The focus of the research program was then altered to reduce aluminum levels from all plants to that of the facility with the lowest values. Many factors were evaluated as possible contributors of aluminum to the final product: raw materials, water purification system, mixing, pumping, filtering, filling, sterilization, container material, and time of storage.

Experimental

Instrumentation

Two graphite furnace atomic absorption spectrophotometers (GFAAS), an inductively coupled plasma atomic emission spectrophotometer (ICP-AES), and two spectrofluorometers were used in the work reported here. The Perkin-Elmer Model 603 atomic absorption spectrophotometer with HGA 2100 graphite furnace included a Model AS-1 autosampler. The excitation source was the 309.3-nm line from an aluminum hollow cathode lamp (Perkin Elmer) set at 12-ma current and was adjusted to a slit width of 0.7 nm. To correct for background interferences to the aluminum signal caused by the high salt and organic matrix of the dialysis fluids, a deuterium back-

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ground correction was used. Nonpyrolytic graphite tubes (Perkin Elmer #0290-1633) were found to be more sensitive than the pyrolytically coated ones (Perkin Elmer #B0091-504). The temperature program used with the HGA 2100 graphite furnace was ramp 30 s to 120 °C, hold for 20 s, ramp 25 s to 1200 °C, hold for 35 s, then atomize at 2650 °C for 9 s. Argon flow through the tube was 30 cc/min except at atomization when the Argon interrupt mode was turned on. The peak height was used for the absorbance measurement with the signal monitored for 5 s during atomization.

Later work involved the use of a Perkin-Elmer Model Z5000 spectrophotometer with an HGA 500 graphite furnace and AS-40 autosampler. The background was compensated by using Zeeman effect background correction. With the availability of this instrument the assay was modified to use a pyrolytically coated graphite tube (Perkin Elmer #B0109-322) with a L'vov platform (Perkin Elmer #B0109-324). An aluminum hollow cathode lamp set at 15-ma current was used as in the 603/2100 system. The temperature program for the Model 5000 furnace was ramp 25 s to dry at 110 °C, hold 20 s, ramp 20 s to char at 350 °C, hold 15 s, ramp 30 s to char at 500 °C, hold for 10 s, ramp 20 s to char at 1500 °C, hold 25 s, atomize at 2700 °C, hold for 7 s, ramp 1 s to cool at 20 °C for 15 s. The Argon flow through the tube was 300 cc/min except at atomization when the Argon interrupt mode was used. Peak area was used for absorbance measurements over a 6-s integration time.

Inductively Coupled Plasma-Atomic Emission Spectroscopy (ICP-AES) was used as an alternate method for measuring aluminum levels in certain raw materials. The specific instrument used was the Model JY38P (Instruments S.A.), which includes a Model ICP 2500 Inductively Coupled Plasma source, maximum power 2.5 mW at 27.12 MHz (Plasma Therm, Inc.), a Czerny-Turner 1-meter monochromator with 2400 g/mm holographic grating, and a spectral range of 190–750 nm using the Hamamatsu R928 photomultiplier tube as a detector. Emission intensities for aluminum were measured at the 396.15-nm line. Due to the low aluminum concentrations present in the CAPD solutions and the low sensitivity of ICP-AES as compared with GFAAS, the inductively coupled plasma technique was used only for those raw materials which contained greater than 0.1 µg/g of aluminum.

The determination of aluminum down to the µg/liter level could be performed by the aluminum-lumogallion fluorescence method similar to one previously described by Hydes and Liss (28). The instrument used for these experiments was the Perkin-Elmer 650-105 Fluorescence Spectrophotometer. Excitation and emission wavelengths used for the aluminum-lumogallion complex were 472 nm and 568 nm, respectively. The method of multiple standard additions was used in this case since the competition of the lactate ion with lumogallion for complexation of aluminum resulted in low aluminum values when determined with external standards. The actual procedure used is summarized in the following description.

Four 50-mL aliquots of the sample were added to polyethylene containers along with 0.5 mL of an acetic acid

(Baker, Ultrex)/sodium acetate (Fisher) buffer (4 M in acetate and adjusted to a pH of 5), and either 0.25 or 0.5 mL of 0.02% lumogallion (Pfaltz and Bauer) solution. The bottles were spiked with 0, 5, 10, and 20 ppb of an aluminum standard, respectively, for a multiple standard additions determination. A fifth bottle containing 50 mL of deionized distilled water, to be used as a reagent blank, was similarly treated. The bottles were transferred to a water bath held at 80 °C for 1.5 hr. The samples were then allowed to cool to room temperature and analyzed with a spectrofluorometer within 24 hr of preparation. The straight line obtained by a regression analysis was extrapolated back to zero emission intensity where the Al concentration of the sample could be obtained once correcting for the reagent blank.

Contamination Control

Avoiding aluminum contamination is essential to any assay with measurements at the µg/liter level. A critical factor for successful aluminum contamination control was the use of properly cleaned plastic labware for sample handling and storage. Samples and standards were allowed to contact only suitably cleaned plastic labware. The following cleaning procedure was found most effective in removing aluminum from plasticware. The labware was soaked in a low-metal content laboratory detergent (NRS-250 Norell, NJ), rinsed with distilled water, then soaked for at least 2 hr in a 10% nitric acid bath, thoroughly rinsed with deionized distilled water, dried face down on rubber matting, and stored in sealed plastic bags. The plasticware was rinsed again with deionized distilled water just prior to use. Containers were left open for as short a time as possible to avoid airborne contaminants. Eppendorf micropipettors with disposable plastic tips were used throughout. The polystyrene autosampler cups (Perkin Elmer) were also soaked in acid and rinsed with deionized distilled water.

Standards

A 1000-mg/liter aluminum stock solution was prepared by dissolving Al(NO₃)₃·9H₂O (J. T. Baker, 99.1% by EDTA titration) in deionized distilled water. This was stable for at least 6 months. Intermediate and calibration standards were prepared in polymethylpentene (PMP) volumetric flasks (Nalgene). A reagent blank and 2-, 5-, 10-, and 20-µg/liter aluminum standards were typically employed, although standards as high as 100 µg/liter of aluminum also have been used. For use with PE603/2100, all samples and standards were made 0.5% v/v in nitric acid (Baker Ultrex grade). In a later modification of the assay for use with the Perkin Elmer Z5000 instrument, in addition to the nitric acid, recrystallized Mg(NO₃)₂ (Fisher Scientific ACS grade) was also added as a matrix modifier at a concentration of 0.05% w/v similar to the procedure described by Manning et al. (29, 30).

Sample Preparation

Preparation of CAPD solution samples for aluminum determination required special procedures to avoid contamination. The solutions are distributed either in glass

bottles or VIAFLEX® (polyvinyl chloride) bags. For the VIAFLEX bag, one port was cut open with a razor blade or scalpel. Some of the contents were allowed to drain to waste to clean the port and minimize external contamination of the small aliquot used. The aliquot was then transferred to a small polyethylene bottle. Initially, only nitric acid, and later, nitric acid plus $Mg(NO_3)_2$, were added as matrix modifiers as with the standards.

For glass bottles, the cap and rubber septum were first removed from the bottle stopper. The bottle was then inverted and some solution allowed to drain to waste. To avoid contamination, aliquots were taken without interrupting the sample stream. The aliquots were then treated similarly as VIAFLEX container samples. As can be seen by some of the data shown later in this article, e.g., see Table III, samples with very low aluminum concentrations and present in either glass or VIAFLEX containers can be assayed by this technique without incurring outlier values because of contamination.

A similar sample preparation technique was used for liquid raw materials such as sodium lactate solutions. These may be diluted if necessary to fall within the range of the working standards.

Sample Transportation and Storage

For those samples that were to be stored, or transported in other than their original containers, treatment was required to insure against losses of aluminum by adsorption onto the container surfaces. Polyethylene bottles were used to transport these samples. These bottles, as described previously under "contamination control," were further soaked in a concentrated EDTA solution then rinsed with deionized-distilled water. Sufficient EDTA (EDTA, disodium salt, Mallinckrodt AR Grade) solution was then added to allow a final concentration of 0.01% of EDTA in the filled bottle.

An experiment was performed to determine whether 0.01% EDTA biased the analysis. Both water and DIANEAL® samples with and without added EDTA were spiked with 0, 10, and 50 $\mu\text{g/liter}$ of aluminum. Results of the aluminum analysis gave $100 \pm 5\%$ recovery at both spiked levels and indicated no bias for the unspiked samples. The samples could be stored for at least 1 week without any noticeable losses of aluminum.

Unopened samples were stored at ambient temperature in their original containers. Opened solutions were transferred from their original containers to the polyethylene bottles and EDTA added. Whenever possible, these solutions were also refrigerated if storage for more than 1 day was required.

Results

Evaluation of the GFAAS Method

An instrument-to-instrument comparison was one of the techniques used to evaluate the GFAAS methodology. The Perkin-Elmer 603 with HGA 2100 and Perkin-Elmer Zeeman 5000 with HGA 500 furnace described above were used for this comparison. In addition, somewhat different experimental procedures were used with each

instrument. A common set of DIANEAL solutions was analyzed each day on each instrument over a period of 3 days. A composite of the results of this study is given in Table I-A. DIANEAL solutions containing approximately 2.5 and 5.0 $\mu\text{g/liter}$ of aluminum were used along with these same samples spiked with 9.9 $\mu\text{g/liter}$ of added aluminum. Recoveries of 52–68% for the 9.9- $\mu\text{g/liter}$ spiked samples were obtained on the 603 instrument, while recoveries of 98–104% were obtained on the Zeeman 5000 when determined against external aluminum standards. Using the spike recoveries to correct the assay values obtained on the Model 603, however, resulted in aluminum concentrations that agreed quite closely with the values obtained directly on the Zeeman 5000 instrument. This study showed that accurate aluminum results could be obtained with the 603/2100 system if a standard addition technique was used. The graphite furnace methodology was also compared with other analytical procedures used for the determination of aluminum concentrations. The GFAAS procedure was compared with Inductively Coupled Plasma-Atomic Emission Spectroscopy (ICP-AES). This comparison was performed on solutions containing higher aluminum levels ($>50 \mu\text{g/liter}$) since the detection of aluminum by ICP-AES was 30 $\mu\text{g/liter}$. Results of this comparison are also given in Table I-B.

The GFAAS method was also compared to an aluminum-lumogallion fluorescence technique. Comparisons of the two techniques were for DIANEAL solutions with aluminum concentrations varying between 1 and 25 $\mu\text{g/liter}$. The results of the comparison are given in Table I-C. The fluorescence technique had a precision of 7.4% at the 6- $\mu\text{g/liter}$ level with a detection limit of 1 $\mu\text{g/liter}$. The range was calculated from the standard error of the regression analysis at 95% confidence.

An interlaboratory comparison was also performed, involving Travenol Laboratories Research and Development facilities in the United States (Morton Grove, IL)

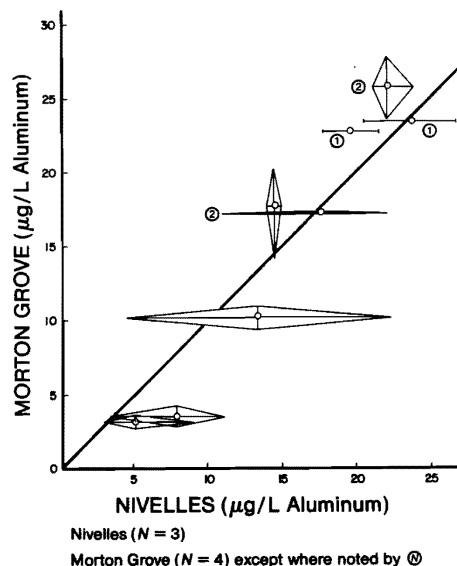


Figure 1—Interlaboratory comparison of aluminum levels determined in several lots of peritoneal dialysis solution.

TABLE I. Assay Validation—Comparisons Between Methods

IA: GFAAS (Zeeman 5000) versus GFAAS (603 W/D₂)

Instrument	Sample	Al ($\mu\text{g}/\text{liter}$)	Precision CV (%)	% Recovery ^a (9.9 $\mu\text{g}/\text{liter}$ Al)	Precision (CV%) Of Standard Addition	Detection Limit ($\mu\text{g}/\text{liter}$ Al)
Zeeman 5000	1	4.7	3.66	100.6	1.7	0.35
	2	2.5	6.07	102.7	1.9	0.35
Model 603/2100	1	5.3 ^b	11.7	63.3	8.3	1.2
	2	2.8 ^b	39.3	62.9	4.5	2.2

I-B: GFAAS (Zeeman 5000) versus ICP-AES—12 Lots Lactic Acid Raw Material

	Added ($\mu\text{g}/\text{liter}$ Al)	Found ($\mu\text{g}/\text{liter}$ Al)	% Recovery	Precision (CV%)
GFAAS	0.5	0.463	92.6	5.5
	1.0	1.009	100.9	6.1
	1.5	1.586	105.7	0.9
ICP-AES	0.5	0.536	107.2	1.0
	1.0	1.059	105.9	1.0
	1.5	1.596	106.4	1.6

I-C: GFAAS (Zeeman 5000) versus Fluorescence (Al—Lumogallion Complex)

Lot of CAPD	$(\mu\text{g}/\text{liter}$ Aluminum)						Lot of CAPD	$(\mu\text{g}/\text{liter}$ Aluminum)							
	1	2	3	4	5	6		1	2	3	4	5	6		
Fluorescence							GFFAS								
Mean range ^c (\pm)	1.44	1.44	2.62	6.85	6.09	24.43	Mean range (\pm)	1.80	1.36	3.20	5.95	5.70	22.80		
	2.0	2.0	0.8	4.2	^d	3.5		^e	0.8	^e	3.2	0.8	^e		^e

^a Versus external standards^b Percent recoveries were used to correct sample concentrations^c Range is 2X standard deviation of the x-intercept of the standard additions curve (blank corrected).^d Coefficient of variation for a sample analyzed on 5 different days was 7.4%: the ranges were from 0.8 to 4.^e Single sample analyzed (otherwise, n = 2 for graphite furnace).

n = 4 for fluorescence.

and Belgium (Nivelles). The Nivelles group used a Pye Unicam Model SP-9 graphite furnace atomic absorption spectrophotometer with a Model SP4-01 autosampler. The method was similar to the one described for the Perkin Elmer 603/2100 instrument, which the Morton Grove group used for this study, except a pyrolytically coated graphite tube was used and no matrix modifier was added. Spike recoveries ranged from 82 to 119%; therefore, no correction for recovery was required. The detection limit for this method was 3 $\mu\text{g}/\text{liter}$. Both laboratories analyzed multiple units from eight lots of CAPD solution. Data for each lot are presented as a rhombus (Fig. 1), the axes of which span the range of values found by each laboratory on the respective units analyzed. Optimally, values should lie on a straight line 45° from each axis. The Nivelles group analyzed three samples per lot and, except where indicated by circled number in Figure 1, the Morton Grove Group analyzed four samples per lot.

Interplant Survey

With the development of reliable analytical methodology, a comprehensive study was undertaken to measure the aluminum content of a wide variety of CAPD solutions produced by Travenol. This included solutions produced at fourteen of Travenol's international manufacturing facilities. A summary of the results can be found in Table II.

The range as well as the mean values of aluminum concentration in CAPD solutions produced at each facility are given.

TABLE II. Aluminum Content of CAPD Solutions—Interplant Survey

Facility	Method	Number of Lots (Samples per Lot)	Al ($\mu\text{g}/\text{liter}$)	
			Mean (Per Lot)	Range (All Units Tested)
A	2	7 (2)	1.98	1.3–3.0
B	3	2 (4)	14.0	11.2–18.2
C	1	2 (3)	10.4	2.6–20.8
D	3	3 (3)	19.0	14.6–23.8
E	1,2	2 (2)	21.5	17.2–27.9
F	2	2 (1)	23.0	22.7–23.4
G	2	7 (1)	3.97	2.6–5.9
H	2	10 (3)	3.48	1.5–7.2
I	2	10 (3)	3.05	1.3–9.2
J	2	1 (2)	3.2	3.1–3.4
K	2	2 (1)	1.3	1.1–1.5
L	2	3 (2)	2.7	1.7–4.1
M	2	10 (1)	0.54	<0.3–2.6
N	1,2	3 (1)	8.57	5.5–11.3

Method 1. P & E 603/2100 with D₂ background correction.

Method 2. P & E 5000 with Zeeman background correction.

Method 3. Pye Unicam SP-9 (Nivelles, Belgium)

Production Process Study

To determine the contribution of various phases of the production process to overall aluminum levels in a CAPD solution, a study of the entire process was undertaken. Facility A of Table II was chosen for this purpose because solutions with very low levels of aluminum were consistently produced there.

Figures 2 and 3 show the principal components of the process and aluminum levels found at each. Figure 2 describes the most important features of the water purification system used at this location, which is typical of most Travenol locations. Duplicate samples were taken at each of 10 different sampling locations along the water purification process. These sampling points included the incoming raw water, various storage and pumping stations, the filter and ion exchange sections, and before and after distillation. In addition, this sampling procedure was performed at two separate times in a single day, once near the beginning of a work period and once near the end. Figure 2 summarizes the salient features of the system along with the corresponding aluminum levels. The values indicated in Figure 2 represent mean aluminum values of a minimum of 4 samples taken at that point in the water purification system.

Figure 3 shows the CAPD solution production and filling line. Samples were taken of the batch water, all raw materials, and batch CAPD solution following mixing and at various points in the filling line. Samples of this solution were also taken following autoclaving in PVC bags. Duplicate samples were taken at six different locations along the filling lines with a summation of the results shown.

Container Study

CAPD solutions are distributed in two types of container, glass bottles and VIAFLEX plastic bags. VIAFLEX plastic, a formulation of polyvinyl chloride, has been in use as a container by most of Travenol's facilities. Table III contains results of a comparison of the aluminum

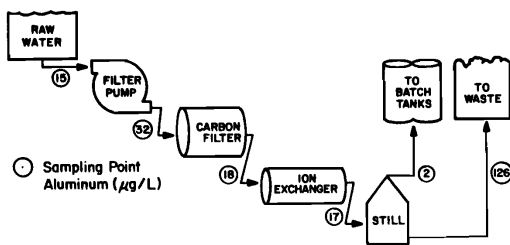


Figure 2—Analysis of water purification system for aluminum.

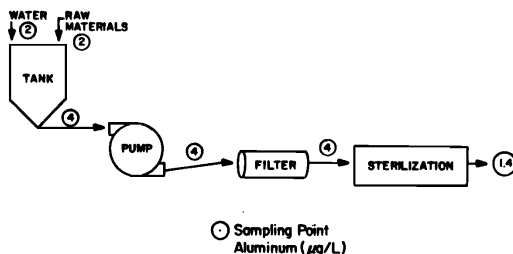


Figure 3—Analysis of process line for aluminum.

TABLE III. Aluminum Levels ($\mu\text{g}/\text{liter}$) for 20 Different Lots of CAPD Solutions Stored in Glass versus VIAFLEX® Containers

VIAFLEX®		Glass	
Mean ^a	SD	Mean ^a	SD
8.37	1.0	3.42	0.45
3.33	0.51	3.97	0.47
2.87	0.21	2.43	0.23
1.83	0.15	1.83	0.06
1.47	0.21	2.37	0.42
1.57	0.21	1.73	0.25
2.67	0.21	4.53	0.35
2.40	0.20	2.30	0.30
2.80	0.0	5.13	0.17
2.93	0.15	7.10	0.17

^a Three units of each lot were averaged.

content of DIANEAL solutions stored in VIAFLEX and glass containers. The analysis was performed by the P & E Zeeman 5000 method. Three units each of 10 lots of CAPD solutions from each of two facilities, one facility using glass and the other VIAFLEX containers, were compared.

Raw Materials

The raw materials used to prepare a typical CAPD solution code are given in Table IV.

For this study the solid raw materials obtained from six facilities (A–F from Table II) were combined in the required proportions to form a laboratory-prepared CAPD solution representing each facility. This permitted analysis by a method already validated for a CAPD matrix. The results for these solutions, as obtained using the Perkin Elmer 603/2100 instrument, ranged from 1 to 4 $\mu\text{g}/\text{liter}$ of aluminum. The only raw materials missing from the solutions were the plant water and sodium lactate.

Sodium lactate was analyzed separately from the other components of CAPD solution since it existed in liquid form and is itself manufactured from two raw materials, sodium hydroxide and lactic acid. Of all the raw materials, sodium lactate has the greatest potential to cause aluminum contamination. Samples of sodium lactate in pretreated containers were obtained from facilities producing CAPD solutions that consistently showed very low aluminum levels, as well as from facilities which produced solutions with somewhat higher aluminum levels. Figure 4 contains results obtained from two such facilities. The results were obtained using both GFAAS and ICP procedures prior to use. A study was performed to determine at what point the aluminum may enter the sodium lactate production. Samples of sodium lactate taken before and

TABLE IV. Components of CAPD Solution with 3.86% w/v Dextrose

Component	Contents per Liter (g)
Dextrose (anhydrous)	38.6
Sodium chloride	5.7
Calcium chloride	0.26
Magnesium chloride	0.15

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