Determination of Aluminum in Serum by Electrothermal Atomic Absorption Spectrometry with Prior Sample Pretreatment by Microwave Digestion and Solid-Phase Extraction

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A microwave-assisted digestion system enabling simultaneous treatment of thirty-six samples with oxidizing acidic media in double-closed vessels was devised. Aluminum was subsequently separated from the digested solution through solid phase extraction by prior reacting with 8-quinolinol and adsorbing onto the simple XAD-4 resin column. In the separated fraction, Al, being in a state free from matrix interferences, can be directly determined with electrothermal atomic absorption spectrometry (ETAAS) simply by an external calibration standard. With meticulous control of analytical blank introduced in the sample pretreatment steps, a good performance of analytical accuracy and limit of detection can be achieved. The proposed method, featuring freedom from potential interference in ETAAS and relatively fast sample throughput, can conveniently serve as a reference and/or an alternative method for the direct ETAAS method commonly used for routine clinical purposes.

Keywords Aluminum, serum, microwave digestion, solid-phase extraction, electrothermal atomic absorption spectrometry

The syndrome of aluminum toxicity has been suggested as one cause of Alzheimer's disease and recognized as the causative agent in patients with renal failure, particularly in those treated by haemodialysis.¹⁻³ Therefore, the potential biological function of aluminum and level of its concentration in biological samples has received a significant amount of attention.³

To meet analytical requirements in various applications, a number of instrumental methods for determination of aluminum, such as neutron activation analysis (NAA)^{4,5}, inductively coupled plasma atomic emission spectrometry (ICP-AES)^{6,9}, inductively coupled plasma mass spectrometry (ICP-MS)¹⁰, and electrothermal atomic absorption spectrometry (ETAAS)¹¹⁻¹⁴ have been attempted. Among these methods, ETAAS is the most widely used one to determine aluminum in biological materials, owing to its fairly high sensitivity, availability and rapidity.

For direct determination of aluminum in serum by ETAAS, the main difficulty encountered comes from the interferences of anions (e.g. Cl^-)^{15,16}, cations (e.g. K^+ , Na^+ , Ca^{2+} , Mg^{2+})^{4,16,17}, and organic substances (e.g. protein)^{17,18} present in the matrix solution. Aside from nonspectral interferences caused by the physical nature of the sample, some investigators attributed part of the interferences to the formation of AlCl_3 in the vapor phase through interaction of chloride with aluminum during

the atomization step;¹⁵ others observed signal changes occurring in the presence of Ca and Mg.^{4,15,16} To solve these matrix interference problems, several matrix modifiers such as H_3PO_4 , $\text{Mg}(\text{NO}_3)_2$ and $\text{NH}_4\text{H}_2\text{PO}_4$ have been commonly used to eliminate the major interference caused by the presence of chlorides in the sample. In addition, tetraammonium salt of EDTA was also proposed as a suitable additive to remove the chloride interference because of its ability to coordinate aluminum and other cations; the ammonium chloride produced can thus be easily volatilized.¹⁶

Beside the use of matrix modifiers in ETAAS as a direct method for Al determination in blood samples, other researchers¹⁹⁻²¹ preferred to apply a multistage-combined procedure to achieve this analytical purpose. In the latter method, the serum sample is first subjected to sample decomposition and chemical separation of Al prior to instrumental analysis. With such sample pretreatment process, the potential interferences of the matrix constituents can be effectively eliminated, but this may, in the meanwhile, inevitably introduce the risk of contamination. Consequently, special care should be taken in order to minimize contamination during the whole sample pretreatment process.

In order to enable this multistage procedure to be a practically applicable method, all precautions to obtain a stable and low blank value, such as use of minimum amount of high-purity reagents, use of small vessels made of inert material, performing experiments in a clean

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laboratory *etc.*, should be strictly followed.²² The use of closed-vessel microwave-assisted digestion procedures can meet most of these basic requirements. This is mainly because with this sample digestion method the acid required for digestion can be reduced and the exposure of the sample to laboratory air can be avoided.

With an attempt to further reduce the analytical blank and to facilitate increasing sample throughput rate, the commercially-available microwave digestion vessel was modified into a double-closed vessel system in this work. This was accomplished by placing three small Teflon-PFA vials (7 ml) into each digestion vessel (120 ml). A total of 12 vessels provided by the manufacturer can thus accommodate 36 samples for simultaneous digestion in a batch.

Following the digestion process, direct determination of Al by ETAAS may suffer from the presence of large amounts of interfering inorganic constituents still remaining in the digested sample.^{23,24} Consequently, an analytical procedure involving chemical separation prior to instrumental determination is necessary. Here, a solid-phase extraction procedure was used, based on the formation of Al-oxinate complexes, followed by adsorption on the laboratory-made XAD-4 column, which successfully extracted trace Al from the digested solution. In this work, optimization of the proposed sample pretreatment and ETAAS measurement was also investigated in some detail.

Experimental

Apparatus

A commercially available microwave digestion system (MDS-81D, CEM, USA), featuring programmable time and variable power (maximum power 630 W, maximum tolerable pressure 120 psig), was used for serum digestion. The system provides 12 Teflon-PFA digestion vessels (each 120 ml). Additionally, screw-capped Teflon-PFA vials (7 ml, Saville, Minnesota, USA) were used as inner vessels. The double-closed vessel was constructed by coupling three vials (7 ml) in sequence into each digestion vessel, as shown in Fig. 1.

Analyses of Al were performed with a Perkin-Elmer Model 4000 atomic absorption spectrometer (Norwalk, CT, USA) with deuterium lamp background correction, equipped with an HGA-300 graphite furnace atomizer, an AS-40 autosampler, and a Rikadenki Kogyo Model R-52 strip chart recorder. An aluminum hollow cathode lamp was used as a radiation source and pyrolytic graphite coated grooved graphite tube (Perkin-Elmer, Part No. B0109322) with pyrolytic graphite coated graphite platform (Perkin-Elmer, Part No. B0109324) was used in the Al determination. The peak height of Al atomization profiles was used exclusively for signal evaluation. Table 1 lists the instrumental parameters and the temperature-time program.

Fig. 1 Scheme of double-closed vessel system for microwave-assisted digestion. (a) vent tubing; (b) outer vessel cap; (c) relief valve; (d) outer Teflon-PFA vessel (120 ml capacity); (e) inner Teflon-PFA vessel (7 ml capacity).

Table 1 Instrumental parameters and graphite furnace program

Wavelength	309.3 nm			
Slit width	0.7 nm			
Lamp current	Al hollow cathode lamp at 9 mA			
Mode	peak height			
Sample volume	20 μ l			
Temperature-time program				
Step	Temp./ $^{\circ}$ C	Ramp/ s	Hold/ s	Ar flow rate/ ml min ⁻¹
Drying	120	10	50	300
Ashing	1700	10	30	300
Atomization	2400	0	5	0
Cleaning	2600	1	5	300
Cooling	20	10	10	300

Containers and reagents

All laboratory wares and digestion vessels used were made of polypropylene, Teflon-PFA or polytetrafluoroethylene (PTFE) and were thoroughly cleaned by soaking in nitric acid (1+1) for at least 24 h. Immediately prior to use, all acid-washed wares were rinsed with deionized, doubly distilled water.

Water used for both preparation and cleaning purposes was treated by reverse osmosis (Milli-RO 10 Plus, Millipore, USA) and mixed-bed ion exchange deionization (Milli-Q SP, Millipore, USA), followed by double distillation in a quartz still equipped with an immersion heater (Destamat, Heraeus, Germany).

All chemicals were of analytical grade except ammonium solution, which was E. Merck ultra pure NH_3 (Darmstadt, Germany). High-purity hydrochloric acid, nitric acid and perchloric acid were prepared by sub-boiling distillation in a quartz still from reagent grade

Table 2 Microwave-assisted heating parameters

Acid mixture	Step 1		Step 2		Step 3	
	Power, %	Time/min	Power, %	Time/min	Power, %	Time/min
HNO ₃ -H ₂ O ₂	100	1	50	30	—	—
HNO ₃ -HClO ₄	100	2	60	5	100	0

feed stocks. Hydrogen peroxide (30%, E. Merck) was used without further purification. The Merck Titrison stock standard solution (1000 mg l⁻¹) of Al was diluted to the desired concentrations with 1 M HCl solution.

Amberlite XAD-4 resin (Fluka AG, 20–50 mesh) was used without further purification. 8-Quinolinol, however, was purified as follows prior to use. An appropriate amount of 8-quinolinol was dissolved in dilute hydrochloric acid solution and passed through a laboratory-made XAD-4 resin column (ca. 0.7 mm × 35 mm). The column on which 8-quinolinol adsorbed was sequentially rinsed with 5 ml of 1 M HCl and 20 ml of deionized, doubly distilled water. About 2 ml of methanol pre-purified by sub-boiling distillation was employed to elute the adsorbed 8-quinolinol from the XAD-4 resin, and the effluent was further evaporated to dryness with vacuum suction at room temperature to obtain the purified 8-quinolinol. 1% (w/v) 8-quinolinol solution was prepared in dilute hydrochloric acid.

Column preparation

The laboratory-made column (Fig. 2) used for matrix separation of the digested sample solution was prepared by coupling two micropipette tips (1 ml) of which the tip ends had been melted and sealed by heating. In the center of the bottom part of the upper tip (a) was drilled a hole with a heating needle to act as solution inlet; while in the lower tip (b), a hole was drilled in the side end part to allow flowing out of the effluent (c). The lower tip was first packed with XAD-4 resin (ca. 0.5 g) and then coupled to the upper tip. Immediately prior to use, the prepared column was washed with 5 ml of methanol, 5 ml of 1 M HCl and then with deionized, doubly distilled water. The column of the same design was used throughout the experiments.

Sample treatment and analysis

Samples and solutions used in this work were prepared in a clean room equipped with laminar flow clean benches, providing a class 100 working environment.

Two microwave digestion procedures, using HNO₃-H₂O₂ and HNO₃-HClO₄ as acid mixtures, were tested to dissolve the serum samples. Typically, 0.5 ml of serum was allowed to be digested with the selected acid mixture in the small Teflon-PFA vial as an inner vessel. In the HNO₃-H₂O₂ acid mixture digestion procedure, 0.5 ml of serum, 0.5 ml HNO₃ and 0.5 ml H₂O₂ were used, while in the HNO₃-HClO₄ procedure, 0.5 ml serum, 0.5 ml HNO₃ and 0.2 ml HClO₄ were used. The inner vial was tightly capped by hand, and then three vials were put into an

Fig. 2 Scheme of the laboratory-made XAD-4 resin column: a & b, modified pipette tip; c, drilled hole for solution outlet; d, 0.5 g XAD-4 resin.

outer vessel (120 ml), as shown in Fig. 1. The cap of each outer Teflon-PFA vessel was closed by an automatic capping station (CEM). Next, twelve vessels each containing three sample vials were placed on the turntable, inserted into the microwave oven cavity, and digested according to the stepwise heating program shown in Table 2.

After digestion with full capacity of the 36 samples, vessel caps were removed by the capping station and by hand, respectively. The digested solution in the Teflon-PFA vials was then evaporated to dryness under the irradiation of an infrared lamp. The dried residue was dissolved in 1 ml of 0.1 M HCl, and to the same vial was added 0.1 ml of 1% (w/v) 8-quinolinol solution. After adjustment of pH to about 9 with ammonia solution, the resulting solutions were passed through a parallel sequence of XAD-4 resin columns. Then the columns were washed with 5 ml of deionized, doubly distilled water. The aluminum ions were then eluted from each column by introducing 1 ml of 1 M HCl at the flow rate of 0.25 ml min⁻¹ and collected in a polypropylene sample cup for determination by ETAAS.

After eluting out the 8-quinolinol chelating agent adsorbed on XAD-4 resin with the use of about 2 ml of purified methanol, the resin column can be ready for use for the next experiments.

Aluminum standards in the range of 2 to 40 ng ml⁻¹ were prepared by diluting the stock solution using micropipettes and polypropylene volumetric flasks. The concentration of aluminum in the treated serum sample was determined against these aqueous standard solutions.

Results and Discussion

Optimization of microwave sample decomposition

In attempts to facilitate sample decomposition in microwave digestion process, emphasis has to be put on how to reduce the analytical blank introduced, to improve the effectiveness in matrix decomposition and to increase the throughput rate of sample digestion. These were all properly investigated to a certain extent in this work.

Owing to the ubiquitous nature of Al element and its low concentration (it occurs in serum at the part-per-billion level)¹⁻³, aluminum contamination has been regarded as a significant problem in trace Al analysis, particularly when wet decomposition process is employed. In a previous paper²⁵, Skelly and his co-worker indicated that using a closed-vessel microwave digestion system and a clean room for sample preparation can effectively control the contamination problem by reducing Al concentration in digestion blanks from 10 ng ml⁻¹ to 1 ng ml⁻¹. They also demonstrated that the relatively large container surface brought into contact with the sample often led toward unacceptably high and/or random procedure blanks. This finding suggests that Al most probably leached from the vessel's surface could seriously affect the blank values. In this work, we employed an improved technique to reduce this type of contamination by using small-volume (7 ml) Teflon-PFA vials to facilitate the digestion of serum samples. With this proposed double-closed digestion system as shown in Fig. 1, a lower Al blank can be effectively achieved by reducing the amount of oxidizing acids required for digesting the small size of sample and by reducing the contact surface of the sample with the smaller digestion vessel. The data shown in Table 3, which lists the blank values of this analysis method, can clearly demonstrate this benefit. The blank values as low as sub-ng ml⁻¹ with both digestion mixtures can be generally achieved. In addition, the fact that thirty-six samples can be simultaneously treated as a batch in less than 30 min (as will be seen later in the heating programs) is undoubtedly an obvious advantage when comparing with the conventional digestion system, in which a maximum of only 12 samples is possible.

The heating program of microwave digestion is known to be one of the key factors affecting the efficiency of sample digestion. A full power microwave heating can be selected in order to facilitate sample digestion in a shorter time, but this would not be applicable in our present case. The reason is simply because under a violent digestion reaction at full power heating, the leakage of the digested solution from the inner vials may occur. Consequently, a mild digestion process and prolonged digestion time was adopted in this study for the digestion of serum samples. Table 2 shows the microwave digestion parameters used for simultaneous digestion of 36 serum samples employed in this study.

A variety of acids including HNO₃, HNO₃-H₂O₂ and

Table 3 Analytical blank levels and detection limits for aluminum

Digestion mixture	<i>n</i>	Blank level (mean±sd/ng ml ⁻¹)	Detection limit/ng ml ⁻¹
HNO ₃ -H ₂ O ₂	10	0.96±0.24	0.72
HNO ₃ -HClO ₄	12	0.28±0.11	0.33

HNO₃-HClO₄ were tested for digestion of serum samples. Experimental results indicated that, after digesting serum with HNO₃ alone, the digested solution contained still some turbid substances, while with HNO₃-H₂O₂ and HNO₃-HClO₄ mixtures, the final digestate could be in a completely clear solution. This reveals that both digestion mixtures can be equally applicable for the decomposition of serum samples under the mild digestion condition, as shown in Table 2.

In order to understand the blank introduction from the acid mixtures used, the concentrations of Al determined in various acids as a function of acid volume were investigated. The results indicated that only a negligible amount of Al can be found both in the sub-boiling distilled HNO₃ and HCl, but a blank concentration as high as 0.3 ng ml⁻¹ Al in HClO₄ and 2.3 ng ml⁻¹ Al in H₂O₂ was found. By following the established procedure for digestion of 0.5 ml serum sample, using 0.5 ml HNO₃/0.5 ml H₂O₂ and 0.5 ml HNO₃/0.2 ml HClO₄ each as digestion mixtures, the blanks so obtained were 0.96±0.24 and 0.28±0.11 ng ml⁻¹ respectively. From the viewpoint of blank consideration, HNO₃-HClO₄ acid mixture as an oxidizing agent is obviously superior to HNO₃-H₂O₂, and is thereafter employed for serum digestion throughout this study.

On the basis of the above discussion, the optimized conditions for the simultaneous microwave digestion of 36 samples as a batch can be accomplished with the procedure depicted in Table 2. The optimum heating program in HNO₃-H₂O₂ process is 100% power (630 W) for 1 min followed by 50% power for 30 min, and that in HNO₃-HClO₄ process is 100% for 2 min, then 60% for 5 min and followed by 100% for 8 min. The latter process consumes less time and its lower blank introduction is more desirable for analytical purposes.

Matrix separation by solid-phase extraction

As mentioned above, serum can be dissolved by utilizing the double closed-vessel microwave-assisted digestion technique. However, some difficulty may still exist in determining trace Al in the digested serum by ETAAS due to the high salt contents, including both positive and negative ions, in the digested solution. Figure 3 shows the relative absorbance of Al as a function of the concentration of alkali chloride and alkaline earth chloride solutions. As indicated in this figure, Al signal is significantly suppressed with increasing the concentration of alkali chlorides and alkaline earth chlorides, indicating the interference effect

Fig. 3 Recovery of Al (1 ng) in solutions containing various concentrations of Na, K, Mg and Ca ions.

of Al by the presence of these matrix substances in sample solution. This interfering trends for Al measurements by ETAAS correlate well with those obtained by Matsusaki and co-workers.¹⁶ By referring to the salt contents in normal serum sample¹², the concentrations of Ca and Mg in the final solution after pretreatment of the sample with the proposed process lie in the range of 20–100 $\mu\text{g ml}^{-1}$, of which a significant depression of Al signal can be observed in Fig. 3. In view of possible interference effect by the inorganic salts as discussed above, it is considered that a proper measure for removal of matrix constituents prior to ETAAS determination is of importance.

For eliminating the interference effect caused by the sample matrix of serum sample, aside from the possibility of applying matrix modification techniques as described above, a pretreatment procedure based on prior isolation of Al followed by ETAAS determination is also feasible. Recent interest has grown in solid-phase extraction technique, which is versatile and has been successfully used for the concentration of analytes together with removal of the major interfering constituents.^{26,27} Because 8-quinolinol is a well-known characterized reagent which can react with almost sixty metal ions to form metal chelates, we propose a solid-phase extraction method involving the complexation of Al ion with 8-quinolinol followed by adsorption on XAD-4 resin. This method can be used to extract trace Al from the digested solution. Figure 4 shows the results for the Al extractability as a function of pH. The results indicate that the extraction of Al gradually increased from pH 3 to 8 and then levelled off. Hence, trace Al can be quantitatively separated from the digested matrix when the extracted pH is higher than 8. Finally, pH=9 was selected throughout this study. The sequestered Al on XAD-4 resin can be subsequently eluted from the column simply by using 1 M HCl solution. To prevent the pos-

Fig. 4 Extraction of Al-oxinate complexes as a function of pH with XAD-4 resin column.

sible loss of Al during solid phase extraction procedure, the flow rates of digested solutions and 1 M HCl eluant are always kept as low as possible ($\leq 0.25 \text{ ml min}^{-1}$) during the entire extraction procedure. The total times taken for parallel treatment of a batch of 36 samples, including sample adjustment and column separation procedure, can be normally completed in less than one hour.

Analytical performance

Owing to the effect of sample matrix, one generally needs to use standard addition methods in the direct ETAAS methods^{18,28} in order to accurately determine trace Al in serum sample. Obviously, quantitation by standard addition method is always tedious and time-consuming. With the use of the proposed methods

Table 4 Recovery tests carried out by spiking deionized, doubly distilled water and pooled sera with Al

Al added/ ng ml ⁻¹	HNO ₃ -H ₂ O ₂		HNO ₃ -HClO ₄	
	Found/ ng ml ⁻¹	Recovery, %	Found/ ng ml ⁻¹	Recovery, %
D.D. water				
50	53.0	106	48.5	97
50	50.0	100	51.5	103
20	19.3	97	19.6	98
20	20.1	100	21.0	105
Pooled sera				
20	19.0	95	19.4	97
20	19.8	99	19.8	99
20	21.0	105	20.8	104
20	21.2	106	20.5	103

D.D. water: deionized, doubly distilled water.

The initial Al content in the pooled sera is about 15 ng ml⁻¹.

Table 5 Analytical results for Al in reference materials

	Certified value	Value found
Seronorm trace elements serum (SNT 112.1)	70 ng ml ⁻¹	68.5 ± 1.8 ng ml ⁻¹
Bovine liver (NIST SRM 1577a)	(2) µg ml ⁻¹	2.15 ± 0.25 µg g ⁻¹
Freeze-dried urine (NIST SRM 2670)	(180) ng ml ⁻¹	179 ± 5 ng ml ⁻¹

Results presented are mean ± standard deviation ($n=3$). The data in parentheses are reference values provided by the producer.

comprising microwave digestion and solid-phase extraction, Al can now be free from organic and inorganic matrix interferences. Consequently, one can use a standard calibration function instead of the standard addition technique to determine the trace Al in the digested serum sample. This can be validated by the recovery tests of the serum samples as shown in Table 4. The table summarizes both the results for recovery of Al from deionized, doubly distilled water and the pooled sera spiked at total concentrations of 20 ng ml⁻¹ and 50 ng ml⁻¹, respectively. From the quantitative recoveries (95–106%) of Al both in the pure water as well as in the serum sample by following the proposed sample preparation procedure, one may conclude that the possible interference effects caused by sample matrix can be effectively eliminated.

Further evaluation of analytical reliability of the proposed method has also been tested with the use of certified sample of Seronorm Trace Elements Serum SNT 112.2 (Nycomed Pharma, Norway). For testing whether the proposed method can be equally applicable to other biological samples, two materials: NIST SRM 1577a Bovine Liver and NIST SRM 2670 Freeze-Dried Urine from National Institute of Standard and Technology, were also analyzed in this work. Table 5 summarizes the analytical results obtained. From the reasonably good agreement between the certified values of the serum and other samples with those obtained by the proposed method, the feasibility as well as reliability of this method can be further ascertained.

The limit of detection of the proposed method, defined as three times the standard deviation of the blank, was estimated by analyzing at least ten samples of the digested blanks. This affords the limits of detection of 0.72 ng ml⁻¹ and 0.33 ng ml⁻¹, respectively, with HNO₃-H₂O₂ and HNO₃-HClO₄ as digestion mixture (as shown in Table 3). Because the concentration range of Al in serum of normal population lies in the range of 2–10 ng ml⁻¹,^{13,18} the detection sensitivity achievable in the proposed method can satisfactorily meet the requirement for routine monitoring purposes.

In summary, the proposed multistage combined procedure, based on simultaneous microwave digestion of multiple serum samples with double-closed vessel

system, followed then by solid-phase extraction with XAD-4 column, can effectively result in separation of Al from sample matrix. The analyte in the separated solution, being in a state free from potential interfering organic and inorganic matrix substances, can be directly determined with ETAAS by external calibration with aqueous standard solution. By taking advantages of digestion of the samples in small vials of the double-closed vessel system, a significant decreasing of analytical blank can be achieved through use of lower amounts of acid mixture and reducing container surface contact with the sample. With meticulous control of analytical blank, the limit of detection of the proposed method can be extended to lower ng ml⁻¹ levels of Al in serum, which is well below the average concentration of normal population. The method has been proven to be efficient and sensitive for determination of Al in serum samples and can thus serve as a comparative and/or alternative means for direct ETAAS method currently used for clinical analytical purposes.

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