

## Analytical Methods

## Kinetic-spectrophotometry method for determination of ultra trace amounts of aluminum in food samples

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## ABSTRACT

A simple and sensitive kinetic-spectrophotometry method is developed for the determination of trace amounts of aluminum in food samples based on its catalytic effect on the oxidation of Nile Blue A by potassium bromate in sulfuric acid medium. The absorbance is measured at 595.5 nm with the fixed-time method. The optimization of the operating conditions regarding concentrations of the reagents, temperature and interferences are also investigated. The calibration curve is linear over the concentration range 0.07–0.9  $\mu\text{g ml}^{-1}$  of aluminum with good precision and accuracy and the detection limit was down to 0.034  $\mu\text{g ml}^{-1}$ . The relative standard deviation for a standard solution of 0.4  $\mu\text{g ml}^{-1}$  of aluminum is 1.73% ( $n = 10$ ). The proposed method proved highly sensitive, selective and relatively rapid for the assay of aluminum at ultra trace level without any pre-concentration and separation step. The method was applied to the determination of aluminum in food samples (rice, tea and potato). The analytical results of the real samples were in good agreement with the standard method.

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## 1. Introduction

As an essential element, it is believed that aluminum may be toxic and tolerance levels of toxicity of aluminum vary considerably with species. On the other hand, the toxicity of aluminum is much acute in aquatic species. Most biologically active and environmentally significant aluminum forms are the monomeric positively charged hydroxyl ions. These species have been found to be primarily responsible for aluminum toxicity. The toxicity of aluminum discovered when an abnormal amount of aluminum has been detected in the brain of Alzheimer's disease, Shaver's disease Parkinson disease, and dialysis encephalopathy (Campbell, Becaria, Lahiri, Sharman, & Bondy, 2004; Exley & Korhazhkina, 2001; Falten, 2001; Lukaszewski & Blevins, 1996; Mc Lachlan, 1995; Tabrizi, 2007; Takabatake & Shimmen, 1997; Zatta, Lucchini, Van Rensburg, & Taylor, 2003; Wang, 1985; Wang, Lu, & Setiadji, 1993; Wang, Percio, & Farias Mahmoud, 1985; Wang, Lei, Bi, Gan, & Wei, 2001). However the mechanism for which aluminum is toxic have not been fully established. It is not longer in question that aluminum is a toxic element. Aluminum may enter the human body by mouth, intravenous infusion and by environment, drinking water, food and pharmacological products. Since the analysis and monitoring of aluminum in environmental, biological and food

samples is extremely problematic. The problem of determination of trace quantities of aluminum has engaged the attention of chemists for many years. High priority was assigned to this problem following the publication of WHO which specified the concentration value 0.2  $\text{mg L}^{-1}$  of aluminum is not assessment of risk to the health. The most commonly used technique for the determination of trace level of aluminum is atomic absorption spectroscopy AAS (Campillo, Vinas, Lopez, & Hernandez, 1999; Goncalves, Alves, & Aparecida, 2002; Luccas, Nobrega, Dliveira, & Krug, 1999), and inductively coupled plasma (ICP) (Mauras & Allain, 1985), flow-injection chromatography (Albendin, Manuel-vez, Moreno, & Garcia-Vargas, 2003; Lian et al., 2004) and spectrofluorimetry (Bao, Guo, Wu, & Zhu, 2004; De Armas, Miro, Caldera, Estela, & Cerda, 2002). An interesting electrochemical method for determination of trace elements is stripping voltammetry. This technique is based on accumulation of an element on the hanging mercury drop electrode followed by electrochemical reduction of the element. Determination of aluminum by stripping voltammetry is reported by (Qiong, Lirong, Danli, & Guanghan, 2006; Wang, 1985; Wang, Lu, & Setiadji, 1993; Wang, Percio, & Farias Mahmoud, 1985; Wang, Lei, Bi, Gan, & Wei, 2001). However the above noted technique employ expensive instruments and/or materials, and high purity solvents. Also some spectrophotometrically methods are reported for determination of aluminum (Ahmed & Hossan, 1995; Gong & Zheng, 2003; Li, 2004; Ranau, Oehlenschlager, & Steinhart, 2001). However spectrophotometric method can be used

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if combined with a pre-concentration step. The kinetic method is one of the most attractive procedures in terms of sensitivity and the fact that no expensive or special equipment is required (Wang, Zhang, Sun, Liu, & Liu, 1996). In the present work, a kinetic method for determination of low levels of aluminum in the food samples is described which the reagent is Nile Blue A (NBA) ( $C_{20}H_{20}N_3OHSO_4^-$ ). It was found that, bromate could oxidize NBA slowly in acidic media. Aluminum has a strong catalytic effect on this reaction. The difference in absorbance of NBA at 595.5 nm, between unanalyzed (in the absence of aluminum) and catalyzed reaction (in the presence of aluminum) or ( $\Delta A$ ) is directly proportional to the concentration of aluminum.

## 2. Experimental

### 2.1. Apparatus

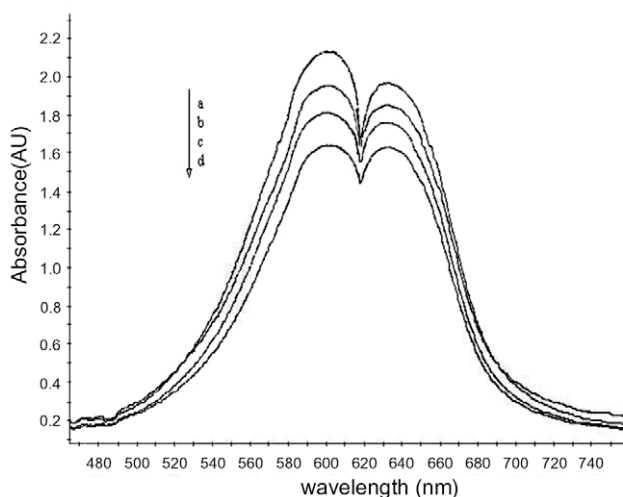
All absorbance measurements were carried out on a Scinco's PDA UV-Vis. Spectrophotometer (photodiode array) equipped with 1.0-cm quartz cells. All pH measurements were made with a 780 pH-meter (Metrohm Switzerland) with a combined glass-calomel electrode. All voltammetric measurements were carried out with a 746 VA-Trace Analyzer (Metrohm, Switzerland) and equipped with a 747 VA stand (Metrohm, Switzerland).

### 2.2. Reagents

All chemical used were of analytical grade or higher grade. De-ionized water was used throughout. The Al stock solution ( $1 \mu\text{g ml}^{-1}$ ) was prepared every week. The working solution ( $0.1 \mu\text{g ml}^{-1}$ ) was prepared daily by diluting a stock solution kept in a refrigerator.  $KBrO_3$  solution (0.2 M) was prepared by dissolving an appropriate amount of the reagent in de-ionized water. The sulfuric acid (0.2 M) was used. ICP standard solution of aluminum ( $1000 \text{ mg L}^{-1}$ ) was purchased from Aldrich. A 260 ppb of the solution was prepared.

### 2.3. Procedure

In a 25-ml comparison tube were placed 0.5 ml of 0.2 M sulfuric acid, 0.9 ml of  $10^{-3}$  M NBA solution, 2 ml of 0.2 M bromate solution. After diluting to volume with de-ionized water, the solution



**Fig. 1.** Effect of aluminum concentration on the color development of NBA. Conditions: 0.5 ml sulfuric acid 0.2 M, NBA 0.09 ml of  $10^{-3}$  M and 2 ml  $BrO_3^-$  0.2 M and (a)  $0 \mu\text{g ml}^{-1}$  aluminum, (b)  $0.2 \mu\text{g ml}^{-1}$  aluminum, (c)  $0.4 \mu\text{g ml}^{-1}$  aluminum, (d)  $0.6 \mu\text{g ml}^{-1}$  aluminum.

was put in a 1.0 cm quartz cell. The initial absorbance ( $A_i$ ) at 595.5 nm was recorded immediately. After addition of different amounts of aluminum standard solution, the mixture was equilibrated at room temperature for 30 s. Then the final absorbance ( $A_f$ ) was recorded at 595.5 nm (Fig. 1). The absorbance difference was defined as  $\Delta A_{595.5} = A_f - A_i$ .

The samples were analyzed by the stripping voltammetry as reference method as follows: the sample solution pipetted out into a 50 ml volumetric flask, 10.0 ml of  $2.0 \text{ mol L}^{-1}$   $(NH_4)_2SO_4$ , 3.50 ml of  $3.2 \times 10^{-3} \text{ mol L}^{-1}$  cupferron solution, diluting to 50 ml. after 10 min, the solution transferred to the electrolytic cell. Measurements were carried out after an accumulation step while the solution was stirred for a given time. The accumulation potential of  $-0.95 \text{ V}$  (vs. SCE) was applied. After a rest period of 10 s, the response curve was recorded. For more details see (Qiong, Lirong, Danli, & Guangan, 2006).

### 2.4. Sample preparation and determination

Rice, black tea and potato were selected for analysis. Potato and rice were obtained from Iranian agricultural farms and tea was brought in tea shop.

#### 2.4.1. Determination of aluminum in rice

Twenty gram of rice or flour sample was accurately weighed and placed into quartz crucible. Ten milliliter of concentrated sulfuric acid was added to it and evaporated to near dryness; then 10 ml of nitric acid (1 + 1, volume ratio) was added and evaporated to dryness. Under the heating conditions, concentrated hydrogen peroxide was added by drop till the solution clearness and evaporated. Water was added and continued to heat to remove the hydrogen peroxide. The residue cooled and was transferred into a 50-ml calibrated flask and diluted to the mark with water. Volumes of 2.00 ml of each of the aliquots were taken for the determination of aluminum via the recommended procedure under the established optimum conditions. The results are presented in Table 2.

#### 2.4.2. Determination of aluminum in tea

Tea sample (3.9883 g) was accurately weighed and placed into ceramic crucible. Six milliliter of concentrated nitric acid and 2 ml of concentrated hydrochloric acid were added. After 20 min, the sample was gently heated to digest till near dryness. It is transferred into muffle furnace for ashing at 600 for 1 h. The sample was taken out to cool and 5 ml of nitric acid (1 + 1, volume ratio) was added and evaporated to near dryness by gently fire heating. Two gram of ammonium peroxydisulfate was added to cover the residue. The sample was transferred to muffle furnace at  $800^\circ\text{C}$  for ashing for 1 h. It cooled and was taken out. Ten milliliter of nitric acid (1 + 99, volume ratio) was used to dissolve the residue and transferred to a 100-ml calibrated flask and diluted to the mark with water. Volumes of 2.00 ml of each of the aliquots were taken for the determination of aluminum; the results are presented in Table 2.

#### 2.4.3. Determination of aluminum in potato

The potato samples were washed and cut into bar. Then the samples were dried at  $110^\circ\text{C}$  for 4 h. Ten grams of the dried samples were accurately weighed and placed into ceramic crucible. The samples were put into muffle furnace for ashing at  $650^\circ\text{C}$  for 8 h. After the ashing was completed, the furnace was opened and the samples cooled and were taken out. After the samples cooled to a room temperature, a few drops of water were added and 4–5 ml of hydrochloric acid (1 + 1, volume ratio) was added to dissolve the residues. The above substances were transferred to a 50-ml calibrated flask and diluted to the mark with water. Volumes of 2.00 ml of each of the aliquots were taken for the determination of aluminum via the

recommended procedure under the established optimum conditions. The results are presented in Table 2.

### 3. Results and discussion

NBA is a dye which has been used as indicator. This reagent is slowly oxidized to a colorless compound by potassium bromate; however, in the presence of aluminum the rate of oxidation strongly increases. In the non-polar solution, NBA has a maximum absorbance band at 634 nm (Fig. 2) and in the polar solutions the main absorbance band of NBA split into two absorbance bands at 595.5 nm and 634 nm (Fig. 3) (Davis, Hannah, & Hetzer, 1966). Because of high sensitivity of 595.5 nm absorbance, it used for further studies. This work is based on the catalytic determination of low levels of aluminum in the presence of NBA. Since, aluminum addition has a catalytic effect on the  $\text{KBrO}_3$ –NBA reaction system. In the other word; the oxidation reaction of NBA by bromate in acidic media is very slow whereas in the presence of trace amount of aluminum, it undergoes a rapid reaction rate. The role of aluminum as a catalyst in the presence of bromate for the oxidation of NBA can be described by the following reaction:

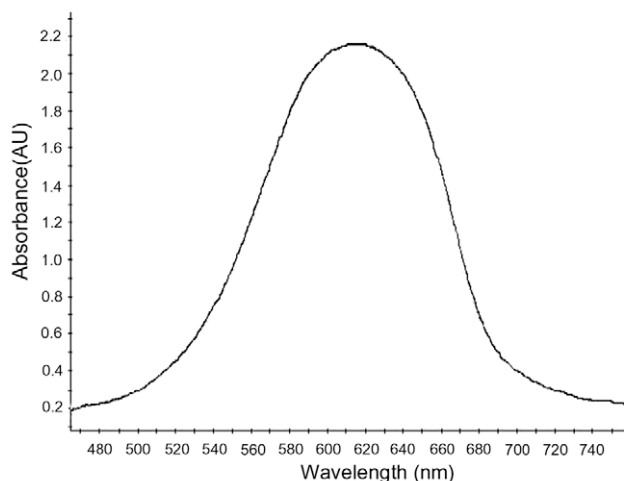
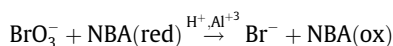


Fig. 2. The maximum absorbance of NBA in a non-polar solution. The absorbance accurate at 634 nm.

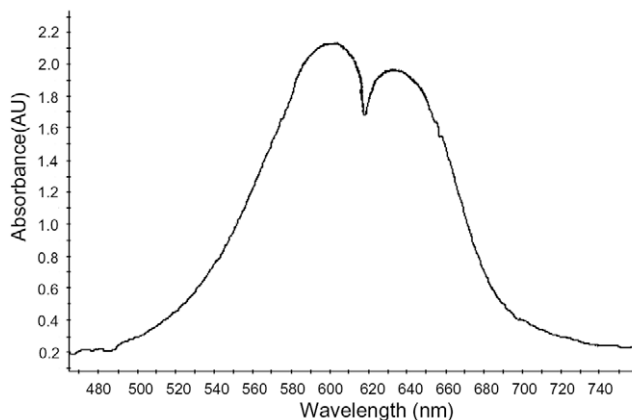


Fig. 3. The maximum absorbance of NBA in a polar solution. In the polar solution the maximum absorbance is split into two absorbance. The absorbance accurate at 634 nm and 595.5 nm.

where red and ox are the reduced and oxidized form of NBA, respectively.

#### 3.1. Effects of variables

The experimental variables were optimized as below.

##### 3.1.1. Effect of sulfuric acid concentration

The effect of various acid types, with the same concentration such as sulfuric acid, hydrochloric acid, phosphoric acid, citric acid and nitric acid was studied. The result shows that sulfuric acid gives greater sensitivity. The optimum value of sulfuric acid concentration is obtained in the presence of 1 ml NBA  $10^{-3}$  M and 2 ml of bromate 0.2 M. The results show that 0.5 ml of sulfuric acid 0.2 M is the best. The greater amount of acid decreases the sensitivity. This effect is due to the fact that in the presence of higher concentration of acid, the NBA is protonated and thus reducing the rate of oxidation reaction.

##### 3.1.2. Influence of NBA concentration

The effect of NBA concentration on the catalytic system is explored. The data obtained were used for the plot of  $\Delta A$  versus concentration of NBA. The results show that 0.9 ml of NBA  $10^{-3}$  M is the best. Greater amount of the NBA cause a decrease in the reaction rate. In the higher concentrations of NBA in the acidic media, the NBA may be coagulated and precipitated in the solution.

##### 3.1.3. Effect of bromate concentration

The effect of bromate concentration is probed. The results demonstrate that  $A_{595.5}$  value increased with an increase in bromate concentration from 0 ml to 2 ml of bromate concentration. Greater amount of bromate decreases the sensitivity. This is due to the fact that at higher concentration of bromate, the rate of blank reaction is very fast and the net reaction rate signal was diminished.

##### 3.1.4. Effect of reaction temperature

The effect of temperature was studied in the range of 0–70 °C. With increasing the temperature up to 20, the change of absorbance is increased, whereas at higher temperatures, the change in the absorbance decreased.

#### 3.2. Calibration curve

The calibration data for aluminum were prepared by the procedure described above under the optimum experimental conditions. There was a linear relationship between  $A_{595.5}$  and aluminum concentration in the range of 0.07 to 0.9  $\mu\text{g ml}^{-1}$  with a correlation coefficient of  $R^2 = 0.9981$  and calibration sensitivity 0.1488. The regression equation was  $A_{595.5} = 0.1488C (\mu\text{g ml}^{-1}) + 0.0051$ , where  $C$  is the aluminum concentration.

#### 3.3. Limit of detection (LOD)

The limit of detection, expressed as the concentration,  $c_L$ , or the quantity,  $q_L$ , is derived from the smallest measure,  $x_L$ , that can be detected with reasonable certainty for a given analytical procedure. The value of  $x_L$  is given by the equation  $x_L = \bar{x}_{bl} + kS_{bl}$ , where  $\bar{x}_{bl}$  and  $S_{bl}$  are the mean and standard deviation for  $n$  measure of the blank, respectively and  $k$  is a numerical factor chosen according to the confidence level desired. In analytical chemistry, the detection limit, lower limit of detection (LOD) is the lowest quantity of a substance that can be distinguished from the absence of that substance (a blank value) within a stated confidence limit (generally 1%). The detection limit is estimated from the mean of the blank, the standard deviation of the blank and some confidence factor. Another consideration that affects the detection limit is the accu-

**Table 1**

Tolerance ratio for the determination of  $0.4 \mu\text{g ml}^{-1}$  of aluminum in the presence of interfering ions.

Species	Tolerance limit ( $w_{\text{ion}}/w_{\text{Al}^{3+}}^*$ ) <sup>a</sup>
Hg <sub>2</sub> <sup>+</sup> , Ag <sup>+</sup> , NO <sub>2</sub> <sup>-</sup> , IO <sub>3</sub> <sup>-</sup> , Na <sup>+</sup>	200
Fructose, methanol, ethanol, glucose, tartaric acid	150
Zn <sup>2+</sup> , Cd <sup>2+</sup> , K <sup>+</sup> , Mn <sup>2+</sup> , Ba <sup>2+</sup> , Ce <sup>3+</sup> , Li <sup>+</sup> , HCO <sub>3</sub> <sup>-</sup> , Pb <sup>2+</sup> , Cr <sup>3+</sup> , Ca <sup>2+</sup> , Ni <sup>2+</sup> , Sn <sup>2+</sup> , Co <sup>2+</sup>	100
Fe <sup>3+</sup> , Cu <sup>2+</sup> , I <sup>-</sup> , Br <sup>-</sup> , F <sup>-</sup> , CN <sup>-</sup> , Cl <sup>-</sup>	10

<sup>a</sup> The relative weight of interfering ion to the of Al<sup>3+</sup>.

racy of the model used to predict concentration from the raw analytical signal. The LOD were calculated according to the recommended formula by International Union of Pure and Applied Chemistry (IUPAC) as  $\text{LOD} = \frac{3SD}{K}$  where SD is the standard deviation of the blank measurements and K is the slope of the calibration curve. For the method reported here the LOD was  $0.034 \mu\text{g ml}^{-1}$  on the basis of 10 blank measurements.

### 3.4. Effect of interference

To study the selectivity of the proposed method, the effect of foreign species on the determination of  $0.4 \mu\text{g ml}^{-1}$  of aluminum was tested. The tolerance limit was defined as the concentration at which the species caused an error less than  $3\sigma$ . The results are given in Table 1 which show that most of the coexisting substances in the foodstuff samples had no interference on the determination of aluminum. The interference of Fe<sup>3+</sup> and Cu<sup>2+</sup> can be greatly diminished by addition of 0.1% EDTA (0.25 ml) solution. Chloride, bromide and iodide interference may be diminished by addition of 5 M nitric acid (0.5 ml) as well as boiling the solution (Ensafi & Abbasi, 1997). In the acidic media, there are no any interference of anions such as sulfate and phosphate, etc.

### 3.5. Application

To investigate the applicability of the proposed method to real samples and based on similar determination of aluminum concentration in various food samples such as tea and rice, etc. (Gramiccioni, Ingrao, Milana, Santaroni, & Tomassi, 1996; Muller, Anke, & Illing Gunther, 1998; Oniawa, Ikadeh, & Nweze, 1997) and because rice, tea and potato are the most common foodstuff used by Iranian peoples so control of aluminum in these samples are most important. Furthermore, we have used the (Anbar boo) rice and (Golestan) black tea samples for analysis. The results obtained for the analysis of real samples using the proposed method and the stripping voltammetry method as reference methods (Qiong et al. 2006) are summarized in Table 2. As shown in Table 2, the results of both methods are in very good agreement. The result of determination of aluminum in rice, black tea and potato by this method accord with the results reported in (Gramiccioni et al., 1996; Muller

**Table 2**

Determination of aluminum in real samples.

Sample	Proposed method (ng/g) (n = 5)	Reference method (stripping voltammetry) <sup>†</sup> (ng/g) (n = 3)
Rice (Tarem, Anbar boo)	92.33 ± 0.12	92.07 ± 0.16
Black tea (Golestan)	174.86 ± 0.08	175.11 ± 0.11
Potato	134.07 ± 0.13	134.00 ± 0.09
Certified standard Al <sup>3+</sup> of ICP <sup>‡</sup>	260.21 ± 0.06	260.07 ± 0.07

<sup>†</sup> (Qiong et al., 2006).

<sup>‡</sup> 260 ppb of the certified standard material of Al<sup>3+</sup> for ICP was prepared.

**Table 3**

Recovery for the determination of aluminum in real samples (n = 3).

Sample	Added (ng/g)	Found (ng/g)	Recovery (%)
Rice	0	92.19	–
	5	96.85	99.6
	8	100.21	100.0
Tea	0	174.86	–
	5	180.22	100.0
	8	182.06	99.5
Potato	0	134.11	–
	5	139.04	99.9
	8	141.84	99.8

et al., 1998; Ni, Huang, & Kokot, 2007; Oniawa et al., 1997; Qiong et al., 2006; Scancar, Stibilj, & Milacic, 2004) (see Table 3).

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