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Graphene Modified Glassy Carbon Electrode for Determination of Trace Aluminium(Ⅲ) in Biological Samples

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A graphene modified electrode for voltammetric determination of concentrations of aluminium(III) in the presence of alizarin has been reported. The modified electrode was characterized by cyclic voltammetry(CV), transmission electron microscopy(TEM), infrared spectrum(FT-IR) and alternating current(AC) impedance experiments. This voltammetric sensor exhibited strong catalytic effect to the oxidation of alizarin in a Tris-HCl buffer solution (0.08M, pH7.5). The measurements were carried out from underaerated solutions with an accumulation time of 2 mins. Other metal ions in low concentration did not interfere with the analysis of Al(III) ions. The decrease of the differential pulse voltammetry(DPV) anodic peak(−0.65V versus SCE) current for alizarin ligand was linear with increasing of aluminum concentrations. The response was linear over the 0.12–1.2μM range with a detection limit of 9×10^{-8}M. The relative standard deviation at 1×10^{-6}M was 3.2% (n=8). The proposed procedure was validated in the course of Al(III) determination in biological samples.

Keywords: Chemically modified electrode, Alizarin, Graphene, Aluminum.

1. INTRODUCTION

Aluminum has been proposed to be involved in the etiology of dialysis encephalopathy, Alzheimer’s disease, Parkinson’s disease, diabetes, anemia and cancer[1-4]. The relationship between these diseases and abnormal accumulation of aluminum is an important public health issue. Thus, the determination of aluminum in environmental and biological system is of current interest and the development of a simple, reliable, and accurate method to determine trace aluminum is in great demand. The electrochemical methods are simple and reliable techniques for detecting metal ions in trace levels, but the direct determination of Al ion by voltammetry is seriously complicated due to interference by H^+, alkali metal ions, and alkali earth metal ions because of the similarity of their reduction potentials. However, aluminum(III) can be determined voltammetrically after complexation with electroactive ligands such as incorporating o,o'-dihydroxyazo, di-o-hydroxy groups and so on. Meanwhile, electroanalysis exploiting the complexation of Al(Ⅲ) with all kinds of electroactive ligands has been reported[5-11]. Furthermore, electroanalytical methods have some merits such as low detection limits, simple operation, portable equipments, low cost, and short time over spectrometries. In this paper, alizarin as an organic chelator for aluminum was usefulness for the indirect determination of aluminum, and alizarin was extracted from natural dye madder belonging to anthraquinone derivatives. This red dyes with the anthraquinone structure is a kind of photosensitizer with widely existed in the nature and is also an important part of anti-cancer drugs, due to the benzoquinonyl and hydroxyl group in the molecule, which can react with Al(III), and the central quinone functionalities of the alizarin undergo quasireversible reaction[12-16]. The study on the interactions between Al and alizarin on the molecule level will help to improve our understanding of the detailed mechanism of Al toxicity and facilitate the design of new therapeutic drugs. Some literatures have been reported to use chemically modified electrode(CME) and glassy carbon electrode to study the interactions between alizarin and Al(III). But until now, there has been no report about the research of the interaction between alizarin and Al on graphene modified electrode. In order to elevate the selectivity and sensitivity of determination, chemically modified electrodes which prepared by adsorption of ligand are also developed. The chemical reduced graphene (CRG) has attracted much attention in the perspective of electrochemical sensor design. Because of the excellent electrical properties, the ultrahigh ratio of surface area to volume, and the extreme sensitivity of its surface atoms to any surface adsorption reactions. The CRG/GC has the ability to mediate electron-transfer reactions with an electroactive species in solution and has the merits including refreshing its surface easily, repeating excellently and biocompatibility when used as electrodes. Besides, it shows wide potential window, relative inert electrochemistry and electrocatalytic activity for a variety of redox reactions. CRG conducts electricity faster at room temperature than anything else[17-20]. Therefore, we choose the chemically modified electrode which immobilized CRG onto the glassy carbon(GC) electrode, the electrochemical behaviors of alizarin in the absence and presence of Al(III) at the chemically modified electrode have been studied and applied to the practical analysis[21-25]. The novel carbon nanomaterial has become one of the hottest topics in the fields of materials science, chemistry, and nanotechnology[26-28]. Additionally, it is recognized that the speciation of aluminum is important on its toxicity, fate and behavior[29]. Its chemistry property and speciation in aqueous solution are an area of intensive study because of the possible biological and environmental toxic properties. Previous investigation have revealed that the species of Al was dominant influenced by pH, ionic strength and ligands[30-33]. Due to the impact of aluminum on the human body depends strongly on the nature and concentration of its various chemical forms. Therefore, useful analytic methods for measurement of Al in natural samples should be the target the biologically significant fraction(for example: serum and cerebrospinal fluid. pH7.35-7.45). The advantages of this new procedure are the fast complexation kinetics which obviate the need to pretreatment the solution after addition of biological reagent, moreover, it is very important to study real aluminum concentration by on-site determination in physiological conditions. These studies not only enrich the methods of determining Al, but also explore effective practical applications with advanced carbon nanomaterial.

2. EXPERIMENTAL

2.1 Chemicals

Analytic purity alizarin(1,2-dihydroxyanthraquinone) was bought from Shanghai Reagent Co., Ltd. The stock solution of alizarin(0.01M) was prepared with methanol and kept darkly. Graphene oxide(GO, 5mg/mL) was obtained from Shenzhen Nanotech Port Co., Ltd. (Shenzhen, China)and CRG was obtained by the GO reduction of hydrazine hydrate. Al stock solution (0.01M) was prepared by dissolving super-purity (99.99%) aluminum powder in 1:4 HCl solution and diluting to the proper volume.Various concentration and pH Tris-HCl buffer were prepared by dissolving an appropriate amount of tris(hydroxymethyl)-aminomethane and then adjusting the pH value with concentrated HCl. The methanol and ethanol were purchased from Beijing Chemical company. Biological samples were provided by The second people hospital WuHu. All chemicals were of analytic grade unless stated otherwise and double-distilled water was used throughout.

2.2 Apparatus

CHI 660B electrochemical workstation (Shanghai Chenhua Instrument, Inc., China) was used for DPV, CV and AC impedance experiments. A conventional three-electrode system was used throughout the experiment. The CRG modified electrode served as the working electrode; the auxiliary electrode was a platinum wire and a saturated calomel electrode (SCE) was used as the reference electrode. All potentials in this paper refer to the SCE. A transmission electron microscope(TEM) (Hitachi S-480) was used to observe the morphology of the CRG. FTIR absorbance data were acquired with a Nicolet Nexus 670 FTIR spectrometer.

2.3. Preparation of graphene modified glassy carbon electrode

The glassy carbon electrode was polished on abrasive paper with Al_2O_3 of 0.05 μm, sonicated in ethanol and twice-distilled water for 5 min, respectively, dipped 4μL graphene oxide on prepared blank electrode, the electrode was kept at room temperature to dry it. Then put it in tube with 100μL hydrazine hydrate for 6 hours at 70 degrees centigrade. The CRG modified glassy carbon electrode was obtained by cleaning and drying it. After the pretreated glassy carbon electrode was placed in the buffer fluids, a cyclic voltammetric scanning (70 mV s^{-1}) over a range from -1.0V to 0.20V was performed until the current response reaching a steady state. The reproducibility of the current response was good due to treating with ethanol after every experiment.

2.4 Procedures

In all voltammetric experiments, the working electrode compartment was filled with ca. 3 ml of the solution to be studied .The three-electrode system was set in a 10 ml electrolytic cell. After stirred for 2 min the DPV mode was applied and the anodic peak current (Ipalizarin) of alizarin was recorded. Then, Al(III) was spiked and the mixture was stirred for 2 min, the DPV mode was applied and the anodic peak current (IpAl, $_{add}$) was recorded again. The above operations were repeated for every further spiking of Al(III). The decrease of the anodic peak current was denoted ΔIp = IpAl, $_{add}$ − Ipalizarin. All the experiments were performed at ambient temperature.

3. RESULTS AND DISCUSSION

3.1 Characterization of modified electrodes

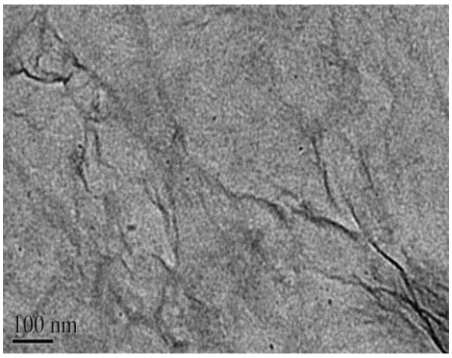

Figure 1. The TEM image of CRG.

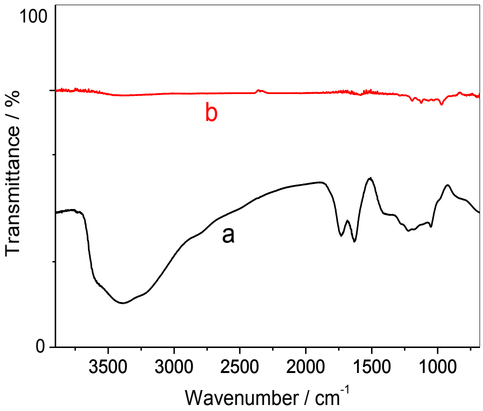

Figure 2. FT-IR spectra of GO/GC(a) and CRG/GC(b)

The TEM image of CRG was showed in Fig.1. CRG films dispersed on the GC electrode surface exhibited a curly morphology consisting of a thin wrinkling paperlike structure[17], which spontaneously produced ripples to stabilize itself special a thermodynamically impossible structure[34]. Also they rippled and entangled with each other, and resembling crumpled silk. Fig. 2 showed the FT-IR spectra of CRG/GC (a) and GO/GC (b), in the FT-IR spectra of CRG and GO, the bands around 3480,1750,1580,1230, and 1050 cm^{-1} are attributed to the oxygen-containing functional groups on GO. GO surface existed -COOH(1750cm^{-1}) , -COO^{-} (1580cm^{-1}), -OH (3480cm^{-1}) group . The peak at 1750cm^{-1} corresponding to the γ(C=O) spectrum, the peak at 3480cm^{-1} corresponding to the γ(-OH) spectrum and the peak at 1050cm^{-1} corresponding to the γ(C-O-C) spectrum. After the reduction, the residual oxygen functionalities were less present on the modified electrode surface[17]. Additionally, in AC impedance experiments (Fig.3). The sequence of the values of charge-transfer resistance(Rct) for different electrodes is GO/GC electrode > GC electrode(346Ω) > CRG/GC electrode(94Ω), this demonstrates the CRG/GC electrode has higher electrochemical activity than GC electrode[35].

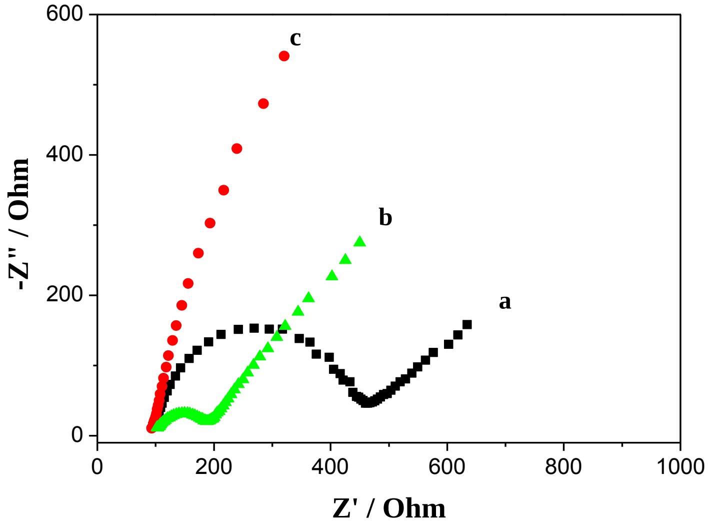

Figure 3. Nyquist plot of Faradic impedance obtained at bare GCE (a), CRG-modified GCE(b) and GO-modified GCE (c). In 5mM $Fe(CN)_6^{3-/4-}$ containing 0.1M KCl. The frequency range is from 1Hz to 10 kHz

3.2 Effects of alizarin at modified electrode in the absence and presence of Al(III)

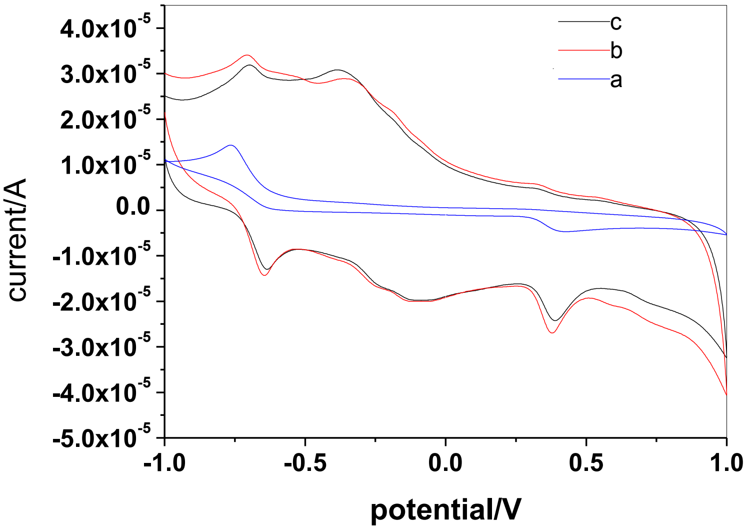

Figure 4. The cyclic voltammograms of alizarin(9×10^{-4}M) at different electrode: (a)a bareGC; (b)a CRG/GC; (c)b+1×10^{-7}mol/LAl(Ⅲ); 0.08mol/L pH=7.5 Tris-HCl buffer solution, scan rate:70mV/s

A quasi-reversible redox reaction wave appeared at −0.69 V/−0.65V, which corresponded to the redox reaction of the quinone functionality of alizarin molecules. The weaves appeared at 0.40V corresponded to the oxidation of the dihydroxyl functionality of alizarin molecules (Fig.4). The central quinone functionalities of the alizarin molecule underwent quasi-reversible redox reaction. The superior electrocatalytic activity at the CRG/GC electrode to alizarin exhibits a better analytic performance for the detection of alizarin compared with GC electrode . The result showed that there was a well-defined DPV peak at -0.65 V for CRG/GC electrode(-0.4V for GC electrode) in the voltammogram of the solution, which belonged to the oxidation of the quinone group in alizarin. After in addition of aluminium, the oxidative peak current of alizarin decreased without the shift of the oxidative peak potential, and no new peak appeared (potential range -1000 to 200mV), which was due to the formation of alizarin-aluminium complex in the solution. It suggested that the addition of Al(III) resulted in the decline of the concentration of the electroactive species on the electrode surface. Furthermore, the corresponding oxidative peak current of alizarin decreased proportionally to the additional amount of aluminum. Therefore, the peak was chosen as the analytic signal. In the case of the CRG/GC electrode, the anodic potential moved negatively with enhanced peak current compared with GC electrode, which meaned the electrode interface of the CRG/GC electrode had higher electrocatalytic activity to alizarin oxidation. The CRG films made the electron transfer easier. High density of edge-plane-like defective sites on CRG may provide many active sites. Furthermore, it was found alizarin in modified electrodes of oxidation peak current (Ipa) increased within scan speed 10~100 mV/s range for CV test with and without aluminum, the relationship between the anodic peak currents of alizarin and the CV scan rates showed in Fig.5 indicated that the anodic peak current Ipa was proportional to the scan rate. Thus the oxidation currents showed adsorptive characteristic. The results indicated that the electrochemical behaviors of alizarin at the CRG modified electrode in the absence and presence of Al(III) showed irreversible electrode processes. According to these, the CRG modified electrodes reaction on the electrode process was under the control of irreversible adsorption process, which was not affected by aluminum.

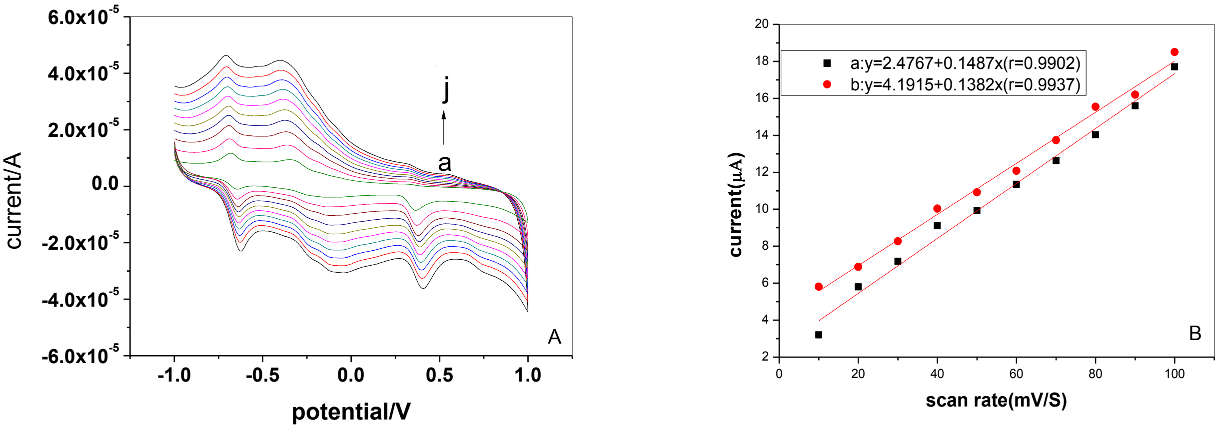

Figure 5. Cyclic votammograms obtained at the CRG modified electrode in different scan rates, scan rates: a.10. b.20. c.30. d.40. e.50. f.60. g.70. h.80. i.90. j.100mVs^{-1} in Tris – HCl buffer solution(0.08M, pH= 7.5), 9×10^{-4} M alizarin, 1×10^{-7}mol/L Al(Ⅲ) (A). The i~V curve, a: obtained 0.1μmol/L Al， b: without Al (B).

3.3 Optimization of experimental conditions

The method demanded quite complicate optimization procedures includes the optimization of various experimental parameters affecting the response of the alizarin/aluminum, because the adsorbed current depended not only on the level of the aluminum, but also upon the concentration of alizarin and buffer solution. Meanwhile, accumulation step also is a simple and effective way to enhance the sensitivity. The process of adsorptive accumulation of alizarin/aluminum depends on many parameters. One of them is the accumulation time. Being too long it would cause competitive saturation of the electrode surface with free ligand and decreases the effectiveness of Al determination. In alizarin /aluminum solution, the peak current increased with the accumulation time from 60s-120s, then it kept constant, indicating that the adsorption was saturated after 120s. So the accumulation time was selected as 120s. However, the accumulation potential during -1.0V to -0.8V has no effect on the peak current, which is a character of an adsorption process.

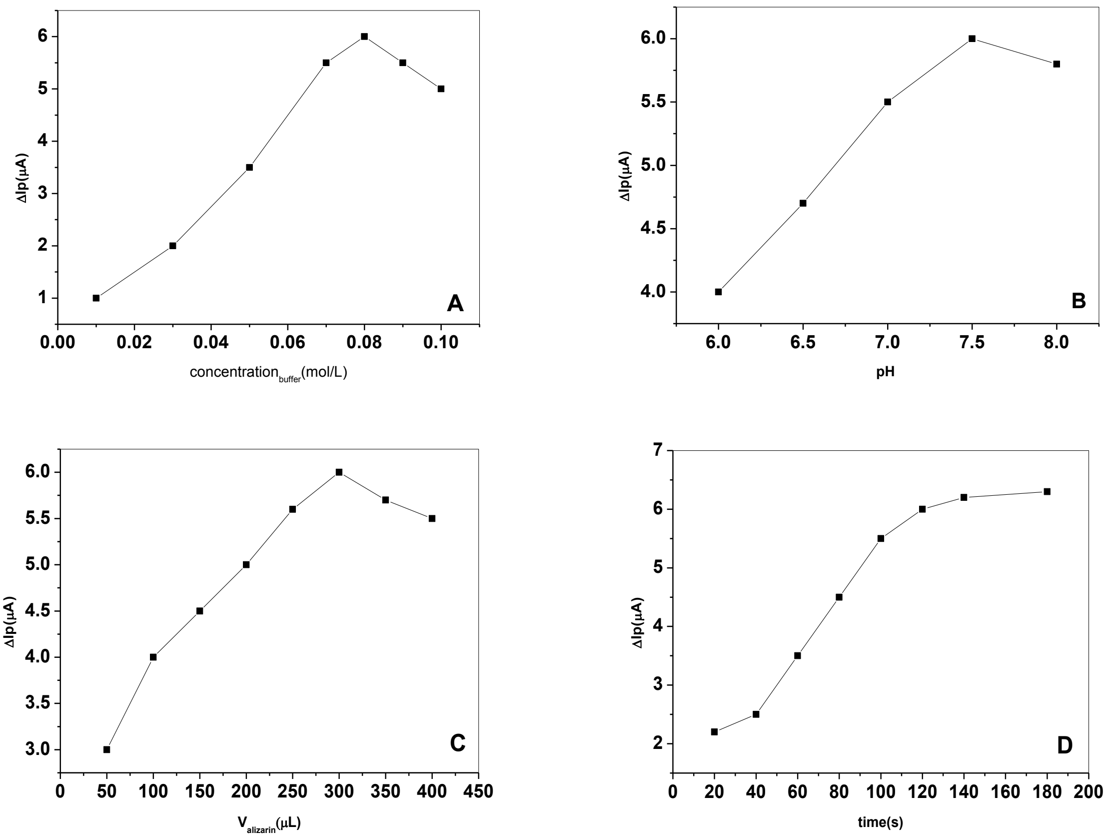

Figure 6. Optimization of experimental conditions: (A) the effect of the concentration of buffer solution (pH=7.5 Tris-HCl, 1×10^{-6}mol/L Al(Ⅲ) and 9×10^{-4}mol/L alizarin). (B) the effect of pH (0.08mol/L Tris-HCl buffer solution ,1×10^{-6}mol/L Al(Ⅲ) and 9×10^{-4}mol/L alizarin). (C) the effect of the concentration of alizarin(1×10^{-6}mol/L Al(Ⅲ), pH=7.5, 0.08mol/L Tris-HCl buffer solution). (D) the effect of the accumulation time.(1×10^{-6}mol/L Al(Ⅲ), 9×10^{-4}mol/L alizarin,pH=7.5, 0.08mol/L Tris-HCl buffer solution).

Fig.6 showed the difference of peak current ΔIp dependence on the pH value, accumulation time, concentrations of both buffer solution and alizarin. Based on these results, the optimal conditions for the determination of Al as follows. The accumulation time was 2min, and pH was 7.5 with 0.08mol/L Tris-HCl buffer solution and then concentration of alizarin was 9×10^{-4}mol/L. The value of pH was critical factor to detect aluminum accurately. Both the redox properties of alizarin and the formations of Al-alizarin complexes were influenced by the changes of pH. We choose Tris-HCl buffer solution for its excellent peak height and peak type of ligand oxidation. The decrease of current at lower buffer solution and alizarin concentrations was due to the increase in solution resistance, whereas at the higher concentrations was due to the hydrolysis of Al(III). The competitive saturation of the electrode surface with free ligand and complex also decrease the effectiveness of Al determination.DPV mode was used under the optimum experimental conditions, and the linear relationship between ΔIp and the concentrations of Al(III) was established in Fig.7. The linear working range for the CRG-modified electrodes was 0.12-1.2 μM for Al. In the range of the linear equation was ΔIp (μA) = 5.4280 - 0.2559 $C_{Al}\times10^{-6}$ mol/L, correlation coefficient was 0.9976(r=0.9976), detection limit was 9×10^{-8}mol/L for CRG/GC electrode. Eight replicate analysis measurements of a 1 μM Al standard solution gave a relative standard deviation (RSD) of 3.2% (n=8) for the method.

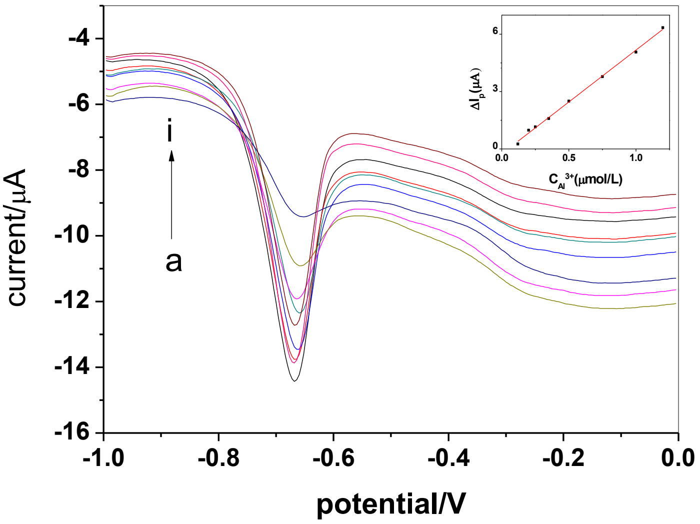

Figure 7. DPV responses of alizarin with (i) and without Al(Ⅲ) (a) in CRG/GC electrode system at 9×10^{-4}mol/L alizarin, pH=7.5, 0.08mol/L Tris-HCl buffer solution. (a)without Al(Ⅲ). (b) 0.12×10^{-6}mol/L Al(Ⅲ). (c) 0.2×10^{-6}mol/L Al(Ⅲ). (d) 0.25×10^{-6}mol/L Al(Ⅲ). (e) 0.35×10^{-6}mol/L Al(Ⅲ). (f) 0.5×10^{-6}mol/L Al(Ⅲ). (g)0.75×10^{-6}mol/L Al(Ⅲ). (h) 1.0×10^{-6}mol/L Al(Ⅲ). (i) 1.2×10^{-6}mol/L Al(Ⅲ). (Inset: plot of ΔIp vs. concentration of aluminum)

3.4 Applications of proposed method to determine Al(III) concentration in real biological samples

The results of interference studied often give very important information on real samples detection. Human body fluids including as serum, cerebrospinal fluid and so on, have sophistic component, such as all kinds of protein, organic acid, inorganic ion. Some of them may adsorb onto the electrode surface and yield electrochemical responses. Therefore, many related anions and cations were chosen purposely for interference study. The interfering ions were titrated step-wise to higher concentrations while monitoring the electrochemical signals(peak current change<3%). With 1×10^{-6}mol/L Al(III), 150-fold of citrate, tartrate, arginine and leucine, 100-fold of aspartic acid, serine, oxalic acid and alanine do not interfere. 100-fold of Zn(II), Ca(II) and Mg(II) do not interfere. These was due to aluminum as a lewis acid has a stronger reaction with free radical of anthraquinone compared with zinc and magnesium. A large amount of Na^+, K^+, Cl^-, NO_3^-, SO_4^{2-} have no interfere. 20-fold of Fe(III), Cu(II), Fe(II) do interfere, but they could combine with proteins and the concentration in body fluid were low, which displayed no affected . Due to modified electrode strong hydrophobic interaction, the hydrophilic body fluids proteins do not interfere. These results indicated that CRG/GC electrode has an excellent selectivity for alizarin, and it might be applied to determine aluminum in real samples. In order to validate feasibility, the CRG-modified electrode was used for quantitative analysis of aluminum in biological samples. The standard addition method was used. Although the suggested electrode is simply fabricated and inexpensive, the measurements data showed a good agreement with those obtained by standard ICP-AES (Inductively coupled plasma-atomic emission spectroscopy) for the investigated real samples. The results were listed in Table 1.

Table 1.

Al concentration(10^{-7}mol/L)				
Sample	Detected±SD	Added	Found±SD	Recovery(%)±SD
Serum (CRG/GC) (ICP-AES)	10.1±0.3 10.0	2	11.9±0.3	90±1.4
Cerebrospinal fluid (CRG/GC) (ICP-AES)	10.1±0.2 10.0	2	12.0±0.3	95±1.7

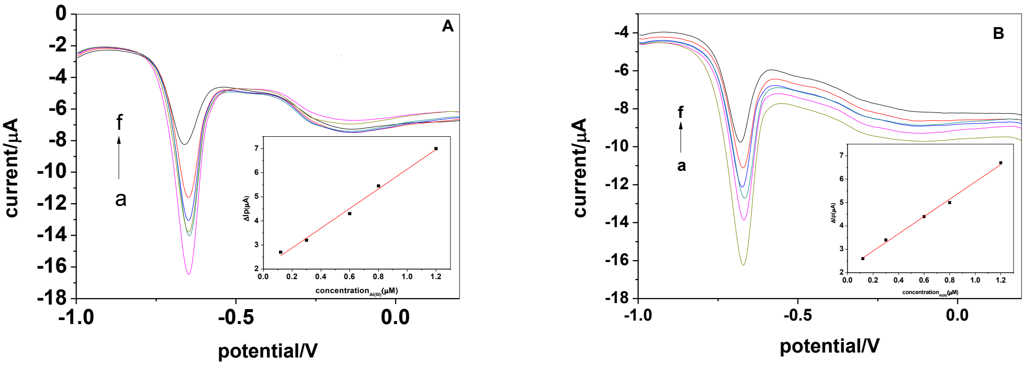

Figure 8. DPV responses of alizarin with (f) and without (a) Al(Ⅲ) in CRG/GC electrode system at the serum(A) and cerebrospinal fluid(B). (a)without Al(Ⅲ). (b) 0.12×10^{-6}mol/L Al(Ⅲ). (c) 0.3×10^{-6}mol/L Al(Ⅲ). (d) 0.6×10^{-6}mol/L Al(Ⅲ). (e) 0.8×10^{-6}mol/L Al(Ⅲ). (f) 1.2×10^{-6}mol/L Al(Ⅲ). (Inset: plot of ΔIp vs. concentration of aluminum).

We determined above two biological samples for five times, respectively, the concentration of aluminum in cerebrospinal fluid was 1.01μmol/L and standard deviation(SD) was 0.02, then 0.2μmol/L standard solution of aluminum was added separately, the concentration of aluminum in cerebrospinal fluid was 1.20μmol/L and SD was 0.03 by the proposed method. And similar processes were carried out in serum. The excellent recoveries and precisions indicated that the method was reliable for determining Al(III) in real biological samples. As showed in Fig. 8, the CRG modified electrode detected trace aluminum in biologic samples, which the linear equations for Al from 0.12μM to 1.2μM were $\Delta Ip(\mu A)=2.0619+4.0662C_{Al}\times10^{-6}$mol/L (r=0.9900) for serum and $\Delta Ip(\mu A)=2.1865+3.6978\ C_{Al}\times10^{-6}$mol/L (r=0.9948) for cerebrospinal fluid.

3.5 The probable mechanism of determination aluminum with alizarin by modified electrode

The complexation reaction might occurred between alizarin and Al by takes place on the alizarin (O,O) ends. alizarin and Al(III) could cooperate stable hexatomic ring structure(Fig.9), which have more coordination ability than oxalic acid and citric acid and so on. Therefore, the Al(III)-alizarin formation were used to detected Al(III) in the biologic fluids. The CRG-modified electrode greatly increased the oxidation signal of alizarin due to its very high surface area, strong adsorptive ability and a high concentration of active sites[36,37]. Also, the π-π conjugated bonds between graphene and alizarin and hydrophobic interaction can facilitate electron transfer from alizarin to electrode. So the modified electrode showed excellent electrocatalytic properties with obvious decrease of overpotentials and enhancement of oxidation currents. As a result, the novel carbon nanomaterial modified electrodes electron transfer kinetics could be possibly ascribed to the synergetic effects of the surface chemistry (e.g., C/O ratio, presence of quinone-like groups, surface charge, and surface cleanness) and conductivity of the materials[19]. Based on these results, a sensitive and simple electrochemical method was developed for the determination of aluminum, this method possesses high sensitivity and rapid response.

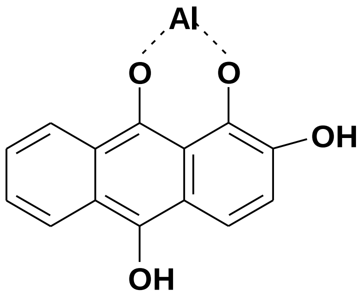

Figure 9. Probable Molecular structure of alizarin coordinate with aluminum

4. CONCLUSIONS

Sensitive, selective and accurate methods are required for the determination of aluminum in biological and environmental matrices. Electroanalytical techniques have important advantages that include speed, high selectivity and sensitivity, low detection limit, relative simplicity, and low equipment cost compared to other techniques. In comparison with the conventional adsorptive stripping voltammetry, the proposed method is characterized by high selectivity, a short time consuming, and less accumulative process acquired at physiological pH. The greatest advantage of the aluminum determination using alizarin as complexing agent is the elimination of biological fluids digestion with high concentrated acid. For aluminum speciation can change with sample storage and pretreatment, on-site determination of reactive aluminum is expected to give the most meaningful data, meanwhile on-site measurements have the important advantage of yielding results at once and reducing the sample handling and transport requirements. It is expected to be adopted to the clinic diagnosing of the concentrations of Al(III) in vitro, it also might play a role for developing effective electrochemical methods for practical applications with advanced carbon-based nanomaterial.

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