Analytical Methods

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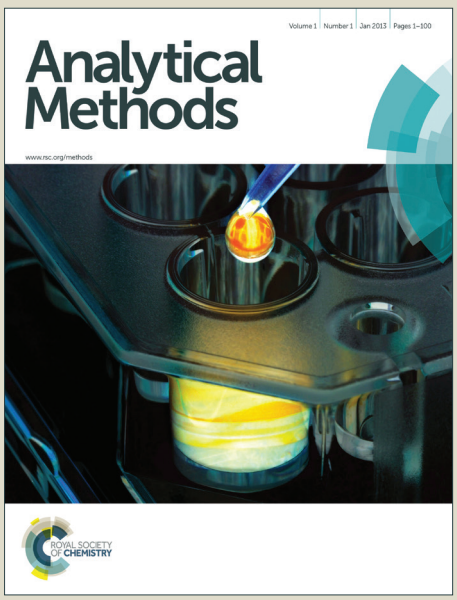

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ARTICLE

Glass carbon electrode based on graphene quantum dots (GQDs) for simultaneous detection of Acetaminophen and Ascorbic Acid

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In this present work, graphene quantum dots (GQDs) was handily prepared with citric acid by pyrolysis method and used to fabricate an electrochemical sensor for the simultaneous determination of acetaminophen (AC) and ascorbic acid (AA) in compound preparation. The electrochemical properties of the GQDs modified glass carbon electrode (GCE) were characterized by differential pulse voltammetry and cyclic voltammetry. The electrochemical response of the modified electrode for AC and AA was separately investigated and had a good linear relation in the ranges of 5~80 μ mol· L^{-1} and 25~1350 μ mol· L^{-1}, respectively. The concentration range for the simultaneous determination of AC and AA were 1~200 μ mol· L^{-1} and 25~1400 μ mol· L^{-1}, respectively. The GQDs modified GCE showed high sensitivity, good selectivity and acceptable precision for AC and AA. The experimental result indicated that proposed method can be applied for pharmaceutical analysis about AC and AA, especially for compound preparation.

Introduction

In recent years, it has been considerably attractive to develop highly sensitive, commendably selective, well reliable and very low-cost analytical method for the detection of physiological related species, and it is still challenging to construct sensitive, fast and simple electrochemical biosensor for simultaneous detection of two-component systems.

Acetaminophen (N-acetyl-p-aminophenol, AC), an effective analgesic and antipyretic drugs, is extensively employed to relieve arthralgia, neuralgia, cephalagra, cancer pain, headache, backache and postoperative pain [1, 2] in the world. AC seems to be remarkably safe and appears to have no toxic and side effect for human health, if the recommended doses are not exceeded [3]. AC can be used even in infant [4], according to the reasonable dosage. However, acute overdoses of AC can result in the accumulation of toxic metabolites to cause liver and kidney damage [5]. Ascorbic acid (Vitamin C, 2-(1,2-dihydroxyethyl)-4,5-dihydroxyfuran-3-one, AA), a water-soluble vitamin, is well-known as antioxidant in human diet, pharmaceutical formulation and cosmetic application [6]. AA, as an essential component in biological fluids, plays a significant role in the prevention and treatment of scurvy, common cold, cancer and AIDS [7, 8]. AC and AA, the two substances, are main ingredients and associated in some pharmaceutical formulations, because AA can heighten the pesticide effect of AC and relieve the hepatotoxicity and nephrotoxicity of AC [9]. Therefore, it is excellently important to determinate simultaneously the AC and AA for quality control of bulk pharmaceutical and pharmaceutical formulation in pharmaceutical industry.

At present, several analytical methods, such as high performance liquid chromatography [10], spectrophotometry [11] and capillary electrophoresis [12], was used for the detection of AC and AA. However, these methods are faced with several inevitable disadvantages, such as high costs, necessary sample pretreatment, toxic organic solvents and consuming times. Thus, it is requisite to develop low-cost, simple and convenient, environmentally friendly and time-saving analytical technology for the simultaneous determination of AC and AA in the pharmaceutical industry. Compared with the above analytical methods, electrochemical techniques possess satisfactory advantage, including high sensitivity, easy simplicity, low cost and rapid response [13]. Hence, in recent years electrochemical techniques attract more and more attention, and have been proposed for the detection of AC [14] and AA [15]. Because AC and AA are electroactive compounds, the both can simultaneously be detected through the appropriate modified electrode [16, 17]. It is very important to select a suitable material to improve the performance of modified electrode. Currently, the nanomaterials were widely used to modify electrode due to its large specific surface area, acceptable biocompatibility and ideal catalytic activity.

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Graphene quantum dots (GQDs), a new member of carbon-based nanomaterials family, attract more and more attention and have been used to modify electrode, for example, GQDs modified pyrolytic graphite electrode to fabricate electrochemical biosensors [18]. In this work, GQDs were used to modify GCE to obtain simple electrochemical sensor. The obtained sensor could successfully overcome overlapping oxidation peaks because of unmodified electrodes suffering from lack of selectivity for simultaneous detection of AC and AA, and showed satisfactory sensitivity, selectivity and reproducibility. The proposed sensor is superior to the previous reports about the simultaneous detection of AC and AA [19]. The prepared sensor in the paper has several remarkable merits, such as simple fabrication process, ideal selectivity, speedy response and miniaturized potential. And the sensor can be utilized to detect AC and AA levels of the compound preparation (Vitamin C Yiniqiao Tablets and Ganmaoling Granules) in phosphate buffered saline (pH=6.5).

Experimental

Reagents and apparatus

Citric acid ($C_6H_8O_7$, CA), sodium hydroxide (NaOH), hydrochloric acid (HCl), dibasic sodium phosphate ($Na_2HPO_4 \cdot 12H_2O$), sodium dihydrogen phosphate ($NaH_2PO_4 \cdot 2H_2O$), potassium chloride (KCl) and sodium chloride (NaCl) were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Acetaminophen and ascorbic acid were purchased from Sigma–Aldrich Company (China). Vitamin C Yiniqiao Tablets and Compound Ganmaoling Granules were purchased from the drugstore. Ultrapure water (18.2 M$\Omega \cdot$cm) purified from Millipore water purification system was used throughout the experiments. All the other reagents were of analytical grade and used as received without any purification.

The preparation method of phosphate buffer saline (PBS) with pH=6.5 is as follows: precision weighing 2.1373 g of $NaH_2PO_4 \cdot 2H_2O$ and 2.2563 g of $Na_2HPO_4 \cdot 12H_2O$, the both of which were dissolved in 100 ml of ultrapure water.

The measurements of differential pulse voltammetry (DPV) and cyclic voltammetry (CV) were carried out at CHI 660C electrochemical workstation (Shanghai ChenHua Instruments Co., China) in PBS (10 ml, pH=6.5) with conventional three-electrode system that was composed of the modified GCE as working electrode, Ag/AgCl electrode (saturating with KCl) as reference electrode and platinum wire as auxiliary electrode. Transmission electron microscopy (TEM) images were obtained with a JEOL JEM-1400 Microscope. The fourier transform infrared spectroscopy (FTIR) spectrum was collected on Nicolet Avatar 360 spectrometer. The related experimental parameters of DPV and CV were given, according to the concrete experiment. All the experiments were carried out at room temperature.

Preparation of GQDs

At this work, graphene quantum dots (GQDs) was synthesized by CA which was pyrolyzed at a high temperature ($>200℃$). The specific process was as follows: first, 2 g CA was heated at 200 ℃ for 30 min in autoclave in which the CA crystals gradually turned into orange liquid, signifying the formation of GQDs; Next, the orange liquid was slowly mixed with 100 ml NaOH (10 mg/ml) solution; last, the pH of the GQDs solution was adjusted to 8.0 with HCl to make the GQDs stable. The obtained GQDs solution was stored at 4 ℃ with keeping out of the sun, when not to use.

Preparation of GQDs modified GCE

Prior to modification, the glass carbon electrode (GCE) was polished successively with 0.3 and 0.05 μm aluminum oxide aqueous slurry on the microcloth pad, and then adequately sonicated in HNO_3 solution (volume ratio of HNO_3 to water is 1:1), ethanol (95%) and deionized water for 3 min, respectively. After the processed GCE was dried under nitrogen, 5 μL of the above GQDs solution was dropped onto the surface of the GCE and dried at room temperature for 24 h. The surface of GCE could form a homogeneous GQDs film and was named as GQDs modified GCE.

Sample preparation of commercial samples

Vitamin C Yiniqiao Tablets, a kind of Chinese patent medicine for the influenza, contain many different kinds of components, such as acetaminophen, ascorbic acid, chlorpheniramine maleate and some traditional Chinese medicine. One tablet (0.54 g), containing 105 mg AC and 49.5 mg AA, was put in the 100 ml volumetric flask and diluted with ultrapure water to scale. Compound Ganmaoling Granules, an over the counter (OTC) for the influenza, are composed of acetaminophen, chlorpheniramine maleate, caffeine and some traditional Chinese medicine. One bag (10 g) containing 0.2 g of AC, was placed in 1000 ml volumetric flask and diluted with ultrapure water to scale. The two samples were stored at 4 ℃, when not to use.

Results and discussion

Characterizations of TEM and FTIR

GQDs prepared by the above method were characterized by TEM and FTIR. The image of TEM (Fig. 1A) and grain diameter distribution map (Fig. 1B) clearly shows the diameters of GQDs mainly distributed in range of 4~6 nm. The results illustrate the grain size of the prepared GQDs is uniform. As can be seen fom Fig. 1A, the GQDs could be uniformly distributed. FTIR analysis provided some information on the functional groups in the formation of GQDs, as shown in Fig. 1C. The peaks at about 1583 and 1404 cm^{-1} were assigned to C=O and C-O, that illustrated the existence of C=O and C-O in the GQDs [20].

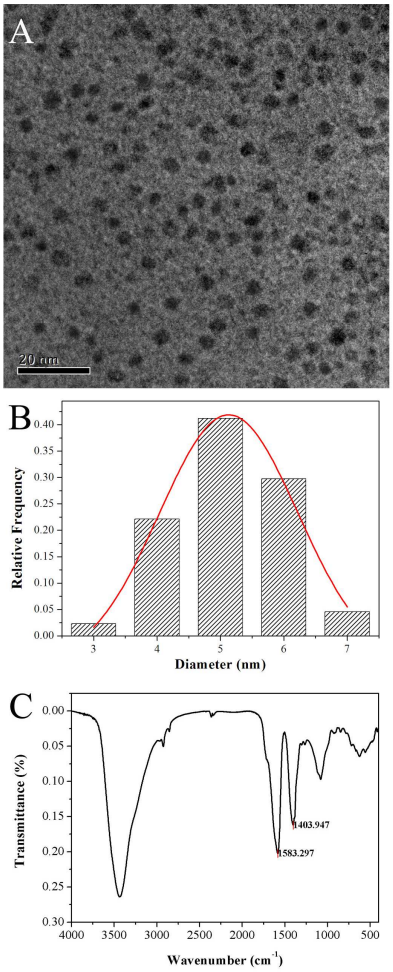

Fig. 1 TEM image (A), grain diameter distribution map (B) and FTIR spectrum (C) of GQDs

Effect of the pH values

The effect of the PBS pH for the DPV peak current was investigated, which was showed in Fig. 2. The response current of AC (curve a) on the modified electrode slowly decreased in the pH range of 5.0~6.0, maintained a balance from 6.0 to 7.0, and inchmeal decreased in the range of 7.0~9.0. The DPV peak current of AA (curve b) first increased step by step in the pH range from 5.0 to 6.5, after that the current gradually decreased from 6.5 to 9.0. When the pH value of PBS was 6.5, the response current of AA reached maximum. Considering both AC and AA should have the maximum response, the 6.5 of pH was chosen as the optimal pH value PBS.

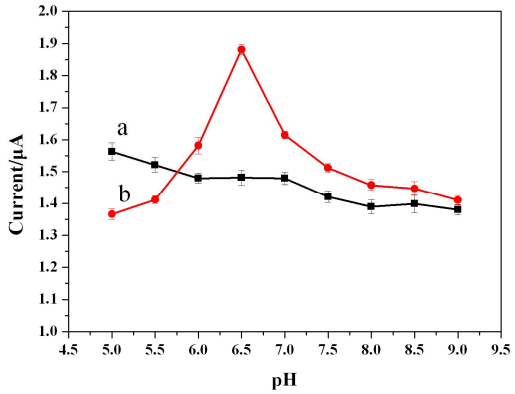

Fig. 2 Broken line graph of pH effects for DPV peak current of AC (a) with 5 μ mol· L^{-1} and AA (b) with 100 μ mol· L^{-1}; pH values were 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5 and 9.0, respectively.

Electrochemical behaviors of AC and AA at GQDs modified GCE

The electrochemical performance of GQDs modified GCE decides its application for the detection of AC and AA. The differential pulse voltammetry (DPV) was used to detect the AC and AA on the GQDs modified GCE. As can be seen from Fig. 3A, the DPV (curve a) of GQDs modified GCE in PBS (pH=6.5) was without AC and AA in PBS (pH=6.5), and had no anodic peak at 0.20 and 0.44 V. Curve b and curve c showed that AA (100 μ mol· L^{-1}) or AC (5 μ mol· L^{-1}) in PBS (pH=6.5) was solely detected and DPV of AA or AC had an obvious anodic peak at 0.20 or 0.44 V on GQDs modified GCE. Curve d showed that AA (100 μ mol· L^{-1}) and AC (5 μ mol· L^{-1}) in PBS (pH=6.5) were simultaneously measured and DPV had two clear anodic peaks at 0.20 and 0.44 V. And the two anodic peaks of DPV with AC and AA were clearly separated and had no mutual influence at 0.20 and 0.44 V. But curve e illustrated that the two anodic peak of DPV for simultaneous determination of AC and AA were not completely separated and the overall current of DPV on the bare GCE was smaller than the GQDs modified GCE (curve d), which meaned bare GCE could not be applied to simultaneously detect AC and AA in the mixture. The result demonstrated that AC and AA could be simultaneously detected in PBS (pH=6.5) by the GQDs modified GCE.

The influence of different scan rate for the electrochemical response of AC and AA was also investigated through cyclic voltammetry (CV) on GQDs modified GCE. Namely, AC and AA were simultaneously detected in PBS (pH=6.5). As shown in Fig. 3B, the CVs of GQDs modified GCE had two anodic peaks which were assigned to AC and AA, respectively, and the anodic peak current of AC and AA increased with the increase of scan rate. As can be seen from Fig. 3B insert, the peak current value of AC and AA had a good linear relation with the square root of scan rate in the range of 0.01~0.3 V/s. The corresponding linear regression equation of AC and AA were I_{pa}=-0.4409+90.29 $\nu^{1/2}$ (R=0.9993) and I_{pa}=-1.754+72.63 $\nu^{1/2}$ (R=0.9978), respectively. The results testified the oxidation of AC and AA on the GQDs modified GCE was a typical diffusion-

controlled process, the relation of which accords with Cottrell equation. The results in Fig. 3 fully indicated the GQDs modified GCE could be used for the simultaneous analysis of AC and AA.

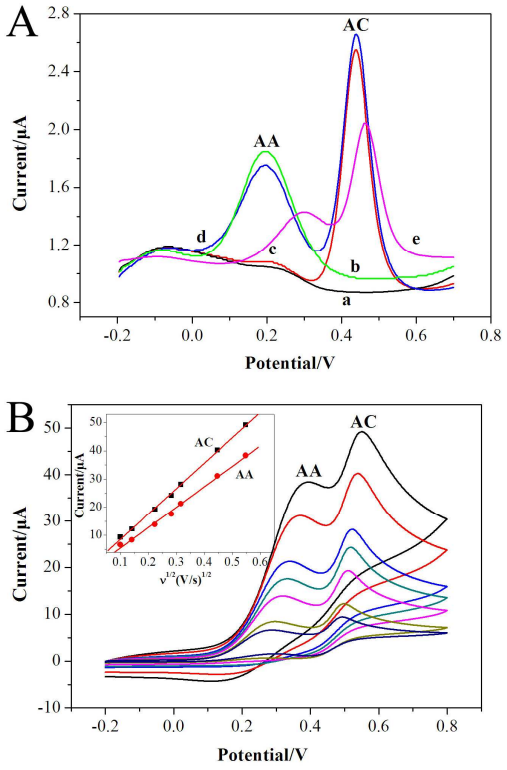

Fig. 3 (A) DPVs of GQDs modified GCE in PBS (pH=6.5) was curve a (black) without AA and AC, curve b (green) with AA, curve c (red) with AC and curve d (blue) with AA and AC, respectively. DPVs of bare GCE in PBS (pH=6.5) was curve e (magenta) with AA and AC; (B) CVs of GQDs modified GCE in PBS (pH=6.5) with AC and AA at various scan rates (from inside to outside: 0.01, 0.02, 0.05, 0.08, 0.1, 0.2, 0.3 V/s), insert showed the linear relation between anodic peak current (AA and AC) and the square root of scan rate.

Determination of AC and AA

Because of AC and AA coexisting in the pharmaceutical formulation, the detection of AC and AA in the commixture was especially measured in PBS (pH=6.5), and the experimental results were displayed in Fig. 4. Fig. 4A showed that DPVs of different concentration AA in the presence of AC (5 μ mol· L^{-1}) were obtained by GQDs modified GCE. Obviously, the peak current of DPV gradually increased with the increasing of AA concentration from 25 to 1350 μ mol· L^{-1}, but the DPV peak current of AC at 0.44 V was almost no significant increase. The linear regression equation between the peak current value and AA concentration was I_p(μ A)=1.625+0.00634C_{AA}, and the correlation coefficient (R) was 0.9967. As shown in Fig. 4B, when the concentration of AA maintained 100 μ mol· L^{-1}, the DPV peak current of AC increased with the AC concentration ranging from 5 to 80 μ mol· L^{-1}, and the linear regression equation was I_p(μ A)=1.891+0.0782C_{AC} with R=0.9963. Meanwhile, the anodic current peak of AA at 0.20 V didn't appeared obvious increases. Fig. 4C notably showed that the DPVs of the mixture with AC and AA on GQDs modified GCE were obtained through simultaneous increasing concentrations of AC and AA. The DPV peak current of AC was proportional to the concentration ranging from 1 to 200 μ mol· L^{-1}, and the linear regression equation was I_p(μ A)=1.941+0.0595C_{AC} with R of 0.9967. For AA, the DPV peak current linearly increased with the increase of AA concentration in the rang of 25 to 1400 μ mol· L^{-1}, the linear regression equation was I_p(μ A)=2.067+0.00434C_{AA} with R of 0.9984. The limit of detection were 0.15 μ mol· L^{-1} and 10 μ mol· L^{-1} for AC and AA, respectively, with a signal to noise ratio of 3. The results of Fig. 4 fully demonstrated the GQDs modified GCE could be used for the simultaneous quantitative detection of AC and AA.

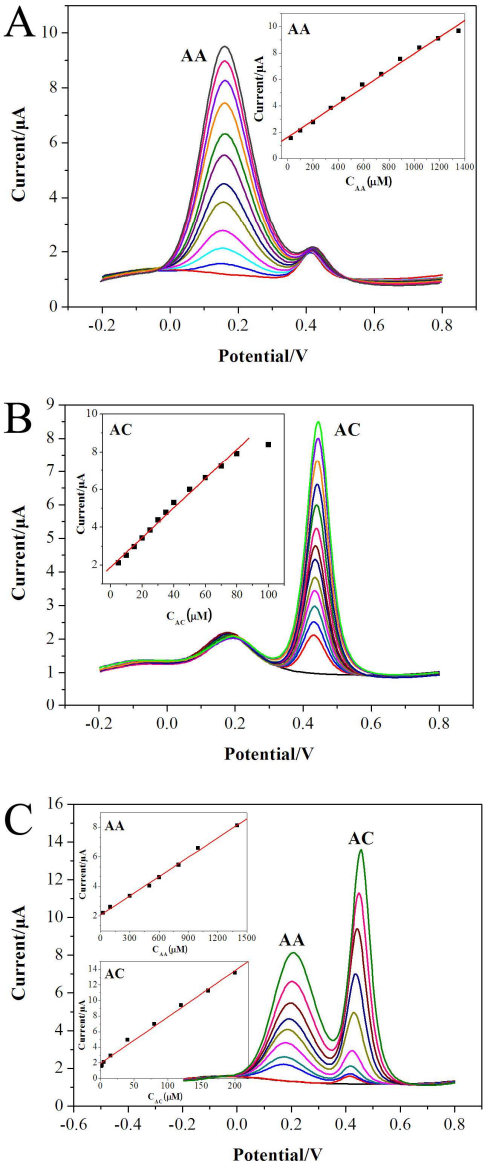

Fig. 4 DPVs of varying concentrations of AA (from bottom to top: 25 to 1350 μmol • L^{-1}) in the presence of AC (5 μmol • L^{-1}) (A); varying concentrations of AC (from bottom to top: 5 to 80 μmol • L^{-1}) in the presence of AA (100 μmol • L^{-1}) (B); varying concentrations of AA (from bottom to top: 25 to 1400 μmol • L^{-1}) and AC (from bottom to top: 1 to 200 μmol • L^{-1}) (C) in PBS (pH=6.5). Inserts severally showed standard curves.

Detection of AC and AA in real samples

The electrochemical sensor was used to detect AC and AA in compound preparation, including Vitamin C Yinqiao Tablet and Compound Ganmaoling Granules. 100 μl of Vitamin C Yinqiao Tablet stock solution were added into the PBS (10 ml, pH=6.5) for the detection AC and AA. 100 μl of Compound Ganmaoling Granules stock solution were used for the detection of AC in the same solution. As shown in Table 1, it was to obtain by the proposed method that the contents of AC and AA in Vitamin C Yinqiao Tablet were 107.86 mg and 49.5 mg, respectively, and the content of AC in Compound Ganmaoling Granules was 197.84 mg. The labeled amount of AC and AA in in Vitamin C Yinqiao Tablet were 102.7% and 104.1%, respectively. The labeled amount of AC in Compound Ganmaoling Granules was 98.9%. The above results met the standards of Chinese Pharmacopoeia (Chinese pharmacopoeia prescribed labeled amount range of preparations is 95%~105%). The determined results for Vitamin C Yiniqiao Tablets and Compound Ganmaoling Granules indicated that the proposed method can be used for the simultaneous detection of AC and AA in the compound preparation.

Table 1 Detection results of AC and AA in compound preparation based on the proposed sensor

Compound preparation	Content (mg)		Fine content [a] (mg)	Standard deviation	Labeled amount (%)
Vitamin C Yinqiao Tablet	AC content per tablet	105	107.86	0.0138	102.7
	AA content per tablet	49.5	51.54	0.0402	104.1
Compound Ganmaoling Granules	AC content per pack	200	197.84	0.0256	98.9

[a] The data represent the average value of three independent determination.

Conclusions

In this work, GQDs was simply prepared with citric acid by pyrolysis method and used to modify GCE to obtain an electrochemical sensor. The fabricated sensor exhibited good electrochemical property and was successfully applied to simultaneously determinate AC and AA of compound preparation in PBS (pH=6.5) without the influence of other interferents. And the experiment results showed the resulting sensor had high sensitivity, good selectivity and wide linear ranges. The study offers another new choice for the simultaneous determination of AC and AA using the electrochemical technology for pharmaceutical analysis and expends the application of GQDs in electrochemical analysis. We hope the novel nanomaterial will obtain more applications in analysis field.

Acknowledgements

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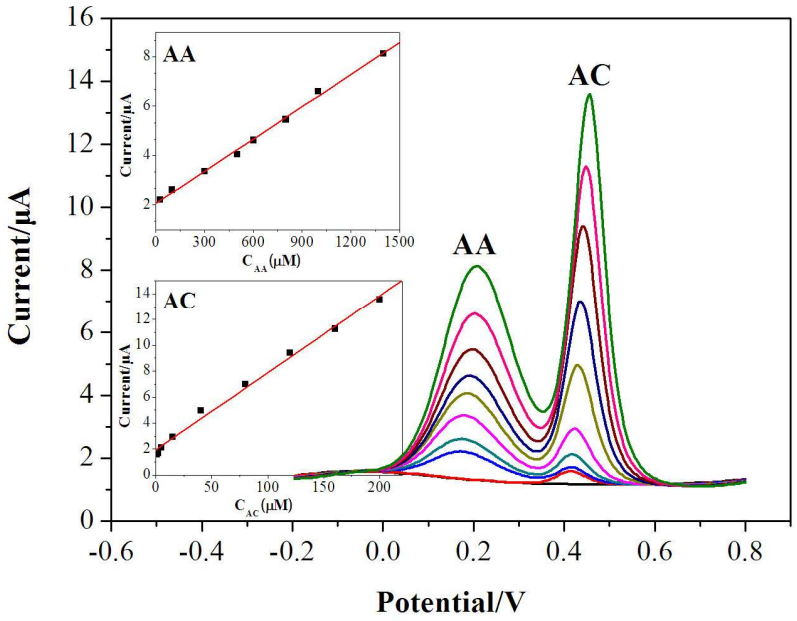
Graphical Abstract



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