

Electrochemical biosensor based on reduced graphene oxide and CMC/silica sol-gel hybrid membranes for the detection of VB₆

Meng Cui¹, Likun Long¹, Yanhui Wu¹, Di Gao², Yandong Wang¹

¹Research Center of Material Science and Engineering, Jilin Institute of Chemical Technology, 132013 Jilin, China

²Jilin Thermal Power Plant, National Energy Group, 132013 Jilin, China

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A novel platform based on graphene oxide (GO) and hybrid membranes of silica and carboxymethyl cellulose (CMC) (short for GO-SiO₂-CMC) was constructed for the fabrication of an electrochemical sensor. The morphology of the composite was tested with SEM. Electrochemical methods including electrochemical impedance spectroscopy and cyclic voltammetry were used to study the composite with an Electrochemical Workstation. The results show that the modified electrode has increased sensitivity, for example, to oxidation of Vitamin B₆ (VB₆), high detection sensitivity (31.5 $\mu\text{AmM}^{-1}\text{cm}^{-2}$), low limit of detection (10^{-7} M), small R_{et} value (252 Ω) and good stability. This may be due to high conductivity and large surface-to-volume ratio of GO, which can facilitate the direct transfer of electrons between the electrode surface and VB₆. The results obtained allow us to propose a simple, sensitive and flexible method for the quantitative determination of vitamin B₆ using electrochemical technology.

Keywords: electrochemical biosensor, graphene oxide, CMC/silica sol-gel hybrid membranes.

Електрохімічний біосенсор на основі відновленого оксиду графену і гібридних золь-гелевих мембран КМЦ/силікагель для виявлення VB₆. *Meng Cui, Likun Long, Yanhui Wu, Di Gao, Yandong Wang*

На основі оксиду графену (GO) і гібридних мембран SiO₂/CMC (GO-SiO₂-CMC) створено нову платформу для виготовлення електрохімічного сенсора. Досліджено морфологію композиту, його електрохімічні властивості методами спектроскопії електрохімічного імпедансу і циклічної вольтамперометрії. Результати дослідження показали, що модифікований електрод має підвищену чутливість до окислення вітаміну B₆ (VB₆) (31,5 $\mu\text{AmM}^{-1}\text{cm}^{-2}$), низьку межу виявлення (10^{-7} М), невелике значення R_{et} (252 Ω) і хорошу стабільність роботи. Це може бути пов'язано з високою провідністю і великим співвідношенням поверхні до об'єму GO, що може сприяти прямому переносу електронів між поверхнею електродів і VB₆. Наведені результати пропонують простий, чутливий і гнучкий метод кількісного визначення вітаміну B₆ за допомогою електрохімічної технології.

На основе оксида графена (GO) и гибридных мембран SiO₂/CMC (GO-SiO₂-CMC) создана новая платформа для изготовления электрохимического сенсора. Исследованы морфология композита, его электрохимические свойства методами спектроскопии электрохимического импеданса и циклической вольтамперометрии. Результаты исследования показали, что модифицированный электрод имеет повышенную чувствительность к окислению витамина B₆ (VB₆) (31,5 $\mu\text{AmM}^{-1}\text{cm}^{-2}$), низкий предел обнаружения (10^{-7} М), небольшое значение R_{et} (252 Ω) и хорошую стабильность работы. Это

может быть связано с высокой проводимостью и большим соотношением поверхности к объему GO, что может способствовать прямому переносу электронов между поверхностью электродов и VB_6 . Приведенные результаты предлагают простой, чувствительный и гибкий метод количественного определения витамина B_6 с помощью электрохимической технологии.

1. Introduction

Recently, there has been significant interest in graphene due to its extraordinary properties, including large surface area, outstanding mechanical strength, excellent electrical conductivity and good biocompatibility [1, 2], which shows promising application in sensors [3], electrochemical devices [4], various nanocomposites [5] and so on. Novel graphene composites have been widely developed, in which graphene is combined with polymers [6, 7], semiconductors [8, 9], and metal nanoparticles [10, 11]. Due to its 2D structure, all the delocalized p -conjugated electrons are effectively available on the surface which makes its electronic structure very sensitive to the local chemical environment [12]. Thus, graphene provides an ideal platform to prepare electrochemical sensors and biosensors [12, 13].

VB_6 is a compound of great biomedical interest due to its significant role among the B vitamins. It is involved in many human metabolic reactions such as protein digestion, fat absorption. The lack of VB_6 would lead to diseases such as anemia, arthritis. As a common drug, VB_6 can be used to treat diseases such as arteriosclerosis, mental retardation, leukopenia. Therefore, it is very important to study the possibilities of VB_6 determination. At present, the means to determinate VB_6 are mainly spectrophotometry [14], fluorescence [15], high efficiency liquid chromatography [16], etc. The electrochemical method, as an alternative to the traditional detection techniques, has been recognized as a promising method for on-site tracking and analysis of VB_6 due to its portability, high sensitivity, low cost, and availability.

VB_6 has electrochemical activity, which can be determined by the electrochemical method. However, due to the large overpotential of VB_6 on the solid electrodes, the sensitivity of unmodified solid electrodes to VB_6 was low. The chemical modification can effectively reduce the overpotential, increase the mass transfer rate of the electrode reaction, and can effectively adsorb and concentrate the analyte selectively at the expense of the electrode surface. At present, there are still few domestic and for-

eign reports on the electrochemical behavior of VB_6 studied by the method of chemically modified electrode [17–21]. Therefore, there is great interest in this area, and for this purpose various modified electrodes have been designed. Various modified electrodes have been used to quickly determine VB_6 , including polymer, nanoparticles, self-assembled monolayers, and metal oxides. To create a biosensor based on a nanocomposite film, an appropriate support material is required. However, the one-component material has inherent disadvantages such as cracking and less biocompatibility. The hybrid materials can effectively eliminate the brittleness of pure inorganic materials and the swelling property of some pure hydrogel polymers. Meanwhile, cellulose ether composite materials have undergone extensive research [22]. Among them, silica/CMC has generated wide interest due to its exciting properties such as controlled porosity and hydrophobicity, and mechanical stability [23]. Here, we have developed an environmentally friendly, simple and inexpensive approach to making a modified GN and silica/CMC electrode for LA determination, which combines the advantages of silica/CMC (good adhesion and biocompatibility) and GN (huge surface area, high electron transfer rate and good biocompatibility). The proposed electrochemical sensor exhibited good selectivity and acceptable reproducibility and could be used for the detection of VB_6 in real samples.

2. Experimental

2.1 Chemicals and materials

Graphite powder and H_2SO_4 (98 %) were obtained from Tianjin Kaixin Chemical Industrial Co. Ammonia solution (25 wt. %) was obtained from Tianjin Yongda Chemical Reagent Co. NaOH, H_2O_2 (30 %) and KCl were purchased from Tianjin Damao Chemical Reagent Factory. Polydiallyldimethylammonium chloride (PDDA) was purchased from Aladdin Industrial Corporation. KMnO_4 was obtained from Tianjin Beifang Tianyi Chemical Reagent Factory. Isopropyl alcohol was obtained from its group chemical reagent Co., LTD. Tetraethyl orthosilicate (TEOS) was obtained from Tianjin Beilian Fine chemicals development Co.,

LTD. NaNO_3 , lactic acid, potassium hexacyanoferrate were purchased from Tianjin Reagent Chemicals Co., LTD. Dialysis tube with molecular weight of 3500 was purchased from Beijing Dingguo Changsheng Biotechnology LLC. Glassy carbon electrodes with 3 mm diameter glassy carbon core were purchased from Audin, USA. CMC was home-made [22].

All reagents were of analytical-grade and used without any further purification. All solutions were prepared with distilled water. VB_6 solutions were freshly prepared before measurements.

2.2 Apparatus

Electrochemical characteristics were investigated on a CHI 660E Electrochemical Workstation (Shanghai Chenhua Instruments, China). A conventional three-electrode system was used. The working electrode with a diameter of 6 mm was a bare, pretreated or $\text{GO-SiO}_2\text{-CMC}$ modified GCE; the auxiliary electrode was a platinum wire and a calomel electrode saturated with KCl as the reference. All potentials in this paper refer to this reference electrode.

The SEM images were obtained at an instrument EVOMA 10 (Carl Zeiss, Germany). The FT-IR spectra of the samples were obtained at an apparatus Nicolet IS10 (USA). The XRD patterns were obtained using a DX-2700 (China). The AFM images were obtained on a CPM5000 (China).

2.3 Preparation of GO, SiO_2 nanoparticles and $\text{SiO}_2\text{/CMC}$ composite material

GO was prepared in a typical process. 11.5 mL H_2SO_4 (98 %) was cooled in an ice-water bath. 0.5 g graphite powder and 0.5 g NaNO_3 were slowly added into the solution until the temperature was 0°C . The necessary step was stirring. Then KMnO_4 (3.75 g) was slowly added in the above mixture and controlled so that the reaction temperature did not exceed 20°C . To complete the reaction, the mixture was stirred in an ice-water bath for 2–4 h. After stirring, the mixture became viscous and dark green. Then the mixture was stirred at 35°C for 1 h. During the stirring process, the viscosity of the mixture gradually decreased. Then the slurry was poured into 230 mL of deionized water, and checked that the reaction temperature was below 98°C . After that, the slurry turned dark brown. Then the slurry was poured into 480 mL of deionized water. 15 mL H_2O_2 was added dropwise into it and the mixture turned golden yellow. The golden yellow suspension was stirred for 2 h. After 12 h of settling,

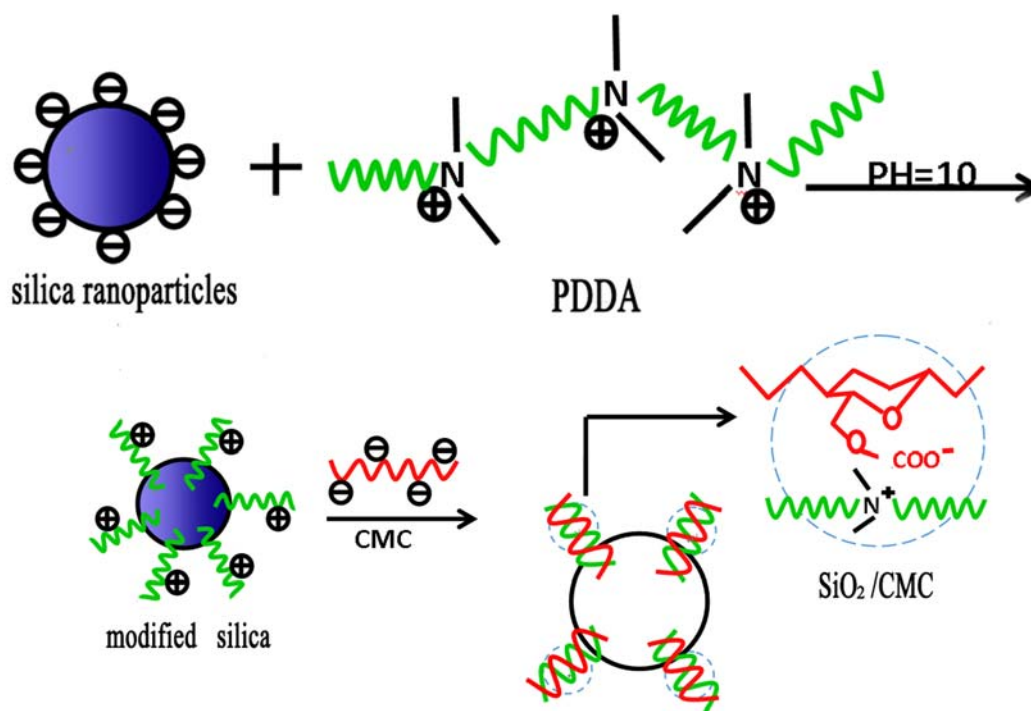
the upper clear liquid was decanted, and the precipitate was poured into a dialysis tube. To get GO, the dialyses was carried out for 7 days until the pH of the suspension was close to neutralization. The final GO product was collected and stored for further use.

SiO_2 nanoparticles were prepared by a typical method. Briefly, isopropanol, ammonium hydroxide and deionized water were mixed well at a certain ratio. The mixture was sonicated and condensate water passed through. After the temperature was stabilized, TEOS was added in the mixture. The solution reacted continuously for an additional 30 min until the reaction was complete. Then, the mixture was centrifuged and washed alternately with deionized water and absolute ethyl alcohol until the pH becomes neutral. After drying, the powder was collected and stored for further use.

To get a CMC solution, 1.25 g of CMC was dissolved in 100 mL of distilled water. The SiO_2 solution was prepared by dissolving 0.25 g of SiO_2 nanoparticles in 50 mL of distilled water. The pH of the mixture was adjusted with NaOH. After the pH reached 10, the mixture was sonicated. 10 mL of PDPA was added to 8 mL of distilled water. Similarly, the pH of the solution was adjusted to 10. Thereafter, the mixture was dispersed with ultrasound for 15 min. PDPA was added to the SiO_2 system slowly, and the system was sonicated for another 15 min. After separation in a centrifuge, the precipitate was collected and 30 mL of distilled water was poured in. After stirring, SiO_2 was dispersed well, and the solution was sonicated for 15 min. 25 mL of distilled water was poured in the well-dispersed SiO_2 solution. The mixture was stirred and ultrasonically dispersed. After the pH of the mixture was adjusted to 10, the mixture was added slowly in the CMC solution above. To get the $\text{SiO}_2\text{/CMC}$ composite material, the obtained solution was centrifuged, and upper clear liquid was poured out. The typical procedure of constructing the $\text{SiO}_2\text{/CMC}$ composite material is schematically shown in Scheme 1.

2.4 Fabrication of the modified electrodes

1 mg GO and 1 mg $\text{SiO}_2\text{/CMC}$ were dispersed in 10 mL distilled water and the mixture was sonicated for 30 min to obtain a homogeneous suspension. Before the start of the electrochemical experiments and modification procedures, GCE was polished to a mirror-like surface with $0.3\ \mu\text{m}$ $\alpha\text{-alumina}$ slurries and sonicated for 2–3 min in ethanol and water separately. Then, 10 μL



Scheme 1. Schematic illustration of SiO_2/CMC composite material.

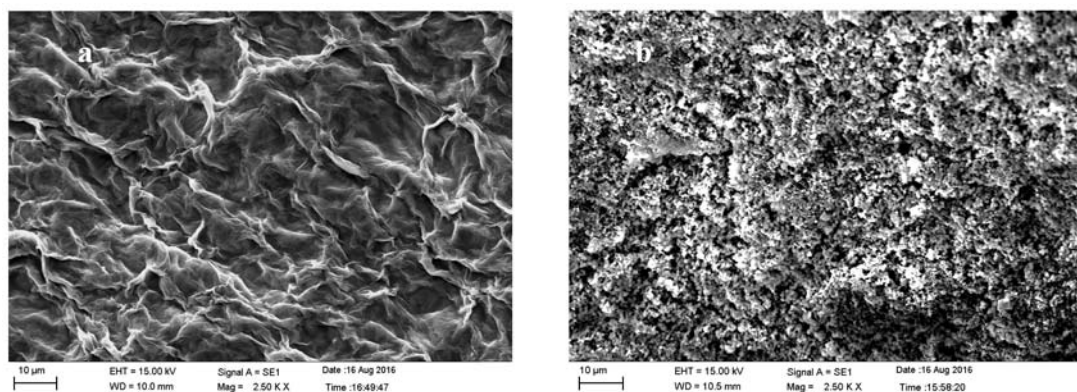


Fig. 1. SEM and AFM images of GO (a) and GO-SiO₂-CMC (b).

of the mixture was dropped onto the surface of GCE with a microinjector. Finally, the electrode was air dried to form GCE-modified GO-SiO₂-CMC, designated as GO-SiO₂-CMC/GCE.

3. Results and discussion

3.1 Characterization of GO and GO-SiO₂-CMC

The surface morphology of GO and GO-SiO₂-CMC was characterized by SEM as shown in Fig. 1. Fig. 1a and 1c show that GO nanosheets with a thickness of 20–30 nm are transparent and look like flakes with folds, which can facilitate immobilization of more SiO₂/CMC. Fig. 1b demonstrates that the GO nanosheets are uni-

formly distributed in the polymer (SiO₂/CMC). It was shown that the GO was attached to the GO-SiO₂-CMC composite. From Fig. 1 we also notice that there are many small holes in the GO-SiO₂-CMC film, which can be caused by the formation of gas bubbles [24] during the reduction treatment of GO-SiO₂-CMC.

The XRD patterns of the pristine graphite, GO, and GO-SiO₂-CMC are shown in Fig. 2. Pristine graphite exhibits a sharp diffraction peak at $2\theta = 26.5^\circ$ (curve a) corresponding to an interplanar spacing $d = 0.336$ nm according to Bragg's law. Compared with the pristine graphite, the characteristic diffraction peak of GO (curve b)

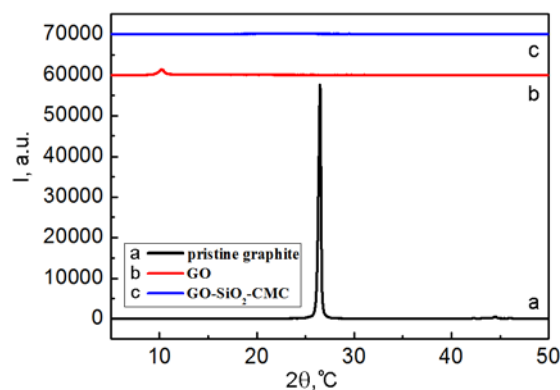


Fig. 2. The XRD patterns of pristine graphite (a), GO (b) and GO-SiO₂-CMC (c).

appears at 10.01° since the AB... stacking order is still observed in graphite oxide with an interplanar spacing $d = 0.892$ nm. This value is larger than the d -spacing (0.336 nm) of pristine graphite ($2\theta = 26.5^\circ$) (curve *a*) due to the introduction of oxygenated functional groups into carbon sheets. After electrochemical reduction of the GO composite in SiO₂/CMC, the peak located at 9.9° (curve *c*) disappeared, confirming the reduction of GO.

Fig. 3 shows FT-IR spectra of CMC, GO, SiO₂/CMC and GO-SiO₂-CMC. In Fig. 3b, there are peaks which belong to oxidizing functional groups. This illustrates that during the oxidation reaction, a large number of oxidizing functional groups were trapped in graphite. As can be seen from Fig. 3b, peaks at 1052, 1728, 3400 cm⁻¹ correspond to the presence of C–O, C=O, O–H. In Fig. 3c, the peaks appeared at 3435.35 and 1427.81 cm⁻¹ belong to O–H, and the peaks appeared at 2924.67 and 1587.04 cm⁻¹ belong to –CH₂. In addition, the characteristic peaks at 1005.87 and 1637.72 cm⁻¹ correspond to C–O–C and –COO, respectively. We also have found peaks at 1089 and 473 cm⁻¹, which belong to the Si–O group in Fig. 3c. After introduction of GO into the GO-SiO₂-CMC, the peaks of GO and SiO₂/CMC were observed in curve *d*, confirming that the GO was effectively immobilized by SiO₂/CMC film.

3.2 Electron paramagnetic resonance (EPR) analysis

EPR spectra of SiO₂/CMC and GO-SiO₂-CMC are shown in Fig. 4 (*a* and *b*). In Fig. 4, the characteristic peaks at 1700 G belong to the Fe³⁺ which exists in the sample cells. And the strong peak at 3500 G is from solvent water. In SiO₂/CMC, there are no characteristic EPR resonances at room tempera-

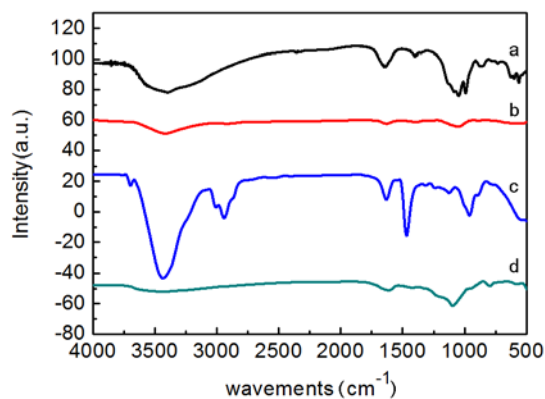


Fig. 3. FTIR spectra of CMC (a), GO (b), SiO₂/CMC (c), GO-SiO₂-CMC (d).

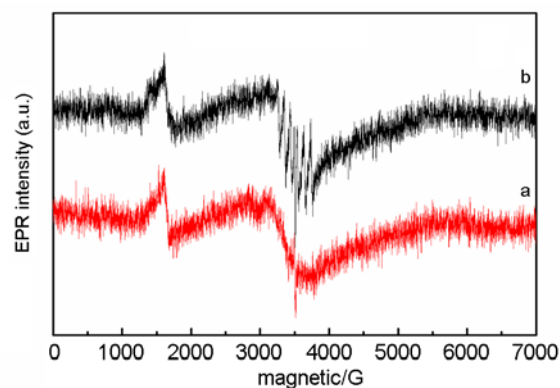


Fig. 4. Room temperature EPR spectra of GO (a) and GO-SiO₂-CMC (b).

ture. This means SiO₂/CMC cannot generate the EPR signal observed in the sample. However, after combining with GO, a paramagnetic spin $S = 5/2$ with isotropic g -value at 2.002 is observed with linewidth of 3500 G. The six distinguished multiplets (sextet) are assigned to the presence of manganese [Mn(II), $S = 5/2$] ions in GO sample, which are encapsulated in the GO sheets during the synthesis process. The easy spin software was used to simulate the experimental EPR spectrum of GO using the effective spin-Hamiltonian with Zeeman and hyperfine interaction terms. This result was similar to that of FT-IR.

3.3 Electrochemical characteristics of electrochemical sensor

The electron transfer properties of electrodes after various surface modifications were characterized by EIS which was used to monitor the electrode modification process. The impedance spectra include a semicircle portion and a linear portion. The semicircle diameter at higher frequencies corresponds to the electron-transfer resis-

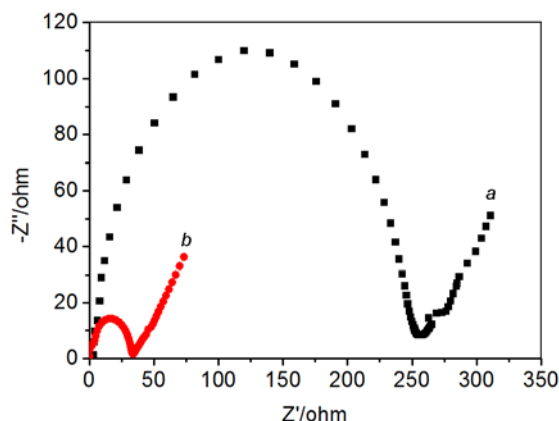


Fig. 5. Electronic transfer properties of GO (a) and GO-SiO₂-CMC (b).

tance (R_{et}), and the linear part at lower frequencies corresponds to the diffusion process. Fig. 5 shows the impedance spectra corresponding to the bare glassy carbon electrode (GCE) (a) and GO-SiO₂-CMC modified electrode (b). It can be seen from Fig. 5 that the EIS of bare GCE displays a small well defined semicircle at higher frequencies, indicating low interface impedance. However, after immobilization of GO-SiO₂-CMC on the GCE, a smaller R_{et} is observed. Based on the results, it was suggested that GO-SiO₂-CMC is an excellent electrically conductive material that can accelerate electron transfer and lead to a decrease in R_{et} .

Fig. 6 shows the voltammetric responses of the bare GCE (a), SiO₂/CMC/GCE (b), GO-SiO₂-CMC/GCE (c) of 0.01 M PBS towards VB₆. With the bare GCE (a), VB₆ shows no apparent peaks. However, when using SiO₂/CMC/GCE, a pair of redox peaks was observed. It indicates excellent catalytic ability of SiO₂/CMC in the oxidation of VB₆. With GO-SiO₂-CMC/GCE (c), the current of the VB₆ oxidation peaks increases obviously compared to uncoated GCE. A pair of symmetrical redox peaks with significantly increased peak currents and less peak separation for VB₆ strongly indicate excellent catalytic ability of GO-SiO₂-CMC in the oxidation of VB₆.

3.4 The performance of the GO-SiO₂-CMC modified electrochemical sensor towards different concentration of VB₆

To accurately determine VB₆ with GO-SiO₂-CMC/GCE, DPV (Differential Pulse Voltammetry) analysis was carried out in the potential range of -0.1 to 0.5 V in PBS (PH = 7.0) for VB₆ of various concentrations. As shown in Fig. 7, the electrochemical response of VB₆ increases linearly with

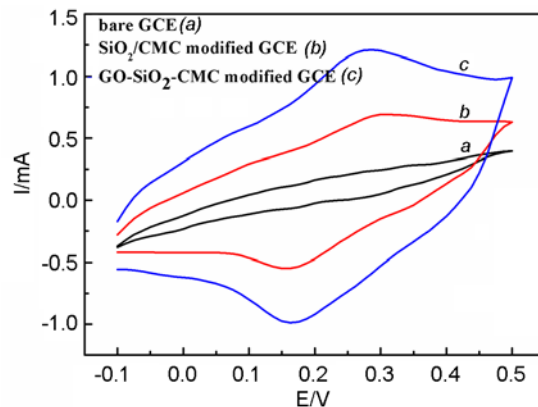


Fig. 6. CV of bare GCE, SiO₂/CMC modified GCE, and GO-SiO₂-CMC modified GCE.

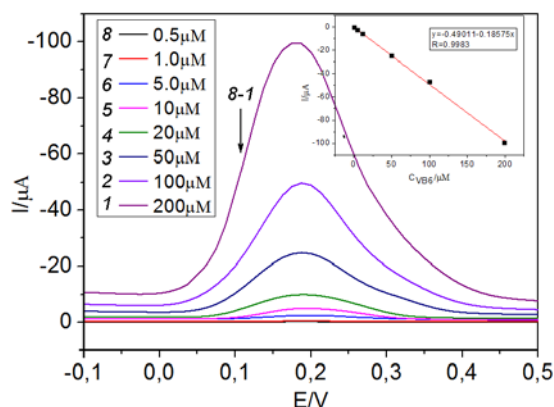


Fig. 7. DPV profiles at GO-SiO₂-CMC/GCE in PBS (PH = 7.0) with different concentrations of VB₆ from 0.5 to 200 μ M.

an increase in the VB₆ concentration. The linear range for the determination of VB₆ is 0.5–200 μ M. The linear equation is with a statistically significant correlation coefficient of 0.9993 and a slope of 31.5 μ AmM⁻¹cm⁻²; the limit of detection is as low as 10⁻⁷ M ($S/N = 3$), which is obviously better than that of RGO-AuNPs-CSHMs/GCE (0.05–0.08 μ M) [25].

3.5 Interference, reproducibility and stability

Selectivity and anti-interference properties were also investigated by the chronoamperometric method. As shown in Fig. 8, the additions of 0.1 mM lactic acid (LA) and 0.1 mM uric acid (UA) resulted in very weak responses, compared to the large increase in current following the addition of 2 mM VB₆. It was evident that the influence of the tested interfering particles on the VB₆ response was negligible, indicating a high selectivity of the GO-SiO₂-CMC electrochemical sensor. Ten repetitive measurements were carried out in 1 mM VB₆ and a

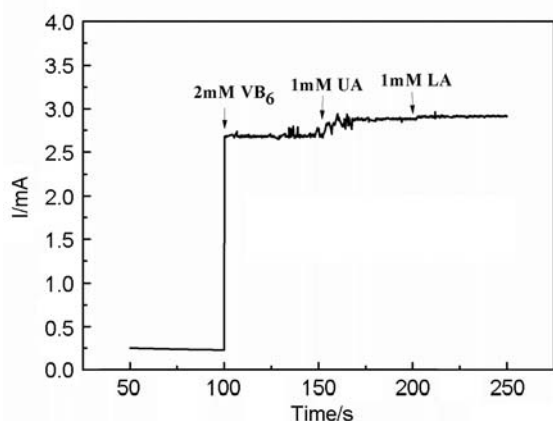


Fig. 8. Time-dependent current responses at GO-SiO₂-CMC modified electrode to additions of 2 mM VB₆, 0.1 mM LA and 0.1 mM UA in PBS (pH = 7.0).

relative standard deviation of 6.8 % for the current was observed indicating acceptable reproducibility of the electrochemical sensor.

4. Conclusions

In this work, we developed an environmentally friendly, simple, and inexpensive approach to fabricating a new composite that was obtained by alloying GO into a SiO₂/CMC composite. The as-synthesized composite film was used as a model to construct biosensors for VB₆ detection. The modified electrode exhibited a higher sensibility (31.5 $\mu\text{A nM}^{-1}\text{cm}^{-2}$), a wider linear range (0.5–200 μM), a lower detection limit (10^{-7} M) and better reproducibility and stability. The proposed strategy and electrochemical reduction may be potentially useful for the preparation of other biomolecular sensors.

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