Carboxymethyl cellulose assisted preparation of water-processable halloysite nanotubular composites with carboxyl-functionalized multi-carbon nanotubes for simultaneous voltammetric detection of uric acid, guanine and adenine in biological samples

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ABSTRACT

Uric acid (UA), a waste product of purine derivatives catabolism, is one of the main culprits of gout. In this paper, a simple and sensitive method for the simultaneous voltammetric detection of uric acid (UA) and the most common purine derivatives like guanine (G) and adenine (A) in human serum and lettuce samples was successfully realized using glassy carbon electrode (GCE) modified with the water-processable nanotubular composites consisting of both halloysite nanotubes (HNTs) and carboxyl-functionalized multi-walled carbon nanotubes (*f*MWCNTs) that were well dispersed by carboxymethyl cellulose (CMC). A water-processable HNTs hybrid nanotubes with *f*MWCNTs were successfully prepared using CMC assist, and the CMC-HNTs-*f*MWCNTs composites were characterized by scanning electron microscopy, fourier-transform infrared spectroscopy, transmission electron microscope, electrochemical impedance spectroscopy, cyclic voltammetry and chronocoulometry. CMC-HNTs-*f*MWCNTs/GCE displayed enhanced electrode stability in water, effective surface area, adhesive property and adsorption capacity, and electrocatalytic activity for the oxidation of UA, G and A, which could simultaneously detected three purine derivatives in linear ranges from 1 to 130 μM with a limit of detection (LOD) of 0.5 μM for UA, 0.05 to 9 μM with LOD of 0.018 μM for G, and 0.3 to 25 μM with LOD of 0.19 μM for A, respectively. The as-fabricated sensor displayed high sensitivity, low detection limit, good sensing stability, remarkable feasibility, and satisfactory practicality.

Keywords Electrochemical sensor · Purine derivatives · Nanotubes · Uric acid · Adenine · Guanine

1. Introduction

Gout is a kind of inflammatory disease with repetitive relapse, which is caused by accumulation of urate crystals in synovial joints, slippery capsules, cartilages and other tissues due to the hyperuricaemia. Numerous's studies have confirmed that the increasing metabolism of the purine biosynthesis, the production of the excess uric acid (UA), and the hyperuricaemia because of abnormal excretions, which easily result in the gout[1, 2]. In addition, the synthesis in vivo, nucleic acid decomposition, and food intake are important sources of purine derivatives in the human body, while the UA is a waste product of purine derivatives catabolism[3]. Guanine (G) and adenine (A) are the most common purine derivatives in vivo, and they are mainly derived from the degradation of nucleic acid. Therefore, the determination of UA content in the tissue fluid is of great significance in the clinical medicine, and the detection and monitor of the most common purine derivatives in agro-food and blood is also very necessary.

The existing methods like high performance liquid chromatography [4], mass spectrometry [5], liquid chromatography-tandem mass spectrometric [6, 7], ion-pairing liquid chromatography [8], flow injection-chemiluminescence [9] and capillary electrophoresis [10] have been established for the determination of biomolecules. Although they display unique advantages in accuracy and selectivity in mixtures with real samples, these methods still exist complex and time-consuming sample pretreatment, tedious sample purification steps, strong interference resulting from colored substances or impurities present in real samples, low sensitivity, and the high cost of equipment, reagent, and operator training. Sensors based on chemically modified electrode were employed for the electrochemical detection of purine derivatives, even further employed for the electrochemical detection of purine

derivatives in real samples. More and more literatures have been widely reported for the determination of purine derivatives due to the combination of superior properties of various materials ([Table 1\[11-20\]](#)). Electrochemical sensors based on various nanocomposites have significantly enhanced synergistic effects in sensing performances like sensitivity, selectivity, accuracy due to the combination of nanomaterials[\[21\]](#).

Nanomaterials and their nanocomposites have recently been attracting considerable attention of scientists and engineers owing to their surface and interface effect, small size effect, quantum size effect, macroscopic quantum tunneling effect, this have been extensively used in chemically modified electrodes due to their large surface area, superior catalytic activity, and good adsorption capacity, etc[\[22-29\]](#). Carbon nanotubes (CNTs), one of the most excellent nanomaterials and most commonly used carbon nanomaterials, which can foresee their promising applications in electroanalytical chemistry for constructing CNT-based sensors[\[30\]](#) in virtue of their unique structural, high surface-to-volume ratio, good electronic and mechanical properties, excellent electrocatalytic ability, high electrical conductivity, good biocompatibility and chemical stability[\[23, 31-33\]](#). Hydrophilic groups like carboxyl, hydroxy and amino functionalized carbon nanotubes (*f*CNTs) have obtained much attention in recent years because of their ability to reinforce properties, enhance interfacial interactions, improve the solubility[\[24-27, 29, 34, 35\]](#). In addition, nanoclay materials have attracted intense interest in today's materials research for applications in catalysis and sensors as the important characteristics of surface area, particle size, particle shape, adsorption, surface chemistry and low cost[\[36\]](#). However, the main intrinsic problem of nanoclay materials in sensing application is their insolubility in water (extremely poor solubility in most of common solvents) and bad

adhesion towards substrate electrode (easy to peel off from the surface of substrate electrode during use). Though different solvents, biopolymers, and surfactants have been employed to improve their dispersion, and various materials like nafion, ionic liquid, and chitosan were introduced to enhance adhesion between the coating and the substrate electrode. There was a challenge for a large-scale production of nanoclay aqueous dispersion with good adhesive property using simple, green, facile, and low-cost approaches.

In our previous work, carboxymethyl cellulose (CMC), a good water-soluble and environment friendly biopolymer with high hydrophilicity, superior biocompatibility, excellent film forming ability, and good adhesive property, could well disperse layered nanoclay MMT, single-walled CNT and their nanocomposites[37], improve long-term electrode stability in water of PEDOT:PSS and its derivatives[38, 39]. Moreover, CMC improve synergistically electrocatalytic ability, adhesive ability, and flexibility, these are very favorable for application in electrochemical sensors[37, 40].

In this work, a simple, green, facile, and effective strategy for a large-scale production of the water-processable nanotubular composites consisting of both halloysite nanotubes (HNTs) and carboxyl-functionalized multi-walled carbon nanotubes (μ MWCNTs) were proposed, and CMC could well disperse two nanotubes and their composites in water. The as-fabricated nanotubular composite modified electrode was successfully employed for the simultaneous determination of UA, G and A in biological samples.

2. Experimental

2.1. Chemicals and materials

Sodium salt of CMC was purchased from TCI (Tokyo, Japan). HNTs were bought from Sigma Aldrich. μ MWCNTs were obtained from Chengdu Institute of

Organic Chemistry, Chinese Academy of Sciences. Nafion D-521 dispersion (5% w/w in water and 1-propanol, ≥ 0.92 meq g⁻¹ exchange capacity) was purchased from Alfa Aesar (Tianjin) Chemical Co. Ltd. UA, G and A were obtained from Aladdin Co., Ltd. Phosphate buffer solution (PBS, 0.1 M) with different pH values was prepared using 0.1 M Na₂HPO₄, 0.1 M KH₂PO₄ and 0.1 M H₃PO₄. All experimental reagents were prepared with the doubly distilled water.

2.2. Apparatus

Linear sweep voltammetry (LSV), electrochemical impedance spectroscopy (EIS), and cyclic voltammetry (CV) measurements were performed using a CHI660E electrochemical workstation (Chenhua Instrument Co., Shanghai, China). The conventional three-electrode system consists of a platinum wire (Pt), a glassy carbon electrode (GCE), and a saturated calomel electrode (SCE). Fourier-transform infrared spectroscopy (FTIR) spectra were recorded with a Fourier spectrometer. The surface morphology was measured by scanning electron microscopy (SEM) with a JSM-6701F microscope (JEOL Ltd., Japan). Transmission electron microscope (TEM) was used by TECNAI G2 F20 S-TWIN microscope (FEI, America). The pH value was adjusted by the CT-6023 portable pH meter.

2.3. Preparation of CMC-HNTs-*f*MWCNTs/GCE

Before modification, the GCE was carefully polished on a chamois leather with 0.05 μm Al₂O₃ slurry, then sonicated in sequence with double distilled water, anhydrous alcohol, and double distilled water for five minutes, respectively, and dried at room temperature. 2 mg HNTs and 1 mg *f*MWCNTs were dispersed in 0.3 mg mL⁻¹ CMC aqueous solution with ultrasonic treatment for 0.5 h. Subsequently, 4 μL nafion-water mixed solution (0.3 %) was added to enhance the stability of the modified electrode. The CMC-HNTs-*f*MWCNTs/GCE was prepared by drop-coating

the composite solution on the surface of the bare GCE with the flnnpipette and dried in infrared lamp.

2.4. Experimental measurements

A certain concentration of UA, G and A solution were transferred into the electrochemical cell containing 5 mL of 0.1 M PBS (pH 7.0) by flnnpipette at room temperature. The CMC-HNTs-*f*MWCNTs/GCE was immersed into 0.1 M PBS containing UA, G and A with agitation for 50 s. The simultaneous detection of UA, G and A was studied by LSV, and the scan rate of LSV was 100 mV s⁻¹.

2.5. Preparation of real samples

The serum sample was collected from healthy volunteer. The lettuce was purchased from the supermarket near Jiangxi Agricultural University. The lettuce sample was obtained by cutting it into small pieces, using a mortar and pestle to crush, and then filtering with qualitative filter paper. Subsequently, the lettuce sample was diluted 200-fold, and the serum sample was diluted 250-fold, all sample solutions were adjusted to pH 7.0 with 0.1 M PBS. Finally, 5.0 ml different sample solutions were added into the electrochemical cell to simultaneous determination of UA, G and A by LSV, respectively.

3. Results and discussion

3.1. Water dispersibility

[Fig. 1](#) presents the water dispersibility of HNTs (bottle a), *f*MWCNTs (bottle b), and CMC-HNTs-*f*MWCNTs (bottle c) with ultrasonic treatment. HNTs or *f*MWCNTs could not disperse well in the water, only a small amount of HNTs or *f*MWCNTs were dispersed in the water, which were attributed to their hydrophilic group like hydroxy and carboxyl group in nanotubular structures. Apparently, most of them were precipitated at the bottom, especially after half a month of static cultivate ([Fig. 1a and](#)

b), which was ascribed to bad water processability of HNTs or *f*MWCNTs, while CMC-HNTs-*f*MWCNTs were well dispersed in the water, even after half a month (Fig. 1c), indicating that CMC as the excellent dispersant and stabilizing agent promoted the interaction between HNTs and *f*MWCNTs.

3.2. Characterization of CMC-HNTs-*f*MWCNTs

3.2.1. SEM

SEM was used to investigate surface morphological structures of raw HNTs, pure *f*MWCNTs, and CMC-HNTs-*f*MWCNTs composite. It could be seen that raw HNTs have a hollow nanotubular structure, and there was no the phenomenon of sealing end, curling and casing (Fig. 2A), while the surface morphology of original *f*MWCNTs was bended or entangled with each other and had an inattentive filamentous structure (Fig. 2B). As reported in our literature, the original CMC was generally a regular, smooth, compact and homogeneous structure[37, 39, 40] . As shown in Fig. 2C, HNTs were covered onto *f*MWCNTs or embed/incorporated into *f*MWCNTs, and CMC were well adhered their surface, and all of them were got together tightly with each other and formed nanotubular network.

3.2.2. TEM

Fig. 3 shows typical TEM images of the as-prepared CMC-HNTs-*f*MWCNTs composite with different magnifications. As reported in previous literature, the raw HNTs or pristine *f*MWCNTs tend to form aggregates in bundles[41-43], It could be observed that HNTs was wrapped with randomly arranged *f*MWCNTs, less bundled nanostructures are observed for the HNTs-*f*MWCNTs composite, which was because CMC results in the reduction of non-covalent interactions among composite or themselves. CMC facilitate the dispersibility in water of HNTs-*f*MWCNTs composite, and the formed nanotubular network revealed that two kinds of one dimensional

nanomaterials in the presence of CMC exhibit the ability to mutually improve their own dispersibility.

3.2.3. FTIR

FTIR spectrum of raw HNTs (curve a), *f*MWCNTs (curve b), CMC-HNTs-*f*MWCNTs (curve c) are shown in Fig. 4. It could be seen from the spectrum of HNTs, there were two strong peaks at 3694 and 3622 cm^{-1} , which were came from hydroxyl stretching vibrations. The peaks at 541 and 465 cm^{-1} were the flexural vibration characteristic absorption peak of Si-O, the 1110 cm^{-1} was assigned to Si-O stretching vibration, and the 1032 cm^{-1} was based on Si-O-Si asymmetrical stretch vibration. The flexural vibration peak of Al-OH was at 910 cm^{-1} . In the infrared spectra of *f*MWCNTs, the broad peak at 3434 cm^{-1} belonged to O-H stretching vibrations. The band at 1637 cm^{-1} was ascribed to C=O stretching vibrations for the carbonyl group, and the stretch vibration of C-O at 1086 cm^{-1} . For the spectrum of CMC-HNTs-*f*MWCNTs composite, the characteristic absorption peak of HNTs and *f*MWCNTs intensity becomes weaker, especially for HNTs. This indicated that there was a strong interaction between HNTs and *f*MWCNTs with the presence of CMC.

3.2.4. EIS

In order to exhibit the interface characteristic of different modified electrodes, EIS was executed at the open circuit potential of 0.2 V and the frequency extent from 0.1 Hz to 100 kHz in a redox probe of 5 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ containing 0.1 M KCl. It was known clearly that the semicircle diameter was used to estimate the electron-transfer resistance (R_{ct}) of different modified electrode. As shown in Fig. 5A, the diameter of the semicircle increased when CMC (curve b) or CMC-HNTs (curve c) was drop-coated onto the surfaced of GCE compared with the impedance of bare GCE (curve a), this was because the electron transport was blocked by non-

conductive CMC, and made it difficult to transfer the interfacial charge. However, the R_{ct} decreased rapidly when CMC-*f*MWCNTs (curve d) was modified onto the surface of GCE, indicating that high conducting *f*MWCNTs increased the conduction path between the electrode and the electrolyte, and enhanced the diffusion of potassium ferricyanide towards to the surface of electrode. The R_{ct} of CMC-HNTs/GCE (curve c) and CMC-HNTs-*f*MWCNTs/GCE (curve e) were larger than that of CMC/GCE and CMC-*f*MWCNTs/GCE, respectively, indicating that non-conductive HNTs also had a certain impact on the electron-transfer.

3.2.5. CVs

CVs of corresponding electrodes were shown in Fig. 5B. Obviously, the peak to peak separation (ΔE_{pa}) of the redox couple between the anodic and cathodic peak potential at CMC/GCE (curve b) and CMC-HNTs/GCE (curve c) was bigger than that at bare GCE (curve a), while their peak currents compared with bare GCE were decreased obviously, which further revealed that the non-conductive CMC hindered the electron transfer, and HNTs also had a certain effect on the electron-transfer. It was clear that the ΔE_{pa} at CMC-*f*MWCNTs/GCE (curve d) and CMC-HNTs-*f*MWCNTs/GCE (curve e) was smaller than that at other modified electrodes, but their response currents was increased clearly in comparison with other GCEs, which was because the high conductivity of *f*MWCNTs accelerated the transfer of electrons. Therefore, all results of CVs were in accordance with that of EIS mentioned above.

3.3. Electrode stability

The water stability of the film electrode and the adhesion of the film towards the electrode were very important in the electrochemical application of the modified electrode. Therefore, the water stability of CMC-HNTs-*f*MWCNTs/GCE was investigated by performing 100 cycles of CVs tests at a scan rate of 50 mV s⁻¹ (Fig.

6A). It could be known that the relative standard deviation (RSD) values of redox peak currents were 1.17% and 0.67%, respectively, indicating that the CMC-HNTs-*f*MWCNTs/GCE had a better cycle stability and a higher repetitive charge/discharge reversibility. The results further demonstrated that the CMC-HNTs-*f*MWCNTs/GCE film owned an excellent electrode adhesion and the stability in water solution on the surface of GCE. The stability and adhesion of CMC-HNTs-*f*MWCNTs/GCE was preserved in the double distilled water for eight days, and investigated by CVs every day (Fig. 6B). The RSD values of redox peak currents were 1.34% and 1.98%, respectively, and revealing that both CMC and nafion enhanced synergetically the stability and adhesion of the composite modified electrode, and made CMC-HNTs-*f*MWCNTs a promising electrode material for the electrochemical application of high performance film electrode.

3.4. Effective surface area

The large effective surface area of the modified electrode can lower the limit of detection (LOD), increase the electrochemical active sites, enhanced the electrochemical response and improve sensitivity of sensor. Hence, the electrochemically effective surface areas of different electrodes were determined by chronocoulometry with a model complex of 5 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ (Fig. 7A). According to the Anson equation[44, 45]:

$$Q(t) = 2nFAcD^{1/2}t^{1/2}\pi^{-1/2} + Q_{\text{dl}} + Q_{\text{ads}}$$

where A is the effective surface area, c is the concentration of substrate, D is diffusion coefficient, n is the electron number, Q_{dl} is double layer charge which could be eliminated by background subtraction, v is the scan rate and Q_{ads} is Faradic charge, F , π have their usually meanings. For $[\text{Fe}(\text{CN})_6]^{3-/4-}$, the D is $7.6 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$, n is 1. The linear equation of Q vs $t^{1/2}$ curves in different electrode was shown in Fig. 7B. It

is considered that the slope of the linear equation between Q and $t^{1/2}$ could be used to estimate the effective surface area of the modified electrode[46], when the CMC-HNTs-*f*MWCNTs was modified on the surface of GCE, while its A was obviously increased (the A of bare GCE was 0.056 cm^2), the A value was calculated to be 0.13 cm^2 . In addition, CVs were also used to estimate the A of the constructed electrode (Fig. 7E and F), and the A value of CMC-HNTs-*f*MWCNTs was calculated to be 0.11 cm^2 , which was closed to the calculated result by chronocoulometry, suggesting that CMC-HNTs-*f*MWCNTs enlarge the A .

3.5. Absorption capacity

The absorption capacity (Γ_s) of CMC-HNTs-*f*MWCNTs/GCE was also determined by chronocoulometry (Fig. 7C and D), and it could be estimated by the Anson equation ($Q_{\text{ads}} = nFA\Gamma_s$), and the Γ_s of CMC-HNTs-*f*MWCNTs/GCE can be obtained as $8.34 \times 10^{-10}\text{ mol cm}^{-2}$.

3.6. Electrochemical behaviors

CVs of UA, G and A in 0.1 M PBS (pH 7.0) using bare GCE (curve a), CMC/GCE (curve b), CMC-HNTs/GCE (curve c), CMC-*f*MWCNTs/GCE (curve d), CMC-HNTs-*f*MWCNTs/GCE (curve e) were shown in Fig. 8. Two broad and weak oxidation peaks appeared at the bare GCE, CMC/GCE and CMC-HNTs/GCE, respectively, while oxidation peaks of A at three electrodes almost invisible, indicating that the electrocatalytic activity of CMC or HNTs was pretty poor for the oxidation of UA, G and A, especially for the oxidation of A. However, when CMC-*f*MWCNTs were modified onto the GCE surface, it could obtain three obvious oxidation peaks, which was ascribed to the excellent electrocatalytic ability of *f*MWCNTs for the oxidation of UA, G and A. Furthermore, a remarkable increase of oxidation peak currents (I_{pa}) of UA, G and A when CMC-HNTs-*f*MWCNTs were

coated onto the surface of GCE. The CMC-HNTs-*f*MWCNTs/GCE displayed the strongest and sharpest peak shape, the highest peak current, and a relatively lower peak potential compared with all other electrodes, revealing that the synergistic effect among different components improved their voltammetric responses. Therefore, CMC-HNTs-*f*MWCNTs/GCE can be used to fabricate an electrochemical sensor for the simultaneous determination of UA, G and A.

3.7. Effect of scan rates

The effect of scan rates (ν) on electrochemical responses of UA, G and A was investigated by CVs (Fig. 9A). The oxidation peak potentials (E_{pa}) shifted towards a relatively positive potential and the I_{pa} of three compounds increased linearly with the increase of ν (Fig. 9B), indicating that the irreversible electrode reaction was controlled by adsorption. From Fig. 9C, it could be seen a upward bending characteristic curve of I_{pa} versus $\nu^{1/2}$, which was a typical characteristic of adsorption process, manifesting that electrode reactions of UA, G and A on the CMC-HNTs-*f*MWCNTs/GCE were a adsorption-controlled electrode process[20, 47]. The relationship between E_{pa} and $\ln \nu$ was presented by Laviron equation[48]:

$$E_P = E^0 + \left(\frac{RT}{\alpha nF}\right) \ln\left(\frac{RTk^0}{\alpha nF}\right) - \left(\frac{RT}{\alpha nF}\right) \ln \nu$$

Where α is the electron transfer coefficient; k^0 is the rate constant of the reaction; n is the electron-transfer number; ν is the scan rate and E^0 is the formal potential, other symbols have their usual meanings. The linear relationship between E_{pa} and $\ln \nu$ was presented in Fig. 9D, the value of αn was calculated easily from the slope of the equation in Fig. 9D, and its αn was 2.13 for UA, 1.06 for G and 0.93 for A, respectively. In general, α was approximated to be 0.5 in the response of irreversible electrode processes, so n was obtained to be 4 for UA, 2 for both G and A.

3.8. Effect of pH

The effect of pH on oxidation peak of UA, G and A was investigated by LSVs over the pH range from 5.5 to 8.5 (Fig. 10A). It was obvious that the E_{pa} of UA, G and A shifted positively with the decrease of pH value, indicating that protons were involved in the electrochemical oxidation-reduction process of UA, G and A (Fig. 10C)[49]. According to Nernst equation[50], the formula $dE_{pa}/dpH = (-2.303mRT)/(nF)$ was obtained, where m is the number of proton participating in the electrochemical reaction, which could be determined from slopes of E_{pa} vs. pH. As can be seen from Fig. 10C, the slope of dE_{pa}/dpH plots are 57, 57, 67, respectively. Thus the m/n ratios of three analytes were all approximately to 1, suggesting that the number of electrons and protons was equal. Therefore, the electrode reaction of UA oxidation was four electrons accompanied by four protons process, while the electrochemical oxidation of both G and A at CMC-HNTs-*f*MWCNTs/GCE involved a two-electron and two-proton process. In addition, it was also noted that the I_{pa} of UA reached its maximum value at about pH 6.5, while the I_{pa} of both G and A reached their maximum values at approximately pH 7.0, respectively (Fig. 10B), and the distance between ΔE_{G-UA} and ΔE_{A-G} reached their maximum values at approx pH 8.5, respectively (Fig. 10D). Considering different factors, pH 7.0 PBS (0.1 M) was selected as the optimal value of pH for the simultaneous voltammetric determination of UA, G and A.

3.9. Simultaneous detection of UA, G and A

LSVs were used to evaluate the relationship between I_{pa} and concentrations of UA, G and A using CMC-HNTs-*f*MWCNTs/GCE. According to Fig. 11 (A and D), concentrations of both G (7 μ M) and A (6 μ M) kept constant, and a linear response range of UA was 1 – 130 μ M. The equations $LOD = 3s/m$ and $LOQ = 10s/m$ were

used to calculate the LOD and LOQ, where s is the standard deviation of peak currents of the blank (twenty runs), and m is the slope of the linear equation [51, 52], the constructed sensor shows a relatively low LOD of 0.5 μM and LOQ of 1.7 μM for the detection of UA. It can be seen from Fig. 11 (B and E), a wide linear response from 0.05 to 9 μM with a good LOD of 0.018 μM and LOQ of 0.06 μM for the detection of G. In addition, the diversified concentration of A in the presence of 2.4 μM G and 40 μM UA were evaluated in Fig. 11 (C and F), a good linear response range from 0.3 to 25 μM was obtained, and the LOD and LOQ of A were 0.19 and 0.6, respectively. All results suggested that the as-fabricated electrode was likely to be used to detect the content of both A and G in daily food and the content of UA in human body (in general, the range of UA content in human body was male 149 – 417 μM , female 89 – 357 μM). The comparison of sensing performance based on different electrochemical methods and chemically modified materials for the determination of UA, G and A in previous reports during recent three years was shown in Table 1. It could be seen that the constructed electrode was better in some cases compared with the recently reported literature.

3.10. Sensing stability

In order to demonstrate the sensing stability of CMC-HNTs-fMWCNTs/GCE, the repeatability of UA, G and A was evaluated by 30th continuous voltammetric sweep. It could be seen from Fig. 12A, RSD values in current responses were 2.60 % for UA, 0.74 % for G, and 0.79 % for A, respectively, which suggested the good repeatability of CMC-HNTs-fMWCNTs/GCE for their simultaneous detection. In addition, we also evaluated the reproducibility of ten CMC-HNTs-fMWCNTs/GCE by comparing with the difference of oxidation peak currents of UA, G and A, and their RSD values were 2.83 % for UA, 2.27 % for G, and 2.73 % for A, respectively

(Fig. 12B), implying the good reproducibility of CMC-HNTs-*f*MWCNTs/GCE. All results implied that the constructed sensor based on CMC-HNTs-*f*MWCNTs/GCE had a good precision.

3.11. Interference

There are a lot of interfering substances for the detection of UA, G and A in actual samples, such as inorganic ions, organic acids, amino acids, sugars. Hence, it is very important to study the effect of these substances on the electrochemical detection of UA, G and A, but most of substances did not have an obvious interference towards electrode responses owing to no electrochemical activities or oxidation peak potentials relatively far away from the UA, G and A. It could be seen from Fig. 13, glucose (Glc, Fig. 13D) and ascorbic acid (VC, Fig. 13F) did not produce any significant interference, and epinephrine (Ep, Fig. 13B) with higher concentrations produced remarkably interference towards UA, G and A, especially for UA, and cysteine (Cys, Fig. 13A) with higher concentrations began to cause a slightly interference for simultaneous detection of G and A, while tyrosine (Tyr, Fig. 13C) or tryptophan (Trp, Fig. 13E) with higher concentrations produced observably interference towards G and A, which set an obvious limit on its application for the analysis of practical samples.

3.12. Practical application

The practical application of the CMC-HNTs-*f*MWCNTs/GCE was evaluated by the voltammetric determination of UA, G and A in human serum sample and lettuce sample, and analytical results were listed in Table 2. The obtained results yielded good recoveries in human serum sample in ranges from 97.7 % to 108.8 % for UA, 94.0 % to 102.5 % for G, 96.7 % to 107.0 % for A, respectively, and in lettuce sample ranged from 96.9 % to 103.9 % for UA, 101.7 % to 109.0 % for G, 94.4 % to 108.3 %

for A, respectively. Their RSD values for the analysis of practical samples were less than 5%, and they were within the acceptable range. All results revealed that the proposed method was feasible and applicable for the simultaneous analysis of UA, G and A in biological samples, but the anti-interferent ability still needed to be improved to enhance the practicability of the proposed sensor.

4. Conclusions

In this work, a hybrid nanotubular composites consisting of both HNTs and *f*MWCNTs were well dispersed in water by CMC. CMC-HNTs-*f*MWCNTs/GCE displayed good electrode stability in water, large effective surface area, excellent adhesive property and adsorption capacity, and good electrocatalytic activity for the electrochemical oxidation of UA, G and A, which could realize simultaneously voltammetric detection in linear ranges of 1 – 130 μM with a low LOD of 0.5 μM for UA, 0.05 – 9 μM with a low LOD of 0.018 μM for G, and 0.3 to 25 μM with a low LOD of 0.19 μM for A, respectively. The fabricated sensor displayed wide linear range, low LOD, high sensitivity, and remarkable sensing stability, which could be used for the simultaneous analysis of UA, G and A in human serum and lettuce samples. However, the Tyr, Trp and Ep with high concentrations influence the selectivity and practicability of the proposed sensor. The further research work of highly selective detecting UA, G and A in daily diet and clinical medicine is also in progress.

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Caption

Fig.1 The pictures of HNTs (a), fMWCNTs (b), CMC-HNTs-fMWCNTs (c) dissolved in distilled water: the fresh prepared samples (A), and the samples after half a month of static culture (B).

Fig.2 SEM images of HNTs film (A, magnification, 20,000 $\times$, and magnification, 50,000 $\times$), fMWCNTs film (B, magnification, 50,000 $\times$), and CMC-HNTs-fMWCNTs film (C, magnification, 50,000 $\times$).

Fig.3 TEM images of CMC-HNTs-fMWCNTs at different magnifications.

Fig.4 FTIR spectra of HNTs (a), fMWCNTs (b), and CMC-HNTs-fMWCNTs (c).

Fig.5 (A) Nyquist plots of bare GCE (a), CMC/GCE (b), CMC-HNTs/GCE (c), CMC-fMWCNTs/GCE (d), CMC-HNTs-fMWCNTs/GCE (e) in 5 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ containing 0.1 M KCl. (B) CVs of different electrodes in 5 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ containing 0.1 M KCl.

Fig.6 (A) The electrode stability of CMC-HNTs-fMWCNTs/GCE in 5 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ containing 0.1 M KCl. Insert: CVs (100 circles) of CMC-HNTs-fMWCNTs/GCE in 5 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ containing 0.1 M KCl. (B) The electrode stability of CMC-HNTs-fMWCNTs/GCE in 5 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ containing 0.1 M KCl. Insert: CVs of CMC-HNTs-fMWCNTs/GCE in 5 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ containing 0.1 M KCl, and CVs was tested periodically every day for eight days.

Fig.7 The plots of $Q-t$ curves at bare GCE and CMC-HNTs-fMWCNTs/GCE in 5 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ containing 0.1 M KCl (A), and the plots of $Q-t^{1/2}$ curves at two different electrodes in 5 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ containing 0.1 M KCl (B). The plots of $Q-t$ curves at CMC-HNTs-fMWCNTs/GCE in 0.1 M KCl solution or in 5 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ containing 0.1 M KCl (C), and the plots of $Q-t^{1/2}$ curves at CMC-HNTs-fMWCNTs/GCE in 5 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ with the absence of background

charge (D). CVs of CMC-HNTs-*f*MWCNTs/GCE in 0.5 mM $[\text{Fe}(\text{CN})_6]^{3-}$ containing 0.5 M KCl (E), and the plots of I_{pa} and $v^{1/2}$ at CMC-HNTs-*f*MWCNTs/GCE in 0.5 mM $[\text{Fe}(\text{CN})_6]^{3-}$ containing 0.5 M KCl (F).

Fig.8 CVs of UA, G and A at bare GCE (a), CMC/GCE (b), CMC-HNTs/GCE (c), CMC-*f*MWCNTs/GCE (d), CMC-HNTs-*f*MWCNTs/GCE (e) in 0.1 M PBS (pH 7.0). Insert: CVs of UA, G and A at bare GCE (a), CMC/GCE (b), CMC-HNTs/GCE (c) in 0.1 M PBS (pH 7.0).

Fig.9 (A) CVs of UA, G and A at CMC-HNTs-*f*MWCNTs/GCE in 0.1 M PBS with scan rates from 20 to 400 mV s^{-1} (from a to k), and the linear relationship of UA, G and A between I_{pa} and v (B), between I_{pa} and $v^{1/2}$ (C), between E_{pa} and $\ln v$ (D).

Fig.10 (A) LSVs of UA, G and A at CMC-HNTs-*f*MWCNTs/GCE in 0.1 M PBS with different pH values. (B) Effects of pH on I_{pa} for the electro-oxidation of UA, G and A. (C) Effects of pH on E_{pa} for the electro-oxidation of UA, G and A. (D) Effects of pH on ΔE_{pa} for the electro-oxidation of G and UA or A and G.

Fig.11 Linear Sweep Voltammetry of UA, G and A at different concentration levels in 0.1 M PBS (pH 7.0) using CMC-HNTs-*f*MWCNTs/GCE, (A) 6 μM A, 7 μM G and UA with concentration ranges from 1 to 130 μM , (B) 3 μM A, 30 μM UA and G with concentration ranges of 0.05 to 9 μM , (C) 2.4 μM G, 40 μM UA and A with concentration ranges of 0.3 – 25 μM . D, E, F are the corresponding calibration curves of UA, G and A, respectively.

Fig.12 The repeatability of CMC-HNTs-*f*MWCNTs/GCE (A), and the reproducibility of CMC-HNTs-*f*MWCNTs/GCE (B) for voltammetric responses of UA, G and A.

Fig.13 The interference of different substances with different concentrations in simultaneous detection of UA, G and A, and effects of Cys (A), Ep (B), Tyr (C), Glc (D), Trp (E), and VC (F) on voltammetric responses of UA, G and A.

Table 1 Comparison of sensing performances based on different electrochemical methods and chemically modified material for the determination of UA, G and A in previous reports during recent three years

Material	Method	pH	Buffer solution	Analyte	Linear range (μM)	LOD (μM)	Practical application	Reference
Nano-Au/DNA/nanoAu/poly (SFR)/GCE	DPV	5.0	0.1 M PBS	UA	0.09 – 12	0.08	Human serum and urine	[11]
				G	0.009 – 5	0.0005		
				A	0.06 – 0.8	0.004		
Ag-PMel/GCE	SWV	4.5	0.1 M PBS	UA	0.1 – 50	0.1	Human urine	[12]
				G	0.1 – 50	0.008		
				A	0.1 – 60	0.008		
Dpa-melanin CNSs– grapheme/GCE	DPV	4.5	0.1 M HAc-NaAc	A	0.5 – 150	0.03	Herring sperm	[13]
				G	0.5 – 150	0.05		
				UA	3.6 – 249	0.59		
PIlox–GO/GCE	DPV	3.0	0.1 M PBS	G	3.3 – 103	0.48	—	[14]
				A	9.6 – 215	1.28		
				UA	50 – 943	—		
NiCoO ₂ -C modified/GCEs	DPV	12.0	0.1 M PBS + 0.02 M KOH	A	50 – 943	—	bovine serum, urine samples, tablets, Salmon sperm	[15]
				UA	50 – 943	—		
				G	0.2 – 4.0	0.08		
MWCNT/NiFe ₂ O ₄ /GCE	LSV	7.4	0.1 M PBS	A	0.1 – 3.0	0.012	Mouse	[16]
				G	0.1 – 6	—		
				UA	0.1 – 10	—		
TiO ₂ NPs-MgY/ZMCPE	DPV	7.2	0.1 M PBS	A	10 – 100	2.9	Calf thymus DNA	[17]
				G	10 – 100	4.8		
				UA	0.06 – 100	0.02		
PANI/MnO ₂	DPV	7.0	0.1 M PBS	A	0.04 – 100	0.01	Calf thymus DNA	[18]
				G	0.05 – 1.0	0.017		
				UA	0.025 – 1.0	0.008		
PPyox/GR/GCE	LSV	4.0	0.1 M PBS	A	1 – 130	0.50	Human serum and lettuce	This work
				G	0.05 – 9	0.018		
				A	0.3 – 25	0.19		

Table 2 The simultaneous determination of UA, G and A in real samples (n = 3)

Sample	Result of no added (μm)			Added (μm)			Mean ± SD (μm)			Recovery (%)		
	UA	G	A	UA	G	A	UA	G	A	UA	G	A
Serum	271.26	5.58	10.66	3.0	0.40	0.20	274.19 ± 0.037	5.99 ± 0.032	10.87 ± 0.061	97.7	102.5	107.0
				30	0.80	1.5	303.31 ± 0.056	6.39 ± 0.053	12.11 ± 0.11	108.8	101.3	96.7
				50	2.0	5.0	321.04 ± 0.052	7.46 ± 0.054	15.95 ± 0.0057	99.6	94.0	105.8
Lettuce	–	4.59	1.12	20	0.60	2.5	20.78 ± 0.03	5.20 ± 0.074	3.48 ± 0.15	103.9	101.7	94.4
				75	2.0	7.5	74.30 ± 0.31	6.77 ± 0.13	8.68 ± 0.12	99.1	109.0	100.8
				100	4.5	15	96.99 ± 0.162	9.21 ± 0.15	17.36 ± 0.10	96.9	102.7	108.3

Fig.1

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Fig.13

The important sources of UA, G and A and their simultaneous determination using CMC-HNTs-MWCNTs composites modified electrode