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A Silver Hexacyanoferrate-Graphene Modified Glassy Carbon Electrode in Electrochemical Sensing of Uric Acid and Dopamine

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A silver hexacyanoferrate-graphene hybrid modified glassy carbon electrode is put forward in sensing of uric acid (UA) and dopamine (DA) in this work. The structure and composition of the silver hexacyanoferrate was characterized by X-ray diffraction (XRD) and X-ray photoelectron spectroscopy (XPS). Catalysis of the modified material to the electrochemical reaction of UA and DA were verified by cyclic amperometry method. Experiments show that both UA and DA have excellent linear response on the modified electrode in acetic acid buffer solution by chronoamperometry measurements. The sensitivity is 0.102 μ A/ μ M in linear range of 2-80 μ M UA with a detection limit of 0.07 μ M and the sensitivity DA is 0.133 μ A/ μ M in the range of 0.4-28 μ M DA with a detection limit of 0.03 μ M. Furthermore, the developed sensor exhibited a high sensitivity, good reproducibility, stability for the detection of UA and DA.

Keywords: dopamine; uric acid; electrochemical sensor; graphene; silver hexacyanoferrate

1. INTRODUCTION

Sensing and detecting of biomolecules such as dopamine (DA) and uric acid (UA) are important tasks and fast developed in recent years. DA is a well known catecholamine neurotransmitter compound which affects the function of the central nervous and cardiovascular system [1]. Studies have been reported that many diseases such as Parkinson's disease, Alzheimer's disease, epilepsy are caused by parasecretion of DA [2]. UA is a nitrogenous end product of the purine nucleotide

catabolism and it widely exists in biological fluids, such as blood, urine and serum [3, 4]. Abnormal levels of UA may lead to several diseases like gout, Lesh-Nyhan syndrome, renal failure, hyperuricaemia, and physiological disorders [5, 6]. Therefore, the importance of detecting and monitoring the level of DA and UA can never be over emphasized for the vital roles those they play in the functions of human body. Many methods have been developed in detection of DA and UA such as electrochemiluminescence [7, 8], high performance liquid chromatography (HPLC) [9, 10], capillary electrophoresis [11], molecular imprinting polymer [12-14], complementary metal oxide semi conductor (CMOS) capacitive sensors [15, 16], photoelectrochemical sensors [17, 18] and electrochemical methods [19-21]. However, electrochemiluminescence methods are ease of being interfered by many quenchers such as Cu^{2+} , Pb^{2+} and some organic regents which widely existed in samples. HPLC, the strong and traditional separation method, can separate anything in theory provided that appropriate filling material and the mobile phase can be supplied, but it suffers from high cost for expensive columns and organic solvents. Molecular imprinting polymer requires rigorous preparation steps and CMOS capacitive methods need expensive reagents in analytes beneficiation before analysis. In comparison, electrochemical methods have advantages of simpleness, low costs and high sensitivity [22]. Furthermore, the differences of electrochemical redox characteristic between DA and UA permit sensitive and selective determination at modified electrodes [23]. Thus, the detection results lie on the properties of sensitive electrode.

Polynuclear hexacyanoferrates become promissing candidates in electrodes modification for their reversible mixed-valence species between the redox states of the hexacyanoferrates [24]. Many sensors based on polynuclear hexacyanoferrates have been developed in recent years, e.g., sulfite sensor [25], l-cysteine sensor [26], alcohol sensor [27], glucose sensor [28], etc. Among the hexacyanoferrates, silver hexacyanoferrate (AgHCF) would be especially attractive for the excellent electrocatalysis properties of Ag^+ and the counter cations storage capacity of the hexacyanoferrate compounds [29, 30]. However, the poor electron exchange ability of the polynuclear hexacyanoferrates represses the electrochemical current signal on the modified electrodes tremendously, which limits the usage of ferricyanides modification. In order to solve this problem, graphene was introduced to improve the electron and material exchange abilities. Graphene is a marvelous two-dimension carbon material which was first prepared and reported by Novoselov and Geim in 2004 [31]. The special lattice structure gives it excellent electron exchange ability and permits electron and holes coexist at high concentration [32], which may greatly benefit the electrochemical properties of the modified electrodes.

In previous works, we have developed copper hexacyanoferrate and nickel hexacyanoferrategraphene hybrid modified electrodes [33, 34] in photoelectrochemical sensing of hydroquinone and hydrogen peroxide, respectively. As a extension of using polynuclear ferriyanides in sensor construction, we report a silver hexacyanoferrate-graphene hybrid modified glassy carbon electrode for sensing of UA and DA in this work.

2. EXPERIMENTAL

2.1. Materials and chemicals

Silver nitride (AgNO₃), potassium ferricyanide (K₃Fe(CN)₆), acetic acid (HAc), sodium acetate (NaAc), dibasic (K₂HPO₄ 3H₂O) and monobasic (KH₂PO₄) potassium phosphate salts were purchased from Sino Pharma Company. Uric acid (UA) and dopamine (DA) hydrochloride were purchased from Sigma Aldrich Company. All the reagents were of analytical grade and all the solutions were prepared by double-distilled water. Phosphate buffer solution (PBS) was made of 0.1 M K₂HPO₄, 0.1 M KH₂PO₄ and adjusted to pH=7.0. Acetate buffer solution (ABS) was made of 0.1 M HAc and 0.1 M NaAc and pH was adjusted to 4.7.

2.2. Preparation of modified electrode

Graphene was prepared by the oxidation of exfoliated graphite using the modified Hummer's method from graphite powders as mentioned in our previous work [34] and was suspended in N, N-dimethylamide (DMF) in concentration of 1.0 mg·mL⁻¹. Silver hexacyanoferrate was prepared by AgNO₃ and K₃Fe(CN)₆. Specifically, 25 ml 3 mM AgNO₃ solution were dipped into 25 ml 1 mM K₃Fe(CN)₆ solution slowly in ultrasonic bath at 90 °C for 30 min to generate silver hexacyanoferrate (Ag₃Fe(CN)₆). The prepared Ag₃Fe(CN)₆ was isolated by centrifugation and washed with double-distilled water for 3 times to remove any impurities before transmitted into 10 ml ethanol suspension. Then 2 ml ethanol Ag₃Fe(CN)₆ suspension, 4 ml DMF graphene suspension and 4 ml ethanol were mixed and sonicated for 20 min to form Ag₃Fe(CN)₆-graphene suspension.

Glassy carbon electrode (GCE, 3 mm diameter) was polished with 0.3 μ m and 0.05 μ m alumina slurry and cleaned ultrasonically in double-distilled water and ethanol, respectively. 5 μ L Ag₃Fe(CN)₆-graphene suspension was dipped on the GCE and dried under an infrared lamp to prepare Ag₃Fe(CN)₆-graphene/GCE.

2.3. Characteristics of the Ag₃Fe(CN)₆-graphene/GCE

A Bruker D8 advance diffractometer with Cu K α radiation (λ =0.15405 nm) was used to characterize the structures of the Ag₃Fe(CN)₆ on the modified electrode. The X-ray diffraction (XRD) patterns were measured at 40 kV accelerating voltage with 40 mA emission current at 6°/min scanning speed. An X-ray photoelectron spectroscopy (XPS, PerkinElmer PHI-5600 system) was used to assay the composition of the Ag₃Fe(CN)₆ by using monochromatic Al K α radiation (1486.6 eV) operating at 250 W (12.5 kV). The specimens were analyzed using a spherical capacitance analyzer at an electron take-off angle of 45°. The analyzer energy resolution (the energy difference between two recorded data points) was 0.4 eV for survey scans and 0.1 eV for multiplex scans. The peak positions were calibrated against the carbon 1*s* peak at 284.67 eV. The morphology of the modified electrode was observed with scanning electron microscope (SEM, Hitachi SU8010).

The effects of $Ag_3Fe(CN)_6$ and graphene modification to the electrochemical response of DA and UA were studied by cyclic voltammetry (CV) measurements in PBS solution containing 40 μ M DA and 40 μ M UA with a LK2005 electrochemical workstation using a saturated calomel electrode (SCE) as reference electrode, a platinum plate as auxiliary electrode and the $Ag_3Fe(CN)_6$ -graphene/GCE as work electrode. The potential cycling is from -0.2 to +0.8 V (vs SCE) at a scan rate of 0.05 V s⁻¹. The electrolyte solution was degassed by bubbling with high purity nitrogen (99.999%) for at least 10 min before starting measurements. The electrochemical reaction controlling process of DA and UA on the modified electrode was discussed by CV measurements in different scan rate between 0.01 V s⁻¹ and 0.08 V s⁻¹.

2.4. Chronoamperometric measurement of DA and UA

Linear detection of DA and UA were performed by chronoamperometry method in pH 4.7 ABS. According to the CV characteristics of DA and UA, the potential of the $Ag_3Fe(CN)_6$ -graphene/GCE was set at 0.3 V (vs SCE) for detection of DA and at 0.5 V for detection of UA, respectively. In samples containing both DA and UA, two steps of above mentioned measurements have to be carried out, and both the concentration of DA and UA can be calculated according to current response and the sensitivity of the modified electrode to DA and UA. Reproducibility of the $Ag_3Fe(CN)_6$ -graphene/GCE was evaluated by measuring 10 μ M UA and 10 μ M DA samples for seven successive times on six different $Ag_3Fe(CN)_6$ -graphene/GCE prepared under the same conditions. The stability of the $Ag_3Fe(CN)_6$ -graphene/GCE was studied by comparing the current response of 10 μ M UA and 10 μ M DA before and after one month. In addition, practical application of the electrochemical sensor was determined by the standard addition method in human urine.

3. RESULTS AND DISCUSSION

3.1. Characteristic results of the samples



Figure 1. XRD patterns of the Ag₃Fe(CN)₆ used in electrode modification.

Fig. 1 shows the XRD patterns of the prepared $Ag_3Fe(CN)_6$ used in electrode modification. This XRD patterns match the ICSD #173553 card in the Inorganic Crystal Structure Database (ICSD), belonging to rhombohedral structure $Ag_3Fe(CN)_6$ which was first reported by Goodwin and Keenon on Journal of the American Chemical Society in 2008 [35] and was recorded in ICSD in February 2009. The results of XRD patterns prove the success preparation of $Ag_3Fe(CN)_6$.

The composition of the films was also confirmed by X-ray photoelectron spectroscopy (XPS). Fig. 2 (A) shows the XPS survey of Ag₃Fe(CN)₆-graphene/GCE. It is evident that C, Ag, N, O and Fe are present on the modified electrode. Ag 3*d* spectrum of the Ag₃Fe(CN)₆ on the electrode is shown in Fig. 2(B). The binding energies at 368.2 eV and 374.2 eV were assigned to Ag $3d_{5/2}$ and Ag $3d_{3/2}$, respectively [36].



Figure 2. XPS for survey scans (A) of Ag₃Fe(CN)₆-graphene/GCE and high resolution scans of Ag 3d (B), Fe 2p (C), C 1s (D), N 1s (E) and O 1s (F) from the same sample.

The Fe 2*p* XPS high-resolution spectra of the Ag₃Fe(CN)₆-graphene film deposited on GCE are presented in Fig. 2 (C). The peaks of Fe $2p_{3/2}$ and Fe $2p_{1/2}$ at 710.6 eV and 721.4 eV are attributed to Fe (III) of Ag₃Fe(CN)₆ [37]. The carbon peak at 284.6 eV in Fig. 2(D) comes from ions of CN⁻ and graphene in Ag₃Fe(CN)₆-graphene. Fig. 2 (E) shows the high resolution scans of the N 1*s* peak. The observed binding energy of N 1*s* electron at 398.7 eV is in good agreement with the formation of a carbon-nitrogen bond from the cyano-coordinated Fe(III) [38]. Moreover, the O 1s peak at 532.3 eV in Fig. 2F indicates the presence of absorbed oxygen on the surface of modified electrode [39].





Fig. 3 shows the morphologies of the $Ag_3Fe(CN)_6$ (A) and $Ag_3Fe(CN)_6$ -graphene hybrid (B) on GCE surfaces. It is clear that the $Ag_3Fe(CN)_6$ is in nanoscale and uniformly scattered state which can provide large specific surface area. SEM of the $Ag_3Fe(CN)_6$ -graphene complex as shown in Fig. 3(B) indicates that the nanoscale $Ag_3Fe(CN)_6$ attached intensively on the micronscale graphene, which is benefit to the electron and material exchange capacity.

3.2. CV characteristics of the $Ag_3Fe(CN)_6$ -graphene/GCE



Figure 4. Cyclic voltammograms of different modified electrodes (a) bare GCE, (b) Ag₃Fe(CN)₆/GCE, (c) Graphene/GCE and (d) Ag₃Fe(CN)₆-graphene/GCE in pH 7.0 PBS at a scan rate of 50 mV.s⁻¹.

Fig. 4 shows the effects of $Ag_3Fe(CN)_6$ -graphene modification to the electrochemical activity of DA and UA on the modified electrode (curve d) in pH 7.0 PBS. In comparison, bare GCE, $Ag_3Fe(CN)_6/GCE$ and graphene/GCE were also provided in curve a, b and c, respectively. It is clear that DA and UA have very low response on the bare GCE as shown in curve a. With the modification of $Ag_3Fe(CN)_6$, the oxidation peak of UA increases 3 times relative to that on bare GCE, indicating good catalysis to UA electrochemical reaction, while the DA oxdation peak on the $Ag_3Fe(CN)_6/GCE$ increases to double of that on bare GCE. In the case of graphene modification in curve c, it depicts better catalysis to DA than UA. With the recombination of $Ag_3Fe(CN)_6$ and graphene as shown in curve d, the modified electrode demonstrates better catalysis both to DA and UA than any of the onecomponent material modified GCE.



Figure 5. CVs of 20, 40, 60, 80 100 and 140 μ M UA reaction on the Ag₃Fe(CN)₆-graphene/GCE (A) in pH 7.0 PBS, and (B) in pH 4.7 ABS. (C) Linear fitting between the concentration of UA and the peak currents in pH 7.0 PBS, and (D) in pH 4.7 ABS.

Fig. 5A and Fig. 5B represents the CVs of different concentrations of UA reaction on the Ag₃Fe(CN)₆-graphene/GCE in pH 7.0 PBS and in pH 4.7 ABS, respectively. The linear fitting results between the concentration of UA and the oxidation peak current on the CV curves are showed in Fig. 5C and Fig. 5D, respectively. As can be seen, the oxidation peak current is in good linear relationship to the concentration of UA in pH 4.7 ABS while is not in pH 7.0 PBS. So, the quantitative analysis was carried out in pH 4.7 ABS afterwards.

3.3. Effects of scan rate on the CV curves of UA and DA

The effects of scan rate on the CV characteristics are often used to study the reaction controlling process of an electrochemical reaction in pH 4.7 ABS. Fig. 6 A depicts the CVs of UA electrochemical reaction on the modified electrode and Fig. 6 B shows the linear fitting result between the natural logarithm of the oxidation peak current of UA and the natural logarithm of the scan rate. The slope can be obtained to be 0.7661 as shown in Fig. 6B, indicating a combination controlling process of diffusion and absorption [40]. Similar results can be observed on the CVs of DA electrochemical reaction at different scan rate as shown in Fig. 6C and Fig. 6D. The fitting results between the natural logarithm of the oxidation peak current of DA and the natural logarithm of the scan rate show that the electrochemical oxidation process of DA is still a combination controlling process of diffusion and absorption, which is deviated slightly to diffusion process.



Figure 6. Effects of scan rates on the CV characteristics of UA and DA electrochemical reaction on the $Ag_3Fe(CN)_6$ -graphene/GCE in pH 4.7 ABS (A) CVs of UA reaction on the $Ag_3Fe(CN)_6$ -graphene/GCE at different scan rates; (B) the natural logarithm relationship between the oxidation peak current of UA and the scan rate; (C) CVs of DA reaction on the $Ag_3Fe(CN)_6$ -graphene/GCE at different scan rates; (D) the natural logarithm relationship between the oxidation peak current of DA and the scan rate.

3.4. Determination of UA and DA on the $Ag_3Fe(CN)_6$ -graphene/GCE

In order to determine the appropriate potential at which the chronoamperometric measurement could be performed for detection of UA and DA, a cyclic voltammogram of $80 \mu M$ DA and $80 \mu M$

UA in 0.1 M pH 4.7 ABS on $Ag_3Fe(CN)_6$ -graphene/GCE is illustrated in Fig. 7. The DA and UA gave two oxidation peaks at 0.32 V and 0.48 V, respectively. The peak-to-peak separation of DA and UA was 160 mV, which was large enough to determine DA and UA simultaneously without any interference. In other word, controlling the potential at 0.3 V, the DA could be oxidized enough and was not affected by the oxidation of UA.



Figure 7. CV curve of 80 μM UA and 80 μM DA reaction on Ag₃Fe(CN)₆-graphene/GCE in 0.1 M pH 4.7 ABS.

The oxidation peak of UA appears at 0.48V on the cyclic voltammogram no cathodic peak was observed on the reverse scan within the investigated potential range, indicating totally irreversible electron-transfer kinetics for the UA oxidation. So, 0.5 V potential could ensure the UA oxidation at enough speed for the chronoamperometric measurement. As above mentioned, the work electrode potential was set at 0.3 V and 0.5 V for detection of DA and UA, respectively, in the chronoamperometric measurements.

Fig. 8A shows the typical the chronoamperometric currents of UA obtained at the Ag₃Fe(CN)₆graphene/GCE at 0.5 V in 0.1 M pH 4.7 ABS. The current immediately changed after the addition of UA and reached another steady-state current within several seconds. The response current increased stepwise for successive additions of different concentrations, 2 μ M (4 times), 4 μ M (4 times), 8 μ M (2 times), 12 μ M (2 times) and 16 μ M UA. Fig. 8B shows the linear fitting result between the concentration of UA and the response current, showing an excellent linear relationship (R²=0.999) with the sensitivity of 0.102 μ A/ μ M and the detection limit can be estimated to be 0.07 μ M (S/N=3) [41]. Similarly, Fig. 8C shows the chronoamperometric currents of DA in 0.1 M pH 4.7 ABS at 0.3 V. The successive additions of DA concentration were 0.4 μ M (4 times), 0.8 μ M (3 times), 1.2 μ M (3 times), 2.4 μ M (2 times) and 4.8 μ M (2 times). The corresponding linear relationship between the current response and the concentration of DA is indicated in Fig. 8D. A sensitivity of DA detection was 0.133 μ A/ μ M with a detection limit of 0.07 μ M (S/N=3). Comparison with the studies on the linear range and limit of detection for UA and DA is shown in Table 1.



Figure 8. (A) Chronoamperograms of successive addition of UA 2 μ M (4 times), 4 μ M (4 times), 8 μ M (2 times) and 16 μ M into 0.1 M pH 4.7 ABS at 0.5 V; (B) The linear relationship between the current response and the concentration of UA; (C) Chronoamperometric curve of successive addition of DA 0.4 μ M (4 times), 0.8 μ M (3 times), 1.2 μ M (3 times), 2.4 μ M (2 times) and 4.8 μ M (2 times) into 0.1 M pH 4.7 ABS at 0.3 V; (D) The linear relationship between the current response and the concentration of DA.

Table 1. Comparison results of the linear range and limit of detection for UA and DA

Modified electrode	Technique	Linear range	LOD	Linear range	LOD	Dof
		UA(µM)	UA(µM)	DA(µM)	DA(µM)	Ker.
PABS-MWNT/GCE ^a	DPV	6-55	0.44	9-48	0.21	42
Au-NPs/Au electrode ^b	DPV	0.1-850	0.023			43
MWCNT/GCE ^c	Amperometry	5-120	1.5	5-120	4.5	44
MB-MWNT/GCE ^d	DPV	2-200	1.0	0.4-10	0.2	45
a-CD/MWCNT/CPE ^e	SWV	5-165	0.097			46
HBNBH-TiO ₂ /CPE ^f	SWV	8-1400	0.84			47
Ag-PMel/GCE ^g	SWV	0.1-50	0.1	0.1-50	0.01	48
poly(ABB) /GCE ^h	DPV	0.1-1700	0.02			49
ECACE ⁱ	DPV	0.1-15	0.02			50
Cu–PEDOT/Pt ^j	DPV	6-200	6.0			51
(PDAC/UOx)10/PB/ITOk	Amperometry	0.1-0.6	0.15			52
Chitosan/CPB/GCE ¹	DPV	2-600	0.5			53

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Nafion/MWNT/GCE ^m	DPV	0.6-80	0.2			54
PCA/GCE ⁿ	Voltammetry	1-40	0.4			55
GNME °	DPV	0.2-1200	90			56
Ag ₃ Fe(CN) ₆ -GR/GCE	Amperometry	2-80	0.07	0.4-28	0.03	This
						work

a: poly (orthanilic acid)-multiwalled carbon nanotubes/glassy carbon electrode.

b: gold nanoparticles/gold electrode.

c: multi-walled carbon nanotubes /glassy carbon electrode.

d: methylene blue-multiwalled nanotubes/glassy carbon electrode.

e: α-cyclodextrin/multi-walled carbon nanotubes/carbon paste electrode.

f: 2,2'-[1,7-hepthandiylbis (nitriloethylidyne)]-bis-hydroquinone-TiO₂ /carbon paste electrode.

g:Ag ions and melamine monomer/glassy carbon electrode.

h: poly(Adizol Black B)-modified glassy carbon electrode.

i: electrochemically activated glassy carbon electrode.

j: copper crystal-modified poly(3,4-ethylenedioxythiophene)-coated platinum electrode.

k: poly(diallyldimethylammoniumchloride)/uricase) 10-bilayer /Prussian Blue/indium-tin oxide.

1: chitosan incorporating cetylpyridine bromide modified glassy carbon electrode.

m: Nafion/multi-wall carbon nanotubes composite film-modified glassy carbon electrode.

n: poly(caffeic acid)-modified glassy carbon electrode.

o: self-assembled gold nanoparticle films/Gold electrode.

3.5. Interference studies



Figure 9. (A) Chronoamperograms of successive addition of 10 μM DA, 20 μM DA, 10 μM UA, 10 μM H₂O₂, 100 μM AA, 100 μM L-Cysteine and 10 μM DA into 0.1 M pH 4.7 ABS at 0.3 V. (B) chronoamperometric currents of successive addition of 0.4 μM UA, 10 μM UA, 10 μM DA, 10 μM H₂O₂, 100 μM AA, 100 μM L-cysteine and 20 μM UA into 0.1 M pH 4.7 ABS at 0.5 V.

In order to evaluate the suitability of this analytical procedure in more detail, a study of potential interferences was carried by observing the effect of analogues successive addition at a comparative or higher concentration. Fig. 9 A shows chronoamperometric current responses of the Ag₃Fe(CN)₆-graphene/GCE by successive addition of 10 μ M DA, 20 μ M DA, 10 μ M UA, 10 μ M H₂O₂, 100 μ M AA, 100 μ M L-cysteine and 10 μ M DA into 0.1 M pH 4.7 ABS at 0.3 V. Results clearly indicated that only 10-fold AA produced a few interference to the DA oxidation current. Other

analogue, such as, UA, H_2O_2 , 10-fold L-cysteine shows no obvious influence on the current response of the DA. But the results as shown in Fig. 9 B indicated that a comparative DA, H_2O_2 , 10-fold AA exhibited a few interference to the UA oxidation current. Only 10-fold L-cysteine showed no obvious influence on the current response of the UA.

3.6. Reproducibility, stability and practical application

The reproducibility and stability of the Ag₃Fe(CN)₆-graphene modified electrode have been studied. The relative standard deviation (RSD) of the sensor response to 10 μ M UA and 10 μ M DA is 2.7% and 2.5%, respectively, for seven successive measurements in pH 4.7 ABS. The results indicated that the prepared Ag₃Fe(CN)₆-graphene/GCE showed an acceptable repeatability. The fabrication reproducibility was examined at six Ag₃Fe(CN)₆-graphene/GCEs prepared under the same conditions, and the RSD of the sensor response to 10 μ M UA and 10 μ M DA is 5.9% and 5.3%, respectively. The stability of Ag₃Fe(CN)₆-graphene/GCE was also evaluated, and 91.6 and 92.4% of its initial current response to the oxidation of 10 μ M UA and 10 μ M DA, respectively, could be obtained after one month. These results suggest that the modified electrode has good reproducibility and long-term stability.

For preliminary evaluation of the proposed electrochemical sensor, the concentrations of UA and DA were determined by the standard addition method in human urine without pretreatment before measurement. The analytical results were shown in Table 2, indicating that both recovery and RSD of UA and DA were acceptable and that the proposed method could be efficiently employed for determination of UA and DA in human urine.

Added (µM)	Found (µM)	Recovery (%)	RSD [*] (%)	
UA DA	UA DA	UA DA	UA DA	
2 0.5	2.08 0.48	104.0 96.0	2.32 3.64	
4 1	3.96 1.07	99.0 107.0	2.14 2.47	
6 2	6.15 1.93	102.5 96.5	1.73 1.26	

Table 2 Determinations of UA and DA in human urine samples.

* RSD value reported is for n=3. The urine sample was diluted 100 times with ABS (pH 4.7) before measurement.

4. CONCLUSION

A silver hexacyanoferrate-graphene hybrid was used to modify on glassy carbon electrode to prepare an electrochemical sensor for sensing of DA and UA. Study shows that pH 4.7 acetate buffer solution is more applicative in quantitative analysis of DA and UA than pH 7 phosphor buffer solution. The results of scan rate on the CVs show that both DA and UA electrochemical reactions on the Ag₃Fe(CN)₆-graphene/GCE in acetate buffer solution are combination controlling process of diffusion and absorption. The modified electrode can be used in detection of UA and DA by chronoamperometric measurement at 0.5 V and 0.3 V in pH 4.7 acetate buffer solution, respectively.

The sensitivity to UA is 0.102 μ A/ μ M with a detection limit of 0.07 μ M (S/N=3). The sensitivity to DA is 0.133 μ A/ μ M with a detection limit of 0.03 μ M (S/N=3). The resulted sensor exhibited fast amperometric response, low detection limit and considerable linear range to UA and DA detection. In addition, the sensor has good reproducibility long-term stability.

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