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A novel and simple strategy for simultaneous determination of dopamine, uric acid and ascorbic acid based on the stacked graphene platelet nanofibers/ionic liquids/chitosan modified electrode

Xiuli Niu, Wu Yang*, Hao Guo, Jie Ren, Fusheng Yang, Jinzhang Gao

College of Chemistry and Chemical Engineering, Key Lab of Bioelectrochemistry and Environmental Analysis of Gansu Province, Northwest Normal University, Lanzhou 730070, PR China

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ABSTRACT

A promising electrochemical sensor for simultaneous determination of dopamine (DA), uric acid (UA) and ascorbic acid (AA) was fabricated based on the stacked graphene platelet nanofibers (SGNF)/ionic liquid (IL)/chitosan (CS) modified electrode. The SGNF/IL/CS modified electrode possessed excellent electrocatalytic activity towards the oxidation of DA, UA and AA with obvious reduction of over-potential and increased peak current, and the separations of oxidation peak potentials of DA-UA, DA-AA, and UA-AA were of 151, 213 and 364 mV, respectively. Under the optimum conditions, the linear range for the detection of DA, UA and AA were 0.05–240, 0.12–260, and 30–350 μM with the lowest detection limits of 0.05, 0.10 and 14.8 μM for DA, UA and AA, respectively. In addition, the electrochemical sensor showed high sensitivity, excellent selectivity, reproducibility and long-term stability.

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1. Introduction

Dopamine, uric acid and ascorbic acid play important roles in the functioning of the human metabolism, central nervous and renal systems. Abnormal concentration levels of DA, UA and AA can cause health problems such as Parkinson's disease, leukemia, hyperuricaemia and mental illness [1,2]. However, these three compounds usually coexist in the extra cellular fluid of the central nervous system and serum, separate determination of three compounds is a significant problem due to overlapped signals. Though a series of methods have been used for determination of these three compounds, some factors, such as expensive, complicated and time-consuming restricted their application. Recently, electrochemical methods have received considerable attention due to their unique properties such as simple and convenient, fast response, high sensitivity, stability and selectivity. However, the oxidation potentials of DA, UA and AA are too close to be separately determined by the use of bare electrode, it is important to develop a highly sensitivity and selectivity method to solve this problem and achieve determination of DA, UA and AA simultaneously.

Stacked graphene platelet nanofibers are simply stacked graphene nanosheets with an orientation perpendicular to the

long axes of the fiber. Thus, SGNFs contain an unprecedented number of open graphene edges on their surfaces (while having a graphene "basal plane" surface only on the ends of the fibers) [3–6]. SGNF not only possess the excellent properties of graphene, but also possess the unique characteristic, which is a promising candidate material for electrochemical sensors. Ionic liquids (IL) serve as a new kind of green solvents, have a variety of unique physico-chemical properties such as tunable chemical structures, wide electrochemical windows, high ionic conductivity, high thermal stability, good solubility and biocompatibility [7–10]. Therefore, recently they have been applied in the fields of analytical chemistry and electrochemical sensors.

Here, we describe a novel and simple strategy for simultaneous determination of dopamine, uric acid and ascorbic acid based on the stacked graphene platelet nanofibers/ionic liquids/chitosan modified electrode. The modified electrode exhibits excellent sensitivity, selectivity, reproducibility and long-term stability for the determination of DA, UA and AA with lower detection limits.

2. Experimental

2.1. Reagents

Stacked graphene platelet nanofibers were purchased from STREM Chemicals (Newburyport, MA, USA). [BMIM][BF₄] was

* Corresponding author. Tel.: +86 931 7971216.

E-mail address: xbsfda123@126.com (W. Yang).

acquired from Lanzhou Institute of Chemical Physics (Lanzhou, China). Dopamine, uric acid and ascorbic acid were obtained from Sigma-Aldrich (USA). Phosphate buffer solution (PBS) was prepared by mixing the stock solution of 0.1 M NaH_2PO_4 and 0.1 M Na_2HPO_4 and adjusting the pH with 0.1 M H_3PO_4 or 0.1 M NaOH. Other reagents were of analytical grade and were used as received. Double distilled water and 0.1 M PBS pH 6.0 (as supporting electrolyte) were used throughout the experimental work.

2.2. Apparatus

All electrochemical experiments were performed on a CHI-832 electrochemical workstation (CH Instruments, Shanghai Chenhua Instrument Corporation, China). A three-electrode cell was employed with a glassy carbon electrode (GCE, 3 mm diameter) or a modified GCE as a working electrode, a saturated calomel electrode (SCE) and platinum sheet as the reference and auxiliary electrode, respectively. Scanning electron microscopy (SEM) images were determined with a JSM-6701 F field emission scanning electron microscope (Japanese Electron Optics Company).

2.3. Preparation of SGNF/IL/CS modified electrode

Before the modification, GCE was carefully polished to a mirror with 1.0, 0.3 and 0.05 μm alumina slurry on a polishing cloth, rinsed thoroughly with doubly distilled water between each polishing step, and sequentially sonicated in doubly distilled water for 5 min, and dried at room temperature. For the preparation of SGNF/IL/CS modified electrode, 10 mg SGNF was dispersed in 10 mL 0.01% CS solution and then mixed with 50 μL of IL and sonicated for 1 h to form a homogenous mixture. Then, 5 μL of the mixture was dropped on the pretreated GCE with a microsyringe, and then dried for 24 h in air before use.

3. Results and discussion

3.1. Characterization of SGNF/IL/CS composite film

Fig. 1 shows scanning electron microscopic images of SGNF/IL/CS composite film. As shown in Fig. 1, the composite film displayed a well-defined and interconnected 3D porous network and the pore walls was composed of very thin layers of stacked graphene platelet nanofibers. The nanosized composite film

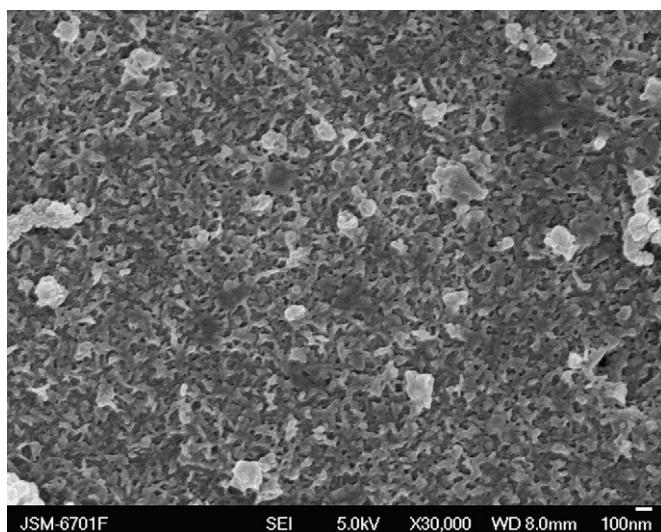


Fig. 1. SEM image of SGNF/ionic liquid/chitosan composite film.

contained an unprecedented number of open graphene edges on their surfaces, which may facilitate the improvement of the electrochemical behaviors. Once deposited on the GCE surface, a well-packed, highly ordered structure film of SGNF/IL/CS was formed, which provides a favorable environment for electrochemical tests of biomolecules.

3.2. Electrocatalytic oxidation of DA, AA and UA

Fig. 2 shows the CVs of the bare GCE and SGNF/IL/CS modified electrode towards single electrocatalytic behavior of DA, UA and AA. At the bare GCE (Fig. 2A), DA showed a pair of small redox peaks with the anodic and cathodic peak potential at 125 mV and 81 mV, respectively (curve a). The peak-to-peak separation (ΔE_p) was calculated as 44 mV and the anodic and cathodic peak current ratio (I_{pa}/I_{pc}) was about 1.12. UA showed irreversibly electrochemical behavior with the anodic peak potential at 251 mV (curve b) while a broad oxidation peak at 176 mV was observed for AA (curve c). The separation of the anodic peak potentials for these three species was not large enough to obtain good selectivity at the bare GCE. However, at the SGNF/IL/CS modified electrode (Fig. 2B), a pair of well-defined peaks with remarkably increased peak current was observed for DA (curve a). The peak potential separation of DA was decreased to 21 mV and the I_{pa}/I_{pc} was about 1.07, which suggested that SGNF/IL/CS/GCE showed good electrocatalytic ability to the electrochemical oxidation of DA. As shown in Fig. 2B (curve b), a quasi-reversible electrochemical behavior for UA appeared with the anodic and cathodic peak potential at 241 mV and 204 mV, respectively. The ΔE_p was found to be 37 mV and the anodic peak current signal was 7.2-fold increments when comparing the bare GCE. For AA, a well-defined anodic peak was observed and the peak potential negatively shifted to -113 mV (curve c). The decrease of overpotential and the increase of anodic peak current demonstrated that the SGNF/IL/CS can facilitate the oxidation of AA greatly. The CVs' changes observed indicated that the modified electrode had a good catalytic activity for the oxidation of DA, UA and AA. The SGNF/IL/CS/GCE can clearly distinguish DA, UA and AA in a large concentration range.

In order to understand the electrocatalytic activity of the SGNF, the electrochemical behavior of mixture of DA, UA and AA at the bare GCE and SGNF/IL/CS/GCE was further studied by CV and DPV, respectively. As shown in Fig. 2C, at the bare GCE (curve a), only two weak and overlapped anodic peaks were obtained and the peak potentials of DA, UA and AA were indistinguishable, indicating the poor selectivity and sensitivity. In contrast, three well-defined peaks with remarkably increased peak current can be observed at the SGNF/IL/CS/GCE (curve b), which indicated that the modified electrode had a good catalytic activity for the oxidation of DA, UA and AA. Fig. 2D shows the DPV responses of DA, UA and AA at the bare GCE and modified electrode. It can be seen from Fig. 2D, two weak peaks were observed with poor selectivity and sensitivity at the bare GCE (curve a). While at the SGNF/IL/CS/GCE (curve b), the three compounds were oxidized with well-defined and distinguishable sharp peak potentials at approximately -160 mV, 53 mV and 204 mV for AA, DA and UA, and the separations of oxidation peak potentials of DA-AA, DA-UA, and UA-AA were of 213, 151 and 364 mV, respectively. The superior electrochemical performance of the modified electrode can be attributed to the fact that the SGNF have an exceptionally large number of accessible graphene-sheet edges on the surface of the nanofibers and improve electron-transfer rates for oxidation of DA, UA and AA. The enlarged separation of the anodic peak potential, together with the increased sensitivity and selectivity, makes it possible to effectively determine DA, UA and AA simultaneously without any interference.

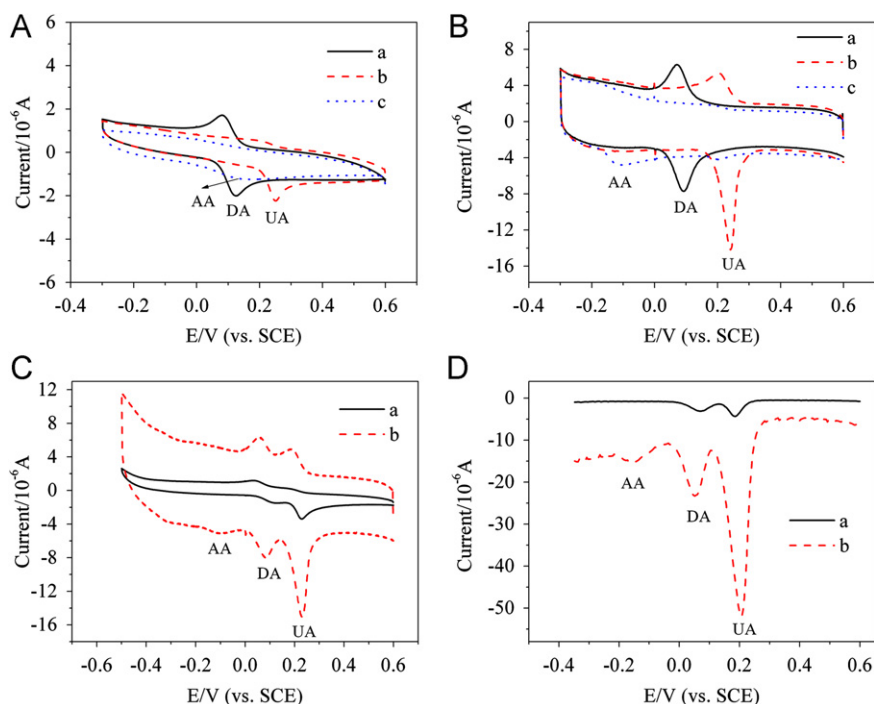


Fig. 2. The CVs of DA, UA and AA at the bare GCE(A) and at SGNF/IL/CS modified electrode (B), respectively. The CVs (C) and DPVs (D) responses of DA, UA and AA in mixture at the bare GCE (curve a) and modified electrode (curve b). Scan rate: 50 mV s^{-1} .

3.3. Effects of solution pH, accumulation potential, accumulation time and scan rate on the electrochemical response of DA, UA and AA

The electrochemical redox behavior of DA, UA and AA at SGNF/IL/CS modified electrode in 0.1 M PBS over the range from pH 4.0 to 8.0 was investigated by DPV. As shown in Fig. 3A, with pH value of the solution increasing, the peak currents of DA, UA and AA increase gradually and then decrease, the bigger peak currents were obtained at pH 6.0. Therefore, pH 6.0 was chosen for further studied. In addition, all the anodic peak potentials for the oxidation of DA, UA and AA shifted linearly to negative direction with the increasing pH (Fig. 3B), which indicates that protons have taken part in their electrode processes.

The influence of accumulation potential and accumulation time on the peak currents of DA, UA and AA was also investigated by DPV. Experimental results showed that peak currents increased gradually and then decreased slightly as the potentials shifted negatively from 0.1 to -0.30 V . Hence, the accumulation potential of -0.2 V was considered as favorable for obtaining the maximal peak currents for DA, UA and AA. In addition, the influence of accumulation time played an important role for pre-concentration. The obtained results showed that the peak current of DA, UA and AA rapidly increased and the maximum peak currents were obtained at 60 s, 90 s and 60 s for DA, UA and AA, respectively. The peak currents of DA, UA and AA were about 1.9, 1.5, and 1.6 times of that without accumulation, respectively. Therefore, an accumulation time of 60 s was chosen as optimum for the mixture of DA, UA and AA to obtain stable peak with higher sensitivity and shorter analysis time.

In order to further investigate the electrochemical behavior of DA, UA and AA at the SGNF/IL/CS/GCE, the effect of scan rate on the oxidative peak potential was studied. Fig. 4 shows the CVs of the DA, UA and AA at the modified electrode at different scan rates. The peak currents of all three compounds linearly increased with the scan rate over the range of $10\text{--}90 \text{ mV s}^{-1}$ and the corresponding plot of peak currents vs. scan rates were shown

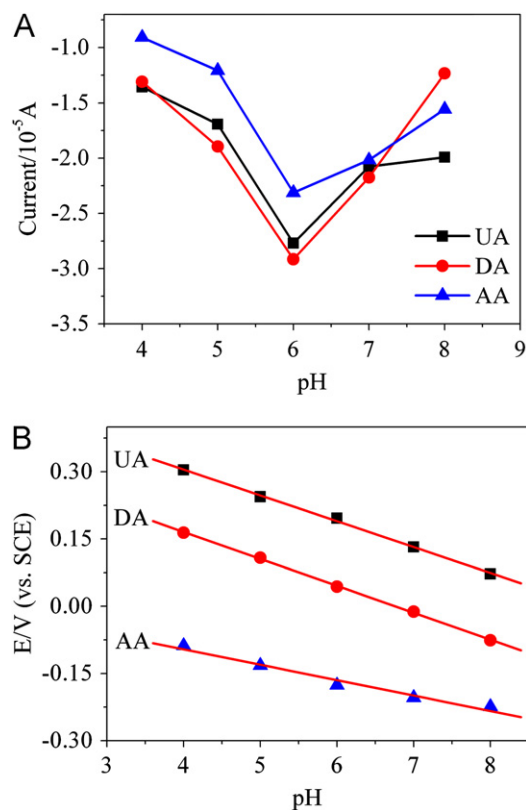


Fig. 3. Effects of pH on the oxidation peak current (A) and potential (B) of UA, DA and AA.

in the inset plot of Fig. 4. This linearity suggests that electrochemical reactions of DA, UA and AA at the surface of SGNF/IL/CS modified electrode are an adsorption controlled process.

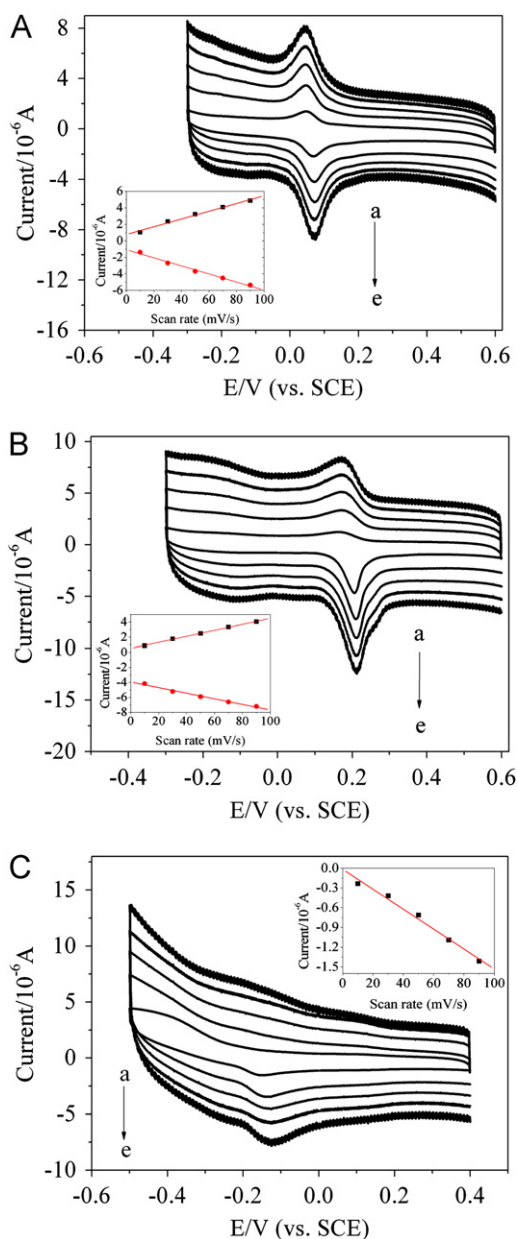


Fig. 4. The cyclic voltammograms of UA (A), DA (B) and AA (C) on SGNF/IL/CS modified electrode at different scan rates in 0.1 M PBS (pH 6.0). Scan rates are 10, 30, 50, 70, 90 mV s^{-1} (from a to e curve). Inset: plots of peak currents vs. the scan rates.

3.4. Simultaneous determination of DA, AA and UA

DPV was used for simultaneous determination of DA, UA and AA because of its higher current sensitivity and better resolution than CV. Under the optimized experimental conditions, a series of DA, UA and AA solutions with different concentration were measured. As shown in Fig. 5, the peak currents of DA, UA and AA increased with increasing their concentration but had no significant influence on the peak currents of two other compounds. As can be seen from Fig. 5, the peak currents of DA, UA and AA were linearly proportional to their concentrations in the concentration ranges of 0.05–240, 0.12–260, and 30–350 μM , respectively. The regression equation was $i (\mu\text{A}) = -13.6119 - 0.1301c (\mu\text{M})$ ($R^2 = 0.9972$), $i (\mu\text{A}) = -4.8113 - 0.1209c (\mu\text{M})$ ($R^2 = 0.9978$), $i (\mu\text{A}) = -6.8191 - 0.0751c (\mu\text{M})$ ($R^2 = 0.9953$), the lowest detection limit was found to be 0.05, 0.10 and 14.8 μM for DA, UA and AA, respectively ($S/N = 3$).

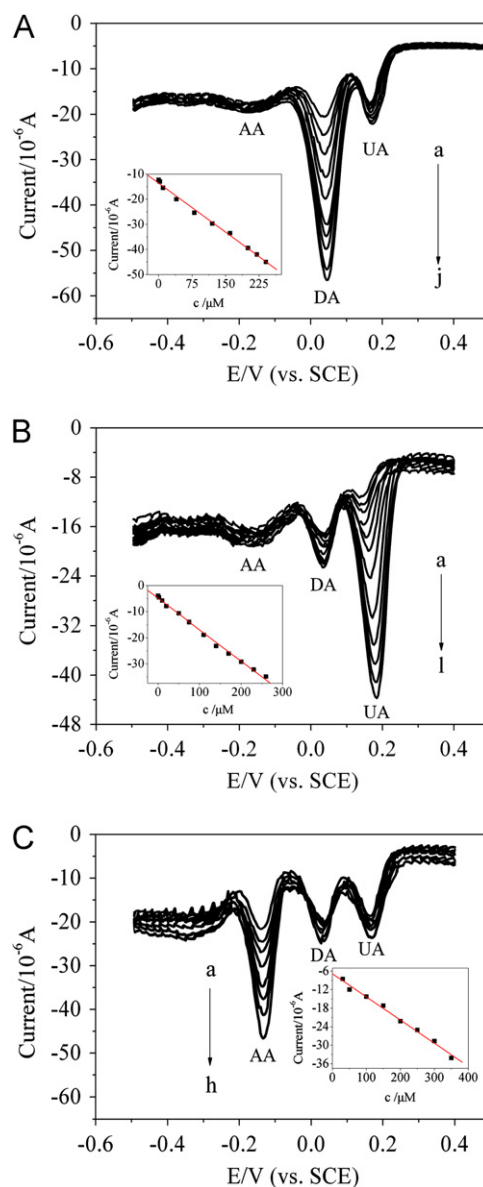


Fig. 5. DPVs at SGNF/IL/CS modified electrode in 0.1 M PBS (pH 6.0) (A) containing 80 μM AA, 50 μM UA and different concentrations of DA (from a to j): 0.05, 3, 10, 40, 80, 120, 160, 200, 220, 240 μM . Inset: calibration plots of the anodic peak current versus different concentration of DA; (B) containing 60 μM DA, 60 μM AA and different concentrations of UA (from a to l): 0.12, 2.5, 10, 20, 50, 75, 110, 140, 170, 200, 230, 260 μM . Inset: calibration plots of the anodic peak current versus different concentration of UA; (C) containing 60 μM DA, 50 μM UA and different concentrations of AA (from a to g): 30, 50, 100, 150, 200, 250, 300, 350 μM . Inset: calibration plots of the anodic peak current versus different concentration of AA; Experimental conditions were pulse amplitude = 50 mV, pulse width = 50 ms and pre-concentration step = 60 s at -0.2 V.

The analytical performances of the proposed biosensor were compared with those of DA, UA and AA sensors based other matrices. The results are shown in Table 1. It can be seen that the SGNF/IL/CS modified electrode exhibited relatively low detection limit, high sensitivity and wide linear range.

3.5. Reproducibility, repeatability and stability of the modified electrode

The reproducibility of SGNF/IL/CS/GCE was estimated by measurement of the DPV response to 60 μM DA, 75 μM UA and 100 μM AA with the same modified electrode. The relative

Table 1
Comparison of the proposed DA, UA and AA biosensor with other biosensors based on differential matrices.

Electrode	Linear range (μM)			Detection limit (μM)			Reference
	DA	UA	AA	DA	UA	AA	
SGNF/IL/CS/GCE	0.05–240	0.12–260	30–350	0.04	0.10	14.8	This work
Pd/CNF-CPE	0.5–160	2.0–200	50–4000	0.20	0.70	15.0	[11]
PGE-DA	1.0–20	2.5–30	25–500	0.11	1.40	13.0	[12]
MCNT/CCE	0.5–100	0.55–90	15–800	0.31	0.42	7.71	[13]
CNF-CPE	0.04–5.6	0.8–16.8	2.0–64	0.04	0.20	2.00	[1]

CNF, carbon nanofibers; PGE-DA, pyrolytic graphite electrode-dopamine; CCE, carbon-ceramic electrode.

Table 2
Determination of DA, UA and AA in human urine samples ($n=3$).

Samples	Detected (μM)			Added (μM)			Found (μM)			Recovery (%)			RSD (%)		
	UA	DA	AA	UA	DA	AA	UA	DA	AA	UA	DA	AA	UA	DA	AA
Urine 1	14.3	–	–	10.0	10.0	40.0	24.9	10.1	39.8	102.5	101.0	99.5	3.5	2.6	2.1
Urine 2	25.8	–	–	20.0	30.0	60.0	45.1	30.5	60.5	98.5	101.7	100.8	3.1	3.4	2.5
Urine 3	30.1	–	–	30.0	50.0	80.0	59.6	49.7	79.1	99.2	99.4	98.9	2.3	2.7	3.0

standard deviation (RSD) for 15 successive determinations was about 2.38%, 3.1% and 2.69%, respectively, indicating an excellent detecting reproducibility. Additionally, five modified electrodes based on the same fabrication procedure were prepared and used for the determination of DA solution, and the RSD was 3.56%, revealing an excellent repeatability of the electrode preparation procedure. The storage stability of the modified electrode was also evaluated. The DPV response current decreased to 94.6% of the initial value after two weeks and 87.2% remained after four weeks storage, demonstrating an excellent long-term stability.

3.6. Analytical applications

In order to evaluate the applicability of the proposed method for the determination of DA, UA and AA in real samples, the SGNF/IL/CS modified electrode was applied to the determination of human urine samples. The sample was diluted 10 times with phosphate buffer solution and then different amounts of urine samples, standard DA and AA solutions were transferred to electrolytic cell for the determination using DPV. The results are shown in Table 2. It can be seen that UA, DA and AA in mixtures could be satisfactory detected with the recovery in the range from 98.5% to 102.5% for UA, from 99.4% to 101.7% for DA and from 98.9% to 100.8% for AA. Additionally, the RSD ($n=3$) is less than 3.5%. These results are acceptable and satisfactory, indicating the applicability and reliability of the proposed method. The results also indicated that the proposed method in paper can apply individually or simultaneously to determine DA, UA and AA without any interference.

4. Conclusions

Combining the unique properties of SGNF and IL, coupled with the dispersion and film-forming abilities of chitosan, a highly sensitivity and selectivity electrochemical sensor was fabricated

and successfully applied to determine DA, UA and AA simultaneously with satisfactory results. The fabricated electrode displayed high electrocatalytic activity towards the oxidation of DA, UA and AA, showing a significantly lower over-potential and a higher peak current. Large peak separation between DA, UA and AA facilitated their simultaneous determination with no mutual interference. The electrochemical sensor showed high sensitivity, excellent selectivity, reproducibility and long-term stability. Therefore, the present method could be efficiently used for the determination of these biomolecules in real samples.

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