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A nonenzymatic sensor for xanthine based on electrospun carbon nanofibers modified electrode

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ABSTRACT

Xanthine (Xa) determination is of considerable importance in clinical analysis and food quality control. Therefore, a sensitive nonenzymatic amperometric sensor for Xa based on carbon nanofibers (CNFs) has been proposed. The CNFs, which were prepared by electrospinning technique and subsequent thermal treatment, were used to modify carbon paste electrode (CNF-CPE) to construct the amperometric sensor device without any oxidation pretreatment. In application to Xa electrochemical determination, the CNF-CPE exhibited high electrocatalytic activity and fast amperometric response. Various experimental parameters, such as pH and applied potential were optimized. Under the optimal conditions, the dynamic linear range of Xa was 0.03–21.19 μM (R = 0.9992) with the detection limit low to 20 nM (S/N = 3). With good selectivity and sensitivity, the present system was successfully applied to estimate the freshness of fish and determine Xa in human urine, which provides potential application in food quality control and clinical analysis.

1. Introduction

Xanthine (Xa) is an intermediate of the purine nucleotide and deoxynucleotide metabolism in humans, and is produced after adenosine triphosphate (ATP) decomposition [1,2]. As the metabolic precursor of uric acid, Xa is the first indicator of an abnormal purine profile, and can serve as a marker of many clinical disorders, including perinatal asphyxia, adult respiratory distress syndrome, cerebral ischemia, tumor hyperthermia and pre-eclampsia [3,4]. Besides, the level of Xa is generally used in the food industry as an index for evaluating the freshness of fish, which is of great significance for the quality control of fish products, because Xa is a major metabolite in the degradation of ATP in the dead fish [5]. Thus, developing a stable, sensitive and selective Xa sensor is highly demanded and plays an important role in clinical analysis as well as food quality control.

Several analytical methods have been developed for Xa determination, in which the most common analytical methods for detecting and quantifying Xa are high-performance liquid chromatography (HPLC) coupled with UV detector [6–11] and electrophoresis (CE) with amperometric detector [12,13] or UV detector [14]. In addition, the enzymatic method for the determination of Xa is promising due to its biocompatibility and sensitivity. Various electrochemical biosensors with xanthine oxidase (XnOx) binding to different matrices, such as polypyrrole [15], layered double hydroxides [16], β-cyclodextrin-branched carboxymethylcellulose [17], calcium carbonate nanoparticles [18] and conducting polymer [19], have been applied to determine Xa. However, the xanthine oxidase can oxidize both hypoxanthine (Hxa) and Xa to uric acid making the specific determination difficult. Additionally, inevitable drawbacks of the enzymatic method, such as instability, high cost and usually involved tedious preparation may limit their analytical application [20]. Nonenzymatic electrochemical approach, on the other hand, is quite sensitive, cheap and rapid [21]. In this sense, special efforts have been devoted to construct enzyme-free electrochemical sensor for Xa, including pretreated carbon paste electrode [22], nanoporous carbon fiber sensor [1], preanodized nontronite-coated screen-printed electrode [21], multi-wall carbon nanotubes film electrode [23] and ionic liquids–carbon nanotubes composite modified electrode [24]. The electrochemical sensors possess the advantages of good sensitivity and stability, but most of the electrodes or electrode materials need pretreatment. Thus, developing a kind of electrode material with good electrocatalytic ability and non-pretreatment can be a shortcut to construct the Xa sensor.

Carbon nanofibers (CNFs) have attracted much attention due to their similar unique electronic and relatively high mechanical properties to CNTs [25]. However, as a kind of new and interesting material, CNFs possess less order and more edge sites on the outer wall, which may lead to more facile electron transfer [26], along with better dispersion and wettability [27,28]. In our previ-

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ous study, it had been demonstrated that the CNFs provided with large specific surface area as well as improved conductivity, and displayed good electrocatalytic ability towards the oxidation of dihydronicotinamide adenine dinucleotide and dopamine [29,30]. Because of their good electrochemical property and biocompatibility, CNFs have also been used to construct many electrochemical biosensors [31–33].

Electrospinning has been proven to be an effective method for generating long polymer fibers with the diameter ranging from tens of nanometers to several micrometers [34]. In this work, we synthesized CNFs by electrospinning and subsequent thermal treatment. To further investigate the electroanalytical application of electrospun CNFs, an electrochemical nonenzymatic sensor for Xa was designed based on CNFs. The electrochemical behavior of Xa was studied and the CNFs showed high catalytic property towards the oxidation of Xa. The practical utility of the present system is demonstrated by measuring the concentration of Xa in fish and urine samples.

2. Experimental

2.1. Chemicals and reagents

Polyacrylonitrile (PAN), dimethylformamide (DMF) and graphite powder (2 μm) were purchased from Aldrich. Mineral oil was obtained from Beijing Chemical Co. (China). Uric acid (UA) was purchased from BBI. Xa and Hxa were obtained from Sigma. All other reagents were of analytical grade and were used without further purification. Phosphate buffer solution (PBS) was prepared by Na_2HPO_4, NaH_2PO_4, H_3PO_4 and NaOH. All solutions were prepared with double distilled water.

2.2. Apparatus

The Scanning Electron Microscopy (SEM) experiment was made on a Hitachi S4800 SEM at an accelerating voltage of 10 kV. Transmission Electron Microscope (TEM) experiment was made on a JEM-2010. Electrochemical impedance spectroscopy (EIS) was performed with an AutoLab Pgstat30 Differential Electrometer. All other electrochemical measurements were performed using a CHI 832 electrochemical workstation (Shanghai, China). A conventional three-electrode system, which consisted of the bare CPE or CNF-CPE working electrode, a platinum wire counter electrode and the Ag/AgCl (saturated KCl) reference electrode, was used for all electrochemical experiments. The cathodic current is assigned to be positive, and the anodic current is assigned to be negative.

2.3. Preparation of CNFs and electrodes

The CNFs were prepared by carbonizing PAN nanofibers which were made via electrospinning process by using a DMF solution containing 8 wt% PAN. The procedure was similar to that reported previously [28]. Briefly, the electrospinning process was performed in an electric field of an order of 100 kV m^{-1}, from a 30 kV voltage applied to a 30 cm gap between the spinneret and the collector. The carbonization of PAN nanofibers was completed in a high temperature furnace.

The carbon paste electrode (CPE) was prepared by packing the carbon paste which was made by mixing graphite powder and mineral oil at the ratio of 70:30 (w/w) in a mortar, into a pipette tube (1-mm diameter; 1-cm depth) tightly. Electrical contact was established with a copper wire inserting into the carbon paste. And then the surface of CPE was smoothed against weighing paper and rinsed with double distilled water. At last, 10 μL CNFs suspension (1 mg mL^{-1}), which had been proved to be the optimum dosage, was dripped carefully onto the smoothed surface of the CPE and allowed to dry at room temperature to obtain CNFs modified CPE (CNF-CPE).

2.4. Preparation of samples

A crucian carp (about 0.5 kg in weight) was killed and a small piece of fish meat (2.5 g) was homogenized for 30 min in 2 mL of 10% HCl and then diluted to 50 mL with double distilled water. After centrifugation at 7000 rpm for 10 min, the supernatant was filtered through a filter membrane (0.22 μm pore size) for the fish extraction. Then, 200 μL of the fish extract was used for fish freshness analysis. All sample solutions were prepared immediately prior to each experiment.

The urine samples were obtained by volunteers in laboratory. The urine samples for experiments were diluted 100 times with 0.1 M PBS (pH 6.0) without any other pretreatment.

3. Results and discussion

3.1. Characterization of CNFs and CNF-CPE

SEM and TEM were applied to characterize the morphology of the as-prepared CNFs. The SEM image (Fig. 1A) shows that the CNFs possess a uniform distribution with a three-dimensional structure.

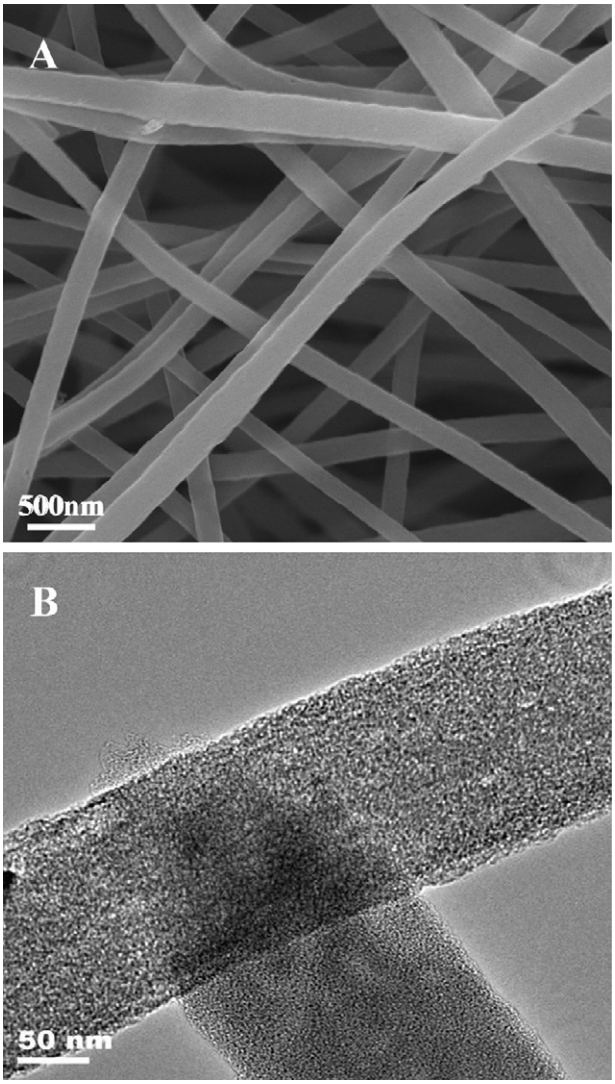

Fig. 1. SEM (A) and TEM (B) images of CNFs.

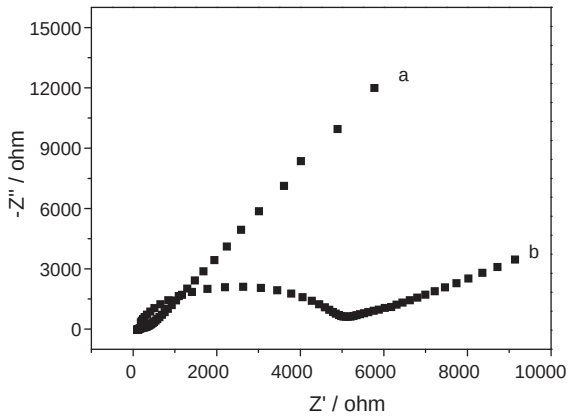

Fig. 2. EIS of CPE (a) and CNF-CPE (b) in 0.5 M KCl containing 5 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$. The alternating current voltage was 5 mV and frequency was between 0.1 and 10^5 Hz.

The diameter of the CNFs ranges from 200 to 400 nm with the length up to tens of micrometers. From the TEM image (Fig. 1B), it can be observed that the CNFs possess a rough and porous surface which could significantly facilitate the electron transfer and increase the effective electrode surface area.

The electron-transfer kinetics at CNF-CPE and CPE was studied by electrochemical impedance spectroscopy (EIS) (Fig. 2) in 0.5 M KCl containing 5 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$. The results illustrated a notable decrease in resistance (R_{ct}) at CNF-CPE (52.6 Ω) (curve a) compared with that at CPE (4.9 kΩ) (curve b), further confirming the ability of CNFs to accelerate electron transfer.

3.2. Electrocatalytic oxidation of Xa

Fig. 3 shows the CVs of CPE (curve b) and CNF-CPE (curve a) in 0.1 M PBS (pH 7.0) containing 1 mM Xa. It was found that the oxidation peak potential for Xa at CPE was 0.85 V, while it shifted to 0.75 V at CNF-CPE due to the presence of CNFs. Moreover, the anodic peak current of Xa at CNF-CPE was two times higher than that obtained at CPE. The decrease of the oxidation overpotential and increase of the peak current indicated that the CNFs film could efficiently catalyze the oxidation of Xa. The electrochemical oxidation pathway of Xa may be similar to the pathway on the carbon fiber electrode as described in the literature [1]. But compared with the carbon fiber, the CNFs can promote the electron transfer and catalyze the oxidation of Xa. The catalytic activity of CNFs should be ascribed

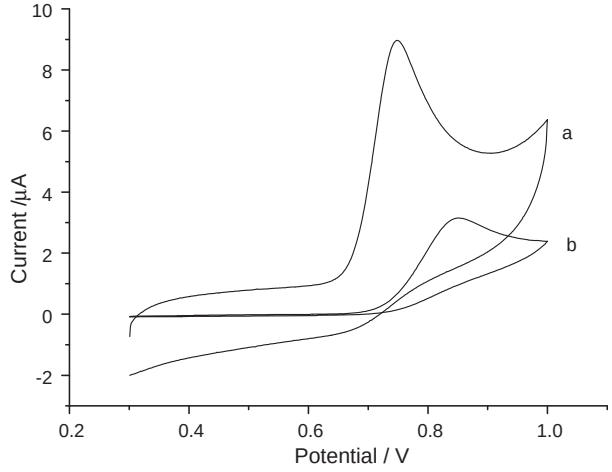

Fig. 3. CVs of 1 mM Xa in 0.1 M PBS (pH 7.0) at CPE (b) and CNF-CPE (a). Scan rate: 50 mV s^{-1}.

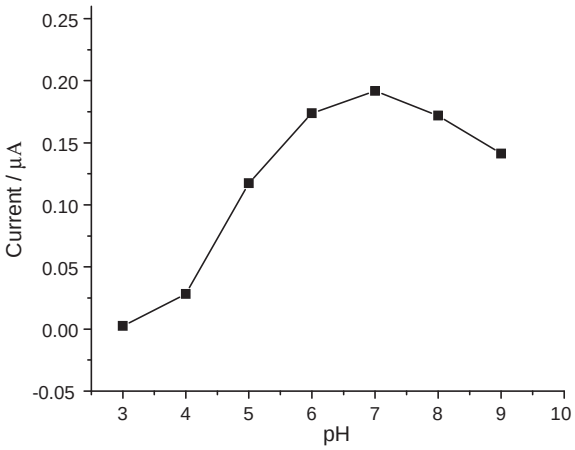

Fig. 4. Effect of pH value on the amperometric response of CNF-CPE towards 10 μM Xa. Solution: 0.1 M PBS under stirring. The potential applied at 0.85 V.

to the nanometer size, three-dimensional and porous structure of CNFs which brings in high surface area and large number of active sites [31].

3.3. Effect of applied potential and pH

The effect of applied potential in the range of 0.6–1.0 V on the CNF-CPE response was investigated in the presence of 10 μM Xa by amperometric *i–t* curve which was used for the following experiments unless otherwise stated. The current response increased with the change of applied potential from 0.6 to 0.85 V, and then decreased with higher potential. Therefore, 0.85 V was selected as the optimal detection potential.

The influence of the pH value on the current response for 10 μM Xa at CNF-CPE was also investigated in the pH range of 3.0–9.0 (Fig. 4). With increasing pH from 3.0 to 9.0, the oxidation current of Xa increased from pH 3.0 to 7.0, but the current decreased with higher pH values. Thus, the highest current value could be obtained at pH 7.0 and this pH was selected as optimum for further experiments.

3.4. Electrochemical determination of Xa

Under the optimized conditions, the electrochemical determination of Xa at the CNF-CPE was studied by successive additions of Xa into 3 mL PBS. Fig. 5 shows the *i–t* curve and the calibration curve (inset) for Xa. The dynamic linear range spanned 3-order concentration of Xa from 0.03 to 21.19 μM ($R = 0.9992$) with the detection limit low to 20 nM ($S/N = 3$). Compared with the enzymatic electrodes [15–19], at which the detection limits of Xa are around or above 0.1 μM, the CNF-CPE possesses a significantly lower detection limit and wider linear range. Compared with other nonenzymatic electrodes, the detection limit of Xa at CNF-CPE is lower than that obtained at pretreated carbon paste electrode [22], nanoporous carbon fiber sensor and preanodized nontronite-coated screen-printed electrode [21].

3.5. Reproducibility and stability study

The reproducibility was examined by the amperometric response to 10 μM Xa and the relative standard deviation (RSD) of current signals for six determinations was 2.4%. The result showed a good reproducibility of the proposed system, which demonstrated that a fresh surface could be obtained and the CNFs possessed the good ability of antifouling. Operational stability of the CNF-CPE for Xa detection was also investigated by comparing the amper-

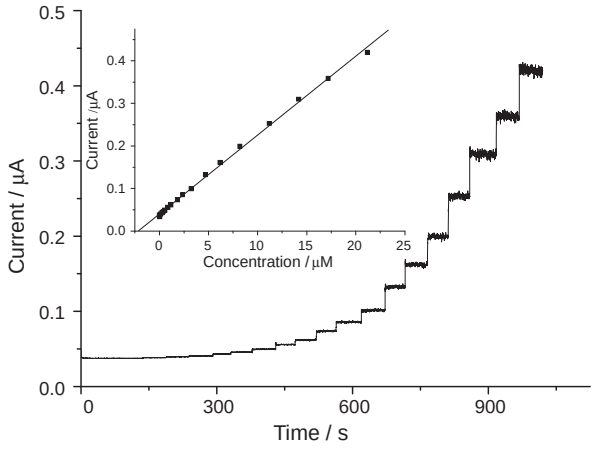

Fig. 5. Current–time response of CNF-CPE upon successive addition of special concentration of Xa to PBS under stirring. Inset shows the calibration curve for Xa determination. Solution: 0.1 M PBS (pH 7.0) under stirring. The potential applied at 0.85 V.

ometric responses of bare CPE and CNF-CPE to 10 μM Xa at 0.85 V over a long period. At CNF-CPE, 85.5% of the initial response was maintained after 1 h, while the bare CPE displayed a rapid current decay and only 31.4% of the initial response was remained. The improved operational stability of the CNF-CPE could be ascribed to the faster electron transfer rate, because the faster electron transfer rate limited the amount of radical intermediates that usually cause electrode surface fouling during the oxidation of Xa [35].

3.6. Interference study

Hxa as one kind of important biological substance often coexists with Xa in human fluid, food processing, pharmaceuticals and clinical analysis. Hxa (50 times content) would not interfere with the determination of 10 μM Xa. The interference of other biological substance which may coexist with Xa, such as glucose, sodium citrate and oxalate was also investigated and all of the substance (500 times content) had no influence on the current response for 10 μM Xa. The results above indicated that the proposed method had good selectivity towards the determination of Xa.

3.7. Freshness of fish evaluation

When a fish dies, it loses freshness through its degradation process. Hxa and Xa are major metabolites of adenine nucleotide degradation and accumulate continuously after death. Therefore, the freshness of fish could be reflected by the concentration of Hxa or Xa [18,36]. Most of the reported enzymatic methods [37,38] estimated the freshness of fish by determining Hxa. But, as we know, no nonenzymatic Xa electrochemical sensor has been applied to the fish freshness evaluation. In this work,

Table 1
Determination of Xa in fish samples.[a]

Sample	Store condition	Detected (μM)	Added (μM)	Found (μM)	Recovery (%)
1	Freshly killed	2.10	5	6.94	92.61
2	5 h	2.46	5	7.27	92.19
3	10 h	3.33	5	8.13	93.90
4	14 h	4.62	5	9.33	93.81
5	24 h	5.97	5	10.55	92.92
6	36 h	8.37	5	12.71	92.17
7	42 h	15.67	5	19.37	91.73

[a] Average value of triplicate.

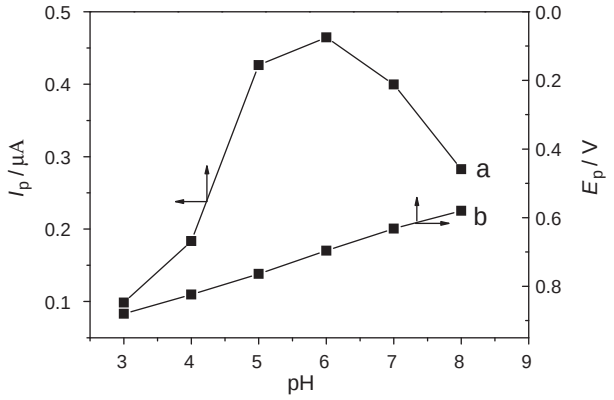

Fig. 6. Effect of pH value on the oxidation peak current (a) and peak potential (b) of 25 μM Xa at CNF-CPE. Solution: 0.1 M PBS. DPV conditions: scan rate, 6 mV s^{-1}; amplitude, 50 mV; pulse width, 100 ms; pulse period, 200 ms.

the proposed nonenzymatic Xa sensing system was used to estimate the freshness of fish by determining the concentration of Xa at 0.85 V, because Hxa has no interference under this potential. The fish meat extract was obtained as described in Section 2. Table 1 shows the determination of Xa in fish samples. After freshly killed and stored at room temperature, the concentration of Xa increased continuously with the extension of storage time, which showed a good agreement with the previous reports, although the detected specific concentrations were a little different from those reported [18]. The difference might be ascribed to the different types of fish and different environments for fish storage. The experimental results indicated that the proposed sensing system could provide expedient measurements in real biological samples.

3.8. Determination of Xa in urine samples by differential pulse voltammetry (DPV)

UA and Hxa are usually present in xanthine samples. Therefore, simultaneous determination of Xa, UA and Hxa was carried out by DPV. The effect of pH value on the determination of 25 μM Xa at CNF-CPE was investigated by DPV in the pH range of 3.0–8.0. It can be seen from Fig. 6 (curve b) that the oxidation peak potential (E_p)

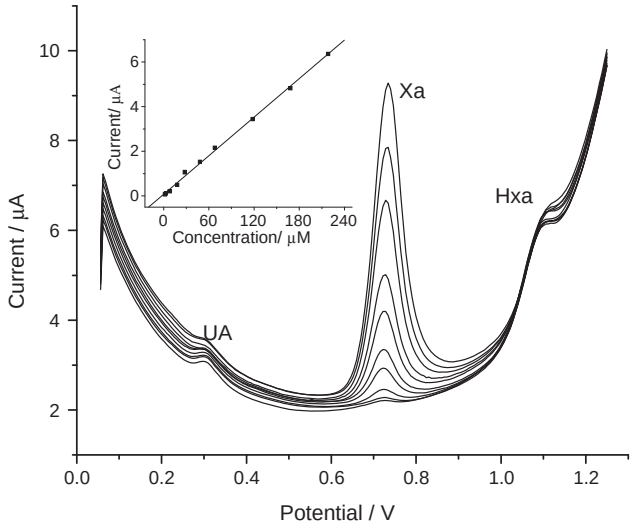

Fig. 7. DPVs of 1.5, 3, 8, 18, 28, 48, 68, 118, 168, 218 μM Xa in 0.1 M PBS (pH 6.0) containing 40 μM Hxa and UA at CNF-CPE. Inset shows the calibration curve for Xa correspondingly. DPV conditions are the same as in Fig. 6.

Table 2
Determination of Xa in urine ($n = 5$).

Sample	Added (μM)	Found (μM)	RSD (%)	Recovery (%)
1	5	5.04	4.31	100.80
2	10	9.78	3.55	97.80
3	15	15.80	4.23	105.33

decreased linearly ($R = 0.9994$) with the increasing pH. While the pH increased, the peak current (I_p) of Xa increased from 3.0 to 6.0 and reached the maximum at pH 6.0, but the I_p decreased with higher pH values (curve a). Therefore, pH 6.0, which is not exactly the same with the optimal pH by *i*–*t* curve, was selected as optimum for further DPV experiments.

Fig. 7 shows the DPVs and the corresponding calibration curve (inset) for Xa at CNF-CPE when coexisting with 40 μM Hxa and UA. Hxa and UA could be oxidized at the detached potentials of 1.0 V and 0.35 V, respectively, which were well separated from the oxidation peak potential of Xa at CNF-CPE. So these three substances could be simultaneously detected, indicating that UA and Hxa have no interference with the determination of Xa. When increasing the concentration of Xa, the peak current was found to increase linearly over the range from 1.5 μM to 218 μM ($R = 0.9990$) with the detection limit of 0.5 μM ($S/N = 3$).

The practical application of this method was further established by the selective measurement of Xa in human urine. Three human urine samples obtained from laboratory personnel were determined by DPV. Before analysis, all the urine samples used for detection were diluted 100 times without any other pretreatment process. The recovery rates of the spiked samples ranged between 97.8% and 105.3% (Table 2). These satisfying results indicated that the detection procedures were free from interferences of the uric sample matrix and could be applied to determine Xa in urine samples.

4. Conclusions

An amperometric sensor for Xa is proposed, which was prepared by electrospun CNFs modified CPE without any enzyme or medium. The CNFs exhibit high electrocatalytic activity towards the oxidation of Xa and improve the analytical performance of CPE. With rapid response, low detection limit, satisfactory linear range, good stability and selectivity, the present system was successfully employed to estimate the freshness of fish and determine Xa in human urine, which is promising in food quality control and clinical analysis.

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