Full Paper

Voltammetric Detection of Ofloxacin in Human Urine at a Congo Red Functionalized Water-Soluble Carbon Nanotube Film Electrode

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Abstract
A simple physical method was developed for the surface modification and the solubilization of MWNTs in water by Congo red. The resulting water-soluble MWNTs (MWNTs-CR) can form stable and uniform films on solid supports when dried, which was used to fabricate MWNTs-CR modified glassy carbon electrodes (MWNTs-CR/GCE). Voltammetric studies showed that MWNTs-CR/GCE exhibited a strong enhancement effect on the electrooxidation of ofloxacin. MWNTs-CR films were also proved to possess overwhelming advantages as electrochemical sensing films over other commonly used MWNTs composite films (e.g., MWNTs-DHP and MWNTs-Nafion), reflected by the higher oxidation current, lower background and stronger accumulation capacity towards less soluble species. The sensitive oxidation of ofloxacin at MWNTs-CR/GCE was used for the determination of ofloxacin. Under optimal conditions, the oxidation current was proportional to ofloxacin concentration in the ranges of $5 \times 10^{-8} - 3.0 \times 10^{-5}$ M. The detection limit of 9×10^{-9} M was obtained for 350 s accumulation at open circuit ($S/N = 3$). This method was applied to the determination of ofloxacin in human urine and the result was satisfying.

Keywords: Multiwalled carbon nanotubes, Noncovalent functionalization, Congo red, Ofloxacin, Electrochemical determination

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1. Introduction

Ofloxacin, (±)-(*S*)-9-fluoro-2.3-dihydro-3-methyl-10-(4-ethyl-1-piperazinyl)-7-oxo-7H-pyrido[1,2,3-de]-1,4-benzoxazine-6-carboxilic acid hemihydrate, belongs to a group of broad spectrum antibiotics called the quinolones. It is effective against several types of bacteria that tend to be resistant to other commonly used antibiotics. It is often used to treat many infections caused by bacteria, such as pneumonia, bronchitis, venereal disease (VD), and prostate, skin and urinary tract infections. It is rapidly and essentially absorbed after oral administration. Ofloxacin undergoes limited metabolism in humans and the most of an administered dose was recovered as unchanged drug in urine within 48 h. So it is essentially to find an effect method to determine ofloxacin in human urine. Many methods were used for the determination of ofloxacin, such as high-performance liquid chromatography (HPLC) [1], UV-visible spectrophotometry [2], fluorimetry [3, 4], anodic stripping voltammetry (ASV) [5], thin-layer chromatography (TLC) [6] and chemiluminescence [7]. The electroanalytical methods have also been reported [8–10]. But there are a few reports about the determination of ofloxacin by cyclic voltammetry. To our knowledge, the studies on the electrochemical behaviors of ofloxacin and its quantitative detection at carbon nanotubes (CNTs) film coated glassy carbon electrode have not been reported.

In the past decades, CNTs have become the subject of intense researches on the basis of their large specific area, their unique architecture, remarkable mechanical and electrical properties [11, 12]. CNTs can significantly improve the electrochemical responses of biomolecules and medicament like dopamine [13, 14], 5-hydroxytryptamine [15], thyroxine [16], NADH [17] and acyclovir [18]. The promoted electron transfer of proteins at CNTs modified electrode was also reported previously [19, 20]. In practice, CNTs are generally treated with acids or heated in air to produce hydroxyl, carboxyl and ketone groups at both the sidewall and the terminus to achieve the surface modification and the solubilization in various solutions. The pretreated CNTs are dispersed in organic solvents, concentrated sulfuric acid, polymer or soluble surfactants before they are cast onto the surface of electrodes. These CNTs suspensions have some inevitable disadvantages due to the weak interactions between CNTs and the dispersing agents, such as low loadings, low stability and the presence of free additives. Recently, we reported a new noncovalent approach for preparing highly water-soluble single-walled carbon nanotubes (SWNTs) by a small planar and conjugated diazoye, Congo red (CR) [21, 22].

In this work, the strong noncovalent adsorption of CR on the surface of carbon nanotubes was used to prepare water-soluble multi-walled carbon nanotubes (MWNTs-CR). Similar to SWNTs-CR, the functionalization of MWNTs by CR can also achieve the solubilization of MWNTs in water with high solubility and form stable and uniform films on glassy carbon electrodes. The prepared MWNTs-CR modified glassy carbon electrode was used for the voltammetric determination of ofloxacin. The result showed that MWNTs-CR films can apparently improve the oxidation response of ofloxacin and show overwhelming advantages as electrochemical sensing films over other commonly used MWNTs composites like MWNTs-DHP and MWNTs-Nafion.

2. Experimental

2.1. Apparatus and Reagents

MWNTs were provided by Timesnano Co. Chengdu, China. Ofloxacin was purchased from Kunshan Pharmaceutical Co., Shanghai, China. Congo red (CR) was obtained from Shanghai Reagent Co., Shanghai, China. Nafion (5 wt%) was purchased from Sigma Chemical Co.

Dihexadecyl hydrogen phosphate (DHP) was obtained from Fluka Chemical Reagent Co. and stored at $-18\,^{\circ}C$. All chemicals were of analytical grade quality and used as received. Phosphate buffer solutions (PBS) with various pH values were prepared by mixing stock standard solutions of Na_2HPO_4 and KH_2PO_4 and adjusting the pH with 0.1 M HCl or NaOH. All solutions were made up of double-distilled water.

Cyclic voltammetry (CV) and the linear sweep voltammetry (LSV) was performed on a CHI 830 electrochemical analyzer (Shanghai Chenhua Co., China) in a three-electrode system with a platinum wire as auxiliary electrode, a saturated calomel electrode as reference and a water-soluble MWNTs coated GCE as working electrode. Electrochemical impedance spectroscopy (EIS) was carried out with the EG&G Model 283 electrochemical workstation and EG&G Model 5210 lock in amplifier (Princeton Applied Research, PAR, USA) powered by the Powersuit software.

2.2. Preparation of the Water-Soluble MWNTs Composite Films Coated GCE

The preparation of MWNTs-CR was similar to that of SWNTs-CR [22]. Briefly, MWNTs were mixed with CR on certain weight ratio (e.g., MWNTs/CR, 5/1) in an agate mortar and ground for 4 h with the addition of little water to avoid agglomeration of the dry CR powder, producing a greenish black mixture (MWNTs + CR). MWNTs + CR was dissolved in water and washed on a polytetrafluoroethylene (PTFE) filter disc of 0.22 μm pore size by water to remove excessive free CR until the filtrate became colorless. The obtained black product had the solubility of about 1.0 mg/mL before being dried. MWNTs-CR was directly dissolved and stored in water immediately after the washing procedure to avoid the rebundling and the solubility loss of the exfoliated nanotubes when dried. 2 mg MWNTs were added to 2 mL 0.1% (W/V) Nafion ethanol solution and then treated in an ultrasonic bath for about 50 min. A black and uniform MWNTs-Nafion suspension was obtained. Five milligrams of MWNTs and 5mg DHP were dispersed together in 5 mL water to form a uniform MWNTs-DHP suspension with the aid of ultrasonic agitation for 20 min.

GCE was first polished with aqueous slurry of 0.05 μm alumina powder on silk. The electrode was thoroughly washed with water and treated in an ultrasonic bath for about 2 min with pure water. Then 1 mg/mL MWNTs-CR was dropped on the surface of the cleaned GCE and air-dried. The prepared electrode was denoted as MWNTs-CR/GCE, which was pretreated by successively scanning in the potential range of 0.0 – 1.2 V until stable voltammograms were obtained.

3. Results and Discussion

3.1. Electrochemical Characterization of Ofloxacin at Water-Soluble MWNTs-CR/GCE by EIS

Figure 1 shows the Nyquist plots (Z″ versus Z′) for the electrochemical impedance spectroscopy (EIS) of ofloxacin at MWNTs-CR/GCE and bare GCE. The Nyquist plot of ofloxacin at bare GCE consists of only a semicircle in the whole frequency range, suggesting that the charge transfer rate is slow for ofloxacin at bare GCE. The Nyquist plot of MWNTs-CR/GCE showed different characteristics and a line part appears at low frequency range besides the semicircle at high frequencies with a smaller diameter,

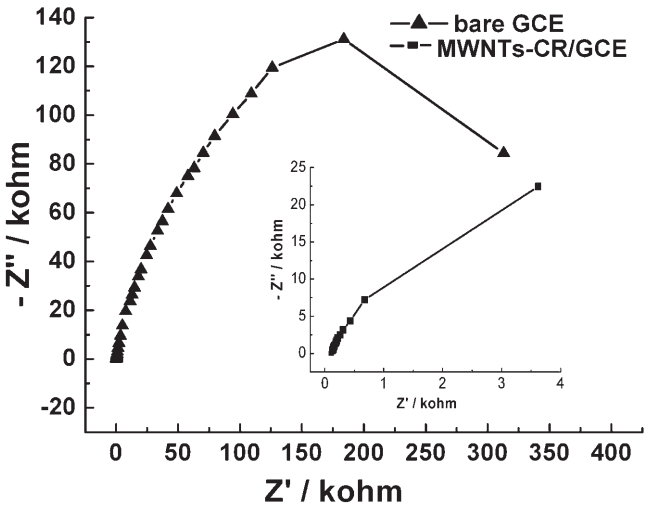

Fig. 1. The impedance responses of 5×10^{-6} M ofloxacin at the MWNTs-CR/GCE (■) and bare GCE (▲). Frequency range used: 100 kHz to 0.1 Hz with single amplitude of 5 mV rms at five steps per decade.

which indicates that the water-soluble MWNTs films can effectively increase the charge transfer rate of ofloxacin.

3.2. Enhanced and Irreversible Oxidation of Ofloxacin at MWNTs-CR/GCE

Figure 2 is the cyclic voltammograms of 5×10^{-6} M ofloxacin in 0.1 M PBS (pH 7.0) at different electrodes following accumulation 350 s at open circuit. Obviously, the response of ofloxacin at bare GCE is rather poor and only a weak oxidation peak is observed at 0.9 V (curve c). For the MWNTs-CR/GCE, the background is apparently enlarged but the baseline remains flat and no any redox peak appears (curve b). When 5×10^{-6} M ofloxacin is added to the solution, a sensitive oxidation peak is observed at about 0.9 V at MWNTs-CR/GCE, which can correspond to the oxidation of ofloxacin. Compared to the response of ofloxacin at bare GCE, the significantly oxidation currents of ofloxacin at MWNTs-CR/GCE confirms that MWNTs show highly effective enhancement to ofloxacin because of its large specific surface area and special electrical properties, which makes for easier adsorption of ofloxacin and provides enough effective reaction sites. Furthermore, the trait of porous of MWNTs-CR makes the adsorption and diffusion of ofloxacin to the electrode surface easily and the concentration of that at electrode increases. No peaks are observed in the cathodic branch, indicating that the ofloxacin oxidation is an irreversible process. The oxidation peak may be attributed to the irreversible oxidation of the piperazine moiety of ofloxacin molecule, in accordance with the redox mechanism postulated by Kauffmann et al. [23] for oxidation of piperazine ring in trazodone molecule. Inset in Figure 2 shows the successive cyclic voltammograms of 1×10^{-5} M ofloxacin in 0.1 M PBS (pH 7.0) at MWNTs-CR/GCE. A well-defined oxidation peak is observed at 0.9 V at the first anodic sweep from 0.0 to 1.2 V. On the reversal scan, no corresponding reduction peak appears. However, during following successive cyclic sweeps the oxidation peak current decreases dramatically and finally disappears. This result confirmed the irreversibility of the ofloxacin oxidation process and it might be resulted from the fact that the electrode surface is blocked by the adsorption of the oxidation products which reduces the effective reaction sites at the modified electrode surface.

Different from free additive-containing carbon nanotubes dispersing systems like MWNTs-DHP and MWNTs-Nafion, MWNTs-CR exhibits its unique properties as electrochemical sensing films in electroanalysis because MWNTs-CR can form three-dimension network structures with plenty of nanopores, which can effectively make full use of the whole films. Moreover, the absence of free additives in the films allows the direct approach of the substrates to the surface of MWNTs and facilitates the electrochemical process. These are demonstrated by the different responses of ofloxacin at different MWNTs composite films (Fig. 3). Clearly, the oxidation current of ofloxacin at MWNTs-CR/GCE was greatly larger than that of ofloxacin at MWNTs-DHP/GCE and MWNTs-Nafion/GCE. In contrast, the background of MWNTs-CR/GCE is apparently lower than MWNTs-DHP/GCE and MWNTs-Nafion/GCE along with a more well-defined peak shape.

The influences of scan rate on electrochemical behavior of 5×10^{-6} M ofloxacin at MWNTs-CR/GCE in pH 6.0 PBS were also investigated (Fig. 4). The oxidation peak currents are proportional to scan rates from 10 to 200 mV s^{-1}. The corresponding linear regression equation can be expressed as follows:

$$I_p\ (\mu A) = 4.7241 + 0.3726\ v\ (\text{mV s}^{-1})\ (R = 0.9972)$$

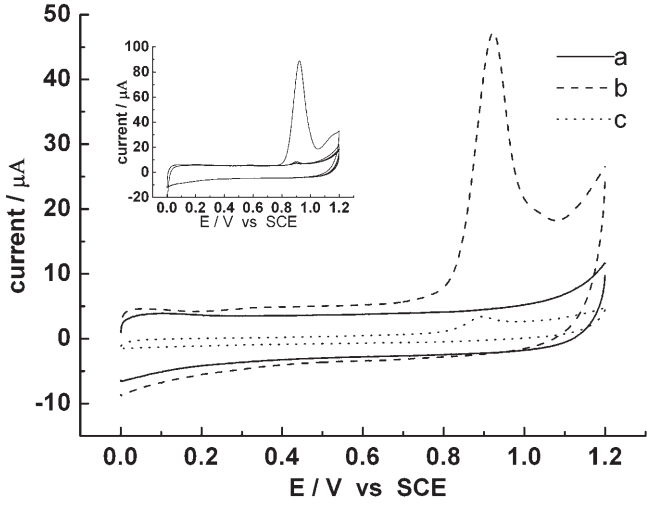

Fig. 2. Cyclic voltammograms of MWNTs-CR/GCE in the absence (curve a) and presence of 5×10^{-6} M ofloxacin at bare GCE (curve c) and MWNTs-CR/GCE (curve b) in 0.1 M PBS (pH 6.0). Scan rate: 100 mV/s. Inset: successive cyclic voltammograms of 1×10^{-5} M ofloxacin in 0.1 M PBS (pH 6.0) at MWNTs-CR/GCE. Scan rate: 100 mV/s.

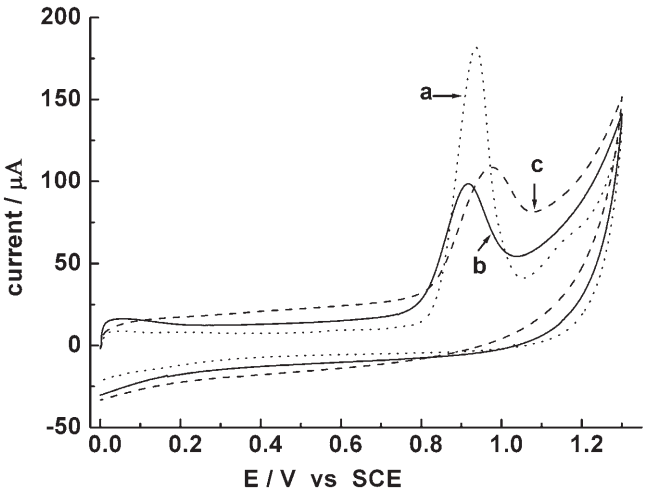

Fig. 3. Cyclic voltammograms of 2×10^{-5} M ofloxacin at MWNTs-CR/GCE (curve a), MWNTs – DHP/GCE (curve b) and MWNTs-Nafion/GCE (curve c) in 0.1 M PBS (pH 6.0). Scan rate: 100 mV/s.

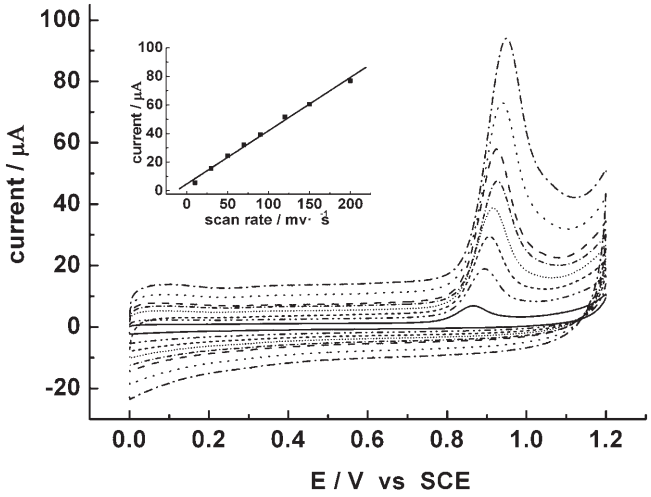

Fig. 4. Influences of scan rate on the electrochemical oxidation peak current of 5×10^{-6} M ofloxacin at MWNTs-CR/GCE in 0.1 M PBS (pH 6.0): 20, 40, 60, 80, 100, 120, 150, 200 mV/s. Inset: plot of the peak current vs. scan rate.

The result indicated that the electrochemical of ofloxacin at MWNTs-CR/GCE was controlled by an adsorption step. The peak current potential shifted to more positive values with the increase of scan rate, which confirmed the irreversibility of the oxidation process.

Cyclic voltammetry of 5×10^{-6} M ofloxacin on different pH solutions showed that the oxidation peak current and potential depended strongly on the solution pH (Fig. 5). The pH of solution would influence the condition of ofloxacin in solution and the consequent electrochemical behaviors on electrode. The oxidation peak potential shifted negatively as pH increased from 4.0 to 8.0, and obeyed the following equation:

$$E_p\ (\mathrm{V}) = 1.2077 - 0.048\ \mathrm{pH}\ (r = 0.9928)$$

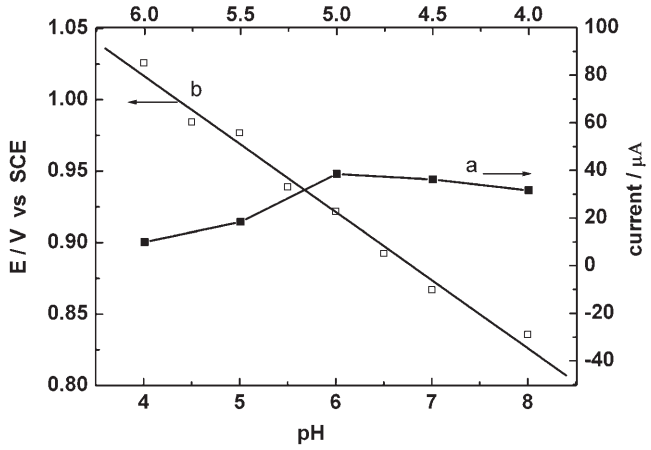

Fig. 5. Dependence of pH on peak current (a) and peak potential (b) of 5×10^{-6} M ofloxacin at MWNTs-CR/GCE in 0.1 M PBS. Scan rate: 100 mV/s.

The value of the slope was close to the expected value 59 mV/pH for one electron and one proton reaction, indicating that the number of electrons transferred in the oxidation of ofloxacin was equal to that of protons. The oxidation peak current was increased with the increase of pH from 4.0 to 6.0 and reached to the maximum at pH 6.0. Subsequently, the peak current decreased slowly with the increase of pH from 6.0 to 8.0. Thus phosphate buffer (pH 6.0) was chosen for supporting electrolyte for investigation of the oxidation of ofloxacin at MWNTs-CR/GCE

3.3. Optimizing of Some Experimental Conditions

3.3.1. Optimization of the Amount of MWNTs

As mentioned above, the electrochemical behaviors of ofloxacin can be enhanced by MWNTs, so the effect of the amount of MWNTs was studied. The oxidation peak current of 5×10^{-6} M ofloxacin increased quickly with the increase of the volumes of 1 mg/mL water-soluble MWNTs-CR solution cast on the GCE surface in the range of 0 – 10 μL. Subsequently, further increasing the volumes of MWNTs-CR solution, the peak current decreased lightly. The increases of the volumes of MWNTs-CR caused the increases of the thickness of the membrane on the GCE, which would go against the electron transfer of ofloxacin based on the unimproved mass transfer. As a result, 10 μL 1 mg/mL MWNTs-CR solution was suitable for the determination of ofloxacin.

3.3.2. Influences of Accumulation Time

The dependence of the oxidation peak current on the accumulation time was also studied. The oxidation peak current of 5×10^{-6} M ofloxacin increased quickly within 250 s and then increased slowly from 250 s to 350 s. The calibration curve gradually tended to a plateau after 350 s, indicating that the adsorption of ofloxacin on MWNTs-CR/GCE was saturated. As a result, the accumulation time was 350 s for the experiment.

3.4. Calibration and Interferences

3.4.1. Calibration Graph

Under the optimized experimental conditions, the calibration curve for ofloxacin in pH 6.0 PBS at MWNTs-CR/GCE was characterized by cyclic voltammetry, and the linear range was comprised from 5×10^{-8} to 3.0×10^{-5} M in terms of the relationship between the concentration of ofloxacin and the oxidation peak current (Fig. 6). The relationship can be described as $I_p\ (\mu\mathrm{A}) = 6.3544 + 6.7032\mathrm{C}\ (\mu\mathrm{M})\ R = 0.9977$. A detection limit of 9×10^{-9} M was obtained for 350 s accumulation at open circuit ($S/N = 3$). After each measurement the modified electrode was refreshed by successive cyclic voltammetric sweeps in blank solution to get a

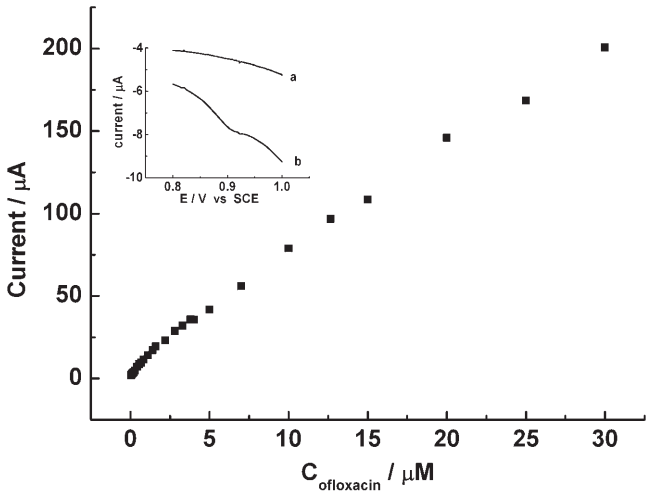

Fig. 6. Plot of the peak current vs. the concentration of ofloxacin. Inset: the voltammograms of MWNTs-CR/GCE in the absence (curve a) and presence of 9 nM ofloxacin (curve b). Scan rate: 100 mV/s.

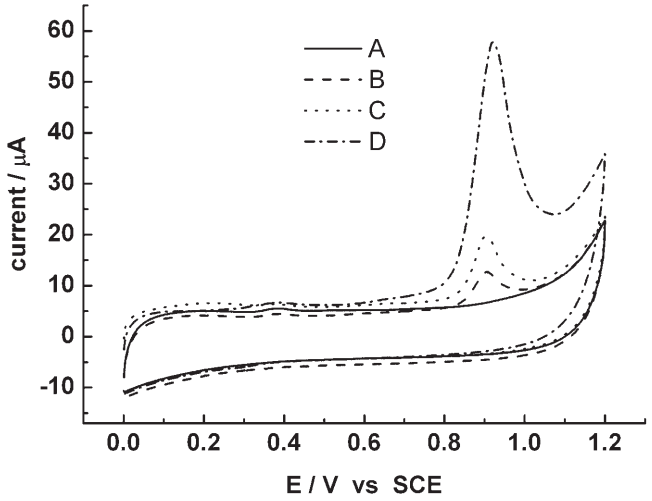

Fig. 7. Cyclic voltammograms of MWNTs-CR/GCE in urine assays without ofloxacin (curve A) and sample A (curve B), sample B (curve C) and sample C (curve D) with expected ofloxacin. Scan rate: 100 mV/s.

reproducible electrode surface. The relative standard deviation (*RSD*) was 3.8% for 8 times parallel detections of 5×10^{-6} M ofloxacin at the same electrode and 4.96% for four times parallel detections of 5×10^{-6} M ofloxacin at different electrodes, indicating that the good reproductivity and repeatability of the modified electrode.

The stability of the modified electrode was studied by the current responses of 5×10^{-6} M ofloxacin at room temperature. The experiment results indicated that the oxidation current response of ofloxacin at the modified electrode was almost unchanged after 10 days, suggesting that the modified electrode possessed good stability.

3.4.2. Interferences

The effect of some biomolecules on the electrochemical oxidation of 5×10^{-6} M ofloxacin has been evaluated. 2.5×10^{-3} M glucose, 5×10^{-4} M uric acid, urea, dopamine, tilidazole, metronidazole and some familiar metallic ion have almost no influences on the responses of 5×10^{-6} M ofloxacin (signal change below 5%) but the influences of 5×10^{-6} M ciprofloxacin and norfloxacin are serious. Because they have the similar electroactive group with ofloxacin, their oxidation potentials are close to that of ofloxacin and affect the responses of ofloxacin.

3.5. Determination of Ofloxacin in Urine Samples

The proposed method was applied to the determination of ofloxacin in urine samples (Fig. 7). The results are showed in Table 1. In our experiments, the concentration of ofloxacin was calculated using standard additions method. The recoveries in our experiment were investigated and the values are varied between 97.25 and 102.2%, which indicated that the determination of ofloxacin using water-soluble MWNTs-CR/GCE was effective and sensitive.

4. Conclusion

Congo red-functionalized water-soluble MWNTs (MWNTs-CR) were prepared on the basis of the strong noncovalent adsorption of Congo red on the surface of MWNTs, which was used to fabricate MWNTs-CR modified glassy carbon electrodes (MWNTs-CR/GCE). Voltammetric studies showed that MWNTs-CR can effectively improve the oxidation response of ofloxacin. MWNTs-CR films were also proved to possess apparent advantages as electrochemical sensing films over other commonly used MWNTs composite films (e.g., MWNTs-DHP and MWNTs-Nafion), reflected by the higher oxidation current, lower background

Table 1. Recovery tests determination of ofloxacin in urine samples.

Sample	Added (mol/L)	Expected(mol/L)	Found (mol/L)	Recovery(%)
A	–	–		0.00
	9.00×10^{-8}	9.00×10^{-8}	9.20×10^{-8}	102.2
B	–	–	1.74×10^{-7}	
	8.00×10^{-7}	9.74×10^{-7}	9.52×10^{-7}	97.25
C	–	–	2.23×10^{-6}	
	4.00×10^{-6}	6.23×10^{-6}	6.18×10^{-6}	98.75

and stronger accumulation capacity towards less soluble species. The enhanced oxidation of ofloxacin at MWNTs-CR/GCE was applied to the determination of ofloxacin in human urine and the result was satisfying.

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6. References

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