

## A simple and sensitive method for determination of Norfloxacin in pharmaceutical preparations

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In this approach, a new voltammetric method for determination of norfloxacin was proposed with high sensitivity and wider detection linear range. The used voltammetric sensor was fabricated simply by coating a layer of graphene oxide (GO) and Nafion composited film on glassy carbon electrode. The advantage of proposed method was sensitive electrochemical response for norfloxacin, which was attributed to the excellent electrical conductivity of GO and the accumulating function of Nafion under optimum experimental conditions, the present method revealed a good linear response for determination of norfloxacin in the range of  $1 \times 10^{-8}$  mol/L– $7 \times 10^{-6}$  mol/L with a detection limit of  $5 \times 10^{-9}$  mol/L. The proposed method was successfully applied in the determination of norfloxacin in capsules with satisfactory results.

**Uniterms:** Norfloxacin/determination. Graphene oxide. Nafion. Voltammetry/differential pulse. Pharmaceutical formulations/quantitative analysis.

Propos-se, por essa abordagem, novo método voltamétrico, com alta sensibilidade e faixa linear de detecção mais ampla, para a determinação de norfloxacino. O sensor voltamétrico utilizado foi fabricado simplesmente por cobertura de camada de óxido de grafeno (GO) e filme de Nafion em eletrodo de carbono vítreo. A vantagem do método proposto foi a resposta eletroquímica sensível para o norfloxacino, atribuída à condutividade elétrica excelente do GO e à função acumulada do Nafion. Sob condições experimentais ótimas, o presente método revelou boa resposta linear para a determinação do norfloxacino na faixa de limite de detecção de  $1 \times 10^{-8}$  mol/L– $7 \times 10^{-6}$  mol/L. O método proposto foi aplicado com sucesso na determinação de norfloxacino em cápsulas, com resultados satisfatórios.

**Uniterms:** Norfloxacino/determinação. Óxido de grafeno. Nafion. Voltametria de pulso diferencial. Formulações farmacêuticas/análise quantitativa.

### INTRODUCTION

Norfloxacin, 1-ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolonecarboxylic acid (NFX), is considered to be the first commercially available member of modern fluoroquinolones (Hooper *et al.*, 1986; Zhang *et al.*, 2013). It is effective against gram-positive and gram-negative bacteria by inhibition of their NAD gyrase, a critical enzyme to bacterial chromosome replication (Kim, Noh, Park, 2013; Marians, Hiasa, 1997). It is also widely used in treatment of respiratory tract and urinary tract infections (Leigh, Smith, Marriner, 1984). Therefore, the widespread

use of NFX in clinic and pharmacological study require fast and sensitive analytical methods for its quantitative determination. For this purpose, some methods have been developed for determination of NFX, such as high-performance liquid chromatography (HPLC) (Samanidou, Demetriou, Papadoyannis, 2003; Delepine, Hurtaud-Pessel, Sanders, 1998), spectrophotometry (Rahman, Ahmad, Hejaz, 2004; Amin, Elsayed, Issa, 1995), spectrofluorimetry (Espinosa-Mansilla *et al.*, 2004; Espinosa-Mansilla *et al.*, 2005) and capillary electrophoresis (CE) (Barrón *et al.*, 2003; Barrón *et al.*, 2001). Although these methods are proven and widely accepted, they require advanced technical expertise and are expensive and time-consuming. By comparison, electrochemical method is less expensive and has additional advantages, such as fast response speed, simple operation, timesaving, high sensitivity, and real-

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time detection in clinical and drug control. Based on these reasons, some electrochemical methods for determination of NFX were developed more recently (Agrawal *et al.*, 2013; Devaraj, Deivasigamani, Jeyadevan, 2013; Moreira, Freitas, Sales, 2011). Shim and co-workers (Agrawal *et al.*, 2013) used a multi-walled carbon nanotube (MWCNT) modified pyrolytic graphite (MPG) electrode for determination of NFX. At this sensor, the detection linear range was from  $1.2 \times 10^{-6}$  mol/L to  $1.0 \times 10^{-3}$  mol/L with detection limit of  $4.06 \times 10^{-8}$  mol/L. Devaraj, Deivasigamani, Jeyadevan (2013) reported a CuO nanoleaves composited MWCNTs film modified electrode for determination of NFX with detection limit of  $3.21 \times 10^{-7}$  mol/L and linear range of  $1 \times 10^{-6} \sim 4.77 \times 10^{-5}$  mol/L. Some of the described methods for determination of NFX possess enough sensitivity, but usually have narrow linear range, and vice versa. So to develop a new electrochemical method for determination of NFX with high sensitivity and wider linear range is still interesting and significant.

Because of its excellent conductivity and good chemical stability (Stankovich *et al.*, 2006; Chattopadhyay *et al.*, 2008), Graphene oxide (GO) is widely used as nanomaterials in chemically modified electrode. As a kind of new voltammetric sensor, it possess excellent properties in sensitive sensing for many analytes, such as dopamine and paracetamol (Cheemalapati *et al.*, 2013), cancer markers (Wu *et al.*, 2014), glucose (Yang *et al.*, 2013) and so on. In experiment, we found that GO composited Nafion film modified glassy carbon electrode (GO/Nafion/GCE) had sensitive voltammetric response for NFX. Based on this property, a new voltammetric method for determination of NFX was proposed with high sensitivity and wider detection linear range. In investigation, the long-time stability of GO/Nafion/GCE was from the good film-forming property of Nafion, which stably fixed the GO on GCE surface. The high sensitivity of detection was from both the good electrochemical response of GO and accumulating action of Nafion for NFX. The wider detection linear range might profit from the large surface area of GO/Nafion film. Finally for appraising the accuracy of proposed method, determination of NFX was performed in NFX capsules with standard content and the results were satisfactory.

## MATERIAL AND METHODS

### Reagents and chemicals

Nafion (5%, w/v in alcoholic solution) was purchased from Alfa and diluted to 0.1% (w/v) with ethanol before use. Norfloxacin (NFX) was purchased from National

Institutes for Food and Drug Control and used as received. Stock solution of NFX ( $1 \times 10^{-3}$  mol/L) was prepared using 0.1 mol/L hydrochloric acid. All other chemicals used were of analytical grade and the experimental water was double-distilled. Sulfuric acid (pH 1.2) was used as supporting electrolyte.

### Apparatus

The electrochemical measurements were performed with a RST5000 electrochemical system (Zhengzhou Shiruisi Instrument Co. Ltd., China), and a CHI650A electrochemical workstation (CHI Instrumental, Shanghai, China) was used for the EIS measurements. All experiments were carried out using a conventional three-electrode system. A bare or modified GCE (working area  $3\text{mm}^2$ ) served as working electrode, a platinum wire and an Ag/AgCl electrode were employed as the auxiliary and reference electrodes, respectively. 5500 Atomic Force Microscopy (Agilent, United States) was employed to characterize the morphology of GO.

### Preparation of film-modified GCE

Graphite oxide was synthesized using graphite power mainly according to the method described in literature (Kovtyukhova *et al.*, 1999). Then exfoliation of graphite oxide to GO was carried out by ultrasonating of the dispersion for 40 min (1000 W, 20% amplitude) according to the reference (Ehret; Baumann; Brischwein, 1997). A homogeneous aqueous dispersion of GO ( $2.3\text{ mg mL}^{-1}$ ) was obtained as a result. For preparation of GO/Nafion modified electrode, GO (0.5 mL) was added in 4.5 mL Nafion ethanol solution (0.1%, w/v) and kept sonicating for 30 min. The GO/Nafion composite solution was obtained. Before modification, a bare GCE was first treated according to standard methods and then cleaned ultrasonically with 1:1 nitric acid, alcohol and double-distilled water sequentially, dried under nitrogen atmosphere. The GO/Nafion modified GCE was fabricated by dropping  $5\mu\text{L}$  GO/Nafion composite solution on the GCE surface and dried naturally. A symbol of GO/Nafion/GCE was denoted for this film electrode, which was preserved in 0.01 mol/L phosphate solution (pH 7.0) under  $4^\circ\text{C}$  when it was not used. For comparison, a Nafion modified GCE was fabricated using the same way and named Nafion/GCE.

### Analytical procedure

Unless otherwise statement,  $\text{H}_2\text{SO}_4$  (pH 1.2) solution

was used as supporting electrolyte in determination of NFX. The accumulation step was carried out under open-circuit while stirring the solution for 120 s, and then the differential pulse voltammetry (DPV) from 0.50 to 1.65 V were performed after 5 s quiet time. Between two determinations, the solution needs to be agitated a while for reviving the electrode surface. Thus, the oxidation peak current in the first anodic sweep was used in all data studies. All measurements were carried out at room temperature ( $25 \pm 1$  °C).

### Procedure for pharmaceutical preparations and sample analysis

The average capsule weights was calculated from the weight of 10 capsules. An accurate weight of sample containing 100 mg NFX was weighed from the homogenized power and then the sample was diluted to 100 mL with 0.01 mol/L HCl. The mixture was filtered, and 1 mL of the filtrate solution was diluted to 50 mL, then 0.1 mL of this dilute sample was transferred to the voltammetric cell containing 10 mL of  $H_2SO_4$  (pH 1.2). The differential pulse voltammetry (DPV) were then performed after 120 s preconcentration time at open circuit condition. The content of NFX in capsule was determined referring to the regression equation.

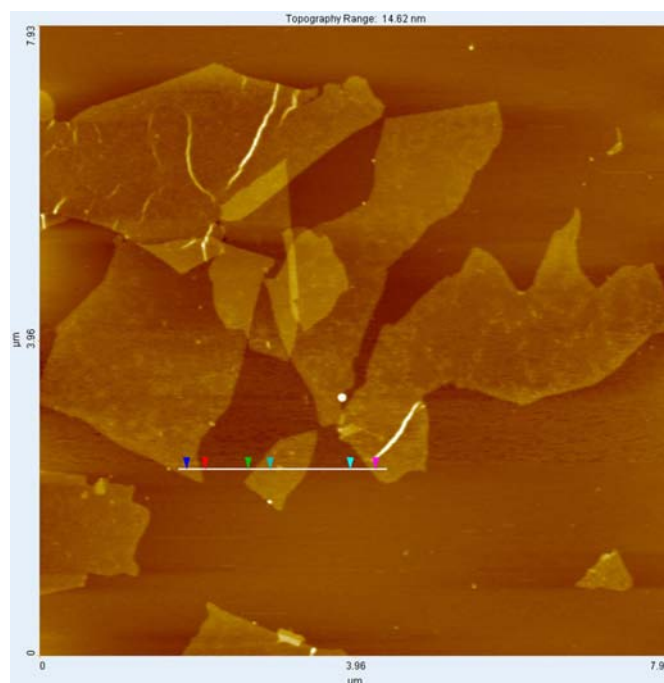
## RESULTS AND DISCUSSION

### The morphology of synthetic GO

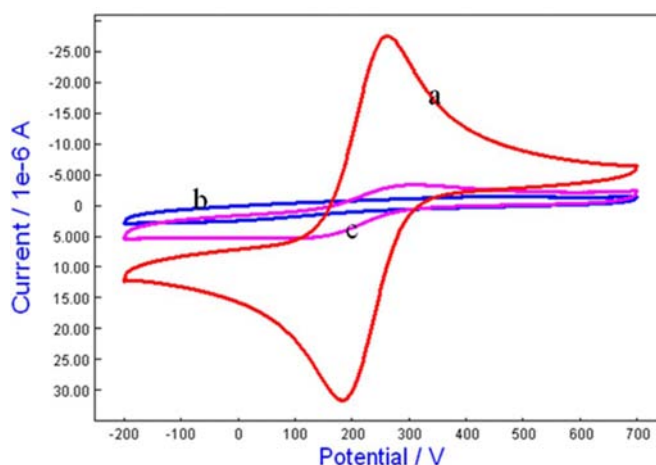
The GO slice was made according to the reference (Kovtyukhova *et al.*, 1999). The structure and morphology of obtained GO was observed by the atomic force microscope (AFM) technique. Figure 1 shows the GO sheet, which was almost single-layer and approximately 1 nm in thickness.

### Characterization of fabricated GO/Nafion/GCE

The  $K_3[Fe(CN)_6]$  is usually used as an electrochemical probe to characterize the properties of modified electrode surface. Figure 2 shows the relative cyclic voltammograms (CVs) of  $K_3[Fe(CN)_6]$  ( $2 \times 10^{-3}$  mol/L containing 0.1 mol/L KCl) with three different electrodes (bare GCE, Nafion/GCE, GO/Nafion/GCE). A pair of well-defined redox peaks was observed at the bare GCE (curve a). After modifying the GCE with Nafion, no recognizable redox peak was observed (curve b), which could be attributed to the electrostatic interaction between negatively charged Nafion and  $Fe(CN)_6^{3-}$ , blocking the  $Fe(CN)_6^{3-}$  reached



**FIGURE 1** - Image of GO from its dilute aqueous dispersion on freshly cleaved mica characterized by AFM.

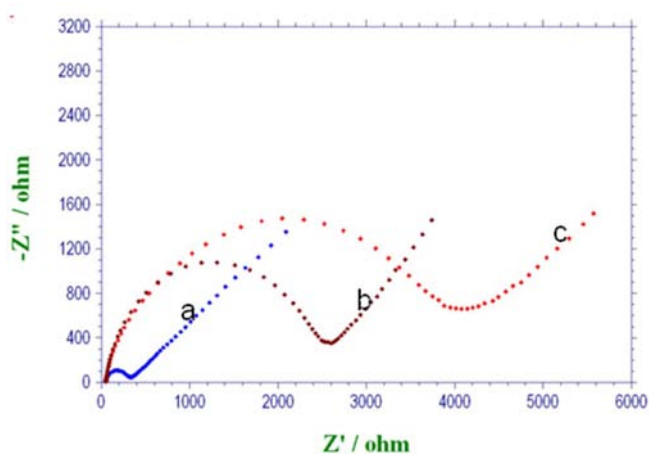


**FIGURE 2** - Cyclic voltammograms of  $K_3[Fe(CN)_6]$  ( $2.0 \times 10^{-3}$  mol/L) at (a) bare GCE, (b) Nafion/GCE, (c) GO/Nafion/GCE. Supporting electrolyte: 0.1 mol/L KCl solution; Scan rate:  $0.1 \text{ V s}^{-1}$ .

electrode surface. However, when little of GO was mixed in the Nafion film (GO/Nafion/GCE), a pair of redox peaks was apparent again (curve c), showing GO excellent electrochemical conductivity toward the rare ferricyanide diffused to the electrode surface.

Next, the electrochemical impedance spectroscopy (EIS) was employed again to evaluate the fabricated electrodes. The EIS is an effective method for probing the features of surface modified electrodes.

Figure 3 shows the Nyquist plots of EIS obtained in  $5.0 \times 10^{-3}$  mol/L  $\text{Fe}(\text{CN})_6^{4-/3-}$  (1:1) containing 0.10 mol/L KCl with bare GCE (a), Nafion/GCE (c) and GO/Nafion/GCE (b) respectively. It can be seen that the electron transfer resistance ( $R_{ct}$ ) of the Nafion/GCE was much larger than that of the bare GCE (a), demonstrating that Nafion blocked the  $[\text{Fe}(\text{CN})_6]^{3-/4-}$  to the electrode surface effectively. However, the  $R_{ct}$  decreased remarkably when a little of GO was mingled in Nafion film, suggesting the GO possessed good conductivity and made the electron transfer easier. By fitting the data using an appropriate equivalent circuit, the  $R_{ct}$  obtained was about 250  $\Omega$ , 3362  $\Omega$ , and 2228  $\Omega$  for GCE, Nafion/GCE and GO/Nafion/GCE, respectively. These data demonstrated that the electrode was modified just as design.



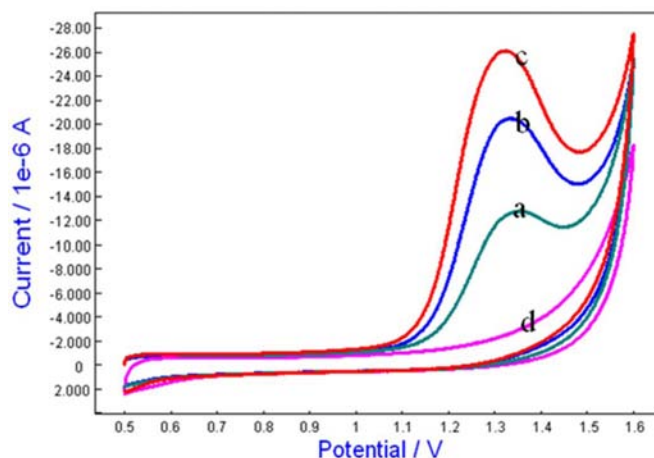
**FIGURE 3** - Nyquist plots of  $5.0 \times 10^{-3}$  mol/L  $\text{Fe}(\text{CN})_6^{4-/3-}$  (1:1) with 0.10 mol/L KCl recorded at bare GCE (a), Nafion/GCE (c) and GO/Nafion/GCE (b).

### Electrochemical behavior of NFX at GO/Nafion/GCE

Figure 4 shows the cyclic voltammetric responses of NFX ( $1 \times 10^{-6}$  mol/L) at bare GCE (Figure 4a), Nafion/GCE (Figure 4b) and GO/Nafion/GCE (Figure 4c) in  $\text{H}_2\text{SO}_4$  medium. Figure 4d is a CV curve of GO/Nafion/GCE in blank solution. Under the same conditions, a relative weak anodic peak of NFX was observed at the bare GCE with peak potential of 1.341 V, and the peak currents increased strikingly at both Nafion/GCE and GO/Nafion/GCE. Especially using the GO/Nafion/GCE, the peak current was significantly higher and the peak potential shifted to 1.325 V. The reasons for the notable sensitivity of the NFX reaction at the GO/Nafion/GCE may be summarized as follows: (1) NFX becomes positively charged (protonation) in the  $\text{H}_2\text{SO}_4$  medium, and the GO/Nafion/

GCE contains the cation exchanger of Nafion, which has an enriched ability due to an electrostatic interaction; (2) the interfusion of GO into Nafion would provide an effective means to improve the sensitivity due to the excellent conductivity of GO. The peak current enhanced and peak potential negatively shifted demonstrate the excellent electrochemical response of GO toward NFX. Without any doubt, the synergetic functions of Nafion and GO make contributions to the higher sensitivity for NFX as well as long-term stability of modified electrode.

It also showed that no reduction peak was observed in the reverse scan, suggesting that the electrochemical reaction of NFX was an irreversible process. For continuous cyclic scan, the best anodic peak was only obtained in the first cycle and the peak current decreased in following cycles, and tends to disappear completely (data not shown). This result demonstrated that the product of NFX oxidation was electro-inactive and adsorbed on the electrode surface to passivate the electrode. To renovate the electrode surface, a very simple method was discovered, that is, agitating the solution for about 1 min after a sweep and the peak current would be revived as the first cycle again in next scan. Thus, the oxidation peak current in the first anodic sweep was used in following experiments.



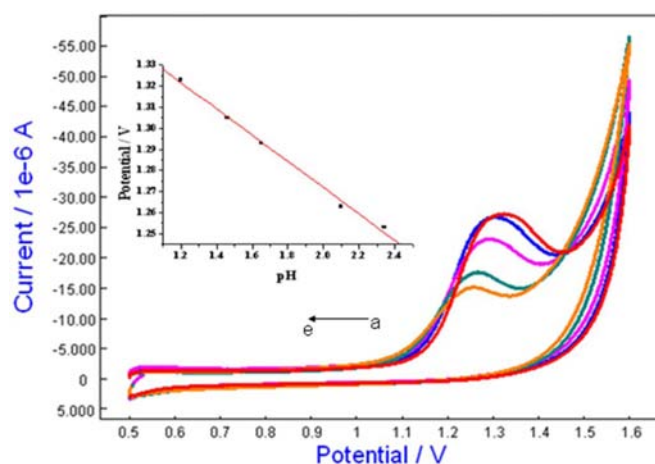
**FIGURE 4** - Cyclic voltammograms of NFX ( $1 \times 10^{-6}$  mol/L) at: (a) Bare GCE; (b) Nafion/GCE and (c) GO/Nafion/GCE. (d): GO/Nafion/GCE in a blank solution. Supporting electrolyte:  $\text{H}_2\text{SO}_4$  (pH 1.2); Scan rate:  $0.05 \text{ V s}^{-1}$ .

### Influence of supporting electrolyte and pH

The electrode response of NFX might be affected by supporting electrolyte and the solution pH. Some electrolytes, such as phosphate buffer, Britton-Robinson, acetate buffer, ammonium-hydrochloric buffer,  $\text{H}_2\text{SO}_4$  and HCl were investigated and the best results was obtained in

H<sub>2</sub>SO<sub>4</sub> solution. Then, the electrode response of NFX was investigated in different pH of H<sub>2</sub>SO<sub>4</sub> solution from 1.20 to 2.56. As shown in Figure 5, the peak currents decreased and peak potentials shifted negatively by increasing of solution pH, meaning that the electrode reaction of NFX oxidation was a losing proton process. pH 1.2 H<sub>2</sub>SO<sub>4</sub> solution was selected as the optimal supporting electrolyte and used throughout following study.

Abstracting the data from Figure 5, the relationship between peak potentials and solution pH was linear with a slope of 0.0624. These data elucidated that the electron number and the proton number taking part in this reaction was equal.



**FIGURE 5** - The cyclic voltammograms of NFX ( $1.0 \times 10^{-6}$  mol/L) at GO/Nafion/GCE in different pH solution of H<sub>2</sub>SO<sub>4</sub> (a → e): 1.20, 1.46, 1.65, 2.10, 2.34; Scan rate: 0.05 V s<sup>-1</sup>. The insert is the  $E_p \sim \text{pH}$  relation.

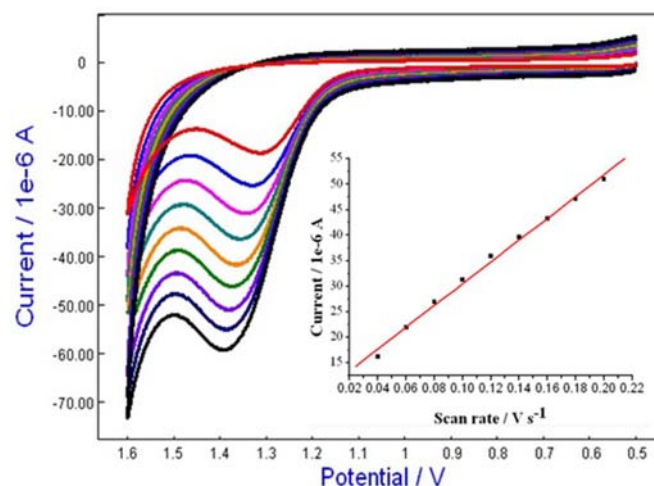
### Influence of scan rates

The effect of scan rate ( $\nu$ ) on the peak currents and potentials of NFX oxidation was further investigated in a wide range. Figure 6 shows the superimposed voltammograms under different scan rates. The solution need to be agitated about 1min between two cyclic scans. Obviously, the anodic peak potential shifted towards more positive and the peak currents enhanced markedly as the scan rate increasing. The peak current showed a linear relationship with respect to the scan rates (inset), indicating the oxidation process of NFX at the GO/Nafion/GCE was adsorption-controlled. Based on Laviron's theory (Laviron *et al.*, 1979), the  $i_p \sim \nu$  relation can be described as following equation:  $i_p = n^2 F^2 \nu A \Gamma / 4RT = nFQ\nu / 4RT$ . From this equation, we know that the electron number ( $n$ ) can be calculated as long as the peak current ( $i_p$ ) and peak area ( $Q$ ) are obtained under certain scan rate ( $\nu$ ). Based on this theory, a value of

$n=2$  was calculated. Similarly, for an irreversible electrode reaction driven by adsorption, the following equation is taken from Laviron theory (Laviron, 1979):

$$E_p = E^0 + \frac{RT}{anF} \ln \frac{RT}{anF} \cdot \frac{K_s}{\nu} \quad (1)$$

From the slope of the straight line of  $E_p$  against  $\ln \nu$ ,  $\alpha n = 0.94$  could be obtained. Thus, the value of  $\alpha$  (charge transfer coefficient) was 0.47. Meanwhile, the value of  $k_s$  of 0.28 s<sup>-1</sup> was calculated from the intercept of  $E_p$  vs.  $\ln \nu$  straight line. The value of  $E^0$  was determined to be 1.045 V from intercept of  $E_p$  versus  $\nu$  plot on the ordinate by extrapolating the line to be  $\nu = 0$ .



**FIGURE 6** - Cyclic voltammograms of NFX ( $1 \times 10^{-6}$  mol/L) at GO/Nafion/GCE in H<sub>2</sub>SO<sub>4</sub> (pH 1.2) with different scan rates. Scan rates (from curve a to i): 0.04, 0.06, 0.08, 0.1, 0.12, 0.14, 0.16, 0.18, and 0.2 V s<sup>-1</sup>. The insert was the  $i_p \sim \nu$  relation.

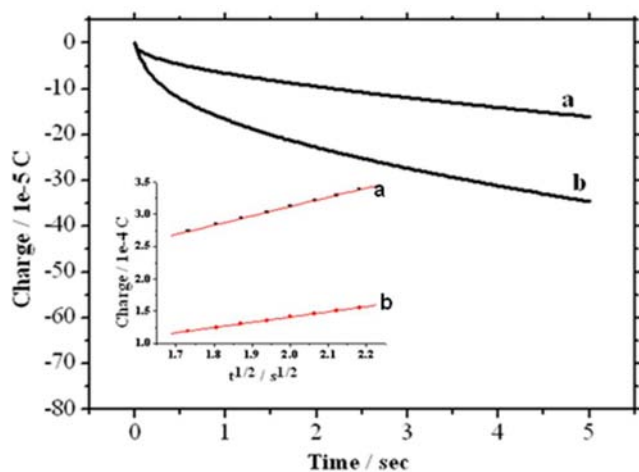
### Determination of saturating absorption capacity

For an adsorptive controlled electrode process, it is necessary to calculate the saturating absorption capacity ( $\Gamma^*$ ) of analyte on the electrode surface, which is related with the maximum peak current in determination. The chronocoulometry is the best technique to do this work. For this system, the GO/Nafion/GCE was immersed in a NFX solution ( $1 \times 10^{-6}$  mol/L) for several minutes to achieve saturated absorption. And then, a step potential from 0.5 V to 1.5 V was applied and  $Q \sim t$  curve was recorded (Figure 7, curve b). For comparison,  $Q \sim t$  curve was also recorded in blank sulfuric acid solution (Figure 7, curve a). The corresponding  $Q \sim t^{1/2}$  plots were also performed and shown as an inset in Figure 7. From the inset, the  $Q \sim t^{1/2}$  curves of a and b were almost parallel, meaning that the diffusion was

non-existent during the NFX electrode process. According to the formula given by Anson (Anson, 1964):

$$Q = \frac{2nFAc(Dt)^{1/2}}{1/2} + Q_{dl} + Q_{ads} \quad (2)$$

$Q_{dl}$  is double-layer charge;  $Q_{ads}$  is the Faradaic charge due to the oxidation of adsorbed NFX, which is the intercept difference of curves a and b. Using Laviron's theory of  $Q = nFA\Gamma^*$ , a value of  $7.2 \times 10^{-11}$  mol/mm<sup>2</sup> was calculated for  $\Gamma^*$ , which was the saturating absorption capacity of NFX at the GO/Nafion/GCE surface.



**FIGURE 7** - Chronocoulometric curves of the background (curve a) and  $1.0 \times 10^{-6}$  mol/L NFX (curve b) at GO/Nafion/GCE; Inset shows the relationship of the corresponding  $Q-t^{1/2}$  plots.

## Analytical applications and methods validation

### Influence of accumulation time

For consideration of adsorption of NFX on the GO/Nafion/GCE surface, an accumulation step could be used for increasing the detection sensitivity. It was important to fix the accumulation time ( $t_{acc}$ ) for an adsorption-driven electrode process. In this investigation, a concentration of  $1 \times 10^{-7}$  mol/L NFX solution was used and the peak currents were linearly increased with  $t_{acc}$  prolonging within 10 s to 110 s. Then, a platform was appearance. However, to obtain lower detection limit, 120 s was selected as the  $t_{acc}$  in this investigation. The potential during the accumulation period was little effect on the peak current. So the  $t_{acc}$  of 120 s was carried out under open circuit for investigating the calibration curve in following experiment.

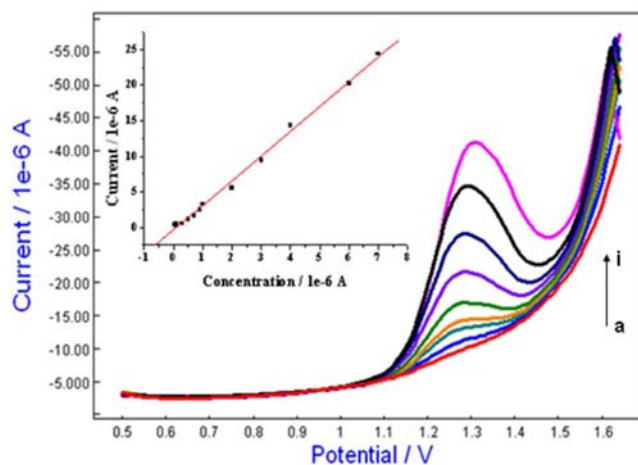
### Calibration curve, stability and reproducibility

In investigating the calibration curve, the differential pulse voltammetry (DPV) was employed during the

stripping process for enhancing the detection sensitivity. Under the optimized conditions mentioned above, a good linear relationship between  $i_p$  and NFX concentrations was obtained in the range of  $1.0 \times 10^{-8} \sim 7.0 \times 10^{-6}$  mol/L. A superimposed DPV curves (part curves shown) was shown in Figure 8 and the linear relation was also shown in Figure 8 as an inset. The linear regression equation and correlation coefficient were:

$$i_p (\mu A) = -0.343 + 3.469 C (\mu M) (R = 0.9978)$$

where  $i_p$  was the oxidation peak current in  $\mu A$  and  $C$  was the concentration of NFX in  $\mu mol/L$ . Based on the signal-to-noise ratio of 3 (S/N=3), the detection limit was calculated as  $5 \times 10^{-9}$  mol/L. The values of  $5 \times 10^{-9}$  mol/L detection limit and  $3.469 \mu A/\mu M$  current response demonstrate the high sensitivity of proposed method.



**FIGURE 8**. Superimposed DPV curves of NFX with different concentrations (a  $\rightarrow$  i):  $1 \times 10^{-8}$  mol/L,  $1 \times 10^{-7}$  mol/L,  $5 \times 10^{-7}$  mol/L,  $1 \times 10^{-6}$  mol/L,  $2 \times 10^{-6}$  mol/L,  $3 \times 10^{-6}$  mol/L,  $4 \times 10^{-6}$  mol/L,  $6 \times 10^{-6}$  mol/L,  $7 \times 10^{-6}$  mol/L. Inset: linear relationship of NFX concentrations versus peak currents.

Compared with some reported analytical methods (Table I), the proposed GO/Nafion/GCE is more sensitive with wider linear range. The comparison of this work with others (Agrawal *et al.*, 2013; Devaraj, Deivasigamani, Jeyadevan, 2013; Moreira, Freitas, Sales, 2011) showed the advantage of GO added. This research demonstrate again that GO is an excellent modification material in voltammetric sensor.

The intra-assay precision of the GO/Nafion/GCE response for NFX was evaluated by assaying one modified electrode for ten replicate determination of NFX ( $1 \times 10^{-6}$  mol/L). The relative standard deviation (RSD) of the peak currents was calculated to be 2.2%. Similarly, the

**TABLE I** - Merits of comparable methods for determination of norfloxacin

Method	Detection limit (mol/L)	Analytical range (mol/L)	Comments	Reference
Spectrophotometry	$3.5 \times 10^{-7}$	$9.4 \times 10^{-6} - 3.1 \times 10^{-5}$	ternary formation with eosin and palladium(II)	(Walily <i>et al.</i> , 1996)
HPLC	$6.8 \times 10^{-6}$	$1.6 \times 10^{-5} - 6.2 \times 10^{-5}$	UV-detection at 278 nm	(Córdoba-Borrego <i>et al.</i> , 1999)
CE	$1.0 \times 10^{-6}$	$3.1 \times 10^{-5} - 1.6 \times 10^{-4}$	UV photo-diode array detection at 301 nm	(Alnajjar <i>et al.</i> , 2007)
Voltammetry	$4.06 \times 10^{-8}$	$1.2 \times 10^{-6} - 1.0 \times 10^{-3}$	multi-walled carbon nanotube modified GCE	(Agrawal <i>et al.</i> , 2013)
Voltammetry	$3.21 \times 10^{-7}$	$1.0 \times 10^{-6} - 4.77 \times 10^{-5}$	multi-walled carbon nanotube modified GCE	(Devaraj <i>et al.</i> , 2013)
Voltammetry	$6.25 \times 10^{-5}$	$7.9 \times 10^{-5} - 2.5 \times 10^{-3}$	molecularly-imprinted materials as sensor	(Moreira <i>et al.</i> , 2011)
Voltammetry	$5.0 \times 10^{-9}$	$1.0 \times 10^{-8} - 7.0 \times 10^{-6}$	Graphene Oxide Modified GCE	This work

inter-assay precision, or fabrication reproducibility, was estimated at five GO/Nafion/GCE, which were modified in parallel. They were performed for determination of NFX ( $1 \times 10^{-6}$  mol/L) in a same solution and the obtained RSD of current response was 2.8%. The current response of NFX at the GO/Nafion/GCE remained 96% of its original response after one month, showing a longer lifetime and stability.

#### Interference

The influence of some foreign species on the determination of NFX was evaluated in detail. Expressly, some species possibly exist in biological sample. In experiment, a fixed amount of  $1.0 \times 10^{-6}$  mol/L NFX spiked with various foreign species was evaluated under the same experimental conditions. The results showed that 50 fold of uric acid, oxalic acid, citric acid, glucose, tartaric acid, vitamin C and 500 fold excess of  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Fe}^{3+}$ ,  $\text{NO}_3^-$ ,  $\text{Cl}^-$  had no interference on the current response of NFX (signal change below 5%). It suggested that the proposed method had good selectivity for the determination of NFX.

#### Determination of NFX in the medicinal capsules

In order to evaluate the practicality of GO/Nafion/GCE for determination of NFX in pharmaceutical and drug test, the content of NFX in capsule (Shanghai Yan'an Pharmaceutical Co., Shanghai, China, with fixed content of NFX) was determined following the procedure described in Section 2.5. The analytical results were shown in Table II. Furthermore, in order to evaluate the present method, the same sample was determined by UV-spectrophotometry at 275 nm and the results were also shown in Table II. From the results shown in Table II, we can see that the present method was accurate and it can be recommended for routine analysis in majority of drug quality control laboratories.

## CONCLUSIONS

In conclusion, the GO/Nafion/GCE was simply fabricated by coating of GO/Nafion suspension on GCE surface. As a voltammetric sensor, it showed sensitive response for NFX. Based on this property, a

**TABLE II** - Results for the determination of norfloxacin in pharmaceutical formulation

Samples		Certified value(mg)	Proposed method (mg)	UV-spectrophotometry (mg)
capsule	A	100	98.84	101.28
	B	100	99.82	99.60
	C	100	101.10	100.81
	D	100	101.93	100.46
	E	100	101.35	100.51
	F	100	102.94	100.45

new voltammetric method for determination of NFX was proposed. The advantages of present method were high sensitivity, wider linear range, good accuracy and precision. The long lifetime stability is another advantage of proposed sensor and it offers a good possibility for extending this method in routine analysis of NFX.

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## REFERENCES

- AGRAWAL, B.; CHANDRA, P.; GOYAL, R.N.; SHIM, Y. B. Detection of norfloxacin and monitoring its effect on caffeine catabolism in urine samples. *Biosens. Bioelectron.*, v.47, p.307-312, 2013.
- ALNAJJAR, A.; ABUSEADA, H. H.; IDRIS, A. M. Capillary electrophoresis for the determination of norfloxacin and tinidazole in pharmaceuticals with multi-response optimization. *Talanta*, v.72, n.2, p.842-846, 2007.
- AMIN, A. S.; ELSAYED, G. O.; ISSA, Y. M. Utility of certain n-acceptors for the spectrophotometric determination of norfloxacin. *Analyst*, v.120, p.1189-1193, 1995.
- ANSON, F. C. Application of potentiostatic current integration to the study of the adsorption of cobalt(III)-(ethylenedinitrilo) tetraacetate on mercury electrodes. *Anal. Chem.*, v.36, n.4, p.932-934, 1964.
- BARRÓN, D.; JIMÉNEZ-LOZANO, E.; BAILAC, S.; BARBOSA, J. Simultaneous determination of flumequine and oxolinic acid in chicken tissues by solid phase extraction and capillary electrophoresis. *Anal. Chim. Acta.*, v.477, n.1, p.21-27, 2003.
- BARRÓN, D.; JIMENEZ-LOZANO, E.; CANO, J.; BARBOSA, J. Determination of residues of enrofloxacin and its metabolite ciprofloxacin in biological materials by capillary electrophoresis. *J. Chromatogr. B.*, v.759, n.1, p.73-79, 2001.
- CHATTOPADHYAY, J.; MUKHERJEE, A.; HAMILTON, C. E.; KANG, J. H.; CHAKRABORTY, S.; GUO, W.; KELLY, K. F.; BARRON, A. R.; BILLUPS, W. E. Graphite Epoxide. *J. Am. Chem. Soc.*, v.130, n.16, p.5414-5415, 2008.
- CHEEMALAPTI, S.; PALANISAMY, S.; MANI, V.; CHEN, S. M. Simultaneous electrochemical determination of dopamine and paracetamol on multiwalled carbon nanotubes/graphene oxide nanocomposite-modified glassy carbon electrode. *Talanta*, v.117, p.297-304, 2013.
- CORDOBA-BORREGO, M.; CORDOBA-DIAZ, M.; CORDOBA-DIAZ, D. Validation of a high-performance liquid chromatographic method for the determination of norfloxacin and its application to stability studies (photo-stability study of norfloxacin). *J. Pharm. Biomed. Anal.*, v.18, n.6, p.919-926, 1999.
- DELEPINE, B.; HURTAUD-PESSEL, D.; SANDERS, P. Simultaneous determination of six quinolones in pig muscle by liquid chromatography-atmospheric pressure chemical ionisation mass spectrometry. *Analyst*, v.123, n.12, p.2743-2747, 1998.
- DEVARAJ, M.; DEIVASIGAMANI, R. K.; JEYADEVAN, S. Enhancement of the electrochemical behavior of CuO nanoleaves on MWCNTs/GC composite film modified electrode for determination of norfloxacin. *Colloids Surf. B: Biointerfaces*, v.102, p.554-561, 2013.
- EHRET, R.; BAUMANN, W.; BRISCHWEIN, M. Monitoring of cellular behaviour by impedance measurements on interdigitated electrode structures. *Biosens. Bioelectron.*, v.12, n.1, p.29-41, 1997.
- ESPINOSA-MANSILLA, A.; MUNOZ DE LA PENA, A.; GONZALEZ GOMEZ, D.; SALINAS, F. HPLC determination of enoxacin, ciprofloxacin, norfloxacin and ofloxacin with photoinduced fluorimetric (PIF) detection and multiemission scanning: Application to urine and serum. *J. Chromatogr. B.*, v.822, n.1-2, p.185-193, 2005.
- ESPINOSA-MANSILLA, A.; MUNOZ DE LA PENA, A.; SALINAS, F.; GONZALEZ GOMEZ, D. Partial least squares multicomponent fluorimetric determination of fluoroquinolones in human urine samples. *Talanta*, v.62, n.4, p.853-860, 2004.
- HOOPER, D. C.; WOLFSON, J. S.; SOUZA, K. S.; TUNG, C.; MCHUGH, G. L.; SWARTZ, M. N. Genetic and biochemical characterization of norfloxacin resistance in *Escherichia coli*. *Antimicrob. Agents Chemother.*, v.29, n.4, p.639-644, 1986.



- KIM, J.; NOH, J.; PARK, W. Insight into norfloxacin resistance of *Acinetobacter oleivorans* DR1: target gene mutation, persister, and RNA-Seq analyses. *J. Microbiol. Biotechnol.*, v.23, n.9, p.1293-1303, 2013.
- KOVTYUKHOVA, N.I.; OLLIVIER, P.J.; MARTIN, B. R.; MALLOUK, T. E.; CHIZHIK, S. A.; VUZANEVA, E. V.; GORCHINSKIY, A. D. Layer-by-Layer Assembly of Ultrathin Composite Films from Micron-Sized Graphite Oxide Sheets and Polycations. *Chem. Mater.*, v.11, n.3, p.771-778, 1999.
- LAVIRON, E. General expression of the linear potential sweep Voltammogram in the case of diffusionless Electrochemical systems. *J. Electroanal. Chem.*, v.101, n.1, p.19-28, 1979.
- LEIGH, D. A.; SMITH, E. C.; MARRINER, J. Comparative study using norfloxacin and amoxicillin in the treatment of complicated urinary tract infections in geriatric patients. *J. Antimicrob. Chemother.*, v.13, p.79-83, 1984. Suppl. B
- MARIANS, K. J.; HIASA, H. J. Mechanism of Quinolone Action: A drug-induced structural perturbation of the DNA precedes strand cleavage by topoisomerase IV. *Biol. Chem.*, v.272, n.14, p.9401-9409, 1997.
- MOREIRA, F. T. C.; FREITAS, V.A. P.; SALES, M. G. F. Biomimetic norfloxacin sensors made of molecularly-imprinted materials for potentiometric transduction. *Microchim. Acta*, v.172, n.1-2, p.15-23, 2011.
- RAHMAN, N.; AHMAD, Y.; HEJAZ, S. N. Kinetic spectrophotometric method for the determination of norfloxacin in pharmaceutical formulations. *Eur. J. Pharm. Biopharm.*, v.57, n.2, p.359-367, 2004.
- SAMANIDOU, V.F.; DEMETRIOU, C. E.; PAPADOYANNIS, I. N. Direct determination of four fluoroquinolones, enoxacin, norfloxacin, ofloxacin, and ciprofloxacin, in pharmaceuticals and blood serum by HPLC. *Anal. Bioanal. Chem.*, v.375, n.5, p.623-629, 2003.
- STANKOVICH, S.; DIKIN, D. A.; DOMMETT, G. H.; KOHLHAAS, K.M.; ZIMNEY, E.J.; STACH, E.A.; PINER, R.D.; NGUYEN, S.T.; RUOFF, R.S. Graphene-based composite materials. *Nature*, v.442, p.282-286, 2006.
- WALILY, A. F.; BELAL, S. F.; BARKRY, R. S. Spectrophotometric and spectrofluorimetric estimation of ciprofloxacin and norfloxacin by ternary complex formation with eosin and palladium(II). *J. Pharm. Biomed. Anal.*, v.14, n.5, p.561-569, 1996.
- WU, D.; GUO, A. P.; GUO, Z. K.; XIE, L.; WEI, Q.; DU, B. Simultaneous electrochemical detection of cervical cancer markers using reduced graphene oxide-tetraethylene pentamine as electrode materials and distinguishable redox probes as labels. *Biosens. Bioelectron.*, v.54, p.634-639, 2014.
- YANG, J.; YU, J. H.; STRICKLER, J. R.; CHANG, W. J.; GUNASEKARAN, S. Nickel nanoparticle-chitosan-reduced graphene oxide-modified screen-printed electrodes for enzyme-free glucose sensing in portable microfluidic devices. *Biosens. Bioelectron.*, v.47, p.530-538, 2013.
- ZHANG, H. Z.; DAMU GURI, L. V.; CAI, G. X.; ZOU, C.-H. Design, synthesis and antimicrobial evaluation of novel benzimidazole type of Fluconazole analogues and their synergistic effects with chloramphenicol, norfloxacin and fluconazole. *Eur. J. Med. Chem.*, v.64, p.329-344, 2013.

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