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Differential pulse voltammetric determination of nimesulide in pharmaceutical formulation and human serum at glassy carbon electrode modified by cysteic acid/CNTs based on electrochemical oxidation of L-cysteine

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Abstract

Carbon nanotubes (CNTs) and cysteic acid based on electrochemical oxidation of L-cysteine (CySH) to form a novel composite thin film material at a glassy carbon electrode (GCE) for electroanalytical determination of nimesulide. The determination of nimesulide at the composite modified electrode with strong accumulation of nimesulide was studied by differential pulse voltammetry (DPV). The peak current obtained at +1.251 V (versus SCE) from DPV was linearly dependent on the nimesulide concentration in the range of 1.0×10^{-7}–1.0×10^{-5} M in 0.05 M H_2SO_4 solution with a correlation coefficient of 0.997. The detection limit (S/N = 3) was found to be 5.0×10^{-8} M. The low-cost modified electrode showed good sensitivity, selectivity, stability and had been applied to the determination of nimesulide in pharmaceutical formulation and human serum samples with satisfactory results.
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Keywords: Carbon nanotube; Cysteic acid; L-cysteine; Nimesulide; Voltammetry; Human serum

1. Introduction

Nimesulide, (Scheme 1) *N*-(4-nitro-2-phenoxiphenyl) methane sulfonamide ($C_{13}H_{12}N_2O_2S$) is a relatively new non-steroidal anti-inflammatory drug (NSAID) that is selective for cyclooxygenase-2 (COX-2). It has the potential to reduce the incidence of formation of gastrointestinal ulcers. It is also well tolerated in comparison to aspirin and is known to exhibit better efficacy than diclofenac and piroxicam [1]. Nimesulide's pK_a value of 6.5 is very important for gastric tolerability, as it avoids the back diffusion of hydrogen ions responsible for tissue damage [2]. As for nimesulide, it is not yet official in any pharmacopoeia and has been determined by spectrophotometry [3,4], capillary zone electrophoresis [5], HPLC with UV [6] or a glassy carbon electrode [7] as a detector. For biological fluid samples, a thin-layer chromatographic method was used for the determination of nimesulide in plasma [8], and two HPLC methods were developed to detect nimesulide in human plasma and urine [9,10]. What's more, many of the methods mentioned above required several time-consuming manipulation steps, sophisticated instruments and special training. For these reasons, a rapid, simple and accurate method with high sensitivity is expected to be established.

Electrochemical detection of analyte is a very elegant method in analytical chemistry. The interest in developing electrochemical-sensing devices for use in environmental monitoring, clinical assays or process control is growing rapidly. Electrochemical sensors satisfy many of the requirements for such tasks particularly owing to their inherent specificity, speed of response, sensitivity and simplicity of preparation. Up to date, there are only a few available electrochemical methods for the determination of nimesulide in the literatures including polarography [11], adsorptive stripping voltammetry with the mercury electrodes [2]. However, the utilization of the mercury electrodes would contaminate the environment because of their environmental toxicity. Catarino et al. proposed

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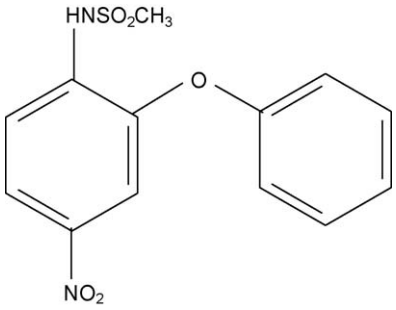

Scheme 1. Nimesulide.

an amperometric method with a glassy carbon electrode for the determination of nimesulide [12,13], and the same linear ranges were 5.0×10^{-5}–3.0×10^{-4} M with the detection limit of 3.1×10^{-6} M. These electrochemical methods were only described for the determination of nimesulide in pharmaceutical formulations. There is no report for quantitative determination of nimesulide in biological media by the electrochemical method.

Since carbon nanotubes (CNTs) were discovered in 1991, they have attracted much attention of researchers [14]. Some progress in application of CNTs as biosensors has been made, which greatly benefits from the ability of carbon nanotubes to promote the electron-transfer reactions of important biomolecules, including enzymes, DNA and proteins et al. [15]. CNTs modified electrodes have been proved to have excellent electroanalytical properties, such as wide potential windows, low background current and good biocompatibility. Different types of CNTs modified electrodes have been reported, including carbon nanotube paste electrodes [16], carbon nanotube-intercalated graphite electrodes [17] and CNTs film coated electrodes [18,19], etc. Recently, conducting polymer/CNTs composites have received significant interest, because the incorporation of CNTs into conducting polymers can lead to new composite materials possessing the properties of each component with a synergistic effect that would be useful in particular applications [20].

In the present paper, we described the use of CNTs and cysteic acid based on electrochemical oxidation of L-cysteine (CySH) to form a novel composite thin film material at a glassy carbon electrode (GCE) for electroanalytical determination of nimesulide in human serum. A sensitive anodic oxidative peak of nimesulide at +1.251 V was used for quantitative determination by using differential pulse voltammetry. A good linear relationship was realized between the anodic peak current and nimesulide concentrations in the range of 1.0×10^{-7}–1.0×10^{-5} M with the detection limit of 5.0×10^{-8} M. Compared with the amperometric method with the glassy carbon electrode [12,13], the detection limit of this method decreased two orders of magnitude. The modified electrode showed significantly enhanced accumulation of nimesulide compared with a bare glassy carbon electrode. This method has the advantages of rapid and simple operation, very low interference and high accuracy in the serum for the determination of nimesulide. This cysteic acid/CNTs composite film is considered to be a promising, low-cost, steady and biocompatible material for the modification of electrodes.

2. Experimental

2.1. Apparatus

Cyclic voltammetry (CV) and differential pulse voltammetry (DPV) experiments were carried out at CHI 660B electrochemical workstation (Chenhua Instruments, China). All electrochemical experiments employed a conventional three-electrode system with a glassy carbon electrode or the cysteic acid/CNTs modified glassy carbon electrode (3.0 mm in diameter) as a working electrode, a platinum wire as an auxiliary electrode and a saturated calomel electrode as a reference electrode. All potentials reported in this paper were referenced to the saturated calomel electrode (SCE). All experiments were carried out at 25 °C.

2.2. Chemicals and solutions

L-cysteine (CySH) was obtained from Shanghai Chemical Reagent Co., Ltd. (Shanghai, China). Nimesulide was purchased from Sigma (Milwaukee, Wisconsin, USA). The multi-wall carbon nanotubes (30–50 nm) were obtained from Chengdu Organic Chemicals Co., Ltd., Chinese Academy of Sciences (Chengdu, China). Nafion (5 wt% in alcohols) was purchased from Fluka (Milwaukee, Wisconsin, USA). All the other reagents used were of analytical grade. Doubly distilled water was obtained by purification through a Millipore water system and used throughout. A stock anhydrous ethanol solution of nimesulide (1.0×10^{-3} M) was prepared, and kept in the dark under refrigeration (below 4 °C). A solution of 0.05 M H_2SO_4 was used as a supporting electrolyte for the determination of nimesulide.

2.3. Fabrication of the cysteic acid/CNTs modified glassy carbon electrodes

Before modification, the glassy carbon electrode was polished, respectively with 1, 0.3 and 0.05 μm α-alumina powder, rinsed thoroughly with doubly distilled water within each polishing step, and then sonicated in 1:1 nitric acid, acetone and doubly distilled water successively. The CNTs was purified according to the previous report [14]: the CNTs was refluxed in the mixture of concentrated H_2SO_4 and HNO_3 for 24 h, then washed with double distilled water and dried in vacuum at room temperature. The CNTs suspension was prepared by dispersing 2.5 mg the CNTs in 25 ml dimethyl formamide (DMF) under sonication for 10 min. A 10 μL aliquot of the black suspension was dropped directly on glassy carbon electrode surface and left it dried under an infrared lamp. The cysteic acid/CNTs/GCE was prepared by cycling scanning the CNTs/GCE between −0.8 and +2.2 V (versus SCE) at the scan rate of 200 mV s^{-1} in 0.04 M HCl solution containing 2.5×10^{-3} M CySH with 20 consecutive cycles (Fig. 1). The modified electrode was then electroactivated by cyclic scanning from +0.6 to +1.5 V in the 0.5 M H_2SO_4

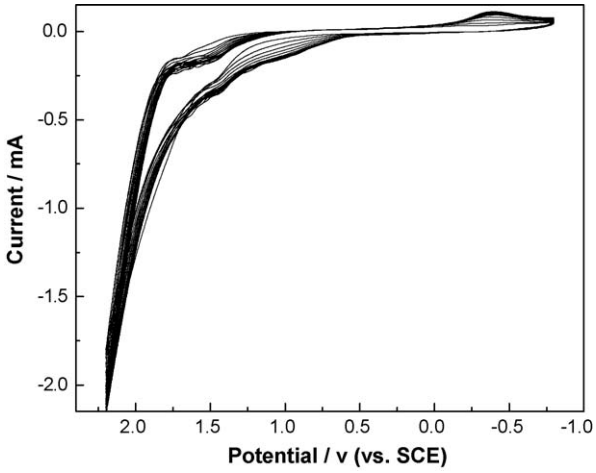

Fig. 1. Cyclic voltammograms of the CNTs/GCE in 0.04 M HCl solution containing 2.5×10^{-3} M L-cysteine (CySH). Scan rate: 200 mV s^{-1}; Scan potential: −0.8 to 2.2 V; consecutive cycle: 20.

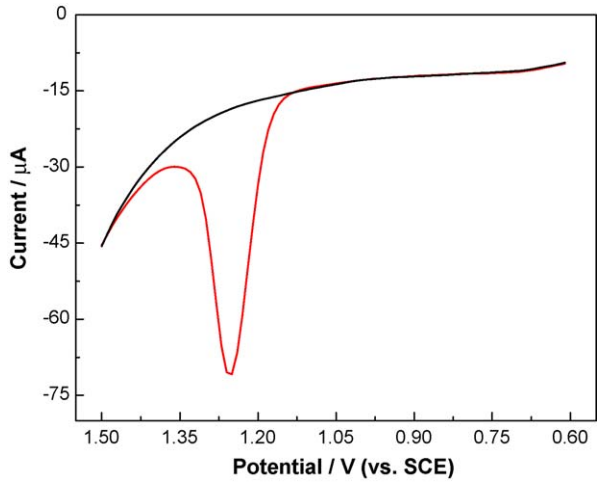

Fig. 2. Differential pulse voltammograms of 6.0×10^{-6} M nimesulide (red line) and blank (black line) at the cysteic acid/CNTs/GCE in the 0.05 M H_2SO_4 solution. Accumulation potential under stirring: + 0.0 V; accumulation time: 120 s; quiet time: 30 s; scan rate: 0.010 V s^{-1}; pulse height: 0.050 V; sampling width: 0.05 s; pulse period: 0.2 s; sensitivity: 1.0×10^{-5} A V^{-1}.

solution until a steady cyclic voltammogram was obtained. Finally, the electrode was dried with a stream of high purity nitrogen.

2.4. Determination of nimesulide

The cysteic acid/CNTs/GCE, the platinum wire counter electrode and the saturated calomel reference electrode were immersed in 20.0 mL 0.05 M H_2SO_4 solution. A certain amount of nimesulide was added into the solution with stirring by a magnetic stirrer. Stirring was stopped after the electrochemical accumulation for 120 s at 0.0 V. Then the differential pulse voltammetry was immediately performed to scan from 0.6 to 1.5 V after quiet time of 30 s. The anodic peak current of nimesulide at +1.251 V was recorded (Fig. 2). The standard addition method was applied for the quantitative determination of nimesulide. After the determination, the renewal of the electrode was accomplished by soaking the modified electrode into the 0.05 M H_2SO_4 solution and cycling its potential between +0.6 and +1.5 V about 20 cycles.

3. Results and discussions

3.1. The role of materials modified on the GCE

The high electroactive surface area and excellent electronic conductivity of CNTs make this material an attractive candidate for use as an active electrode [21]. Fig. 3(A–a) and Fig. 3 (A–b) shows that there is a peak current response at the bare GCE to 3.0×10^{-6} M nimesulide, but it is lower than that at the CNTs/GCE in the absence of cysteic acid, at same oxidation potential with identical experimental conditions. This is attributed to further beneficial effects in terms of branched electrical conductivity coupled to increased electrode surface area of CNTs.

Many electrochemical researchers have been devoted to determination of CySH [22,23]. And it is widely accepted that the reaction occurs by the following oxidation reaction mechanism on electrodes (see [23–26]):

$$\mathrm{CySH} \leftrightarrow \mathrm{CyS^-} + \mathrm{H^+} \quad (1)$$

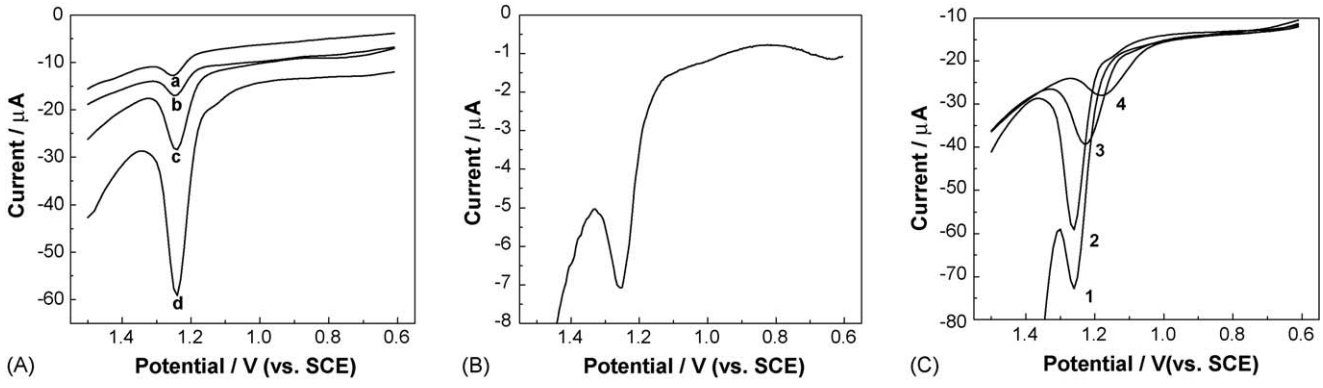

Fig. 3. Differential pulse voltammograms of 3.0×10^{-6} M nimesulide in 0.05 M H_2SO_4 solution. (A) a-the bare GCE; b-the CNTs modified GCE; c-the cysteic acid modified GCE; d-the cysteic acid/CNTs/GCE. (B) The Nafion/CNTs/GCE. (C) The DPV of 3.0×10^{-6} M nimesulide at the cysteic acid/CNTs/GCE in the different base solutions. 1: 0.1 M HCl; 2: 0.05 M H_2SO_4; 3: H_3PO_4 (pH 1.6); 4: PBS (pH 3). The experimental parameters are similar to Fig. 2.

$$CyS^- \rightarrow CyS^{\bullet}_{ads} + e^- \quad (2)$$

$$2CyS^{\bullet}_{ads} \rightarrow CySSCy \quad (3)$$

Herein, electrochemical oxidation of CySH on CNTs/GCE was also investigated by the cyclic voltammetry in the wide potential range of −0.8 to 2.2 V at a sweep rate of 200 mV s^{-1} in 0.04 M HCl solution containing 2.5×10^{-3} M CySH. A typical cyclic voltammogram from Fig. 1 shows that there is an irreversible oxidation peak at ca. + 1.0 V, which implies that CySH is oxidized to L-cystine (CySSCy). And there is another irreversible oxidation peak at ca.1.5 V with further increasing potential toward positive direction. In many cases, cysteic acid ($CySO_3H$) was found as an oxidation product of CySSCy [24–27]. Fei et al. described that the oxidation product of CySH can be oxidized further to chemisorbing molecules (cysteic acid) under high positive potential [26]. Ralph et al. demonstrated that CySH was adsorbed on the electrode by using AC voltammetry on GCE, and its further oxidation to cysteic acid was proposed [25]:

$$RSSR + 3H_2O \rightarrow RSO_3H + 5H^+ + 5e^- \quad (4)$$

Spataru et al. confirmed that the functional group SO_3H of cysteic acid was strongly adsorbed at GCE by using cyclic voltammetric and polarization measurements [23].

In recent years, the incorporation of highly conductive CNTs into Nafion can promote electrochemical responses, and Nafion/CNTs based electrodes have been increasingly used to solve demanding electrochemical problems, further they offer advantages over other types of electrode materials [28–30]. We think that cysteic acid based on the electrochemical oxidation of CySH is similar to Nafion with a sulfonated group, and it can be used as an electrode modifier due to its attractive ion-exchange characteristics. Fig. 3(A–c) shows the peak current response of nimesulide at cysteic acid/GCE is enhanced compared with the bare GCE under above identical experimental conditions. The amine group of nimesulide molecules carries a positive charge in the acid solution. It is a result of accumulation of nimesulide molecule because of electrostatic attraction. Fig. 3(A–d) shows the current response of nimesulide is the most remarkable at cysteic acid/CNTs/GCE compared with cysteic acid/GCE and CNTs/GCE. Obviously, the CNTs (which are now commercially available in high quality and uniformity) can provide high electrical conductivity and high surface area in the cysteic acid film. The higher accumulation effect of the cysteic acid/CNTs modified electrode reflects the synergistic effect of cysteic acid with CNTs. In addition, according to the pervious literature [28], a Nafion/CNTs/GCE was prepared for comparison with the proposed electrode. A 0.1 wt% Nafion solution used in this work was prepared by diluting the 5 wt% Nafion solution in ethanol. CNTs (2.5 mg) was added into 25 mL 0.1% Nafion solution and sonicated for 30 min until a homogeneous dispersion was achieved. A same GCE was coated with 10 μL of the CNTs–Nafion dispersion mentioned above. The solvent was left to evaporate at room temperature in air. Fig. 3(A–d) and Fig. 3B show that the oxidation peak potential at the Nafion/CNTs/GCE is similar to that at the cysteic acid/CNTs/GCE, implying that cysteic acid and Nafion play the same role at the two modified electrodes. Nafion/CNTs/GCE also enhanced the peak current response of nimesulide compared with GCE under above identical experimental conditions, but the current response is lower than that at cysteic acid/CNTs/GCE and decreases one order of magnitude because of poor conductivity of Nafion. On the other hand, Nafion is more expensive than CySH. So cysteic acid/CNTs film has more significant advantages and electroanalytical application than Nafion/CNTs film.

3.2. Optimization of experimental conditions

The highest sensitivity and fine differential pulse voltammograms of nimesulide were obtained in the 0.05 M H_2SO_4 solution. Other supporting electrolytes were also tested, such as HCl, H_3PO_4, HAc–NaAc, NaOH, KCl and phosphate buffers (PBS). All of them were not as favorable as 0.05 M H_2SO_4 for the determination. The pH value of the base solution has a significant influence on the oxidation of nimesulide at the cysteic acid/CNTs/GCE, by altering both the peak current and the peak potential. There is no current response of nimesulide observed in the alkaline solution. Fig. 3C shows the anodic peak current of nimesulide would increase monotonically and peak potential would simultaneously shift toward positive direction with decreasing the pH value. But the background current was too large to facilitate the determination of nimesulide when the acidity was too high. So the 0.05 M H_2SO_4 solution was selected for the determination.

The effects of accumulation potentials and accumulation time on the DPV current response for nimesulide were studied. The accumulation step proceeded in constantly stirred solution and the voltage-scanning step was performed after 30 s of quiet time. The peak current of nimesulide was the highest at +0.0 V as the accumulation potential. The effect of accumulation time was investigated, too. Fig. 4A shows the peak current of nimesulide increased with the accumulation time within 120 s, which indicated that nimesulide was adsorbed onto the modified electrode surface. Further increment of the accumulation time did not increase the absorption of nimesulide on the electrode after 120 s because the peak current remained almost constant, which indicated the surface adsorption saturation. For practical purposes, a 120 s accumulation period was found to be sufficient for the determination of nimesulide.

The peak current response of the cysteic acid/CNTs/GCE to nimesulide is expected to be affected by the amount of the CNTs on the electrode surface, which can be controlled by using same volume (10 μL) of the suspensions with different concentration of the CNTs to prepare the CNTs films. After the cysteic acid film is formed on the CNTs/GCE by electroxidation of CySH, the anodic peak current of 4.0×10^{-6} M nimesulide at these composite modified electrodes was recorded and the relationship between the anodic peak current and the CNTs content on the electrode is shown in Fig. 4B. With the increment of the CNTs concentration, the peak current increased accordingly, implying that higher the CNTs content was, higher the sensitivity of the electrode was. However, it was observed that the background current and noise level also increased with

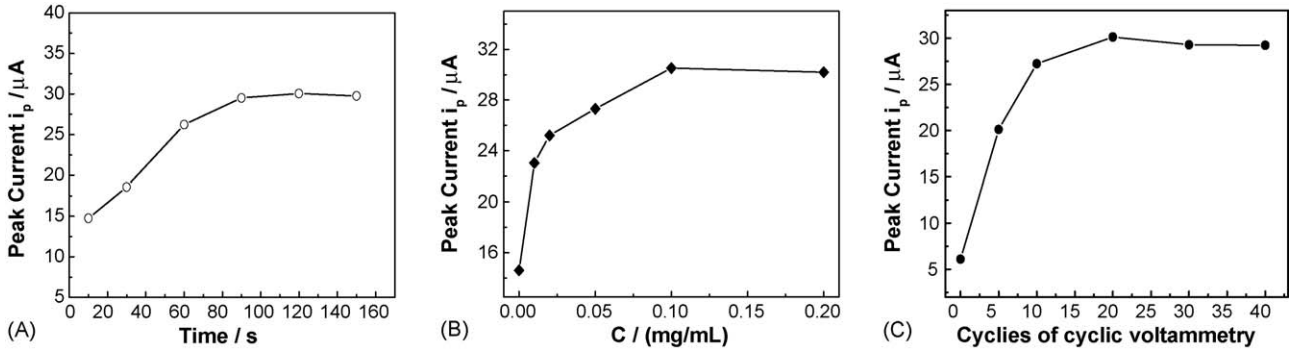

Fig. 4. The DPV current responses of 4.0×10^{-6} M nimesulide in the 0.05 M H_2SO_4 solution at the cysteic acid/CNTs/GCE. (A) Effect of accumulation time on the anodic peak currents. (B) Effect of the CNTs concentration on the anodic peak currents. (C) Effect of sweep circles for electrochemical oxidation of L-cysteine on the anodic peak current. The experimental parameters are similar to Fig. 2 except for the variable objects investigated.

excessive CNTs content in the experiment. Additionally, the cysteic acid/CNTs composite film would not be stable because the CNTs could leave off the electrode surface to decrease the peak current. Therefore, a moderate CNTs concentration of 0.10 mg ml^{-1} was selected for the fabrication of the cysteic acid/CNTs composite modified electrodes in this work.

The anodic peak current of nimesulide was considerably related to the amount of cysteic acid on the electrode, too. When the CNTs/GCE was carried out the sweeps by the cyclic voltammetry, the more cycles of sweep were, the more cysteic acid based on electrochemical oxidation of CySH was adsorbed onto the surface of the CNTs/GCE. Fig. 4C shows the remarkable effect of the cysteic acid amount to the peak current. The anodic peak current gradually increased with the amount of cysteic acid on the CNTs/GCE surface. It might be ascribed to the accumulation of nimesulide. However, the anodic peak current decreased when excessive cysteic acid was adsorbed on to the CNTs/GCE. We considered the reason is that the cysteic acid could leave from the electrode surface to decrease the peak current. So a 20-sweep-circle under the cyclic voltammetry was selected in present experiment.

3.3. Calibration curve and detection limit

Under the optimum detection conditions, the anodic peak current was proportional to nimesulide concentrations in the range of 1.0×10^{-7}–1.0×10^{-5} M (Fig. 5A). The linear equation was I_p (μA) = 1.712 + 7.127·C × 10^6 (C: M) with the correlation coefficient $r = 0.997$. The detection limit of nimesulide was 5.0×10^{-8} M in terms of the role of signal to noise ratio of 3:1 (S/N = 3).

We investigated the relationship between the peak current and the sweep rate in the nimesulide solutions. Fig. 5B shows that the peak current of cyclic voltammetry in 4.0×10^{-6} M nimesulide solution was proportional to the sweep rate. It implied that the electrode process was the oxidation of the adsorbed species.

3.4. Reproducibility

The nimesulide of 4.0×10^{-6} M was determined continuously with the same electrode for seven times, the height of voltammetric peak current decreased by ten percent. It implied that species competed for the adsorbing sites, which influenced

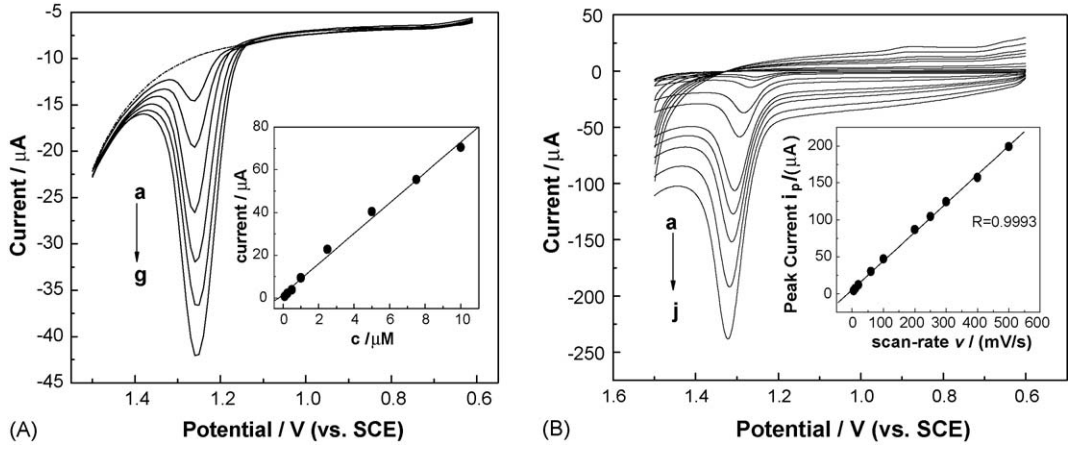

Fig. 5. (A) The DPV of different concentrations of nimesulide in 0.05 M H_2SO_4. (a) Blank solution: dash line, (b)–(g) are 4.0×10^{-7}, 1.0×10^{-6}, 2.0×10^{-6}, 2.7×10^{-6}, 3.4×10^{-6} and 4.0×10^{-6} M. respectively; The experimental parameters are similar to Fig. 2. Inset is the plot of the anodic peak currents versus the concentration of nimesulide. (B) Cyclic voltammagrams of 4.0×10^{-6} M nimesulide on the cysteic acid/CNTs GCE in 0.05 M H_2SO_4 with different scan rates. (a)–(j) are 5, 10, 20, 60, 100, 200, 250, 300, 400 and 500 mV s^{-1}, respectively; Accumulation potential: 0.00 V; accumulation time: 120 s; quiet time: 30 s. Inset is the plot of anodic peak currents vs. scan rates.

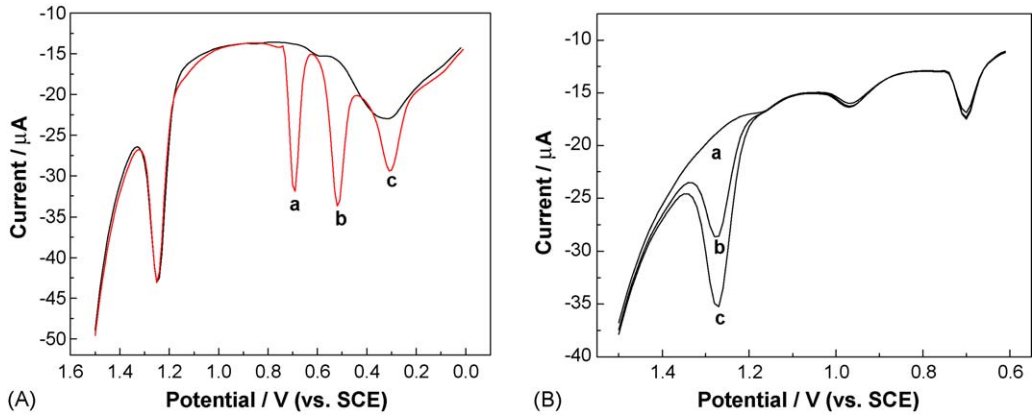

Fig. 6. (A) DPV of 2.5×10^{-6} M nimesulide. Red line is DPV in the presence of 5.0×10^{-6} M uric acid (a), 2.0×10^{-5} M dopamine (b) and 2.0×10^{-4} M ascorbic acid (c). Black line is DPV in the absence above interferents. (B) The DPV of serum sample spiked with different nimesulide concentration. (a) Serum sample, (b) 5.0×10^{-7} M nimesulide, (c) 1.0×10^{-6} M nimesulide. The experimental parameters are similar to Fig. 2.

the height of voltammetric peaks when the analyte adsorbs on the electrode. So it was necessary to renew the electrode surface after every determination, and this process was easily accomplished by soaking the modified electrode into 0.05 M H_2SO_4 solution to sweep between +0.6 and 1.5 V about 20 cycles by cyclic voltammetry. The nimesulide of 4.0×10^{-6} M was determined repeatedly with the same electrode for nine times. The average current was 30.2 μA with the relative standard deviation (RSD) of 2.9%, which indicated that the modified electrode possessed a good reproducibility. The lifetime of the cysteic acid/CNTs/GCE was examined and it demonstrated that the electrode could retain 96.8% of its initial response after four-month-storage. Such electrode stability seemed to be acceptable for most practical applications.

3.5. Recovery test

The recovery tests of nimesulide in the range from 1.50×10^{-7} to 6.50×10^{-6} M were performed. The results were listed in Table 1. The recoveries varied in the range from 96.4 to 105.0% and the RSD was 3.1%.

3.6. Interference

For the following special detection in serum samples, a few possible interferents were examined for their influence on the voltammetric determination of 2.5×10^{-6} M nimesulide. Fig. 6A as comparison voltammograms shows that there are no interferences from uric acid (UA), dopamine (DA) and ascorbic acid (AA) for the measurement of peak height because peak potentials of three components are far away from that of nimesulide. Other possible interferents, such as glucose, oxalic acid, caffeine, DL-tyrosine, citrate, malic acid, Vitamin B_1, Vitamin B_6 and theine were also examined. The effects of some inorganic compounds were also investigated, such as NaCl, KNO_3, $Mg(NO_3)_2$, $Ca(NO_3)_2$ and so on. The results were summarized in Table 2. As could be seen, most of these species did not interfere with the determination of nimesulide.

Table 1
Recovery of nimesulide

Added (M)	Found (M)	Recovery (%)
1.50×10^{-7}	1.48×10^{-7}	98.7
3.00×10^{-7}	3.15×10^{-7}	105.0
6.50×10^{-7}	6.53×10^{-7}	100.5
1.50×10^{-6}	1.47×10^{-6}	98.0
2.50×10^{-6}	2.41×10^{-6}	96.4
6.50×10^{-6}	6.64×10^{-6}	102.2

3.7. Determination of the pharmaceutical preparation samples

The developed DPV method for the nimesulide determination was applied to a commercial preparation (nimesulide tablets: Guangdong Jianlibao Pharmaceutical Co. LTD., China,

Table 2
The influence of the potential interferents on the voltammetric response of 2.5×10^{-6} M nimesulide

Interferent	Concentration (M)	Signal change (i_{NMSL} = 100%)
Uric acid	5.00×10^{-6}	−0.82
Uric acid	1.00×10^{-5}	−2.15
Uric acid	5.00×10^{-5}	−3.52
Dopamine	5.00×10^{-5}	−2.03
Ascorbic acid	2.00×10^{-4}	−1.90
Glucose	5.00×10^{-3}	+1.37
Vitamin B_1	1.00×10^{-3}	−1.68
Vitamin B_6	1.00×10^{-3}	−1.15
Theine	1.00×10^{-3}	+2.31
Citrate	3.00×10^{-4}	−2.76
DL-tyrosine	3.00×10^{-4}	+2.24
Oxalic acid	5.00×10^{-3}	+0.59
Caffeine	5.00×10^{-3}	−1.44
Malic acid	3.00×10^{-4}	−2.19
NaCl	5.00×10^{-3}	+1.72
KNO_3	5.00×10^{-3}	−0.91
$Ca(NO_3)_2$	5.00×10^{-3}	+3.03
$Mg(NO_3)_2$	5.00×10^{-3}	+2.01

Table 3
Determination results of nimesulide in solid tablets at the cysteic acid/CNTs/GCE

Batch number	UV method ($n=5$) (mg/tablet)	Present method ($n=5$) (mg/tablet)	Present method R.S.D[a] (%)
Jianlibao® H19980132	101.6	98.2	3.3

[a] Here R.S.D is relative standard deviation.

Jianlibao®, batch No. H19980132, labeled amount of 0.1 g per tablet). A known number of tablets were ground to fine powder, and an accurate mass of powder (200.0 mg) was transferred into a 50.0 mL calibrated flask. It was extracted for 5 min with 50.0 mL of cold ethanol. Table 3 gives the results of DPV analysis of the commercial preparations. The UV spectrophotometry was employed to compare the validity of the developed method [4]. There was no significant difference between two methods, and a good agreement was achieved. The results show that the proposed methods could be recommended for the determination of nimesulide in solid tablets. The developed method could easily be used in quality control laboratory for the analysis of nimesulide in solid pharmaceutical formulations.

3.8. Detection of nimesulide in human serum samples

The developed DPV method for the nimesulide determination was applied to human serum samples. The serum samples were obtained from volunteers. The recoveries from human serum were measured by spiking drug-free human serum with known amounts of nimesulide. The serum samples were diluted 200 times with the 0.05 M H_2SO_4 solution before analysis without further pretreatments. Fig. 6B shows the differential pulse voltammograms for serum samples detection. A qualitative analysis can be carried out by adding the standard solution of uric acid into the detect system of human serum sample, and then the peak at +0.70 V increased in height. The peak was proved to be response of uric acid in the human serum sample. Another anodic peak appears at 0.95 V attributed to other unknown endogenous chemicals presented in serum, but they do not interfere with the determination of nimesulide. Standard addition method was employed under above optimal experimental conditions. The detection results of six human serum samples obtained were listed in Table 4. And the recovery determined was ranged from 96.3% to 104.5% and the RSD was 3.0%.

Table 4
Determination of nimesulide in serum samples at the cysteic acid/CNTs/GCE

Serum	Spiked (M)	Detected (M)	Recovery (%)
Sample 1	2.00×10^{-7}	2.05×10^{-7}	102.5
Sample 2	2.00×10^{-6}	1.96×10^{-6}	98.0
Sample 3	3.00×10^{-6}	3.03×10^{-6}	104.5
Sample 4	6.00×10^{-7}	5.97×10^{-7}	99.5
Sample 5	4.00×10^{-6}	4.18×10^{-6}	101.0
Sample 6	6.00×10^{-6}	5.78×10^{-6}	96.3

4. Conclusion

The results presented here, demonstrate that the use of CNTs and cysteic acid based on electrochemical oxidation of L-cysteine could form a novel composite thin film material for electroanalytical determination of nimesulide. The success of this strategy suggests that cysteic acid/CNTs film will have significant electroanalytical utility in the future. The novel cysteic acid/CNTs thin film material can be easily applied to other types of substrate electrodes and surfaces, and this will further broaden the potential for applications. Further application of the cysteic acid/CNTs thin film electrode, for example, for the simultaneous detection of DA, UA, AA is under development in our group.

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