Electrochemical studies oxidation of ciprofloxacin at nano-SnO$_2$/PVS modified electrode and its interaction with calf thymus DNA

Yuejuan Cai, Yuzhong Zhang, Shao Su, Shuping Li, Yonghong Ni

College of Chemistry and Materials Science, Anhui Key Laboratory of Functional Molecular Solids, Anhui Normal University, Wuhu 241000, P.R. China

TABLE OF CONTENTS

1. Abstract
2. Introduction
3. Experimental procedures
   3.1. Reagents
   3.2. Apparatus
   3.3. Electrode preparation
4. Results and discussion
   4.1. Preparation and characteristics of nano-SnO$_2$
      4.1.1. The preparation of nano-SnO$_2$
      4.1.2. The characteristics of nano-SnO$_2$
   4.2. Electrochemical behaviors of CFX at the nano-SnO$_2$ modified electrode
      4.2.1. Cyclic voltammograms of CFX at various modified electrode
      4.2.2. Effect of pH
      4.2.3. Determination of CFX
      4.2.4. Electrooxidation mechanism of CFX
   4.3. Studing the interaction between CFX and ctDNA
      4.3.1. Electrochemical investigating the interaction between CFX and ctDNA
      4.3.2. Investigating UV/Vis absorption spectra
      4.3.3. Studying fluorescence spectra
      4.3.4. Agarose gel electrophoresis
      4.3.5. Quenching studies
5. Conclusions
6. Acknowledgment
7. References

1. ABSTRACT

In pH 6.0 phosphate buffer solutions (PBS), a glassy carbon electrode (GCE) modifying by nano-tin oxide/ poly vinyl sulfonic potassium (nano-SnO$_2$/PVS) exhibited an enhanced effectiveness for the oxidation of ciprofloxacin (CFX), which compared with a bare GCE or a nano-SnO$_2$ modified electrode. In addition we also investigated the electrooxidation mechanism of the fluoroquinolone antibiotics (utilizing ciprofloxacin, ofloxacin, sparflloxacin and lomefloxacin) at the modified electrode. Further more, Gel electrophoresis coupled with electrochemistry and spectra techniques were used to study the interaction of CFX and calf thymus DNA (ctDNA). These acquired data showed that the binding mode of CFX and DNA was mainly an intercalation mechanism.

2. INTRODUCTION

Recently nano-materials have attracted much attention and have been widely used in material, electronic, physical fields for various purposes due to their unique properties. Nano-SnO$_2$ possesses excellent photoelectronic properties, high gas sensitivities and a short response time as well as relatively higher conductivity than TiO$_2$ and SiO$_2$ (1-3). These new kinds of inorganic nanomaterials exhibit tunable porosity, high thermal and chemical stability. Up to now, some inorganic oxide nanomaterials have been investigated in fundamental studies of protein-electrode interactions and the development of biosensing devices (4-14). The nanoporous structures of these inorganic oxide films greatly enhance the active surface area available for protein binding. These films facilitate direct electron
transfer process between biomolecules and electrodes. In the work, SnO₂ nanocrystals were synthesized by the hydrothermal method. We used the nano-SnO₂ to modify a GCE and investigated the electrochemical behavior of CFX at the nano-SnO₂ modified GCE.

CFX belongs to the family of fluoroquinolone antibacterial agents that also includes norfloxacin, ofloxacin and some other molecules. These fluoroquinolone antibacterial agents are synthetic derivatives of 6-fluoro-4-oxo-quinoline-3-carboxylic acid. They are fluorinated at position 6 and mostly bear a piperazinyl moiety at position 7. Ciprofloxacin is one of the most potent quinolone derivatives in clinical use with a very broad spectrum of antibacterial activity and is often used as an antibacterial agent of last resort (15). Although the exact mechanism of fluoroquinolone pharmacological action is still unclear, numerous studies had shown that the drug binding to DNA (16–18) and gyrase (19) was essential for antibacterial efficiency of drug-DNA interaction. Fluoroquinolone binding to the gyrase–DNA complex might prevent the religation step (16, 20–23). The mechanism of fluoroquinolone’s inhibition of religation and the role of DNA in drug binding remains to be resolved. Understanding the interactions between fluoroquinolone and DNA might help to elucidate the action mechanism of this class of antibacterial agents. In this paper, we studied the electrochemical behavior of CFX at nano-SnO₂ modified GCE and investigated the interaction of CFX and DNA by electrochemistry, UV/Vis absorbance spectra, fluorescence spectra and gel electrophoresis. These obtained data indicated the binding mode of CFX and DNA was mainly an intercalation mechanism.

3. EXPERIMENTAL PROCEDURES

3.1. Reagents
Calf thymus DNA (sodium salt, type I) was obtained from Sigma (USA) and was used without further purification. Its purity was confirmed by UV/Vis absorption, which produced A₂₆₀/A₂₈₀ value of approximate 1.8-1.9, suggesting the DNA sample was free proteins. The stock solution of DNA was directly dissolved in water and stored at 4 °C. The DNA concentration per nucleotide (DNA-P) was determined spectrophotometrically (24) at 260 nm by using the extinction coefficient 6600 cm⁻¹ M⁻¹. CFX was purchased from Chinese National Institute for the Control of Pharmaceutical and Biological Products (purity >90.0%) and was used without further purification. Poly (vinyl) sulfonic potassium was purchased from Fluka. Other chemicals were analytical reagent. All solutions were prepared with double-distilled water.

3.2. Apparatus
Transmission electron microscopy (TEM) images of nanoparticles were acquired on a Hitachi H-600 (Tokyo) transmission electron microscopy. Ethanol solutions of the nanoparticles were dropped on 50 Å thick carbon-coated copper grids with the excess solution being immediately whisked away.

The X-ray diffraction (XRD) patterns of the nano-SnO₂ were operated on a D/MAX-6000 powder diffractometer. (Rigaku CO, Germany).

Cyclic voltammograms (CVs) were obtained on a CHI 660 electrochemical workstation (Shanghai ChenHua Instruments, China) with a three-electrode system. A platinum wire was used as auxiliary electrode, a saturated calomel electrode (SCE) as a reference electrode, a bare or nano-SnO₂/PVS film modified glassy carbon electrode (GCE) (Ø=3.2 mm) as a working electrode, respectively. All potentials were reported to the SCE.

The UV/Vis absorbance spectra were acquired on U-3010 spectrophotometer (Hitachi, Japan) equipped with a quartz micro-colorimetric vessel of 1cm path length.

Fluorescence spectra and relative fluorescence intensities were measured on a model F-4500 fluorescence spectrophotometer (Hitachi, Japan) equipped with a xenon lamp, dual monochromators, and a 1 cm × 1 cm quartz cell. The slit-width for both excitation and emission was set at 5 nm.

Agarose gel electrophoresis was attained on a general WC horizontal and UV-2000 analysis instrument (Shanghai Tianeng company, China).

3.3. Electrode preparation
A bare GCE was successively polished in the Al₂O₃ slurry from 0.3 to 0.5 µm before modification. Then it was rinsed with double distilled water and sonicated in 1:1 nitric acid, acetone and double distilled water for 10 min, respectively. Then the electrode was activated by continuous cyclic scanning 15 circles in 1.0 M sulfuric acid solution between -0.1 and +1.6 V with a rate of 100 mV/s and allowed to dry at room temperature.

For preparing a nano-SnO₂ and PVS film modified GCE, a nano-SnO₂ suspension was obtained by dispersing 10.0 mg nano-SnO₂ into 10 ml PVS solution. The 6 µL suspension was dropped onto the surface of clean GCE. Then solvent was evaporated in the air, the final electrode was taken as nano-SnO₂/PVS/GCE. A PVS/GCE (dropping 6µL PVS solution), a nano-SnO₂/GCE (dropping 6 µL nano-SnO₂ suspension) and a bare GCE were taken to compare. All measurements were performed in a 10 ml electrolytic cell with 5 ml solutions, where oxygen was removed with high-purity nitrogen for 20 min. All sample solutions were deoxygenated by bubbling N₂ gas before each experiment.

4. RESULTS AND DISCUSSION

4.1. Preparation and characteristics of nano-SnO₂
4.1.1 The preparation of nano-SnO₂
In this paper, nano-SnO₂ was synthesized by the hydrothermal synthesis, using SnCl₄·5H₂O as the precursor. 0.8790 g SnCl₄·5H₂O was dissolved in the mixing solution of 10 ml glycol and 50 ml water, adjusted the solution pH
Electrochemical behavior of CFX and interaction with ctDNA

Figure 1. The TEM image of the nano-SnO₂.

![Figure 1](image1)

Figure 2. X-ray diffraction patterns of the nano-SnO₂.

![Figure 2](image2)

4.1.2. The characteristics of nano-SnO₂

Figure 1 shows the typical TEM image of the nano-SnO₂. The particles are homogeneous with the average diameter of 15–20 nm. Figure 2 shows the X-ray diffraction patterns of the nanoparticles prepared. We could see that the 20 peaks are agreement with the diffraction patterns from (110), (101), (200), (211) and (112) planes.

4.2. Electrochemical behaviors of CFX at the nano-SnO₂ modified electrode

4.2.1. Cyclic voltammograms of CFX at various modified electrode

Figure 3A shows the CVs at the nano-SnO₂/PVS/GCE in pH 6.0 PBS without CFX. No redox peak was observed in blank solution. Figure 3B depicts the CVs of CFX at the nano-SnO₂/PVS/GCE (curve d), nano-SnO₂/GCE (curve c), PVS/GCE (curve a) and bare GCE (curve b) in pH 6.0 PBS, respectively. At a bare GCE (curve b) and PVS/GCE (curve a), the electrochemical responses of CFX were very poor. However at nano-SnO₂ modified electrode (curve c), it shows a good electrochemical response, the peak current was enhanced; meanwhile the oxidation peak negatively shifted. The reason ascribe to the great surface area of nano-SnO₂ (25). At the nano-SnO₂/PVS electrode (curve d), the anodic peak current was higher than that at the nano-SnO₂ modified electrode. The one reason could be that nano-SnO₂/PVS film had negative charge, so it could attract positive charge CFX, because ciprofloxacin exists as a cation below pH 5, when the pH is between 5 and 10, it is as a mixture of anions, cations and zwitterions, and it is as an anion over than pH 10 (26–29). So it could attract more CFX to electrode surface than at bare electrode, so peak current is increased. Another reason was that PVS film could provide a favorable environment for CFX and facilitates the electron exchange (25). From the CVs we could conclude that the electrode reaction of CFX is irreversible at nano-SnO₂/PVS film modified electrode.

In addition, the effect of the scan rate on the peak current of CFX was investigated. The anodic peak currents were proportional to scan rates over the range of 80~260 mV/s. The Linear regression equation was \(i_{pa}(\mu A) = 0.5012 + 0.0062v \) (mV/S) with a correlation coefficient of \(r=0.9980\); meanwhile we also investigated the effect of accumulation time on the peak currents, the experiment result shows that the peak current increases with the accumulation time till five minutes, which suggested that the electrode reaction of CFX at nano-SnO₂/PVS film modified electrode was an adsorption-controlled process.

4.2.2. Effect of pH

The effect of the solution pH on electrochemical response of CFX was investigated over the range of 2.0~8.0. Figure 4A shows that the anodic peak potential negatively shifted with increasing solution pH. The fact indicates protons take part in the electrode process. From Figure 4B, we could also observe the peak current increases with pH value of solution from 2.0 to 6.0. When pH>6.0, the anodic peak current decreases and peak shape became worse. When pH>7.0, the electrooxidation of CFX almost could not happen. From the changes of peak shape and peak current of CFX, we selected pH 6.0 PBS as the supporting electrolyte. These data showed the electrooxidation of CFX easily occurred in the acid solution.

4.2.3. Determination of CFX

The determination of CFX concentration was performed with differential pulse voltammetry (DPV). The oxidation peak current of CFX was selected as the analytical signal. The result shows that the anodic peak current is proportional to the concentration of CFX over the range of \(1.2 \times 10^{-8} \text{ to } 5.0 \times 10^{-5} M\), and the linear regression equation is \(i_{pa}(10 \mu A) = 0.3243 + 0.0065C\) (µM), with a correlation coefficient of \(r=0.9988\). The detection limit
Electrochemical behavior of CFX and interaction with ctDNA

4.2.4. Eletrooxidation mechanism of CFX

To an adsorption-controlled irreversible process, basing on the equation of $E_p$ versus ln (curve a) $E_p = E^0 + \frac{RT}{αnF} \ln \left( \frac{RTK_e}{αnF} \right) + \frac{RT}{αnF} \ln n$. We could obtain the slope of the equation: $0.0257/αn=0.0269$, where $α$, $n$ were electron transfer coefficient and electron number taking part in the reaction, respectively, with a correlation coefficient of $r=0.9987$. Given $α$ to be 0.5, then $n=1.93=2$. Then according to the equation: $dE_p/dpH = -0.059x/αn$, where $x$ was the number of hydrogen ions taking part in the reaction process, we calculated $x$ to be 0.89=1. We could conclude that one hydrogen ion and two electrons probably took part in the electrode reaction. This result was agreement with the values Smyth and Ivaska (30) determined other piperazine moiety, such as flurazepam and chlordiazepoxide. Another study on trazadone and nefazadone with piperazine ring was realized by CVs, DPV and SWV at the GCE, the oxidation process was also similar (31, 32). Taking into account that the CVs of the above molecules closely matched the CVs of CFX, we might postulate that the oxidation steps of CFX were located on the piperazine moiety which represented a typical oxidation system with two electrons oxidation process in acidic media. We might assume that the electrooxidation of the nitrogen occurred when the nitrogen of the piperazine ring, distal to the molecule, was protonated.

For further supporting the electrooxidation mechanism, we investigated other fluoroquinolone antibacterial agents, such as ofloxacin (OFX), sparfloxacin (SPX) and lomefloxacin (LMF). The CVs and schemes of these drugs are showed in Figure 5. We might see the peak potential is similar; furthermore the relationship of pH-dependent is similar under the acidic media condition. When pH > 7.0, the electrochemical oxidation of these drugs almost could not happen. The CVs of SPX was especially and had two anodic peaks, the first peak and the second peak should be responding to the 5-position amidogen and the nitrogen of the piperazine ring oxidation, respectively. In addition piperazine hydrochloric acid also was operated at a bare GCE and had an approximative peak potential. The data more indicate the electrooxidation of fluoroquinolone drugs occur on the piperazine moiety. The electrode reaction (representation by CFX) as follows:

4.3. Studying the interaction between CFX and ctDNA

4.3.1. Electrochemical investigating the interaction between CFX and ctDNA

Fig.7 shows the CVs of CFX at a nano-SnO$_2$/PVS/GCE in pH 6.0 PBS in the absence of ctDNA or in the presence of ctDNA. The oxidation peak current of CFX obviously decreased in the presence of ctDNA. The phenomenon indicates that the interaction of ctDNA and CFX exists. However the peak potential is very stable. The result shows the interaction of  ctDNA and CFX is different from metal ion and organic small molecule produce metal complex (The peak potential of the metal complex usually is different from that of the metal ion).

4.3.2. Investigating UV/Vis absorption spectra

Spectroscopic techniques are very useful tools to gain important information in biological science (33). Spectroscopic investigation of the interaction of CFX and ctDNA may be beneficial to clarifying the interaction mechanism. The interaction of CFX and ctDNA in PBS (pH 6.0) could be further confirmed by UV/Vis spectra. The variation of adsorption spectroscopy is presented in Figure 8. For the spectrum of free CFX, an absorption band was observed at 272 nm and 323 nm (curve a). However, in the presence of ctDNA, the 323 nm absorption peak of CFX red shifted and the absorption intensity
Electrochemical behavior of CFX and interaction with ctDNA

Figure 4. A: Relationship between the peak potential and pH; B: Relationship between the peak current and pH.

Figure 5. Cyclic voltammograms of $2 \times 10^{-5}$ M different fluoroquinolone antibacterial agents and piperazine hydrochloric acid at a bare GCE in 0.1M PBS (pH 6.0).
Electrochemical behavior of CFX and interaction with ctDNA

Figure 6. The electrooxidation mechanism of CFX.

Figure 7. Cyclic voltammograms of 0.1M PBS (pH6.0) containing 1×10^{-5} M CFX in the absence of (b) and presence of 2×10^{-5} M DNA (a).
Electrochemical behavior of CFX and interaction with ctDNA

4.3.3. Studying fluorescence spectra

The interaction of CFX and ctDNA was also examined by fluorescence spectra. The fluorescent emission spectra of CFX and the effect of ctDNA concentrations on the fluorescence emission spectra of CFX are illustrated in Figure 9. CFX exhibited an emission maximum at 449 nm (Fig. 9. a). The fluorescence emission was gradually decreased with increasing amount of ctDNA (curve b-e), showing that the CFX fluorescence was efficiently quenched upon binding to ctDNA. The stronger quenching of ciprofloxacin fluorescence without significant shift could originate in guanine (the oxidation potential of guanine is the lowest in nucleobases). Also, guanine could be an effective quencher of fluorescence through electron transfer from DNA to photo-excited fluoroquinolone 29. Norfloxacin have been well recorded 33, 35-37. When norfloxacin bound to DNA or synthetic polynucleotide, the intensity of fluorescence decreased without changing the shape upon binding to DNA or poly (d(G-C)), however, emission maximum red shifted with an isosbestic wavelength when it bound to poly (d(I-C)) and poly (d(A-T)). From these fluorescence results and other observations, Authors thought norfloxacin bound in the minor groove of native DNA and poly (d(G-C)) which had the possibility of partial intercalation between amine group at the 2-position of guanine base and oxygen atoms of norfloxacin. The chromophore of norfloxacin and CFX is identical; hence similar spectral properties for both compounds are expected if they bind to native DNA with the similar binding mode. This is agreement with that clarified in absorption spectra and the electrochemical methods.

4.3.4. Agarose gel electrophoresis

Agarose gel electrophoresis is a generally separating, identifying, purifying DNA method. For more confirmation the mode of CFX and ctDNA action, an agarose gel electrophoresis had also been done. The patterns are shown in Figure 10. The ctDNA was dyed by the CFX and not ethidium bromide (EB) which was a typical intercalater. The Figure 10 was the patterns of 1.5×10^{-5} M ctDNA and different CFX (2×10^{-6}M, 2×10^{-5} M, 2×10^{-4} M from 1 to 3). The electrophoresis speeds became slower and the patterns were more luminous with the CFX concentration increasing. The reason could be due to much more ctDNA-CFX adduct forming so that the molecular electrophoresis speeds were slower and the dyed ctDNA were more. The experiment indicates CFX is similar to EB and could intercalate into the bases of ctDNA. If the binding of CFX and ctDNA only was an electrostatic interaction or groove binding, positive charge CFX should shift to the cathode of the electrophoresis trough, so ctDNA was not dyed. The result forcefully supported the intercalating mode of CFX and ctDNA.

4.3.5. Quenching studies

Another fact that supported the intercalation mechanism comes from determining the relative intercalating into the ctDNA base pairs. From the observations detailed above, we surmised that the intercalation occurred.
fluorescence intensity of the free and bound CFX when potassium iodide existed. If CFX intercalated into the helix it should be protected, owing to the base pairs above and below. Whereas single groove binding or electrostatic interaction could expose the binding molecules much more than the intercalating species, it should provide much less protection (38). This was consistent with our expectation of intercalation, when the anionic quenchers - potassium iodide were added in the solution; relative fluorescence quenching was found to be much smaller in the presence of ctDNA than its absence. Simple linear behavior was apparent from the quenching curves, this indicated the interaction of CFX and ctDNA was only one major binding mode, which was the intercalation. This was another forceful proof of intercalation mechanism.

5. CONCLUSIONS

In this paper, the nano-SnO$_2$/PVS film was cast onto the surface of GCE and the electrochemical behavior of CFX at the modified electrode was investigated. In addition the electrochemistry, agarose gel electrophoresis and spectroscopic methods were applied to investigate the interaction of CFX and ctDNA, which was an intercalation mechanism.

6. ACKNOWLEDGEMENTS

This project was supported by the Key Nature Science Foundation of Anhui province Ministry of Education (No. 2006KJ040A) and the Education Department (No. 2006KJ006TD) of Anhui Province.

7. REFERENCES


Electrochemical behavior of CFX and interaction with ctDNA


Key Words: Ciprofloxacin, ctDNA, nano-SnO2, Poly (Vinyl) Sulfonylic Potassium, Interaction

Send correspondence to: Dr Yuzhong Zhang, College of Chemistry and Materials Science, Anhui Normal University, Wuhu 241000, P.R. China, Tel: 86-553-3869303, Fax: 86-553-3869303, E-mail: zhangyz65@hotmail.com

http://www.bioscience.org/current/vol12.htm