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Electrochemical studies on the interaction of an antibacterial drug nitrofurantoin with DNA

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ABSTRACT

The electrochemical reduction of an antibacterial drug nitrofurantoin (NTF) at hanging mercury dropping electrode (HMDE) has been studied using techniques. NTF shows one irreversible reduction peak at 1.192 V in Britton–Robinson (BR) buffer of pH 7.1, ionic strength 0.2 mol/dm^3. Cyclic voltammetry (CV), differential pulse voltammetry (DPV) as well as UV/Vis spectroscopy were employed to probe the interaction between NTF and calf thymus DNA. From electrochemical data, the binding constant and binding ratio between DNA and drug was calculated to be 8.22 (±0.05) × 10^6 M^{-1} and 1:3, respectively. Through chronocoulometry experiments, the diffusion coefficients were found as 1.10 (±0.03) × 10^{-6} cm^2 s^{-1} for NTF and 3.67 (±0.03) × 10^{-8} cm^2 s^{-1} for NTF–DNA. Based on electrochemical and spectroscopic results, we concluded that the mode of binding of NTF–DNA was through intercalative binding. The calibration graph for the determination of DNA was obtained by the decrease in the DPV peak current of NTF in the presence of DNA.

1. Introduction

Nitrofurantoin (Fig. 1) {1-(5-nitro-2-furfurylidene amino-hydantoin} is a synthetic, nitrofuranderivative antibacterial agent. This drug is usually bacteriostatic but may be bactericidal in action, depending on its concentration attained at the site of infection and the susceptibility of the infecting organism [1]. It is used in the treatment of initial or recurrent urinary tract infections caused by susceptible organisms. The drug is active against many gram-negative and some gram-positive bacteria including: *Citrobacter*, *Corynebacterium*, *Enterobacter*, *Escherichia coli*, *Klebsiella*, *Neisseria*, *Salmonella*, *Shigella*, *Staphylococcus aureus* and *Enterococcus faecalis*. In general, most susceptible bacteria are inhibited in vitro by nitrofurantoin concentrations of 1–32 μg/ml. Higher concentrations of the drug may be required to inhibit some strains of *Enterobacter* and *Klebsiella* [1].

Deoxyribonucleic acid (DNA) has a central role in life process since it contains all of the genetic information required for cellular function. However, DNA molecules are prone to be damaged under various conditions, especially by interaction with some molecules and this damage may lead to various pathological changes in living organisms. There is growing interesting exploring the binding of small molecules with DNA for the rational design and construction of new and more efficient drugs targeted to DNA as well as in understanding how proteins recognize and bind to specific DNA sequences [2–4].

The binding of small molecules to DNA occurs through primarily in three modes: electrostatic interactions with the negative-charged nucleic sugar–phosphate structure, binding interaction with grooves of DNA double helix and intercalation between the stacked base pairs of native DNA [5]. Recently, growing interest has arisen in electrochemical investigation between nucleic-acid-binding molecules and DNA [6–9]. By observing the electrochemical signal related to DNA–drug interactions, it is possible to propose mechanism of interaction and nature of the complex formed and to evaluate the binding constant.

There has not any report about the detection of the NTF–DNA interaction based on the electrochemical behaviors at HMDE and especially on the change of spectroscopic characteristics. Accordingly, in this work, detailed investigations of the electrochemical behavior of NTF upon addition of DNA were carried out. Morever, the changes in the UV absorption when NTF binding to DNA were used to study the mode of such interaction. The results of electrochemical and spectroscopic techniques confirmed each other. Thus it can be seen, there is a mutual complement between electrochemical method and spectroscopy techniques, which can provide fruitful information about the mechanism of interaction.

2. Experimental

2.1. Materials

Calf thymus (pBR322 plasmid) DNA was obtained from Sigma (200 μg stock solution, 1 μg pBR322 plasmid: 4361 base pairs:

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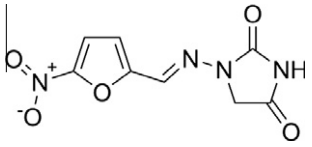

Fig. 1. Structural formula of nitrofurantoin.

0.35 pmol: 2.1×10^{11} molecule) and used as received. Calf thymus (pBR322 plasmid) DNA solution (1 μM) was dissolved in water and stored at 4 °C. Solutions of DNA gave ratios of UV absorbance at 260 and 280 nm (A_{260}/A_{280}) of approximately 1.9, indicating that the calf thymus DNA was sufficiently protein-free [10,11]. Solutions were incubated at 37 °C for 2 h. NTF stock solution (1.0×10^{-4} M) were prepared in MeOH and kept away from light to avoid photochemical decomposition. Working standard solutions were prepared daily by appropriate dilution of the stock standard solutions. If not specially stated, the supporting electrolyte was Britton–Robinson buffer. Britton–Robinson buffers (pH 7.1) were prepared (0.04 M of each of acetic, o-phosphoric and boric acids, adjusted to the required pH with 0.2 M sodium hydroxide) and used as supporting electrolytes. All reagents were of analytical grade quality. Deionized water was used throughout to prepare solutions.

2.2. Instrumentation

BAS 100 B/W model electrochemical workstation was used. The reference electrode was Ag/AgCl and a platinum wire was used as the auxiliary electrode and HMDE was used as the working electrode.

Agilent 8453 single beam UV–visible spectrophotometer was used for spectrophotometric analysis.

All the pH measurements were made with Metler Toledo MA 235.

2.3. Electroanalytical procedure

Prior to the experiments, high purity nitrogen was used to deaeration of 1350 μL supporting electrolyte for at least 10 min. Then 150 μL of 1.0×10^{-4} M stock solution of NTF was placed into the cell to make up 1500 μL mixture solution at a NTF concentration of 1.0×10^{-5} M and N_2 was passed through in 2 s. The voltammograms were recorded with cyclic potential scan between −0.8 V and −1.6 V. During the experiments, nitrogen atmosphere was maintained over the solutions to prevent the reentry of atmospheric oxygen. All experiments were typically carried out at room temperature and were confirmed at least three times.

3. Results and discussion

3.1. Electrochemical reduction of NTF

The electrochemical behavior of NTF at HMDE was investigated employing CV and DPV. A series of supporting electrolyte (Britton–Robinson buffer and phosphate) were tested in the presence of 1.0×10^{-5} M NTF. The results showed that NTF in Britton–Robinson buffer gave higher signal response than in phosphate buffer. So Britton–Robinson buffer was chosen as supporting electrolyte. NTF showed one reduction peak at 1.190 V in BR buffer of pH 7.1 with scan rate of 100 mV s^{-1}. No peak was observed in the reverse scan suggesting that the reduction of NTF at HMDE is irreversible. In addition that the peak potential shifted to negative values when the scan rate was increased (Fig. 2). These results also confirmed that the reduction reaction was irreversible.

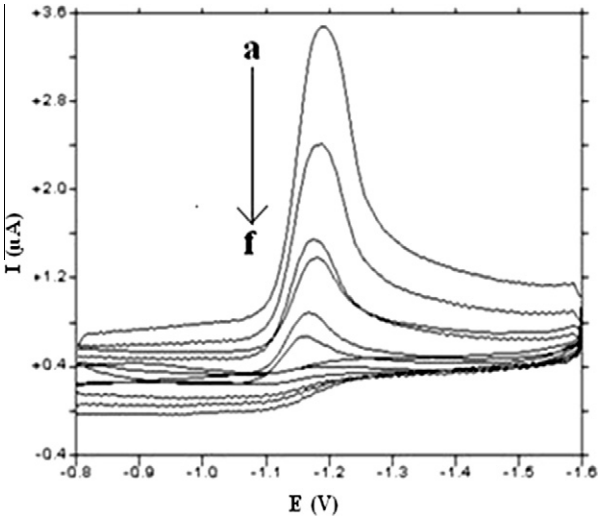

Fig. 2. Cyclic voltammogram of NTF on HMDE Supporting electrolyte: Britton–Robinson buffer (pH 7.1); (a) 25 mV s^{-1}; (b) 50 mV s^{-1}; (c) 100 mV s^{-1}; (d) 200 mV s^{-1}; (e) 300 mV s^{-1} and (f) 500 mV s^{-1}.

The plots of log I_p vs log v in the scan rate range of 25–500 mV s^{-1} yielded a straight line with slope of 0.91. This value is close to the theoretical value of 1.00, which is expected for an ideal reaction condition for adsorption controlled electrode process [12,13]. In addition that the graphs obtained was good linearity between I_{pc} vs scan rate (v) and I_{pc} vs $v^{1/2}$ (Fig. 3a and b). In the range from 25 to 500 mV s^{-1} the cathodic peak currents were proportional to the scan rate. The correlation coefficient was found as 0.9993 ($n = 5$) and 0.9995 ($n = 5$), which indicate the electrode reaction was adsorption controlled [13].

The single reduction peak of nitrofurantoin is attributed to the four electron reduction of nitrofurantoin to the corresponding hydroxylamine [14].

$$R{-}NO_2 + 4e^- + 4H^+ \rightarrow R{-}NHOH + H_2O$$

The electron transfer coefficient 'α' is calculated from the difference between peak potential (E_p) and half wave potential ($E_{p/2}$) according to equation given below [15]:

$$\Delta E_p = E_p - E_{p/2} = (47.7/\alpha)\ mv \text{ (irreversible reaction, at 298 K)} \quad (1)$$

The value of α is calculated to be 0.53. For an irreversible cathodic reaction, we may use the following equation to calculate standard rate constant (k_0) [16,17].

$$E_p = E^0 + (RT/\alpha nF)[\ln(RTk_0/\alpha nF)] - \ln v] \quad (2)$$

where E^0 is the formal potential, R was the universal gas constant (8.314 J K^{-1} mol^{-1}), T (K) was the Kelvin temperature, α was the transfer coefficient, k_0 (s^{-1}) was the electrochemical rate constant and F was the Faraday constant (96,487 C mol^{-1}). The value of E^0 was obtained from the intercept of the E_p vs v plot by the extrapolation to the vertical axis at $v = 0$. The value of k_0 were evaluated from the plot of E_p vs ln v and found to be 1.04×10^3 s^{-1}.

Furthermore, chronocoulometry was performed in the solutions of 1.0×10^{-5} M NTF in BR buffer solution (pH 7.1) as the electrolyte. The diffusion coefficient (D) can be determined according to the formula given by Anson [18].

$$Q(C) = 2nFACD^{1/2}t^{1/2}\pi^{-1/2} + Q_{dl} + Q_{ads} \quad (3)$$

where A (cm^2) was the surface area of the working electrode, C (M) was the concentration of NTF, D (cm^2 s^{-1}) was the diffusion