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Detection of norfloxacin and monitoring its effect on caffeine catabolism in urine samples

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ABSTRACT

A multi-walled carbon nano tube (MWCNT) modified pyrolytic graphite (MPG) electrode is prepared and applied to detect norfloxacin (NFX) based on its electrochemical reduction. The experimental parameters affecting the NFX determination were optimized in terms of MWCNT amount, pH, reaction time, and square wave frequency. The dynamic range for the NFX analysis ranged between 1.2 and 1000 μM with a detection limit of 40.6 ± 3.3 nM. The effect of NFX on the catabolism of caffeine has been studied by determining its concentration in the urine samples after the prolonged administration of NFX using the MPG electrode. The results show that the catabolism of caffeine is inhibited by ~65% after five days of NFX administration, consequently the caffeine concentration in the urine sample is increased, which is reflected in terms of ~2.5 times increase in the peak current of caffeine. The determinations of NFX and caffeine were selective and the method was successfully applied in biological fluids and pharmaceutical tablets for the test compound analysis. In future this method can be useful for the selective determination of NFX and studying its effect on caffeine catabolism.

1. Introduction

Norfloxacin (NFX) [1-ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolene-carboxylic acid] is a synthetic fluoroquinolone antibiotic which exhibits broad-spectrum antimicrobial activity against many pathogenic Gram-negative and Gram-positive bacteria including gentamicin-resistant *Pseudomonas aeruginosa* and methycillin-resistant *Staphylococcus aureus* (Lim et al., 2001). NFX is widely used in the treatment of respiratory and urinary tract infection, ocular and skin infections, gonococcal urethritis, and infectious diarrhea (Chen et al., 2006). Although, NFX is clinically important, it causes several side effects, such as, headache, depression, dizziness, nausea, and vomiting (Chen et al., 2006). The administration of NFX has also been found to affect the metabolism of caffeine through lowering its demethylation (its catabolism) process in the human system. It has also been reported earlier that the prolonged administration of NFX increases the caffeine concentration in the extra cellular fluids of the human body due to its retarted demethylation (Carb et al., 1989, Harder et al., 1988). Additionally, the high concentration of caffeine accumulation in the human body also reflects with numerous clinical disorder including coronary vasospasm and variety of arrhythmias (Chou and Benowitz, 1994; Berger and Alford, 2009). It is also suggested that patients with known history of arrhythmias should curtail intake of caffeine products (Pelchovitz and Goldberger, 2011; Papaioannou et al., 2005).

In the view of such a clinical importance of NFX and its relation with caffeine, it is desirable to develop a single step, sensitive, selective, quick, and less expensive method for the determination of NFX and to monitor its effect on caffeine catabolism in the biological fluids. A number of studies have been reported for the individual determination of NFX and caffeine in biological fluids such as; HPLC (Rao and Nagaraju, 2004); spectrophotometry (More et al., 2009); spectrophotometry with the aid of chemometric (Ni et al., 2008); capillary electrophoresis (Cheng et al., 2007); spectroflourometric (Bian and Jiang, 2006); kinetic spectrophotometric (Rahman et al., 2004). However, rare attempts have been made to monitor the effect of NFX on catabolism of caffeine in biological fluids (Carb et al., 1989). The methods reported for the determination of NFX alone or to monitor its effect on caffeine catabolism require expensive instruments along with complicated time consuming pretreatment and derivatization process. Generally, electrochemical techniques have simplified the testing procedures, including home-use devices (Chandra et al., 2011; Lee and Shim, 2001). Electrochemical methods based on nano material modified electrodes have attracted attention in the last decade for the determination of biomolecules and drugs due to their high electrical and optical property (Abdelwahab et al., 2010; Goyal et al., 2010; Zhu et al., 2010). Thus, the highly conducting characteristics

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of nanomaterials (*e.g.*: MWCNT) can be utilized to develop a label free method for the NFX determination and to monitor its effect on caffeine catabolism by exploring their direct electron transfer processes in the biological fluids. The MWCNT was deposited on the pyrolytic graphite due to its large operational potential window and less background current (Cleary et al., 1981; Kachoosangi et al., 2008). Thus, the present work is focused with two objectives. First, the development of a simple and selective method for NFX detection in the urine samples using a MWCNT modified pyrolytic graphite (MPG) electrode and second, to investigate the effect of NFX on the caffeine catabolism (or demethylation) through its electrochemical determination in urine samples. This is the first report of the selective detection of NFX in the patients urine samples based on its electrochemical reduction using a solid MPG electrode. In this work we have also monitored the caffeine catabolism (or prevention of caffeine demethylation) and its accumulation in the human urine samples for the first time.

2. Experimental

2.1. Instrumentation

The electrochemical experiments were carried out using a computerized BAS (Bioanalytical Systems, West Lafayette, USA) CV-50 W voltammetric analyzer equipped with three electrode cell system. An unmodified pyrolytic graphite electrode (UPG) (~6 mm^2) or MPG electrode was used as working, an Ag/AgCl (3 M NaCl) (Model MF-2052 RB-5B) as reference, and a platinum wire as an auxiliary electrode. The pyrolytic graphite pieces were received as a gift from Pfizer Inc., New York, USA. The JEOL-JSM 7400 field emission scanning electron microscopy (FE-SEM) instrument was used to examine the surface morphology of the MPG electrode. High-performance liquid chromatography (HPLC) studies were performed on Agilent 1100 series system equipped with RP-18e (5 μm) column. The mobile phase was acetonitrile–water (20:80) at the flow rate of 1 mL/min. The urine sample was filtered through a 0.5 μm membrane filter (Millipore) before injection and 5 μL was injected in HPLC. The absorbance of eluent was monitored at 260 nm. The pH of the buffer solutions was measured using Century India Ltd. Digital pH meter (Model CP-901). Ultrasonic machine was used to achieve well dispersed suspension of MWCNT in N,N-dimethylformamide (DMF) solution.

2.2. Chemical and reagents

Caffeine was obtained from Sigma-Aldrich and NFX was obtained from Ishita Drugs and Industries Ltd., Ahmedabad, India [Batch no. 0104/2010] as a gift. Both the compounds were used as received without further purification. MWCNT of $>98\%$ purity was purchased from Bucky, USA. Phosphate buffers of appropriate pH and ionic strength (1.0 M) were used. NFX containing tablets of different companies, Norflox (Okasa Pvt. Ltd., Mfg. Batch no. MV-1050), Norflox—Tz (Okasa Pvt. Ltd., Mfg. Batch no. MV-1061), and Powerflox (Cipla Ltd., Mfg. Batch no. DV-0143) were purchased from the market of Roorkee. All other reagents used were of analytical grade and the double distilled water was used throughout the experiment.

2.3. Preparation of modified pyrolytic graphite (MPG) electrode

The MPG electrode was prepared as follows. At first, the pyrolytic graphite surface was rubbed on an emery paper and then washed with double distilled water followed by softly touching it onto a tissue paper. The suspension of MWCNT was prepared by dispersing 0.5 mg of MWCNT in 1 ml of DMF. To achieve the well dispersed suspension of MWCNT in DMF, the solution mixture was gently agitated for one hour in an ultrasonic bath. The optimized amount of MWCNT was casted onto the UPG and then dried at room temperature for 6 h.

2.4. Analytical procedure

NFX is partially soluble in water but completely soluble in acidic media. Hence, the stock solution (1 mM) of NFX was prepared by dissolving the required amount of NFX using 0.5 ml of HCl (0.1 N) and then using double distilled water. The NFX solutions for the voltammetric experiments were prepared by adding the required volume of the stock solution to the phosphate buffer. The voltammetric studies for NFX determinations were carried out at pH 2.15. The solution was deoxygenated by bubbling high purity nitrogen for 20–30 min before recording the voltammogram. As NFX strongly adsorbs at the MPG electrode, the surface was regenerated by the application of 0.1 V potential for 60 s after each run to remove the adsorbed material. The stock solution of caffeine (2 mM) was prepared by dissolving its required amount in the double distilled water. The urine samples of patients taking caffeine (200 mg, two doses a day) and undergoing treatment with NFX (400 mg, twice daily) were obtained every day. The morning first urine of the patients was collected for 5 days from the Institute Hospital after the permission from ethical clearance committee of the IIT-Roorkee. The AC impedance spectra (charge transfer resistance (R_{ct})) were recorded using a EG&G PAR 273A potentiostat/galvanostat and a lock-in amplifier (PAR EG&G, Model 5210) linked to a personal computer.

3. Results and discussion

3.1. Surface characterization

At first, the surface of UPG and MPG electrode was examined by the scanning electron microscopy (SEM). The results show a well dispersed MWCNT at the MPG electrode surface (**Supporting information (SI)** Fig. S1). In order to confirm the efficacy of surface modification procedure, surface area of UPG and MPG electrode was calculated. For this purpose, cyclic voltammograms (CVs) of 1 mM $K_3Fe(CN)_6$ at different scan rates in 0.1 M KCl as supporting electrolyte were recorded at UPG and MPG electrode. A redox couple was observed due to the Fe^{+3}/Fe^{+2} at both the surfaces, however, an increment in peak current at MPG electrode as compared to the UPG electrode was observed and the peak separation between the redox couple decreased to 70 mV. The surface area was calculated from the slopes of the i_p *vs.* $v^{1/2}$ plots and found to be 0.0744 and 0.2153 cm^2 for the UPG and MPG electrode, respectively. These results clearly indicate the formation of highly conducting MPG electrode with ~2.8 fold larger surface area than the UPG electrode. The modified electrode was also characterized by electrochemical impedance spectroscopy by obtaining the Nyquist plots. The frequency was scanned from 0.1 Hz to 1 MHz at the open circuit voltage with the acquisition of five points per decade in the solution containing 5 mM $[Fe(CN)6]^{3-/4-}$. The **SI-**Fig. S2 shows the Nyquist plots obtained for bare pyrolytic graphite electrode and MWCNT modified pyrolytic graphite electrode. For the bare pyrolytic graphite electrode, the plot showed a semicircle (black line), the R_{ct} was about 3500 Ω, however, the R_{ct} value for the MWCNT modified pyrolytic graphite electrode significantly decreased to 794 Ω. The decrease in the R_{ct} for the MWCNT modified pyrolytic graphite electrode clearly indicates ability of MWCNTs to promote the electron-transfer reactions at the electrode surface.