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ORIGINAL ARTICLE

Voltammetric study of ds-DNA–flutamide interaction at carbon paste electrode

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KEYWORDS

Flutamide;
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Abstract Differential pulse voltammetry (DPV) has been used to develop an electro-analytical procedure for the determination of flutamide and evaluate its interaction with DNA immobilised on the carbon paste electrode (CPE) surface. In 0.3 M phosphate buffer (pH 7.0 ± 0.01) as supporting electrolyte flutamide produced a well defined DPV peak at E_p = −0.75 V vs. SCE. The DPV study on the DNA–flutamide interaction clearly demonstrated that flutamide interacts preferentially with adenine and guanine groups in DNA. Thus, enabling to assign mechanism of action of the anticancer drug, flutamide. The developed method was successfully applied to the determination of flutamide in pharmaceutical formulations. The work has been supplemented by UV spectral study.

1. Introduction

Study on the interaction between drugs and DNA (Oliveira et al., 2009; Xiangqin et al., 2005; Maria et al., 1996; Raufa et al., 2005) is very interesting and significant not only in understanding the mechanism of action of the drug, but also for the design of new drugs (Xiaoquan et al., 2006). However, mechanism of interactions between drug molecules and DNA is still relatively little known. It is necessary to introduce more simple methods for investigating the mechanism of drug–DNA interaction. The understanding of the mechanism of interaction will promote designing of new DNA-targeted drugs. The interaction of some anticancer drugs with DNA has been studied with a variety of techniques (Ravikumar Naik and Bhojya Naik, 2008) and in recent years there is a growing interest in the electrochemical investigation of interaction between anticancer drugs and other DNA targeted molecules and DNA (Yaheng et al., 2008; Nawaz et al., 2006; Kalanur et al., 2009; Chu et al., 1998; Radi et al., 2003; Tiwari and Pitre, 2008; Ye et al., 2005; Wang et al., 2006). DNA biosensor technologies are currently under intense investigation (Ravikumar Naik and Bhojya Naik, 2008) owing to the great promise for rapid and low cost detection of specific DNA sequences in human viral and bacterial nucleic acids. A quantitative understanding of such factors that determine recognition of DNA sites would be valuable in the rational design of new DNA targeted molecules for application in chemotherapy and in the development of roots for biotechnology based on DNA hybridisation.

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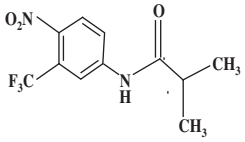

Figure 1 Structure of flutamide.

Flutamide (4-nitro-3-trifluoromethylisobutylanilide) a synthetic antiandrogenic compound with therapeutic use in prostatic cancer has been electrochemically studied to propose a new electroanalytical alternative for its quantitative determination in pharmaceutical forms. Flutamide is shown to be electrochemically reducible in aqueous and mixed media, due to the presence of the nitro aromatic moiety in the molecule. The chemical structure of flutamide is shown in Fig. 1.

Flutamide is used as antineoplastic and antiandrogen drug. It is a powerful nonsteroidal androgen antagonist (Snycerski, 1989) which is used to treat prostate cancer and is believed to block androgen receptor sites. This drug and its primary hydroxyl metabolite decrease the metabolism of C-19 steroids by the cytochrome P-450 system at the target cells in the secondary sex organs.

In the present work we used DNA modified carbon paste electrode in combination with differential pulse voltammetry to obtain information about the interaction of flutamide with DNA. The work is mainly aimed to study the interaction of flutamide with DNA and to propose a suitable mechanism of its interaction.

2. Experimental

2.1. Chemicals and apparatus

All reagents were of AR grade purchased commercially. ds-DNA (Himedia Ltd., Mumbai) and flutamide of Sigma Aldrich were used. Solutions were prepared using deionised double distilled water. Stock standard solution of flutamide was prepared in 90% ethanol.

The voltammetric studies were carried out in exploratory mode on software connected Ω Metrohm 797 VA Computrace (ion analyzer). The electrochemical cell consisted of a three electrode assembly and a stirrer with carbon paste electrode as a working electrode, a platinum wire as auxiliary electrode and Aa/AgCl electrode as reference electrode. The nitrogen gas was purged for 5 min. A systronics digital μpH meter model-361 was used for pH measurements.

2.2. Preparation of carbon paste electrode

Carbon paste was prepared in usual way by hand-mixing graphite powder and mineral oil. The ratio of graphite powder to mineral oil was 70:30. The prepared paste was filled into the Teflon well (Ravikumar Naik and Bhojya Naik, 2008). A Copper wire fixed to a graphite rod and inserted into the Teflon well serves to establish electrical contact with the external circuit. A good reproducibility of electrode response was achieved by simply renewing the surface of carbon paste electrode. New electrode surface was formed by mechanically pressing the carbon paste from the top of the Teflon well smoothening of the electrode surface was done by rolling a smooth glass rod on the electrode surface and finally it was cleaned carefully by distilled water. Each measurement involved fresh carbon paste surface.

2.3. ds-DNA modified carbon paste electrode

The carbon paste electrode was pre-treated by applying a potential of +1.70 V for 1 min in 0.3 M phosphate buffer. ds-DNA was immobilised on pre-treated CPE (Tiwari and Pitre 2008) by applying a potential of +0.50 V for 5 min in 0.1 mg/ml ds-DNA. The electrode was then rinsed with distilled water. The ds-DNA modified CPE was then immersed into 0.3 M phosphate buffer (pH 7.0 ± 0.01) containing flutamide and differential pulse voltammogram was recorded.

3. Results and discussion

3.1. Electrochemical reduction of flutamide at bare CPE

The electrochemical behaviour of flutamide at CPE was investigated employing DPV and CV mode. The cyclic voltammogram for the reduction of flutamide (100 ppm) in 0.3 M phosphate buffer (pH 7.0 ± 0.01) is shown in Fig. 2. In the forward scan, one cathodic peak owing to the reduction of the nitro group was observed and no peak was noticed in the reverse scan suggesting that the reduction of flutamide at CPE is irreversible. Similar in case of DPV, when a solution of flutamide (100 ppm) in 0.3 M phosphate buffer of pH 7.0 ± 0.01, was electrolysed using bare CPE as working electrode, it produced well defined reduction peak at −0.75 V due to reduction of NO_2 group of flutamide as shown in Fig. 3. The linear relation between peak current and drug concentration was observed in the range of 20 ppm to 160 ppm flutamide. The differential pulse voltammograms of flutamide at different concentrations are shown in Fig. 4. Regression data of the calibration line for quantitative determination of flutamide using differential pulse voltammetry are shown in Table 1.

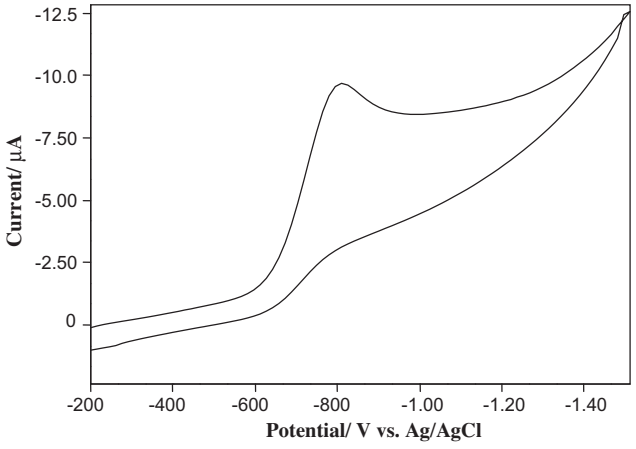

Figure 2 Cyclic voltammogram of 100 ppm flutamide in 0.3 M phosphate buffer (pH 7.0 ± 0.01) at bare CPE, pulse amplitude 50 mV, scan rate 50 mV s^{-1}, current range 10 μA.

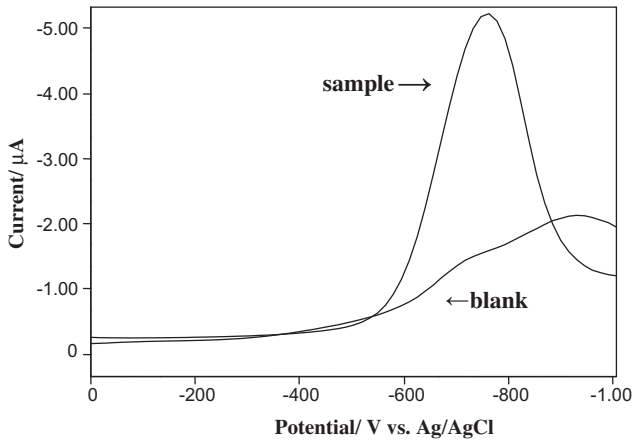

Figure 3 Differential pulse voltammogram of 100 ppm flutamide in 0.3 M phosphate buffer (pH 7.0 ± 0.01) at bare CPE, pulse amplitude 50 mV, scan rate 50 mV s^{-1}, current range 10 μA.

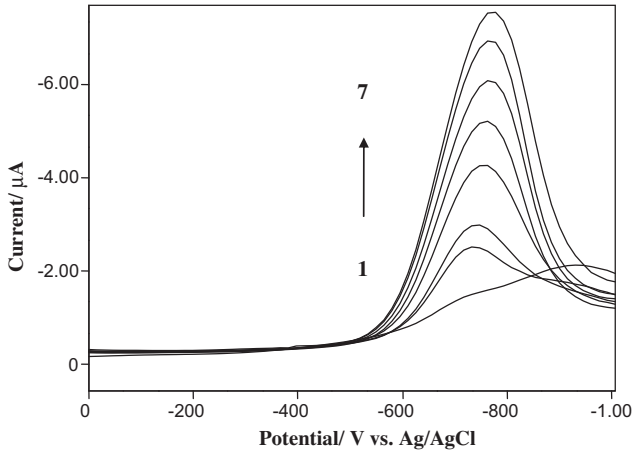

Figure 4 Differential pulse voltammogram of different concentrations of flutamide in 0.03 M phosphate buffer (pH 7.0 ± 0.01) at bare CPE, pulse amplitude 50 mV (1:20 ppm, 2:40 ppm, 3:80 ppm, 4:100 ppm, 5:120 ppm, 6:140 ppm, 7:160 ppm).

Table 1 Regression data of the calibration line for quantitative determination of flutamide using differential pulse voltammetry.

Parameters	Differential pulse voltammetry
Linearity range (ppm)	20–160
Slope (μA/ppm)	0.0416
Intercept (μA)	1.0153
Correlation coefficient (r^2)	0.977
Limit of detection (LOD) (ppm)	1.0
Limit of quantification (LOQ) (ppm)	5.0

3.2. Electrochemical behaviour of ds-DNA at bare CPE

In 0.3 M phosphate buffer of pH 7.0 ± 0.01 the electrochemical oxidation of ds-DNA involves a complex process which occurs at the specific groups adenine and guanine with different electron transfer reaction rates. The differential pulse voltammogram of 100 ppm ds-DNA at the pre-treated CPE gave two anodic peaks (Liping et al., 2006) at +1.107 V and +0.792 V corresponding to the oxidation of adenine and guanine sites of ds-DNA respectively (Fig. 5). For both the peaks the peak current was found to be proportional to ds-DNA concentration.

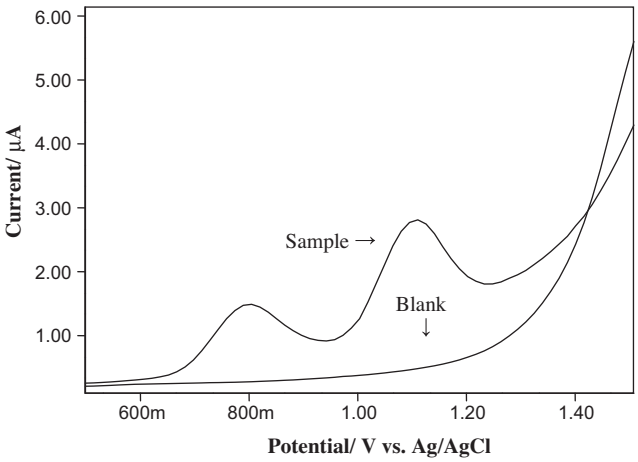

Figure 5 Differential pulse voltammogram for 100 ppm ds-DNA in 0.3 M phosphate buffer (pH 7.0 ± 0.01) at bare CPE, pulse amplitude 50 mV, scan rate 50 mV s^{-1}, current range 10 μA.

3.3. Interaction of flutamide with ds-DNA at bare CPE

After studying the electrochemical behaviour of flutamide at carbon paste electrode, we have been able to study its interaction with ds-DNA. Experimental sets of solutions were prepared by taking a fixed concentration of flutamide (100 ppm) in 0.3 M Phosphate buffer of pH 7.0 ± 0.01 and varying the conc. of ds-DNA from 20 to 60 ppm. The reduction of flutamide was investigated for each set. The first set i.e. without ds-DNA, produced a well defined DPV reduction peak at −0.75 V which shifted to more electronegative potential with increasing concentration of ds-DNA and peak current shortened. The decrease in peak current and shifting in peak potential may be explained on the bases of change of species (Oliveira Brett, 1995; Kalanur et al., 2009; Tian et al., 2008) that is reduced at CPE surface due to the formation of drug–DNA complex.

In the differential pulse voltammetric study, the changes in the peak current due to flutamide reduction without and with ds-DNA are shown in Fig. 6. The results show that peak current due to flutamide at bare electrode was higher (Kalanur et al., 2009; Xia et al., 1998) than the peak current due to the mixture of flutamide–ds-DNA reduction at CPE. It was observed that in recording the DP voltammogram of flutamide alone in forward scan a cathodic peak was observed, which corresponds to the reduction of NO_2 group of flutamide. When calf thymus ds-DNA was added to the solution of flutamide, a marked decrease in the peak current and shift of peak potential to more negative value were observed.

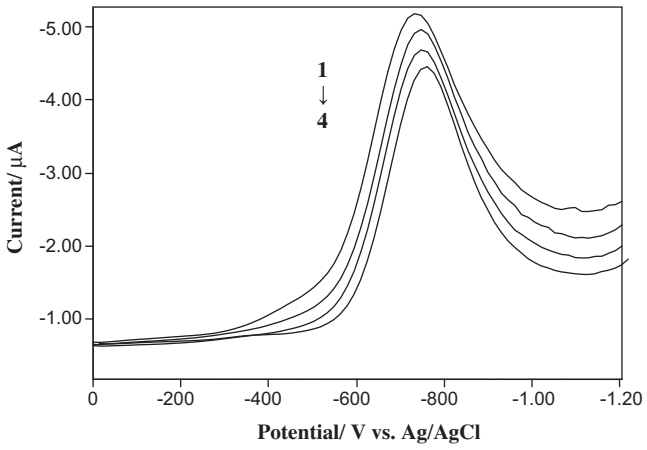

Figure 6 DPV curves of flutamide at bare CPE in 0.3 M phosphate buffer solution (pH 7.0 ± 0.01) curve – 1:80 ppm flutamide curve – 2:20 ppm ds-DNA curve – 3:40 ppm ds-DNA curve – 4:60 ppm ds-DNA.

3.4. Interaction of flutamide at ds-DNA modified CPE

The carbon paste electrode (2 mm diameter) was modified by dipping it in a solution of 100 ppm ds-DNA solution and a potential of +0.5 V was applied for 5 min. The electrode was electrochemically conditioned by application of a DPV potential scan between 0 and +1.5 V in 0.3 M phosphate buffer. The ds-DNA modified CPE was then placed in 0.3 M phosphate buffer of pH 7.0 ± 0.01 containing 100 ppm flutamide and voltammogram was recorded. The change in DPV peak current of flutamide with bare and ds-DNA modified CPE is shown in Fig. 7. Flutamide produced a reduction peak at $E_p = -0.75$ V with bare CPE, whereas using ds-DNA modified CPE it produced a peak with $E_p = -0.78$ V. The peak current with bare CPE is higher (Xia et al., 1998) than peak current obtained with the ds-DNA modified CPE. This indicated the pre-concentration of flutamide at ds-DNA modified CPE electrode, due to the ds-DNA–flutamide interaction. The peak potential for flutamide peak is more negative at ds-DNA modified CPE than that at bare CPE, indicating the intercalative attractions and stacking interactions of flutamide between the base pairs of DNA.

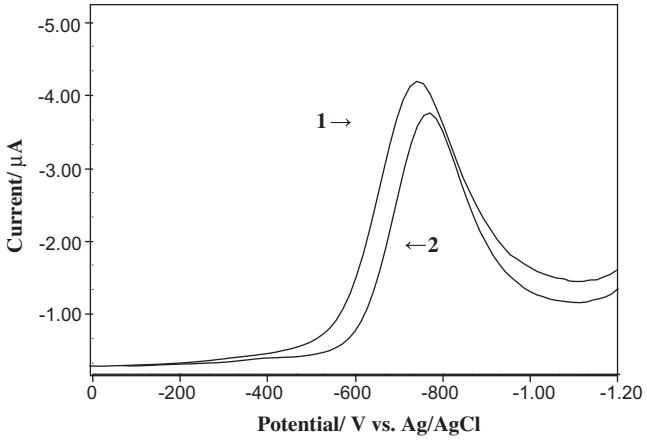

Figure 7 Curve (1) differential pulse voltammogram of 100 ppm flutamide at bare CPE in 0.3 M phosphate buffer (pH 7.0 ± 0.01), curve (2) DPV of flutamide at ds-DNA modified CPE pulse amplitude 50 mV, scan rate 50 mV s^{-1}, current range 10 µA.

3.5. UV–VIS evolution of flutamide–ds-DNA interaction

To determine whether flutamide influences the ds-DNA conformation, the absorption spectra of ds-DNA in the absence and presence of flutamide were monitored. The absorption spectra of 50 ppm flutamide are shown in Fig. 8. The figure clearly shows two absorption bands for flutamide at 225 and 300 nm, respectively whereas ds-DNA produced one absorption band at 260 nm. However, on recording the absorption spectrum of the mixture of ds-DNA–flutamide, only one absorption band at 260 nm was recorded, which ascribes to the combination of ds-DNA and flutamide. The results are in excellent agreement with author's observations using electrochemical study.

3.6. Interaction mechanism of action of flutamide with ds-DNA

Flutamide is a new nonsteroidal antiandrogen useful in the treatment of prostatic carcinoma. When a potential of −0.75 V was applied, flutamide was reduced at the DNA-modified electrode. In this way flutamide generates an anion, this reactive anion may oxidise one of the neighbouring guanine site of the ds-DNA. During this process, electron transfer from guanine moiety to the nitro group of flutamide without hydrogen abstraction is likely to be the predominant reaction leading to the formation of the guanine cation. The cation will undergo hydrolysis forming semiquinone. This semiquinone may undergo further reduction to the fully reduced flutamide.

3.7. Analysis of flutamide in tablet

Flutamide tablet was obtained from local commercial source. An accurately weighed portion of finely powdered tablet was transferred to a 50 ml beaker containing 20 ml doubly distilled water. The solution was filtered and diluted to 50 ml and 0.5 ml of diluted sample was added to electrochemical cell con-

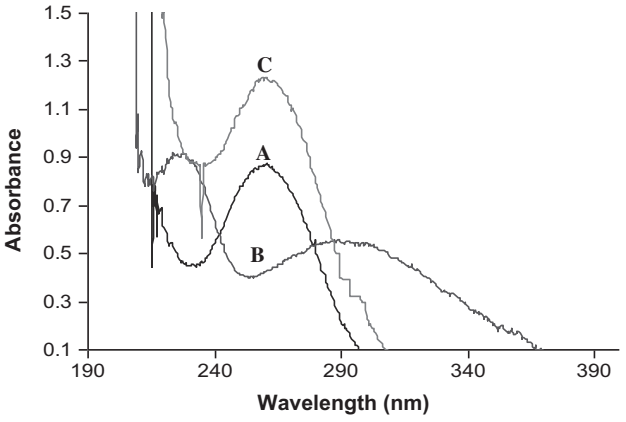

Fig. 8 UV spectra of (A) 30 µg/ml ds-DNA, (B) 30 µg/ml flutamide and (C) mixture of ds-DNA–flutamide (30:30 µg/ml).

Table 2 Assay results of flutamide tablet by DPV and mean recoveries.

Sample	Parameter	Added (mg)	Found (mg)
Cytomid tablet (pharmaceutical formulation)	Amount	Nil	0.452
	%R	0.420	0.871
	R.S.D	99.88	
		0.2	

%R = percentage recovery.
R.S.D. = relative standard deviation.

taining 10 ml phosphate buffer solution (pH 7.0 ± 0.01) and the total volume of the test solution was made to 25 ml and differential pulse voltammogram was recorded using CPE as working electrode. The amount of flutamide was determined by means of the standard addition method. The results obtained using developed method was found to be in good agreement with that reported by its manufacturer for its flutamide content. The percentage recovery and standard deviation of the observed analytical data indicate the accuracy and repeatability of the proposed voltammetric method for flutamide determination (Table 2).

4. Conclusion

The study on the interaction between the anticancer drug flutamide and ds-DNA is very important in the development of a new anticancer drug. This paper investigated the interaction of flutamide with ds-DNA by DP voltammetric and UV spectral studies. The electrochemical behaviour of flutamide at carbon paste electrode was established. The electrochemical process is irreversible. This work has shown experimental evidence of interaction of flutamide with ds-DNA and may contribute to the understanding of the mechanism of action of flutamide as anticancer drug. A simple, rapid and sensitive DPV method is proposed for the determination of flutamide in pharmaceutical formulations.

References

Chu, X., Guo-Li, S., Jian-Hui, J., Tian-Fang, K., Xiong, B., Ru-Qin, Y., 1998. Anal. Chim. Acta 373, 29.

Kalanur, S.S., Katrahalli, U., Seetharamappa, J., 2009. J. Electroanal. Chem. 636, 93.

Liping, W., Lin, L., Ye, B., 2006. J. Pharm. Biomed. Anal. 42, 625–629.

Maria, A., Brett, O., Silvia, H.P., Macedo, S.T.A., Raimundo, D., Helena Merques, M., La-Scaley, M.A., 1996. Electroanalysis 8, 992–995.

Nawaz, H., Rauf, S., Akhtar, K., Khalid, A.M., 2006. Anal. Biochem. 354, 28.

Oliveira Brett, A.M., 1995. J. Braz. Chem. Soc. 6, 97.

Oliveira, S.C.B., Chiorcea-Paquim, A.M., Ribeiro, S.M., Melo, A.T.P., Vivanc, M., Oliveira Brett, A.M., 2009. Bioelectrochemistry 76, 201.

Radi, M.A., Ries, E., Kandil, S., 2003. Anal. Chim. Acta 495, 61.

Raufa, S., Gooding, J.J., Akhtar, K., Ghauria, M.A., Rahman, M., Anwar, M.A., Khalid, A.M., 2005. J. Pharm. Biomed. Anal. 37, 205.

Ravikumar Naik, T.R., Bhojya Naik, H.S., 2008. Int. J. Electrochem. Sci. 3, 409.

Snycerski, A., 1989. J. Pharm. Biomed. Anal. 7, 1513.

Tian, X., Song, Y., Dong, H., Ye, B., 2008. Bioelectrochemistry 73, 18.

Tiwari, S., Pitre, K.S., 2008. J. Chin. Chem. Soc. 55, 1166.

Wang, L., Lin, L., Ye, B., 2006. J. Pharm. Biomed. Anal. 42, 625.

Xia, C., Guo, L.S., Jiang, J.H., Kang, T.F., Xiong, B., Ru-Qin, Y., 1998. Analytica Chimica Acta 373, 29–38.

Xiangqin, L., Jiang, X., Lu, L., 2005. Biosens. Bioelectron. 20, 1709–1717.

Xiaoquan, L., Chen, Y., Chen, J., Zhang, Y., Zhang, L., Li, M., 2006. Int. J. Electrochem. Sci. 1, 130–138.

Yaheng, X.T., Dong, S.H., Ye, B., 2008. Bioelectrochemistry 73, 18.

Ye, B.X., Yuan, L.J., Chen, C., Tao, J.C., 2005. Electroanalysis 17, 1523.