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Full Length Research Paper

Electrochemical reduction of flutamide and its determination in dosage forms and biological media

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Electrochemical reduction of flutamide, non-steroidal anti-androgen was studied at different pH and concentrations at a dropping mercury electrode/hanging mercury drop electrode using different polarographic techniques. Differential pulse polarography was used to establish an electroanalytical procedure for the determination of flutamide in pharmaceutical formulations, urine and serum samples. The cathodic peak observed is attributed to the reduction of nitro group and found to be ph dependent and useful for quantitative estimation. The single step reduction wave/peak observed was found to be irreversible and diffusion controlled. The lower standard deviation of 1.58% with the developed procedure sample analysis can be carried out without pre-treatment or extraction or prior separation of the sample before determination.

Key words: Flutamide, differential pulse polarography, pharmaceutical formulations, urine, serum.

INTRODUCTION

Flutamide (1',1',1' trifluro- 4'- nitroisobuty1-0-mtoluidine) (Figure.1) is a non–steroidal anti-androgen, which has been reported to be useful to improve urine flow in benign prostatic enlargement and gynaecomstia (Katchen et al., 1975; Shutsung et al., 1974; van Winkle et al., 1976). This drug is widely used in India. Nitro group containing drugs and pharmaceuticals have a good biological importance and these are essential for the biological systems for their proper growth and metabolic connections (Lutsky et al., 1975). In recent years, interest in the electrochemical behavior of biologically important compounds has increased.

High-performance liquid chromatography has been used to assay flutamide in pharmaceutical preparations (Nunes et al., 2005). This method utilized extraction procedure followed by direct analysis on packed OV-1 or OV-17 columns. The content uniformity test according to USP XX is required, and specific analytical methods are needed for the determination of single dose content without interference by excipient. In the methods such as spectrophotometry, gas liquid chromatography and sufficiently stable associated with poor polarity are vaporizable and can therefore be detected GLC.recently by HPLC, the active principle involved is extraction before quantitative measurement. The choice of the method of determination of the molecules, drugs Derivation by means of silylating agents often facilitates vaporization. In many cases no foregoing separation procedure is needed if electroanalytical techniques especially DPP are used for assay, because many of the excipient do not disturb the electrode process. DPP can also be employed for the analysis of coloured materials or samples containing dispersed solid particles (Wang et al., 2006). Polarography analysis which is useful for the measurement of urinary excreted drug is usually very polar and often require dramatization to reduce the polarity of the compound and reviews related to the electrochemical behaviour and determination of drugs in pharmaceutical formulations and biological media have been reported (Patriache et al., 1990; Subba et al., 1997; El-Hefnawey et al., 2004; Meites, 1967; Ferreira et al., 1997; Pinilla et al., 1993; Subba et al., 1999; Suresh and Jayarama, 1991; Sree and Jayarama, 1991).

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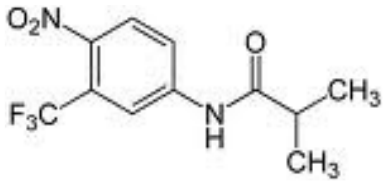

Figure 1. Chemical structure of flutamide.

Electrochemical studies provide useful information about redox reaction in biological systems and also throw light on chemical reactivity of drugs and their metabolites (Xu et al., 2004; Hammam et al., 2006; Mara et al., 2008). To our knowledge, no electroanalytical assay for flutamide has been reported in the literature.

In this paper, the electrochemical behaviour of flutamide is briefly presented by employing advanced electrochemical techniques such as d.c polarography and cyclic voltammeter. The application of DPP is faster and simpler electroanalytical technique for the analysis of the compound form pharmaceutical preparations and biological media (urine and serum) has been described. The chemical structure of flutamide is as shown in Figure 1.

MATERIALS AND METHODS

DC. Polarographic analyzer coupled with BD8 kipp & zonen x-t recorder. A dropping mercury electrode (flow rate 2.48055 mg/s) was used as working electrode, a saturated calomel electrode (SCE) was used as reference electrode and platinum wire as auxiliary electrode. Differential pulse polarographic measurements were performed with a Metrohm E 506 Polarecord connected to an E 612 VA scanner. The three electrode assembly consisted of DME (with an area 0.023 cm^2) working electrode, a platinum wire as auxiliary electrode and a saturated Ag/AgCl (s) Cl^- reference electrode. The cyclic voltammograms were obtained with the digital electronics 2000 x-y/t recorder in conjunction with the above unit (the working electrode was HMDE having an area of 0.0433 cm^2). The I.R. spectra of the compound were taken with the model PYE UNICAM Sp. 3-300 infrared spectrophotometer. The pH measurements were carried out with an Elico digital pH meter. All the experiments were conducted at 25±1°C.

Flutamide was obtained from "Well Come" India Ltd, Bombay (100% chromatographically pure) and was used without further purification. Stock solution was prepared by dissolving required quantity of particular drug in 100 mL double distilled methanol for getting a concentration of 1 mM. The desired concentrations of the solutions were prepared by diluting the stock solution with the supporting electrolyte. 0.05 M citric acid, 0.2 M boric acid and 0.1 M trisodium orthophosphate were used as supporting electrolytes over the pH range 2.0-12.0. All chemicals used were of annular-grade.

Urine samples were obtained from patients at specific time intervals during a single-does administration. 8 h (over night) or 24 h collected urine sample was transferred to 500 mL flaks, an appropriate amount of drug standard was added and flask was made up with urine to the mark. The spiked urine was either analysed immediately or aliquots were put in a deep freezer until measurements were performed. When analyzing, deep frozen samples were first kept at room temperature in dark until they are thermally equilibrated (2 h).

RESULTS AND DISCUSSION

The electrochemical reduction of aromatic nitro group containing compounds has attracted considerable attention in the past and has been reviewed (Smyth, 1979). Flutamide exhibits only one polarography and wave/peak is due to the reduction of nitro group to hydroxylamine with the four electrons addition. Experimental results show the well defined wave/peak of flutamide obtained at pH 4.0. It was observed that with the buffers of pH < 3.0, the wave corresponding to the reduction of nitro group appears at the start of potential and therefore reduction wave/peak is ill-defined. Similarly with the solution of greater alkalinity (pH> 9.0), further reduction of nitro group is not facilitated owing to the non-availability of the protons. The half–wave ($E_{1/2}$) and peak potential (E_p, E_m, and E_s) values are linear function of pH. As the pH of the buffer systems is increased, the reduction potential is found to be shifted towards a more negative value. The electrode processes for the flutamide is found to be free from adsorption and currents are diffusion controlled nature, which is confirmed through the linear plots of i_d (d.c. polarography diffusion current) vs.$h^{1/2}$, i_p (cyclic voltammetric peak current) vs. $v^{1/2}$, i_m (maximum peak current in dpp) vs. $t^{2/3}$, i_m (maximum peak current in dpp) vs. concentration passing through origin (Meites, 1965). The variation of peak potential with scan rate and absence of anodic peak in the reverse scan of cyclic voltammogram show the electrode process to be irreversible. At higher pH values (pH>10.0), a small anodic peak (a_1) is observed in the reverse scan of cyclic voltammogram (Subba et al., 1999). In the 2nd scan another small cathodic peak at more positive potential than c_1 is observed. The anodic peak may be ascribed to the oxidation of (hydroxylamine) reduced product at (c_1) and cathodic peak c_2 to the reduction of (nitroso derivative) oxidised product at a_1 as depicted in (Figure 2). The number of electrons involved in the overall reduction process of flutamide, as determined by millicoulometry at selected pH of the buffer system used, is found to be four.

Analyses

Both standard addition and calibration methods were used for DPP analysis of flutamide. The polarography peak obtained in acidic medium has been utilized in the analytical estimation of the title compound (Suresh and Jayarama, 1991). The main nitro group reduction peak is useful in the DPP analysis of the drugs for the following reasons:

a) It occurs at a small negative potential, where a limited number of other polarographic reduction occurs. Thus the determination of nitro group compounds enjoys a measure of selectivity in the field of polarographic analysis.
b) The peak height is relatively large because of four

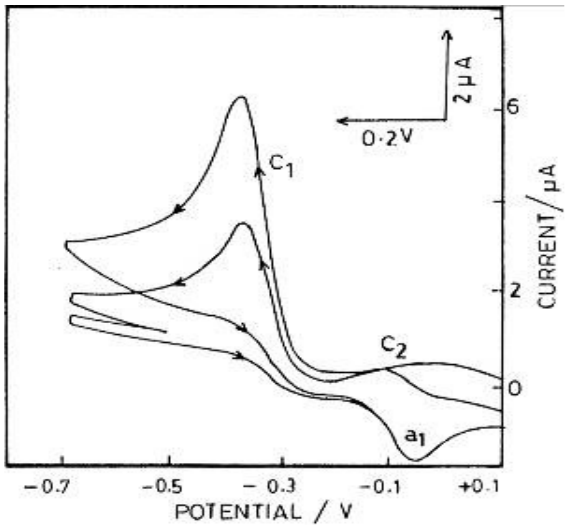

Figure 2. Typical cyclic voltammogram of flutamide in pH 10.0.Concentration: 0.5 mM; Scan rate: 40 mV/s.

Table 1. Differential pulse polarographic data of flutamide in pharmaceutical formulations (Flutamide formulations: Pulse amplitude: 65 mV; Drop time: 2 s).

Sample	Labelled amount	Amount found	Recovery (%)	Standard deviation
Prostamide	10	9.98	99.80	0.012
	20	19.97	99.98	0.012
	30	29.89	99.63	0.014
	40	39.92	99.80	0.024
Sebatrol	10	9.96	99.60	0.018
	20	19.89	99.45	0.020
	30	29.88	99.92	0.015
	40	39.42	99.55	0.011

electron reduction step thus making polarographic determination of nitro group containing drug more sensitive involving lower detection limit of 10^{-9} M.
c) The peak height is unaffected by minor change in pH. For analytical purpose here pH 4.0 or 6.0 is used.

The peak currents of title compound is found to vary linearly with the concentration of the drug over the concentration range $1.5 \times 10^{-5} - 2.0 \times 10^{-9}$ M with the detection limit of 1.72×10^{-9}M. DPP is found to be more suitable at lower concentrations due to its high sensitivity and resolution.

Recommended analytical procedure

Stock solution (1.0×10^{-5} M) was prepared by dissolving appropriate amount of the electroactive compound in methanol. One milliliter of standard solution was transferred into a polarographic cell and was deoxygenated with nitrogen gas for 15 min. Differential pulse polarogram was recorded using optimized conditions. The small increments (0.2 mL) of standard solutions were added and polarogram were recorded after each addition under similar conditions. The optimum conditions for the estimation of flutamide in pH 4.0 are found to be a drop time of 2 seconds, pulse amplitude of 65 mV, and applied potential of -0.18V (vs. Ag/AgC1 (s), $C1^-$). The relative standard deviation and correlation coefficient values for 10 replicates are 1.58% and 0.988 respectively.

Flutamide in pharmaceutical formulations containing 245 mg in total tablet mass of approximately 250 mg has been analysed in order to examine the utility of the method. About 10 tablets were thoroughly grounded and mixed uniformly. Portions equivalent to 10, 20, 30 and 40 mg of compound were accurately weighed, dissolved in pure methanol and transferred in to 25 mL calibrated flasks. A portion of 0.5 mL aliquot of the clear supernatant liquid was diluted to 10 mL supporting electrolyte (pH 4.0) and polarogram recorded. The amount of the compound in portion of the sample taken was estimated by reference to calibration plot. Table 1 represents the corresponding experimental results.

Table 2. Analytical data for flutamide in urine samples.

S/no. of patient	Flutamide administered (mg)	Flutamide excreted (mg)
1	250	1.15
2	250	2.32
3	250	0.89
4	250	1.64
5	250	4.32
6	250	2.56

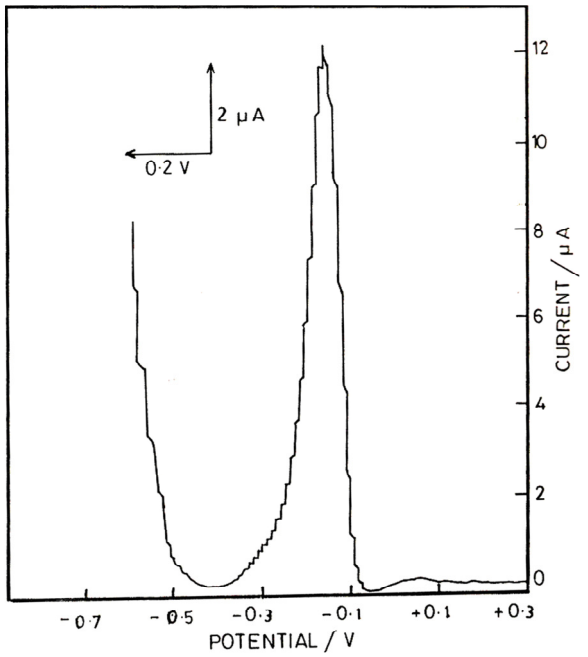

Figure 3. Typical differential pulse polarogram of flutamide in urine in pH 4.0. Concentration: 1.0×10^{-8}M; Pulse amplitude: 65 mV; Drop time: 2 s.

For developing a faster and simple analytical procedure, we had studied the effect of non-degasifying of the flutamide solution with nitrogen gas before polarographic measurements. Our results indicated that previous purging was not required because calibration plots were similar without purging sample solution under investigation. However, the detection limit in this case was about 1.25×10^{-9} M. From the results it was concluded that purging of drug containing solution with nitrogen is not essential for the estimation of either the pure drug or dosage forms, reducing the measurement time by nearly 15-20 min per run.

The reliability of the method for the determinations of flutamide in urine was checked using different spiked urine samples in conjunction with the standard addition method. Six different urine samples were spiked with standards in concentration range at which the unchanged drug is excreted. The recovery was found to be 99.96% with the relative standard deviation of 0.96%.

Urine samples were obtained from the patients at specific time intervals during single-dosage administration. It is known that the portion of an orally administered dose that excreted unchanged in urine is dose dependent. However, it was observed that after administration of single oral dose of 250 mg, the drug concentration in urine increase until it reaches 12% of the initial dose at 4 h and then begins to decrease. Nearly 20-25% of a dose is excreted within 7 h. After 12 h, the polarographic signal disappears and the unchanged drug excreted below the method of detection of method together with polarographically inactive flutamide and corresponding data are tabulated in Table 2. The potential interference of some urine main ingredients was checked without adding electro-active species.

None of the ingredients did affect the potential interferences. Figure 3 represents the typical differential pulse polarogram of flutamide in urine in pH 4.0.

The utility of the proposed method has also been tested for serum samples. 1 mL of serum was denaturated with 0.4 mL/L perchloric acid and was kept in the deep freezer

Table 3. Recovered data for four spiked serum samples in pH 4.0.

S/no.	Labelled amount	Amount found	Recovery (%)	Standard deviation
1	0.2	0.197	99.50	0.012
2	0.2	0.198	99.00	0.008
3	0.2	0.199	99.50	0.016
4	0.2	0.196	98.00	0.010

for 15 min. Then the precipitated protein was centrifuged and the supernatant was neutralized with 0.2 mL 4.0 M sodium acetate solution. To the neutralized serum sample, a standard solution of flutamide was added. The spiked serum samples were diluted with the supporting electrolyte (pH 4.0) and the polarogram recorded immediately. Four different serum samples were spiked with standard solutions. The recovery was found to be 98.2 ± 0.92% and the data in Table 3 also indicates that the method is free from Interferences.

The polarographic method described in this article can be applied for rapid and reliable determination of flutamide in drugs, urine and serum samples without time consuming sample pretreatment, which is capable of application of routine usage. The method is sensitive enough to determine the concentrations as low as those encountered after therapeutic dosage. It has been demonstrated to be applicable to variety of samples media (urine and serum).

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