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Voltammetric-oxidation and Determination of 5-Flurouracil and its Analysis in Pharmaceuticals and Biological Fluids at Glassy Carbon Electrode Mediated by surfactant cetyltrimethyl ammonium bromide

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ABSTRACT

The voltammetric oxidation and determination of 5-Fluorouracil 5-(FU) was studied at a glassy carbon electrode (GCE) in the presence of cetyltrimethyl ammonium bromide (CTAB) by cyclic and differential pulse voltammetry at pH-7. The results indicated that the voltammetric responses of 5-Flurouracil are drastically increased in the low concentration of CTAB, suggesting that CTAB exhibits observable enhancement effect to the determination of 5-Flurouracil. Under the optimal conditions the peak current was proportional to 5-Flurouracil concentration in the range of 2.0 x 10^{-8} to 6.0 x 10^{-7} M with detection limit of 20.13nM by differential pulse voltammetry. The proposed method was applied to the determination of 5-Fluorouracil in pharmaceuticals. The analytical performance of this sensor has been evaluated for detection of analyte in human serum and urine as real samples.

Keywords: 5-Flurouracil, Glassy carbon electrode, Electro-oxidation, Cyclic voltammetry, Surfactant, human serum, Urine.

INTRUDUCTION

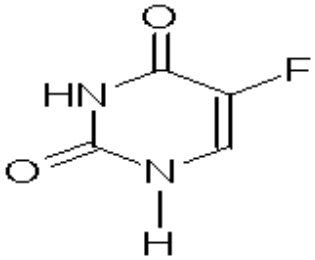
5-Fluorouracil (5-fluoro-1*H*-pyrimidine-2, 4-Dione) (5-FU) (scheme 1). is an antineoplastic agent used for the treatment of solid tumors of the breast and rectum [1]. The polarographic behavior of 5-FU has been examined and it was shown that it undergoes reduction at pH 1.8-11 with the best defined wave at pH 6-8 [2]. Guerrieri et al. [3] studied the formation of an insoluble mercury compound in the presence of 5-FU using different electrochemical techniques. Different methods have been reported for the determination of 5-FU, including cathodic stripping voltammetry [4-6]. This technique is based on the interfacial accumulation of the analyte on the working electrode prior to voltammetric measurements of the surface-bound compound. Liquid chromatography with anodic amperometric detection has also been used for the determination of

5-FU [7]. Although HPLC has been widely applied because of its high sensitivity and selectivity and the ability to minimize interferences, it is time consuming, solvent usage intensive and requires expensive devices and maintenance. Electro chemical detection of analyte is a very elegant method in analytical chemistry. [8-10]

Electrochemical methods have proved to be very sensitive for the determination of organic molecules, including drugs and related molecules in pharmaceutical dosages forms and biological fluids and their oxidisible property [11-12].Carbon electrodes, especially glassy carbon electrodes and paste electrodes, are widely used in the electrochemical investigations because of their low background current, wide potential windows, chemical inertness, low cost, and suitability for detection of various organic and biological compounds. Among these glassy carbon electrode (GCEs), due to unique characteristics such as versatility of chemical modification, have been extensively used.

Surfactants are a kind of amphiphilic ions or molecules with a hydrophilic head compatible with water on one side and long hydrophobic tail compatible with oil on the other side. They have been widely used in field of electrochemical and electro analytical chemistry [13-14]. The surfactant can change the electrochemical process through adsorption at interfaces or aggregation in to supramolecular structure [15]. Many groups successfully employed surfactants for the analysis of some bio molecules in their work [16]. They got very good results which indicated that the electrochemical responses of analysed objects were mark- -ably enhanced in the presence of surfactants.

In the present work, the experimental results showed that the oxidation peak current of 5-fluorouracil was found to increase to a greater extent at the glassy carbon electrode in the presence of a cationic surfactant cetyltrimethyl ammonium bromide (CTAB). The electro analytical method developed under pH-7 has important role in physiological metabolism of the drug. We optimized all the experimental parameters for the determination of 5-fluorouracil and developed an electro analytical method for its determination. This method has the advantages such as fast response, easy repair, good reproducibility and low detection limit. The proposed method was applied to the determination of 5-fluorouracil in the tablets and urine samples.



Scheme1. Structure of 5-flurouracil

MATERIALS AND METHODS

2. Experimental

2.1. Reagents

Pure 5-fluorouracil in powdered form obtained from spectrochem, India and used as received . A stock solution of 5-FU (1.0 x 10^{-3}M) was made in double distilled water. Phosphate buffer

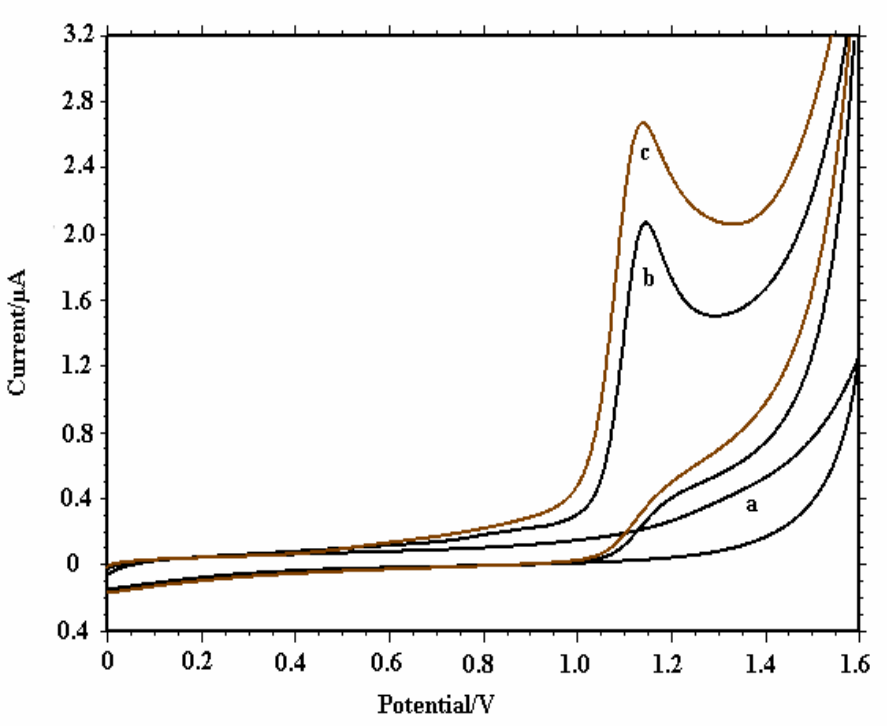
solutions (Ionic strength = 0.2 M) were prepared according to the literature method [17]. The 5-FU containing tablets were purchased from a local pharmacy. All the surfactants obtained from Hi-Media Pvt. Ltd. were dissolved in doubly distilled water to form 1.0 x 10^{-3} M solutions. All other reagents used were of analytical grade.

2.2. Apparatus

Electrochemical experiments were performed on EA-201 Electroanalyzer and were carried out in a 20 ml single compartment three-electrode glass cell with self-made GCE as a working electrode, a platinum wire as counter electrode, and a saturated calomel as reference electrode (SCE) were employed. All potentials were reported vs. SCE. All experiments were carried out at an ambient temperature of 25 ± 0.1 ^{0}C. The pH measurements were made with Elico pH meter model LI120.

RESULTS AND DISCUSSION

3.1. Cyclic voltammetric behavior of 5-FU



The cyclic voltammetric behavior of 5-FU at GCE in the presence of CTAB was investigated using cyclic voltammetry. The results are shown in (Fig.1).

Figure 1. Cyclic voltammograms at the Glassy carbon electrode in phosphate buffer solution (pH 7): (a) in the presence bare pH 7; (b) in the presence of 5-FU; (c) in the presence of CTAB; and scan rate 0.05 Vs^{-1}, accumulation time: 90 s, 5-FU: 1 x 10^{-3} M, CTAB: 1 x 10^{-5} M.

No apparent cyclic signals were observed in the phosphate buffer solution in the presence (curve a) and absence (curve b) of CTAB, which indicate that CTAB is an electrochemically inactive material in the working potential range. The 5-FU exhibits an anodic peak at about 2 V (curve b) at the GCE in the absence of CTAB. After the addition of 1.0 x 10^{-5} M CTAB, the oxidation peak at GCE increases significantly (curve c). This indicates that CTAB can make the electron transfer of 5-FU more easily and show obvious enhancement effect to the oxidation of 5-FU. The peak current enhancement was undoubtedly attributed to the interaction of CTAB with 5-FU and GCE. It is well known that surfactants can be adsorbed on a hydrophobic surface to form surfactant film, which may alter the over voltage of the electrode and influence the rate of electron transfer. In the presence of CTAB, the electrode surface may form a hydrophilic film with positive charge. This hydrophilic layer increase the concentration of 5-FU on the electrode surface.

On the scan, no corresponding reduction peak was observed, indicating that the electrode process of 5-FU is an irreversible one. Nevertheless, it was found that the oxidation peak current of 5-FU showed a remarkable decrease during the successive cyclic voltammetric sweeps. A decrease in the oxidation peak current occurs with the number of successive sweeps. This phenomenon may be due to the fact that the adsorption of its oxidative products occurs at the electrode surface. Therefore, the voltammograms corresponding to the first cycle was generally recorded.

3.2. Effect of accumulation conditions

The two parameters of accumulation step-accumulation time and potential were examined. Open circuit accumulation is widely used in electro analytical chemistry to accumulate analyte and improve the determining sensitivity. The influence of accumulation time ranging from 0 to 200 s on the oxidation of 5-FU at GCE was as shown in (Fig.2).

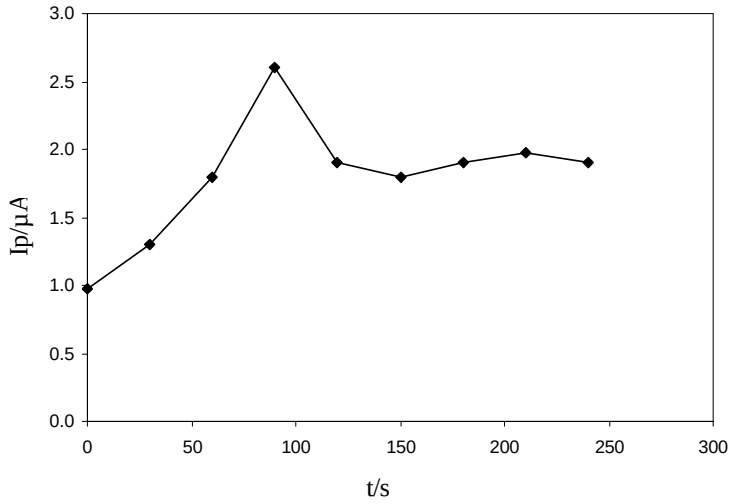


Figure 2. Effect of accumulation time on the oxidation peak current of 1.0 x 10^{-3} M 5-FU. Other conditions are same as in fig.1

The peak current increased gradually as accumulation time increased from 0 to 90 s. However, with further increasing, the accumulation time beyond 90 s the peak current tends to be almost stable. Therefore, the optimal accumulation time of 90 s was employed in further experiments.

With the change of accumulation potential, the peak current of 5-FU varied slightly. So, the accumulation potential has no such effect on the peak current of 5-FU, which in turn indicates that adsorption of CTAB is independent of the charge on the electrode surface. It is consistent with the fact that CTAB is adsorbed on the electrode surface through hydrophobic interaction with paraffin oil. Therefore the accumulation was carried out at open-circuit conditions.

3.3. Effect of concentration of CTAB

Amongst different surfactants used, the CTAB could only effectively promote the oxidation of 5-FU at GCE. The effect of CTAB concentration on the oxidation of 5-FU was as shown in (Fig.3).

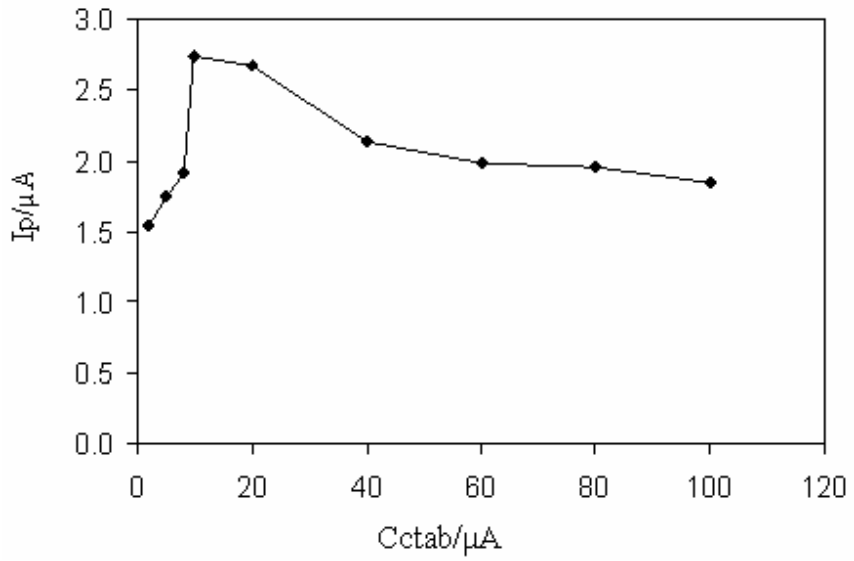


Figure 3. Dependence of the oxidation peak current on the CTAB concentration

When the concentration of CTAB was increased from 0 to 1.0 x 10^{-5} M, the peak current increased to a maximum. However, when CTAB concentration was increased beyond 1.0 x 10^{-5}, the peak current began to decrease. It may be due to the formation of CTAB layer on the electrode surface, which blocks the electron transfer between the 5-FU and the electrode.

3.4. Effect of scan rate

The effect of scan rate on the electro oxidation of 5-FU was examined by cyclic voltammetry (Fig.4).

The influence of the square root of the scan rate on the peak current showed a linear relationship between 0.025 to 0.3 Vs^{-1}, as in (Fig. 5A). which are of typical diffusion controlled currents [18] and the equation can be expressed as follows:

$$Ip = 22.282\ v^{1/2} - 2.081,\ r = 0.9926$$

A linear relationship was observed between log Ip and log v, in (Fig.5B). corresponding to the following equation:

log Ip = 0.5279 log v + 1.1032, r = 0.9976

The slope of 0.52 was close to the theoretically expected value of 0.5 for a purely diffusion controlled current [18], which, in turn, further confirms that the electro oxidation of CTAB was diffusion controlled. With an increase in scan rate, the peak potential shifted to a more positive value, and a linear relationship was observed in the range 0.025 to 0.3 Vs^{-1} as shown in (Fig.5C).

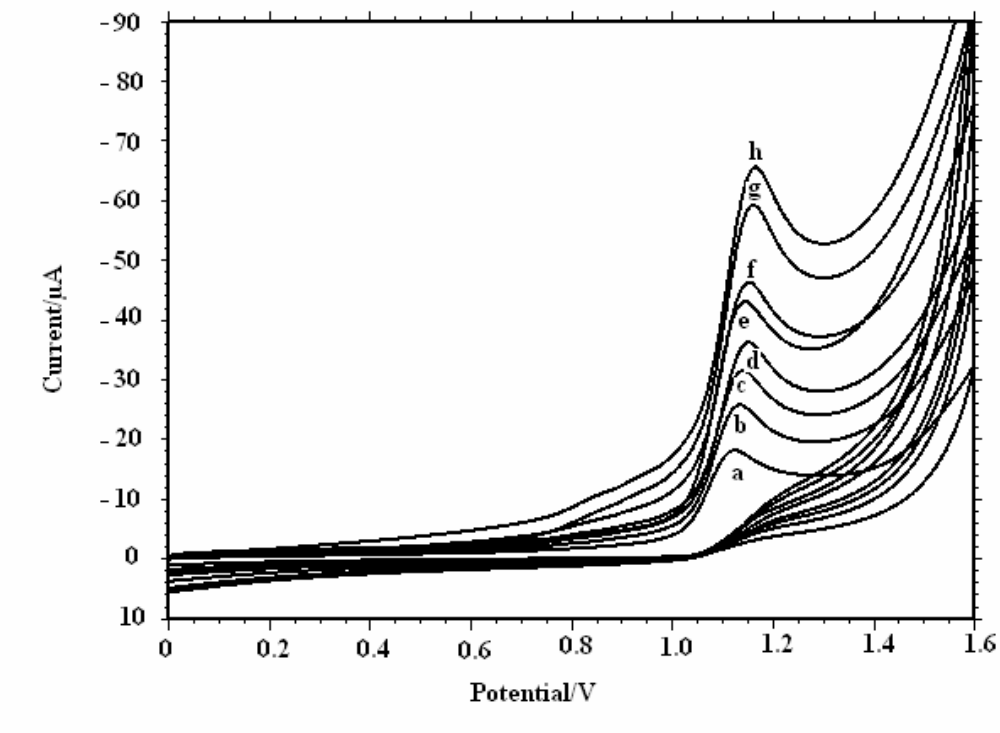
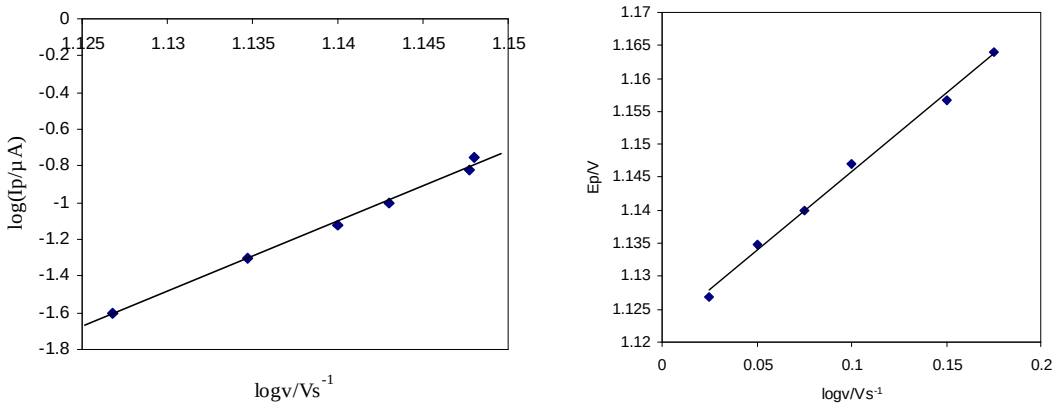
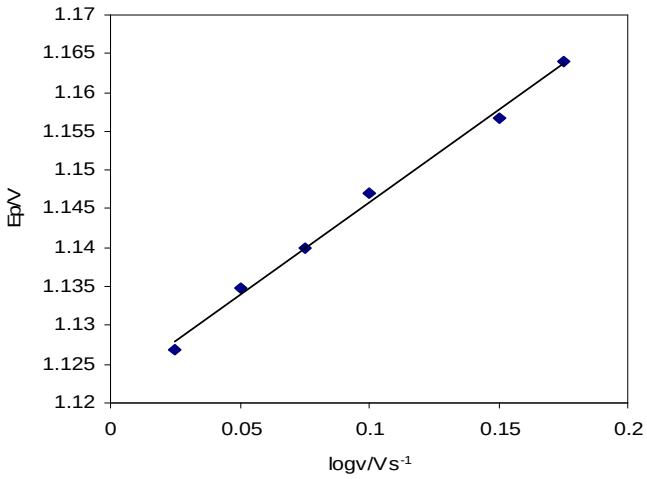


Figure 4. Cyclic voltammograms for the oxidation of 5-FU at different scan rates (a) 0.025 (b) 0.05 (c) 0.1 (c) 0.15 (d) 0.2 (e) 0.25 (f) 0.3 Vs^{-1}.



(A) (B)



(C)

Figure 5. (A) dependence of oxidation peak current on the square root of scan rate. (B) linear relation between logarithm of peak current and logarithm of scan rate. (C) dependence of oxidation peak potential on the logarithm of scan rate

The equation can be expressed as.

Ep = 0.0997 log v + 0.9928, r = 0.9904

As for an irreversible electrode process, according to Laviron, Ep is defined by the following equation [19].

$$Ep = E_0 + \left[\frac{2.303RT}{\alpha nF}\right] \log \left[\frac{RTk^0}{\alpha nF}\right] + \left[\frac{2.303RT}{\alpha nF}\right] \log v \qquad (2)$$

where α is the transfer coefficient, k^0 the standard heterogeneous rate constant of the reaction, n the number of electron transferred, v the scan rate, and E_0 is the formal redox potential. Other symbols have their usual meanings. Thus value of αn can be easily calculated from the slope of Ep vs. log v. In this system, the slope is 0.0997, taking T = 298, R = 8.314, and F = 96480, the αn was calculated to be 0.593. The value of α is 0.5. Further, the number of electron (n) transferred in the electro oxidation of 5-FU was calculated to be 1.19~1. The value of k^0 can be determined from the intercept of the previous plot if the value of E_0 is known. The value of E_0 in eqn. (2) can be obtained from the intercept of E_p vs. *v* curve by extrapolating to the vertical axis at *v = 0* [20]. In our system the intercept for Ep vs. log v plot was 0.9928 and E_0 was obtained to be 0.842, the k^0 was calculated to be 7.48 x 10^2 s^{-1}.

3.5. Effect of pH

The electro oxidation of 1.0 x 10^{-3} M 5-FU was also studied over the pH range 3.0 to 10.0 in phosphate buffer solution by cyclic voltammetry and which is shown in (Fig. 6). The oxidation peaks were appeared strongly between at pH 7.0 we found oxidation peak. With the increase in solution of pH, the peak potential linearly shifts to less positive values and the linear relation between Ep and pH in (Fig. 6 inset.)

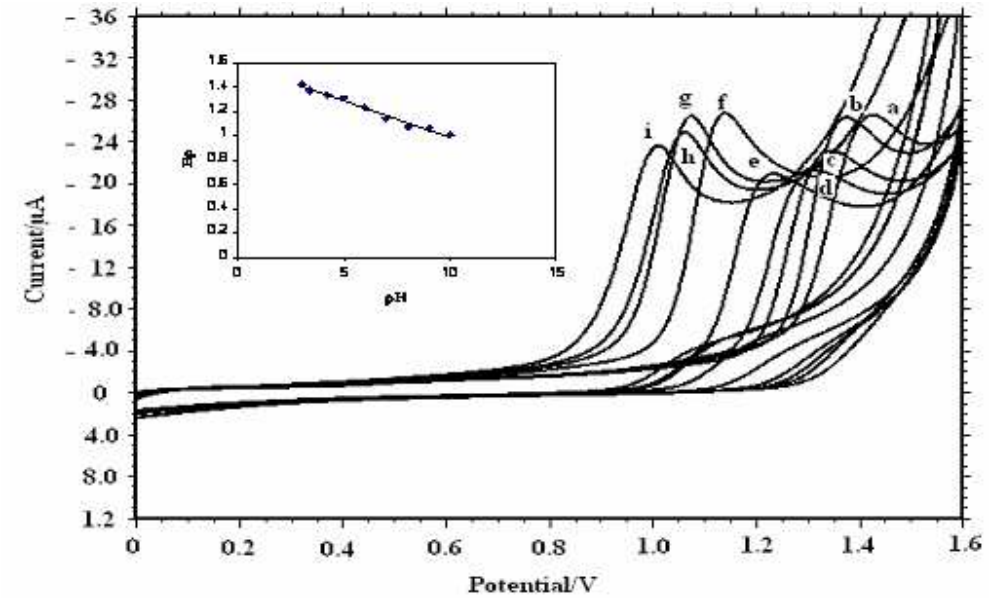


Figure 6. Cyclic voltammograms of 1.0 x 10^{-3} 5-FU at different pH: (a) 3.4 (b) 4.2 (c) 5.0 (d) 6.0 (e) 7.0 (f) 8.0 (g) 9.2(h) 10.4 (i) 11other conditions are same as in Fig. 1. and Inset. Influence of pH on the potential of 1.0 x 10^{-3} M 5-FU on GCE at scan rate of 50 mV s^{-1} in phosphate buffer

The pH of solution influenced the peak current considerably. It is found that the oxidation of peak current increases from pH 3 to 7 then changes very slightly from 8.0 to 10.0 and finally decreases (Fig 7).

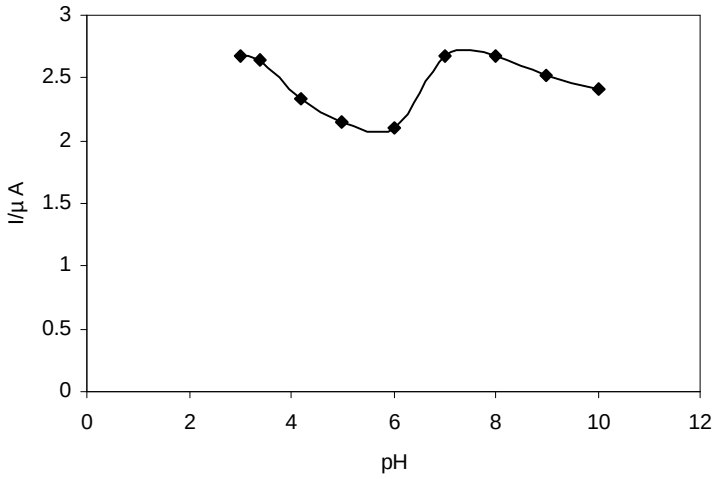
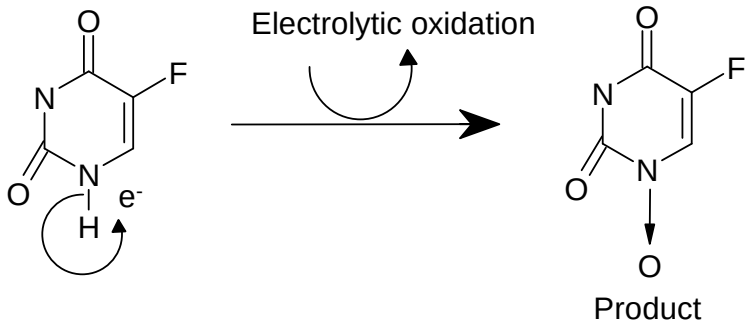


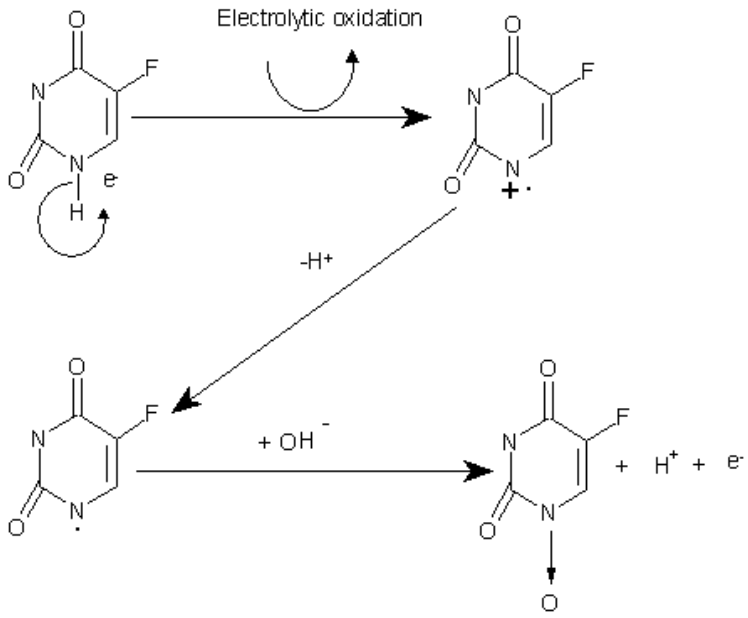
Figure 7 Variation of current with pH of 1.0 x 10^{-3} M 5-FU on GCE at scan rate of 50 mV s^{-1} in phosphate buffer.

Meanwhile, the peak potential shifts towards negative potential with increasing pH value. So, the buffer solution with pH 7.0 was chosen for further experiment.

Mechanism. The one-electron oxidative release of 5-Flurouracil on elecrytic-oxidation to give 5-Fluro-1-(2-oxocycloalkyl) uracils are formed as a product [21](Scheme 2,3).



Scheme 2. Electro oxidation of 5-flurouracil



Scheme 3. Probable Electro-oxidation mechanism of 5-Flurouracil

3.6. Calibration curve

In order to develop a voltammetry method for determining the drug, we selected the differential-pulse voltammetric mode, because the peaks are sharper and better defined at lower concentration of 5-FU than those obtained by cyclic voltammetry, with a lower background current, resulting in improved resolution. According to the obtained results, it was possible to apply this technique to the quantitative analysis of 5-FU. The phosphate buffer solution of pH 7.0 was selected as the supporting electrolyte for the quantification of 5-FU as it gave maximum peak current at pH 7.0. Differential pulse voltammograms obtained with increasing amounts of 5-FU showed that the peak current increased linearly with increasing concentration, as shown in

(Fig.8). Using the optimum conditions described previously, linear calibration curves were obtained for 5-FU in the range of 2.0 x 10^{-8} to 6.0 x 10^{-7} M (Fig. 8. Inset)

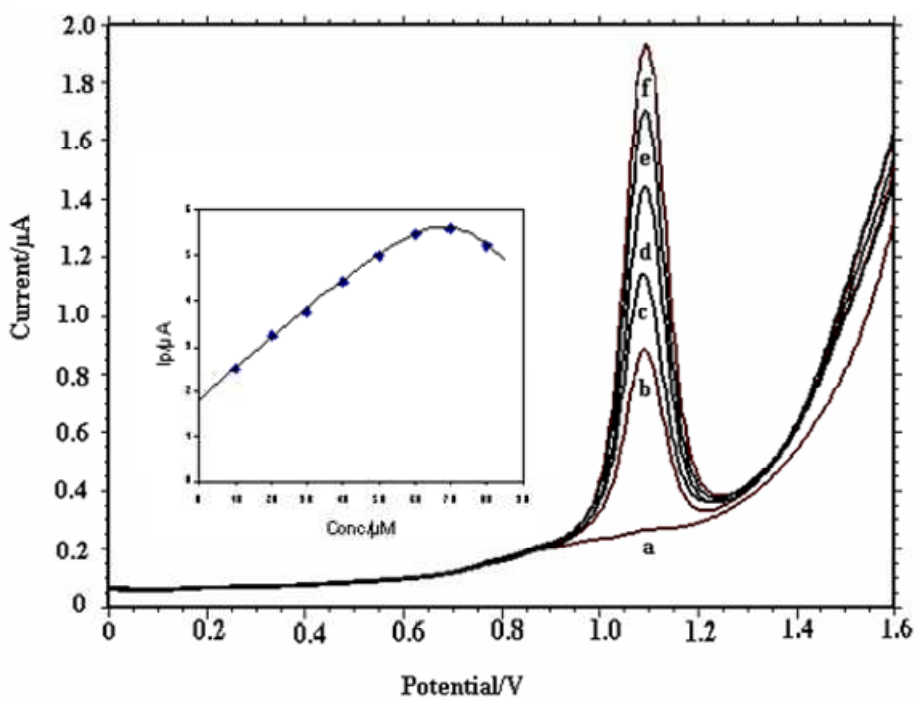


Figure 8. differential pulse voltammograms for increasing concentration of 5-FU: (a) 0.8 (b) 2.0 (c) 8.0 (d) 20.0 (e) 40.0 (f) 60.0 µM. other conditions are same as in fig 1. Inset.Plot of current against the concentration of 5-FU

The linear equation was Ip = 4.7133 + 1.868 (r = 0.9891, C is in µM). Deviation from linearity was observed for more concentrated solutions, due to the adsorption of 5-FU or its oxidation product on the electrode surface. Related statistical data of the calibration curves were obtained from four different calibration curves. The limit of detection (LOD) and quantification (LOQ) were 20.13nM and 7.10 µM, respectively. The LOD and LOQ were calculated using the following equation:

LOD = 3 s / m; LOQ = 10 s / m

Where s is the standard deviation of the peak currents of the blank (four runs), and m is the slope of the calibration curve [22]. Sample solutions recorded after 48 hrs did not show any appreciable change in the assay values.

3.7. Tablet Analysis and Recovery Test

The 5-FU tablets were purchased under the brand name Aduracil from the local market. In order to evaluate the applicability of the proposed method in the real sample analysis, it was used to detect 5-FU in tablets (20 mg per tablet). The procedure for the tablet analysis was followed as described in the procedural section. The results are in good agreement with the content marked in the label. The detected content was 19 mg per tablet with 95.2% recovery.

Table 1. Aduracil Tablet Recovery test of 5-FU

Added (M)	Found (M)	Recovery (%)
4.0×10^{-5}	3.893×10^{-5}	97.3
6.0×10^{-5}	6.025×10^{-5}	100.4
8.0×10^{-5}	7.853×10^{-5}	98.1
1.0×10^{-4}	1.023×10^{-4}	102.3
2.0×10^{-4}	1.95×10^{-4}	97.5

The recovery test of 5-FU ranging from 4.0×10^{-6} to 2.0×10^{-5} M was performed using differential-pulse voltammetry. Recovery studies were carried out after the addition of known amounts of the drug to various pre-analyzed formulations of 5-FU. The results are listed in Table 1. The recoveries in different samples were found to lie in the range from 97.3% to 102.3%, with R.S.D of 1.71%.

Table 2. Influence of potential Interferents on the voltammetric Response of 1.0×10^{-4} M 5-FU

3.9. Detection of 5-FU in urine samples

Interferant	Concentration (10^{-3})	Signal Change (%)
Dextrose	1.0	+3.5
Sucrose	1.0	+3.1
Starch	1.0	+3.2
Citric acid	1.0	-0.67
Oxalic acid	1.0	+4.9
Gum acacia	1.0	+2.8

3.8. Interference

Under the optimum experimental conditions, the effect of potential interferents on the voltammetric response of 1.0×10^{-4} M 5-FU were evaluated. The experimental results (Table 2) shows that ten-fold excess of sucrose, starch, citric acid and gum acacia did not interfere with the voltammetric signal of 5-FU. However, ten-fold excess of dextrose and oxalic acid had apparent influence on the voltammetric signal of 5-FU.

Table 3. Determination of 5-FU in Urine samples and scan rate 0.05 Vs^{-1}, accumulation time: 90 s, 5-FU: 1×10^{-3} M, CTAB: 1×10^{-5} M.

Sr No	Added (M)	Found (M)	Recovery (%)
1	4.0×10^{-5}	3.95×10^{-5}	97.5
2	6.0×10^{-5}	6.0×10^{-5}	100
3	8.0×10^{-5}	7.893×10^{-5}	97.3
4	1×10^{-4}	1.025×10^{-4}	100.4
5	2×10^{-4}	2.023×10^{-4}	102.3

The developed differential voltammetric method for the 5-FU determination was applied to urine samples. The recoveries from urine were measured by spiking drug free urine with known amounts of 5-FU. The urine samples were diluted 100 times with the phosphate buffer solution before analysis without further pretreatments. A quantitative analysis can be carried out by adding the standard solution of 5-FU into the detect system of urine samples, and the peak linearly increased in height. The calibration graph was used for the determination of spiked 5-FU in urine samples. The detection results of four urine samples obtained are listed in Table 3. The recovery determined was in the range from 96% to 103% and the RSD and SD are given in Table 3.

Table 4. Results obtained for 5-FU analysis from spiked human serum sample

Sample	5-FU (M)	Level determined(M)	Recovery (%)	R.S.D (%)
1	8×10^{-5}	7.99×10^{-5}	99.91	1.05
2	6×10^{-5}	6.04×10^{-5}	100.47	0.74
3	3×10^{-5}	2.95×10^{-5}	99.50	0.67

4. Detection of 5-FU in spiked serum samples

The possibility of applying the proposed method for the determination of 5-FU in human serum was tested. Serum samples were spiked with 5-FU to achieve final concentrations of 8.0×10^{-5}, 6.0×10^{-5}, and 3.0×10^{-5}M. The amount of 5-FU in human serum was calculated from the related linear regression equatations (Table-4). The generally poor selectivity of voltammetric techniques can pose difficulties in the analysis of biological samples, which contain oxidizable substances. As can be seen that,no oxidation of compounds present in serum occurs where the analytical peak appears. The percentage recovery of 5-FU was determined by comparing the peak currents of known amount of drug concentrations in serum with their equivalents in related calibration curves. The results of these analyses are summarized in Table 4. Good recoveries of 5-FU were achieved from this type of matrix. Analysis of serum samples by DPV involved only protein precipitation and centrifugation, no time consuming extraction and evaporation steps are required.

CONCLUSION

The electrochemical behavior of 5-FU at a GCE in the absence and presence of CTAB were studied. The results indicated that, CTAB can adsorb at GCE surface via strong hydrophobic interaction and voltammetric responses of 5-FU was facilitated in presence of CTAB. The proposed differential pulse voltammetric procedure can be used successfully to determine 5-FU in tablet, serum and urine samples. This method can be a good alternative for the analytical determination of 5-FU, because it is simple, sensitive, fast, accurate and inexpensive. Furthermore, the present method could possibly be adopted for pharmacokinetic studies as well as clinical and quality control laboratories.

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