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Electrochemical behavior of anticancer drug 5-fluorouracil at carbon paste electrode and its analytical application

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Abstract

Background: A set of pyrimidine nucleobase present in all living systems as a component of nucleic acid constitutes uracil together with thymine and cytosine. A diverse physiological activity is exhibited by many *N*-substituted uracil derivatives. In oncology, 5-FU is widely used as an important anticancer drug.

Methods: Electrochemical behavior was studied using cyclic voltammetric method, and the analytical application was studied using differential pulse voltammetric method. Solution pH has been measured by pH meter.

Results: The process on the surface of electrode was found to be irreversible and diffusion controlled. The charge transfer coefficient, heterogeneous rate constant, and the number of electron transferred were calculated. Possible reaction mechanism taking place on the surface of electrode was proposed. Calibration plot constructed using differential pulse voltammetric technique was used for quantitative analysis in pharmaceutical and human urine sample. Limit of detection (LOD) and limit of quantification (LOQ) were calculated to be 12.25 and 40.8 nM, respectively.

Conclusions: In the present work, we described the electrochemical behavior of anticancer drug and its determination in human urine and pharmaceutical samples. The method shows the development of a sensor for selective and sensitive determination of 5-FU.

Keywords: Electro-oxidation, Carbon paste electrode, 5-fluorouracil, Voltammetry, Pharmaceutical samples

Background

Ribonucleic acid consists of a pyrimidine base called uracil which forms base pair with adenine. For biosynthesis of nucleic acid in tumors, uracil is preferentially used (Rutman R.J. et al. 1954). A drastic change in the biological properties of uracil resulted from the substitution of hydrogen atom at fifth position by halogen atom (Voet D. and Voet J.G. 1995). Amongst the variety of uracil derivatives reported as antitumor and antiviral agent, 5-FU has acquired a position of particular importance. For the treatment of solid tumor of the breast and rectum, 5-FU has been used extensively as an antineoplastic agent (Heidlberg C. and Ansfield F.J. 1963). One of the major mechanisms responsible for antitumor activity of 5-FU is by inhibition of thymidylate synthesis (Hartmann K.U. and Heidelberger C. 1961). Detailed studies have pointed to 5-FU interference

with DNA and protein synthesis, because of conversion to the corresponding ribose nucleoside and substitution into RNA, as an equally important mechanism of toxicity (Myers C.E. 1981). The studies on oxidation—reduction behavior of compounds of biological significance—is of considerable value, as they provide deep insight into the biological relevant redox reactions of these compounds. Although the actual biological redox reactions may be of more complexity due to enzymatic interactions, much more information can be derived from the study of these compounds in aqueous solution of known pH.

Electrochemical methods have proved to be sensitive for the determination of organic molecules, including drugs and related molecules in pharmaceutical dosage forms and biological fluids and their oxidizable property (Padmini V. 2010; Hegde R.N et al. 2008). Carbon electrodes, especially paste electrodes, are widely used in the electrochemical investigations because of their low background current, wide potential windows, chemical

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inertness, low cost, and suitability for detection of various organic and biological compounds (Genxi L. and Peng M. 2013).

Many advantages such as very low background current, low cost, large potential window, simple surface renewal process, and easiness of miniaturization of carbon paste electrode (CPE) are widely applicable in both electrochemical studies and electroanalysis. In addition, easy fabrication of the electrode can be achieved by incorporating different substances during paste preparation which results in the so-called modified electrode with desired composition and predetermined properties (Khoobi A. et al. 2013; Mokhtari A. et al. 2012; Díaz C. et al. 2013; Gholivand M.B. and Mohammadi-Behzad L. 2014; Mazloum-Ardakani M. et al. 2010; Raoof J.B et al. 2007; Dönmez S. et al. 2014).

According to the literature, no works are reported on voltammetric method for the determination of 5-FU at CPE. The focus behind this work is to develop a suitable experimental condition to investigate the electrochemical behavior of 5-FU and its determination in pharmaceutical sample and human urine sample. As compared to the other reported works, the sticking feature for this is, no prior extraction step is needed in urine analysis. In the present work, the electro organic reactions at the surface of electrodes and results of one more such study are presented. Here, we preferred to use bare CPE because of the strong adsorption of nitrogen atom on such material (Scheme 1).

Scheme 1 Chemical structure of 5-FU
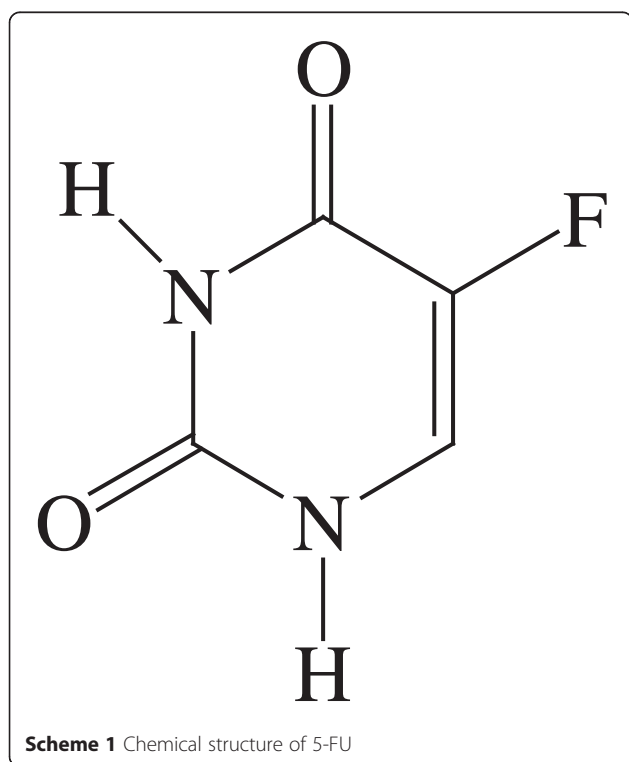
Methods

Apparatus and chemicals

Electrochemical analyzer (CHI Company, D630, USA) was used to study the electrochemical deeds of the drug under investigation at an ambient temperature of 25 ± 0.1 °C. A three-electrode system consisting of carbon paste electrode as working electrode, platinum wire as counter electrode, and Ag/AgCl (3 M KCl) as reference electrode were used in a 10-ml single compartment. In all the measurements, background subtraction was made. The phosphate buffer solutions ranging 3.0–11.2 pH ($I = 0.2$) were prepared according to literature (Christian G.D. and Purdy W.C. 1962), and pH of the solutions was measured by pH meter (Elico Ltd., LI120, India). 5-FU (Sigma-Aldrich, USA) was used to prepare 1.0 mM stock solution in double distilled water (6.5×10^6 Ω). Double distilled water and analytical grade chemicals and reagents without further purification were used throughout the experiments.

Preparation of electrode

The CPE was prepared by mixing 1.0 g of graphite powder and 0.5 ml of paraffin oil in a small agate mortar, and this mixture was then homogenized. A portion of the resulting paste was packed firmly into a cavity of polytetrafluoroethylene tube (PTFE). The surface of the electrode was smoothed against weighing paper and rinsed with water. The paste was carefully removed prior to pressing a new portion in to the electrode after every measurement. The resulting electrode was noted as CPE. Prior to use, the CPE was activated in phosphate buffer solution of pH 7 by cyclic voltammetric sweeps between 0.4 and 1.4 V with a scan rate 50 mVS^{-1} (Malode S.J. et al. 2013).

Randles-Sevcik formula can be used to calculate the electro-active area of the electrode using cyclic voltammetric technique and K$_3$Fe (CN)$_6$ 1.0 mM as a probe at different scan rates in 0.1 M KCl as supporting electrolyte. At T = 298 K and for a reversible process, the equation is as follows (Malode S.J. et al. 2012):

$$\mathrm{Ip} = \left(2.69 \times 10^5\right) n^{3/2} A_0 D_R^{1/2} v^{1/2} C_0 \qquad (1)$$

In Eq. (1), for 1.0 mM K$_3$Fe (CN)$_6$ and 0.1 M KCl as supporting electrolyte, Ip refers to the anodic peak current, n is the number of electron transferred during the electrode reaction equal to 1. A_0 is the surface area of the electrode, D_R is the diffusion coefficient equal to 7.6×10^{-6} cm^2 s^{-1}, v is the scan rate, and C_0 is the concentration of K$_3$Fe (CN)$_6$. From the slope of the plot of Ip vs. $v^{1/2}$, the area of the electrode surface was calculated to be 0.036 ± 0.0014 cm^2.

Sample preparation

To carry out the pharmaceutical analysis, 5-FU tablets were grounded using a mortar and a fraction corresponding to stock solution of 1 mM was weighed and completed to the volume with double distilled water in a 100-ml calibrated flask. After sonication for 10 min, to affect complete dissolution suitable aliquots of the clear supernatant, liquid was taken and diluted with buffer solution of pH 7. The oxidation peak current of 5-FU was measured using differential pulse voltammetric technique. Standard addition method was to study the accuracy of the projected method and the interference from excipients used in dose forms.

Results and discussion

Electrochemical behavior of 5-FU

In order to understand the electrochemical behavior of 5-FU, cyclic voltammetric technique was used. Between the range 3.0–11.2 pH, one well-defined irreversible oxidation peak was observed. In Fig. 1, voltammetric behavior of 5-FU in phosphate buffer solution (pH = 7, I = 0.2) is represented, curve (a) corresponds to buffer solution and (b) anodic peak corresponding to 5-FU oxidation. There was no peak observed on the reverse scan, therefore electrode process is supposed to be irreversible. Since successive cyclic voltammogram showed a decrease in the peak current due to adsorption of 5-FU or its oxidation product, the oxidation peak corresponding to the first sweep was only recorded.

Influence of accumulation time

Peak current can be greatly affected by the adsorption of analyte on the surface of the electrode. It is important to fix the accumulation time to improve the sensitivity of the electrode towards the analyte. Open circuit accumulation with cyclic voltammetric technique was use to study the effect of accumulation time on peak current (Fig 2). The effect of accumulation time was studied in the range of 0–150 s. One hundred twenty seconds was employed as optimal accumulation time in further experiments since maximum peak current was observed at 120 s.

Effect of supporting electrolyte

Electrochemical behavior of the analyte under investigation strongly depends on the pH of the solution. By optimizing the pH conditions, sharper response accompanied with higher sensitivity can be obtained. Hence, phosphate buffer solution over the pH range 3.0–11.2 was used to study the electrochemical behavior of 5-FU (Fig 3). It was observed that the peak potential shifted to less positive values with increase in the pH of the buffer solution (Fig. 3(A)). The linear relationship between Ep and pH can be expressed as follows:

$$\text{Ep} (V) = -0.0595\, \text{pH} (V) + 1.5652 : R^2 = 0.9952$$

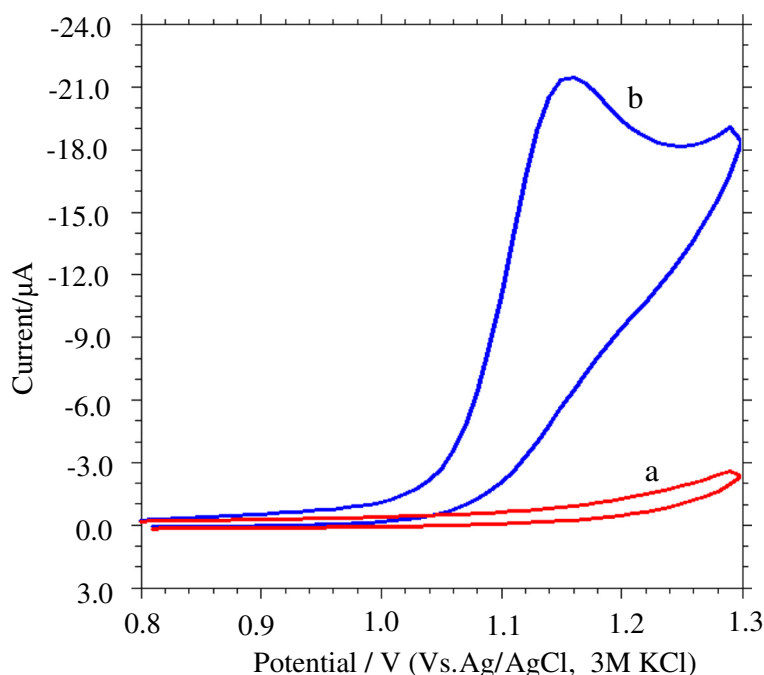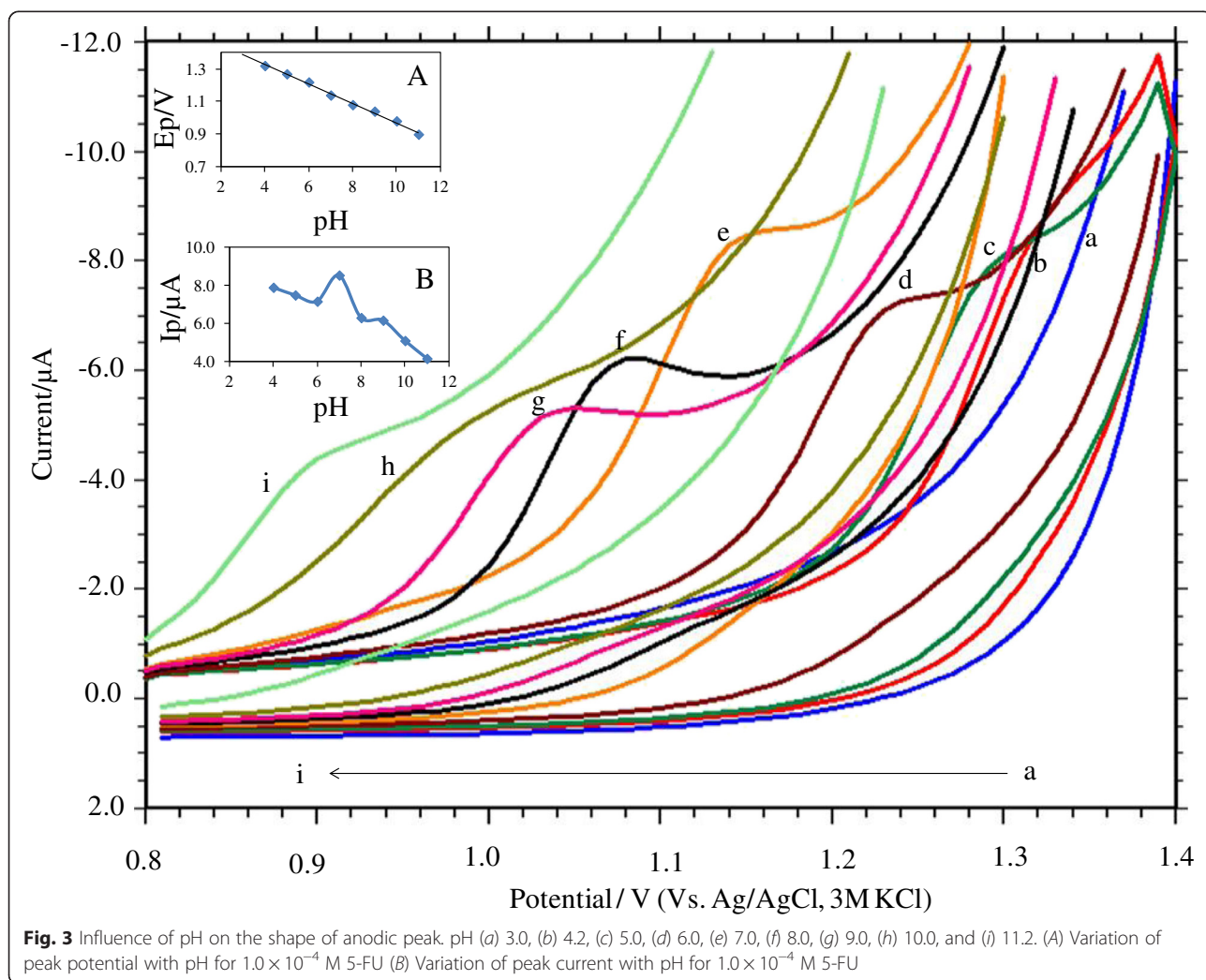From the plot of Ip vs. pH (Fig. 3(B)), it is clear that the best result with respect to sensitivity accompanied

Fig. 1 Cyclic voltammogram behavior of 5-FU, phosphate buffer (pH = 7, I = 0.2 M), (*a*) blank carbon paste electrode, and (*b*) carbon paste electrode 1.0×10^{-4} M; 5-FU scan rate: 50 mVs^{-1}; accumulation time: 120 s (at open circuit)

Fig. 2 Variation of the cyclic voltammetric anodic peak current with accumulation time
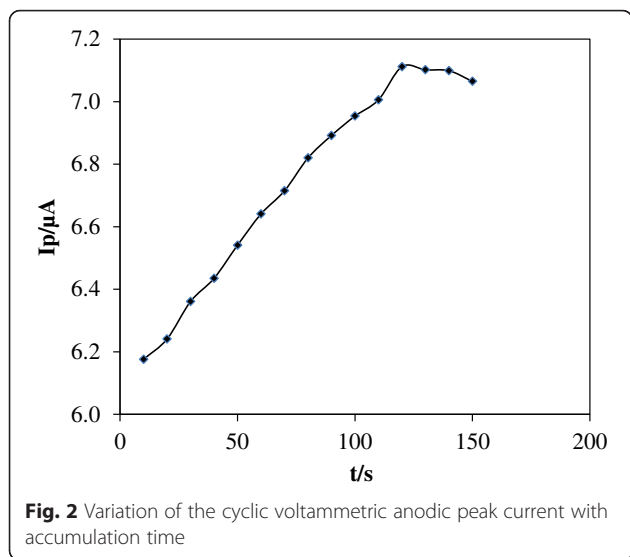
with sharper response was obtained with pH = 7.0, hence, it was selected for further work (Hegde R. N. et al. 2009). The peak current depends on the deprotonation and protonation form of the electro-active species in electrochemical cell. At pH 7, protonated and deprotonated form of 5-FU dominates. And a gradual change of speciation of 5-FU occurs in the pH range 7 to 9. Further, the magnitude of current is directly proportional to the rate of the electrochemical reaction. Hence, it is apparent to conclude that the oxidation of 5-FU is very high at pH 7 (Ioana P. et al. 2005).

Influence of scan rate

Relationship between peak current and scan rate gives constructive information about electrochemical mechanism. At different scan rates, the electrochemical behavior of 5-FU was studied by using cyclic voltammetric technique (Fig. 4). The dependence of the peak intensity Ip (μA) upon the scan rate v (Vs^{-1}) (Fig. 4(A)) was carried out to assess whether the
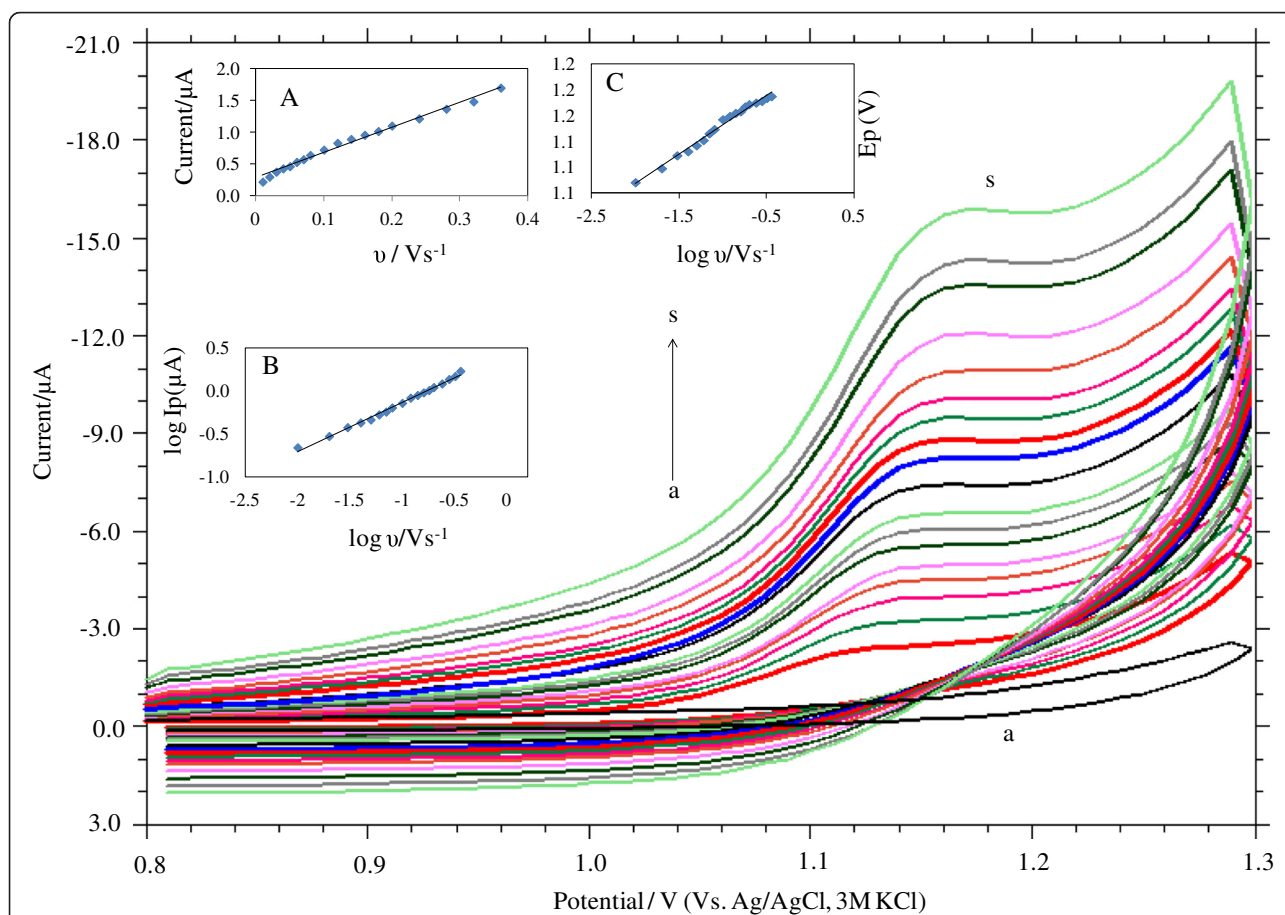

Fig. 3 Influence of pH on the shape of anodic peak. pH (*a*) 3.0, (*b*) 4.2, (*c*) 5.0, (*d*) 6.0, (*e*) 7.0, (*f*) 8.0, (*g*) 9.0, (*h*) 10.0, and (*i*) 11.2. (A) Variation of peak potential with pH for 1.0×10^{-4} M 5-FU (*B*) Variation of peak current with pH for 1.0×10^{-4} M 5-FU

Fig. 4 Cyclic voltammograms of 1.0×10^{-4} M 5-FU in 0.2 M buffer solution at pH 7.0 at scan rates of (*a*) blank, (*b*) 0.01, (*c*) 0.02, (*d*) 0.03, (*e*) 0.04, (*f*) 0.05, (*g*) 0.06, (*h*) 0.07, (*i*) 0.08, (*j*) 0.10, (*k*) 0.12, (*l*) 0.14, (*m*) 0.16, (*n*) 0.18, (*o*) 0.20, (*p*) 0.24, (*q*) 0.28, (*r*) 0.32, and (*s*) 0.36 Vs^{-1}. (*A*) Dependence of peak current on the scan rate ($y = 3.9616x + 0.2793$: $R^2 = 0.9884$). (*B*) Dependence of logarithm of peak current on logarithm of scan rate ($y = 0.571x + 0.4405$: $R^2 = 0.9939$). (*C*) Relationship between peak potential and logarithm of scan rate ($y = 0.0457x + 1.1984$: $R^2 = 0.9877$)

process on carbon paste electrode was under diffusion or adsorption-controlled. A linear relationship which is of typical diffusion controlled process was observed for the influence of square root of scan rate on peak potential, and the equation can be expressed as follows:

$$\text{Ip} \ (\mu A) \ = \ 28433 \ v^{1/2} (\text{mVs}^{-1}) - 0.1465 : R^2 \ = \ 0.9877$$

Straight line with a slope of 0.57 (Fig. 4(B)) closer to the theoretical value of 0.5 for a purely diffusion controlled process was obtained for the plot, logarithm of anodic peak current vs. logarithm of scan rate (Malode S.J (b) et al. 2012), corresponding to the following equation:

$$\log \text{Ip} \ (\mu A) \ = \ 571.7 \ \log v \ (\text{mVs}^{-1}) + 0.4405 : \\ R^2 \ = \ 0.9939$$

And also, the peak potential shifted to more positive values on increasing the scan rate, which confirms the irreversibility of the oxidation process (Brown E.R. and Large R.F. 1964), and a linear relationship between peak potential and logarithm of scan rate (Fig. 4(C)) can be expressed by the following equation:

$$\text{Ep}(V) \ = \ 45.7 \ \log v \ (\text{mVs}^{-1}) + 1.1984 : R^2 \ = \ 0.9877$$

For an irreversible electrode process, according to Laviron (Laviron E. 1979), Ep is defined by the following equation;

$$\text{Ep} = E^0 + \left(\frac{2.303RT}{\alpha nF}\right) \log \left(\frac{RTk^0}{\alpha nF}\right) + \left(\frac{2.303RT}{\alpha nF}\right) \log v$$

Where α is the transfer coefficient, k^0 is the standard heterogeneous rate constant of the reaction, n is the number of electron transferred, v is the scan rate, and E^0 is redox potential. From the slope of E_p versus log v, value of αn can be calculated. Taking $T = 298$ K, $R = 8.314$ JK^{-1}mol^{-1}, and F = 96,480 C mol^{-1}, the value of αn was calculated to be

1.2. According to Bard and Faulkner, (Allen J. B. and Larry R.F. 2004) α can be calculated as

$$\alpha = \frac{47.7}{E_p - E_{p/2}} \, mV$$

Where $E_{p/2}$ is the potential where the current is at half the peak value. From the above equation, value of α was to be 0.56. The number of electrons transferred in electrode oxidation was calculated to be $2.3 \approx 2$. Hence, 5-FU may be assumed to undergo two protons and two electron transfer in the electrode reaction. If the value of E^0 is known, the value of k^0 can be determined from the intercept of the above plot. From the intercept of Ep versus v curve by extrapolating to the vertical axis, at $v = 0$, the value of E^0 can be calculated from Eq. 2 (Shetti N.P. et al. 2012). From the intercept of Ep versus log v which was found to be 1.199, E^0 and k^0 were calculated to be 1.13 and $1.7 \times 10^3 \, s^{-1}$, respectively.

Reaction mechanism

Electrochemical oxidation of 5-FU proceeds with the removal of two electrons and two protons. In the first step, removal of π-electrons from the ring to give species (1)

takes place. As the species (1) is highly unstable, it is readily attacked by OH^- ions to give species (2). Further removal of one proton and one electron gives the final product. The oxidation of C=C depends on the electron density available at π-bond. The introduction of electron withdrawing group at the fifth position may exert a pull on the electrons of π-bond due to inductive effect and therefore the electron density may decrease. However, the mesomeric effect of halogen atom plays an important role, and electron density increases at π-bond and hence oxidation becomes easy. Scheme 2

Concentration variation

Since differential pulse voltammetry technique gave sharper and well-defined peaks even at low concentration of 5-FU, it was preferred to develop a voltammetric technique for determination of 5-FU. The phosphate buffer solution of pH = 7.0 was selected as the supporting electrolyte for the quantitative determination of 5-FU. Differential pulse voltammograms obtained with increasing amounts of 5-FU as shown in Fig. 5. In the range of $1 \times 10^{-7} - 4 \times 10^{-5}$ M linear calibration curves were obtained for 5-FU. The linear equation was

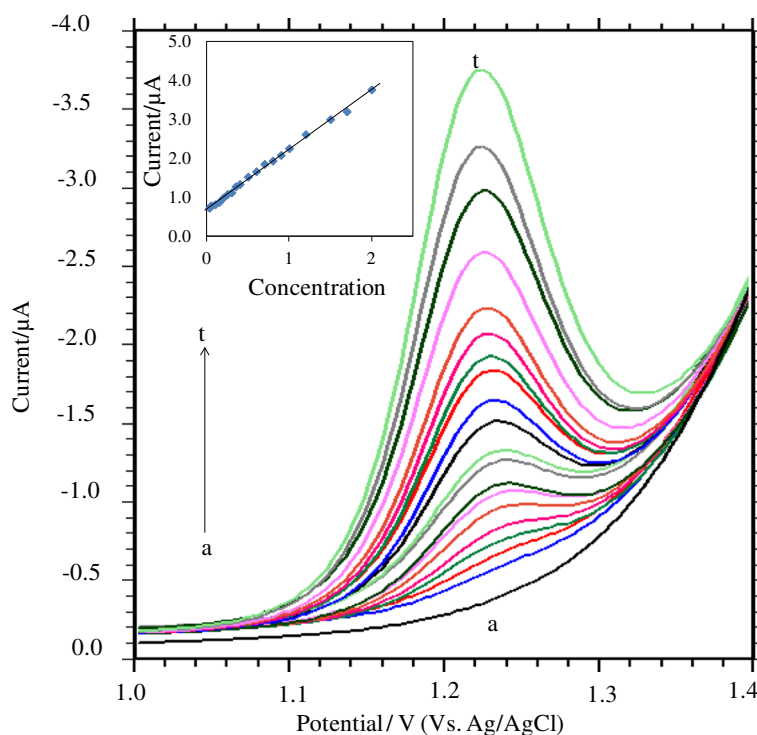**Scheme 2** Possible electrode reaction mechanism of 5-FU
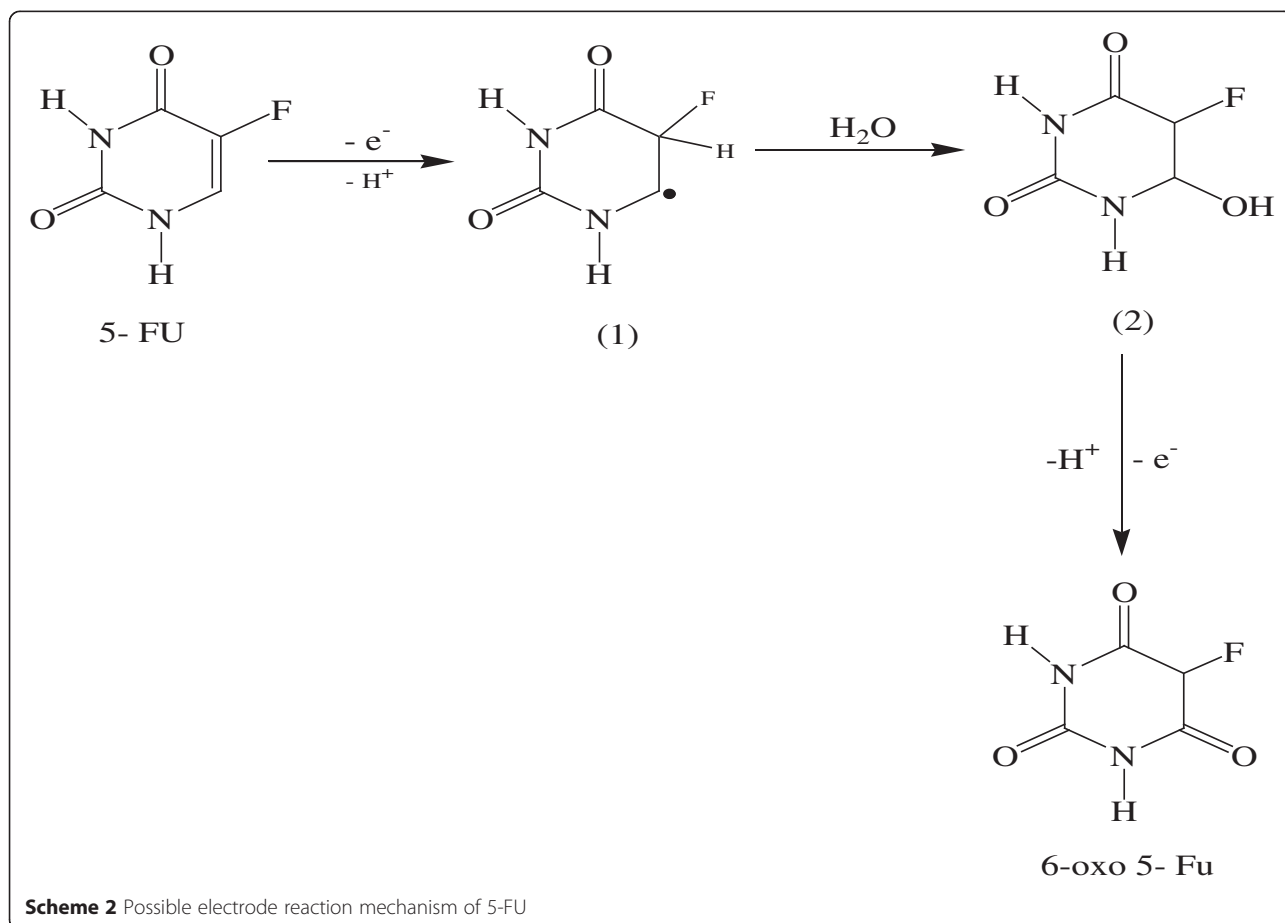
Fig. 5 Differential pulse voltamogramms of 5-FU at carbon paste electrode at different concentrations (*a*) blank, (*b*) 3, (*c*) 5, (*d*) 10, (*e*) 15, (*f*) 20, (*g*) 25, (*h*) 30, (*i*) 35, (*j*) 40, (*k*) 50, (*l*) 60, (*m*) 70, (*n*) 80, (*o*) 90, (*p*) 100, (*q*) 120, (*r*) 150, (*s*) 170, and (*t*) 200 μM. (*A*) Plot of current vs. concentration of 5-FU

$$\mathrm{Ip}/\ \mu\mathrm{A}\ =\ 1.5301\ c\ +\ 0.7042 : R^2\ =\ 0.9974$$

The adsorption of 5-FU or its oxidation product on the electrode surface deviates the linearity for more concentrated solution. Five different determinations were used to develop the statistical data related to calibration curve (Table 1). Limit of detection (LOD) and limit of quantification (LOQ) were calculated to be 12.25 and 40.8 nM, using following equation (Shetti N.P. et al. 2009)

$$\mathrm{LOD} =\ 3\mathrm{S}\ /\ m \cdots; \cdots\ \mathrm{LOQ}\ =\ 10\mathrm{S}\ /\ m$$

S is the standard deviation of the peak currents and *m* is the slope of calibration curve. The detection limits reported at different methods for 5-FU are tabulated in the Table 2. This method was better as compared to other reported methods (Hua X. et al 2013; Sataraddi S. R. and Nandibewoor S. T. 2011; Badea I. et al. 2002; Fars K.A et al. 2009; Tianrong Z. et al. 2011). Repeatability of the electrode renewed every time was studied for every

Table 1 Characteristics of 5-fluorouracil calibration plot using differential pulse voltammetry at carbon paste electrode

Linearity range (M)	1×10^{-7} to 4×10^{-5} M
Slope of the calibration plot (μA M^{-1})	1.530
Intercept (μA)	0.704
Correlation coefficient (r)	0.997
RSD of slope (%)	0.88
RSD of intercept (%)	0.87
Number of data points	19
LOD (M)	12.25 nM
LOQ (M)	40.8 nM
Repeatability (RSD %)	1.6
Reproducibility (RSD %)	2.7

Table 2 Comparison of detection limits of 5-fluorouracil by different reported methods

Method	LOD (nM)	Reference
Glassy carbon electrode modified with bromothymol blue and multi-walled carbon nano-tube	267	Hua X. et al 2013
Glassy carbon electrode mediated by surfactant cetyltrimethyl ammonium bromide	20.1	Sataraddi S. R. and Nandibewoor S. T 2011
Spectrometry	30.69	Badea I. et al. 2002
HPLC	70.89	Fars K.A et al. 2009
Ionic liquid modified carbon paste electrode	13	Tianrong Z. et al. 2011
Carbon paste electrode	12.25	Present work

Table 3 Analysis of 5-fluorouracil in tablets by differential pulse voltammetry and recovery studies

Labeled claim	100
Amount found (mg)[a]	96.72
RSD (%)	3.7
t test of significant	0.43
F test of significant	0.96
Bias (%)	2.3
Added (mg)	1
Found (mg)[a]	0.97
Recovered (%)	97
RSD (%)	1.6
Bias (%)	−3.0

[a]Average of five determinations

several hours within a day, and percentage RSD has been calculated to be 1.6 %. As to the reproducibility between days, it was similar to that of within day repeatability if, the temperature was kept almost unchanged. The reproducibility RSD has been calculated to be 2.7 %.

Tablet analysis and recovery test

Commercially available tablets were used for the analysis of 5-FU recovery test was carried using standard addition method. Grounding the tablets to powder and dissolving it in distilled water, the concentration of 5-FU tablet was prepared in such a way that they fall in the range of calibration plot. The proposed procedure was applied to the analysis of 5-FU in commercially available tablets. Standard addition method was used, and the recovery studies were performed. Identical conditions were employed for the analysis of the tablet as used for plotting calibration plot. Differential pulse voltammetry technique was used, and results obtained were having good agreement with the content marked in the label. In different sample, the recoveries lie between 93.9 and 99.3 % with RSD of 2.1 % (Table 3).

Table 4 Influence of potential interferents on the voltammetric response of 1.0×10^{-4} M 5-fluorouracil

Interferent	Concentration	Signal change (%)
Oxalic acid	0.1	0.87
Citric acid	0.1	2.66
Lactose	0.1	−1.70
Sucrose	0.1	−0.85
Dextrose	0.1	1.75
Glucose	0.1	2.47
Gum acacia	0.1	−3.41
Starch	0.1	0.95

Table 5 Application of differential pulse for the determination of 5-fluorouracil in spiked human urine

Urine spiked (10^{-5} M)	Detected[a] (10^{-5} M)	Recovery (%)	RSD (%)
Urine sample 1	0.1	98.5	2.9
Urine sample 2	0.2	92.8	3.1
Urine sample 3	0.5	99.5	2.9
Urine sample 4	0.8	99.9	2.9
Urine sample 5	1.0	96.6	3.0

[a]Average of five determinations

Effect of interferents

To evaluate the effect of interferents, 0.1 mM 5-FU was used. The Table 4 shows that 100-fold of citric acid, gum acacia, oxalic acid, sucrose, and urea did not interfere with the voltammetric signal of 5-FU. The tolerance limit was less ±5 %. The tolerance limit is defined as the maximum concentration of the interfering substance that caused error less than ±5 % for determination of 5-FU.

Urine analysis and recovery test

For the determination of 5-FU in human urine sample differential pulse, voltammetric technique was used. Drug-free human urine samples were obtained from healthy volunteers who gave their informed consent, filtered through a filter paper, and stored frozen until the assay was carried out. The study was approved by the Institutional Review Committee of K. L. E. Institute of Technology (KLEIT/IRC/2015-16/01). By spiking the drug-free urine sample with known amount of drug, the recovery study was carried out. For the determination of spiked 5-FU in urine sample, calibration graph was used. Five urine samples were used for the detection, and obtained results are tabulated in Table 5. The recovery determination was in the range from 92.8 to 99.9 % with RSD of 2.97 %.

Conclusions

In the present work, oxidation of 5-FU in phosphate buffer solution (pH = 7) was successfully carried out. The electrode process of 5-FU is diffusion-controlled and irreversible. Suitable electrode reaction mechanism was proposed. A differential pulse voltammetric technique was developed for the determination of 5-FU in pharmaceutical dose and human urine samples. As compared to other methods, the proposed method offered an improvement in simplicity and accuracy.

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