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Research Article

ELECTROCHEMICAL BEHAVIOR OF XANTHENE FOOD DYE ERYTHROSINE AT GLASSY CARBON ELECTRODE AND ITS ANALYTICAL APPLICATIONS

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ABSTRACT

Erythrosine (ERT) is a xanthene food dye used in the food industries to enhance the appearance of the food. The electrochemical behavior of ERT at glassy carbon electrode was investigated by cyclic and differential pulse voltammetry. The oxidation peak of ERT was observed in phosphate buffer of pH 5.0. The influence of different pH, scan rate, and concentration were evaluated. The probable reaction mechanism involved in the oxidation of ERT was also proposed. Differential pulse voltammetric method with good precision and accuracy was developed for the determination of ERT dye in real samples. The peak currents were found to be linearly dependent on the concentration range of 1×10^{-5} –6×10^{-4} m. The limit of detection and limit of quantification were noticed to be 1.9×10^{-7} and 6.6×10^{-7} M, respectively.

Keywords: Erythrosine, Electrochemical studies, Glassy carbon electrode, Differential pulse voltammetry, Pharmaceutical formulation.

INTRODUCTION

Erythrosine (ERT) with the chemical structure shown in (Scheme 1) belongs to the class of xanthene dyes, which is highly water-soluble. Initially, it is most popularly used as a food coloring agent and a host of other applications such as printing inks, a dental plaque disclosing agent, a biological stain, cosmetics, cocktails, tinned fruits, biscuits, chocolate, and snack foods. It is highly toxic, causes various types of allergies, thyroid activities, carcinogenicity, anemia, DNA damage behavior, neurotoxicity, and xenoestrogen nature in the humans and animals. Thus, due to the hazardous nature and harmful effects of ERT, it was considered worthwhile to develop a simple method for the determination of ERT in different samples.

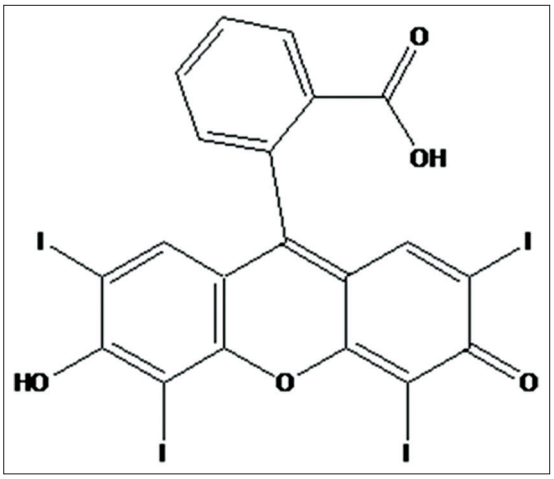
Electrochemical methods, for example, polarography, voltammetry [1], and square wave voltammetry are used to determine either organic or inorganic electroactive species. The limitations of electroanalytical procedures and their advantages compared with high-pressure liquid chromatography (HPLC) and gas chromatography, such as speed, sensitivity, and speciation, are discussed by Zuman [2]. Only few methods are available for the determination of ERT [3-8]. These methods are based on spectroscopy, electrokinetic capillary chromatography, and HPLC. To date, there is no report available on the electrochemical determination of ERT.

The purpose of the present study is to develop a new, rapid, simple, selective, and inexpensive voltammetric method at glassy carbon electrode (GCE) for the direct determination of ERT in real samples without any time-consuming extraction or evaporation steps prior to ERT assay. The GCE has been widely used in electroanalysis for various substrates for a long time because of its stability, wide potential window and fast electron transfer rate. The influences of some interfering species will also be investigated. In addition, an electrochemical behavior of ERT is investigated with cyclic voltammetry and differential pulse voltammetry (DPV).

METHODS

Apparatus

Pure ERT in powdered form was obtained from Sigma - Aldrich and used as received. A stock solution of ERT (1.0×10^{-3} M) was prepared in double distilled water. Phosphate buffer solutions of ionic strength 0.2 m were prepared for variation of pH according to the literature method [9]. Rests of the reagents were of analytical grade, and double distilled water was used throughout the experiment.



Scheme 1: Chemical structure of erythrosine

Instrumentation

Electrochemical measurements were carried out on a CHI 630D electrochemical analyzer (CH Instruments Inc., USA). The voltammetric measurements were carried out in a 10 ml single compartment three-electrode glass cell with Ag/AgCl as a reference electrode, a platinum wire as counter electrode and a GCE as the working electrode. All the potentials are given against the Ag/AgCl (3 M KCl). The pH measurements were performed with Elico LI 120 pH meter (Elico Ltd., India). All experiments were carried out at an ambient temperature of 25°C±0.1°C.

The area of the electrode was calculated using 1.0 mM K_3 (Fe $[CN]_6$) as a probe at different scan rates. For a reversible process, the Randles-Sevcik formula has been used [10].

$$I_p = (2.69 \times 10^5)\, n^{3/2}\, A\, D_0^{1/2}\, \nu^{1/2}\, C_0^* \quad (1)$$

Where, n = number of electrons transferred i.e., 1, A = surface area of the electrode, D_0 = diffusion coefficient, ν = sweep rate (0.1/Vs) and C_0^* = concentration of electro active species (1 mM). The surface area of the electrode was found to be 0.04 cm^2.

Analytical procedure

The GCE was carefully polished using 0.3 μ Al_2O_3 slurry on a polishing cloth before each experiment. After polishing, the electrode was rinsed thoroughly with water. After this mechanical treatment, the GCE was placed in buffer solution and various voltammograms were recorded until a steady-state baseline voltammogram was obtained. The GCE was first activated in phosphate buffer (pH 5.0) by cyclic voltammetric sweeps between 2 V and 3.0 V until stable cyclic voltammograms were obtained. Then electrodes were transferred into another 10 ml of phosphate buffer (pH 5.0) containing proper amount of ERT dye.

Analysis of human urine

Human urine was obtained from four healthy volunteers of similar sex and age. Aliquots were centrifuged at 7000 rpm for 5 min at room temperature (25±0.1°C). These urine samples were analyzed immediately or they were stored at low temperature until analysis.

RESULTS AND DISCUSSION

Voltammetric behavior of ERT

In order to understand the electrochemical process occurring at the GCE, cyclic voltammetry was carried out. The electrochemical behavior of 1.0×10^{-3} M ERT at GCE was investigated at pH = 5.0 (Fig. 1). The potential range is 0.4-1.6 V, with a sweep rate of 0.05 mV/s. In cyclic voltammogram, ERT exhibited two anodic peaks, one anodic peak was sharper at 0.778 mV, and another one was broader at 1.253 mV. No peak was observed in the reverse scan suggests that the oxidation process is an irreversible one. It was found that the oxidation peak current of ERT showed remarkable decrease during the successive cyclic voltammetric sweeps. After second sweep, the peak current decreased and finally remained unchanged. This phenomenon is due to the fact that the adsorption of ERT or its oxidative product occurs at the electrode surface. We considered the sharp peak throughout the experiment.

Effect of pH

The electrochemical oxidation of ERT dye was studied in 0.2 M phosphate buffer as supporting electrolyte. ERT was oxidized on GCE between pH 3.0 and 6.0 of phosphate buffer; producing an oxidation peak thereafter no peaks were observed. This indicates that hydrogen ions were concerned with the electrode reaction. With increasing the pH of the buffer solution, the peak potential shifted to less positive values. This effect of pH on the electrochemical properties of soluble ions in solution can be attributed to the acid-base equilibrium constants of this drug. The linear relationship between E_p and pH (Fig. 2) can be expressed as follows:

$$E_p\,(V) = 0.543 + 0.050\ pH,\ R^2 = 0.774$$

The slopes of 0.050 mV per pH, is close to expected 0.059 mV per pH indicates that the number of protons and electrons involved in the oxidation of ERT are equal. From the experimental results it is observed that highest peak current and better shape of the voltammogram was observed at pH 5.0, suggesting this pH is an optimal pH value; hence, it was selected for further experiments.

Effect of scan rate

Useful information involving electrochemical mechanism usually can be acquired from the relationship between peak current and scan rate. Therefore, the electrochemical behavior of ERT at different scan rates (Fig. 3) was also studied. From this, we observed that increasing the scan rate, the peak potential was shifted to more positive values. Simultaneously, the width of peak increases. This corresponds to the oxidation product formed at the GCE surface. There is a good linear relationship between peak current and scan rate(Fig. 3a). The equation is: $I_p = 18.37\ \upsilon + 1.992$; $R^2 = 0.989$.

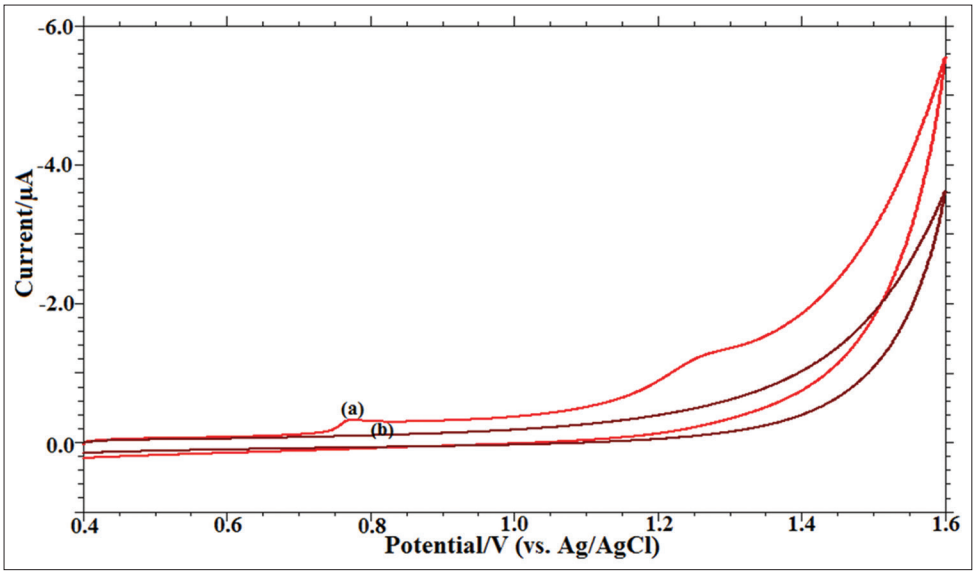


Fig. 1: Cyclic voltammograms of 1.0×10^{-4} M erythrosine on glassy carbon electrode in pH 5.0, phosphate buffer (I = 0.2 M) (a) erythrosine run at 0.05/Vs, (b) blank

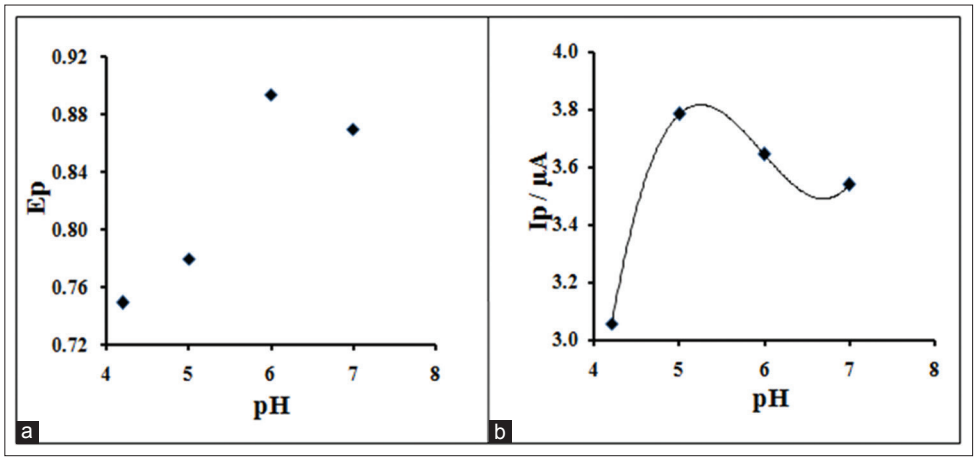


Fig. 2: (a) Influence of pH on the peak potential E_p/V of erythrosine (ERT), (b) variation of peak currents I_p/µA of ERT with pH

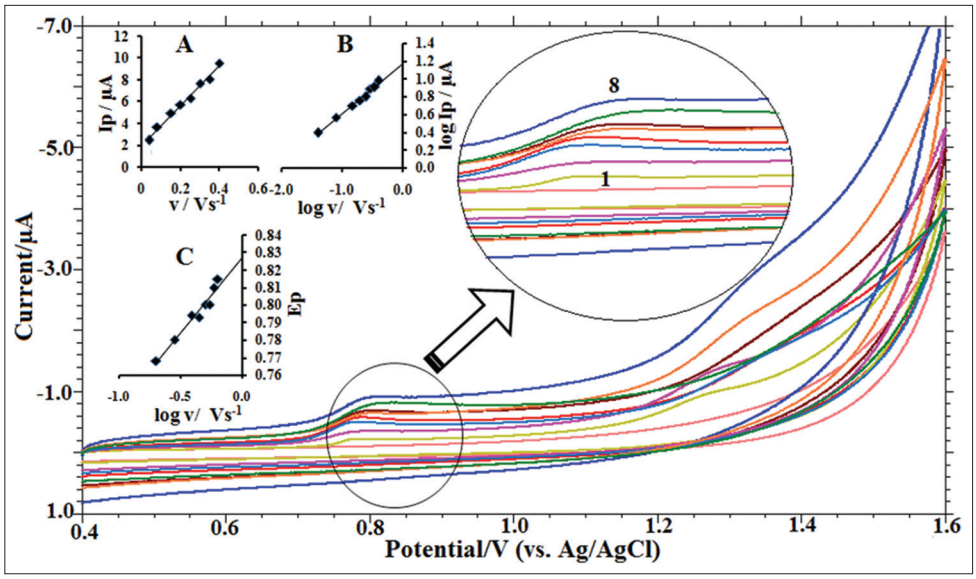


Fig. 3: Cyclic voltammograms of 1.0 × 10^{-4} M erythrosine in pH 5.0 (I = 0.2 M) at scan rate of: (1) blank; (2) 0.04; (3) 0.08; (4) 0.15; (5) 0.2; (6) 0.25; (7) 0.3; (8) 0.35; (9) 0.4 V/s, (a) Dependence of peak current I_p/µA on the scan rate υ/Vs, (b) Plot of logarithm of peak current log I_p/µA versus logarithm of scan rate log υ/Vs (c) Plot of variation of peak potential E_p/V with logarithm of scan rate log υ/Vs

In addition, there was a linear relation between log I_p and log υ (Fig. 3b), corresponding to the following equationog I_p = 0.560 log υ + 1.168; R^2 = 0.989. The slope of 0.56 is close to the theoretically expected value of 0.5 for a diffusion controlled process [11]. The peak potential shifted to more positive values with increasing the scan rates. The linear relationship between peak potential and logarithm of scan rate (Fig. 3c) can be expressed as: E_p = 0.086 log υ + 0.827; R^2 = 0.955.

For an irreversible electrode process, according to Laviron [12], E_p is defined by the following equation

$$E_p = E_0 + \left(\frac{2.303RT}{\alpha nF}\right)\log\left(\frac{RTk^0}{\alpha nF}\right) + \left(\frac{2.303RT}{\alpha nF}\right)\log v \quad (2)$$

Where α is the transfer coefficient, k^0 the standard heterogeneous rate constant of the reaction, n the number of electrons transferred, υ the scan rate, and E_0 is the formal redox potential. Other symbols have their usual meanings. Thus, the value of αn can be easily calculated from the slope of E_p versus log υ. In this system, the slope is 0.054, then αn calculated to be 0.687, taking T = 298 K, R = 8.314/JK/mol, and F = 96480 C/mol.

According to Bard and Faulkner, [13] α can be given as,

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

Where $E_{p/2}$ is the potential where the current is at half the peak value. From this we got the value of α to be 0.5. Further, the number of electron (n) transferred in the electrooxidation of ERT was calculated to be 1.3 ≈ 1. The value of k^0 can be determined from the intercept of the above plot if the value of E^0 is known. The value of E^0 in Equation (2) can be obtained from the intercept of E_p versus υ curve by extrapolating to the vertical axis at υ = 0 [14,15]. In our system the intercept for E_p versus log υ plot was 0.827 and E_0 was obtained to be 0.769, the k^0 was calculated to be 2.1 × 10^3/s.

Oxidation mechanism

ERT showed one well resolved anodic signal in a limited pH range studied. In acid media, the oxidation of ERT at GCE follows a proton-dependent mechanism while in alkaline media protons were not involved in the rate determining step or before. In the acid media, an increase of the peak current with the increase of pH was observed. On the other hand, in the basic media decrease in the peak current with

the increase of pH was observed. By the calculation, we found that the oxidation mechanism involves one proton-one electron at GCE. Based on all these observations we postulated the mechanism as shown in Scheme 2.

Calibration curve

In order to develop a voltammetric method for determining the drug, we selected the differential pulse voltammetric method, because the peaks are sharper and better defined at lower concentration of ERT than those obtained by cyclic voltammetry, with a lower background current, resulting in improved resolution. The pH 5.0 was selected as supporting electrolyte for the quantification of the dye. Differential-pulse voltammograms obtained with increasing amounts of ERT showed that the peak current increased linearly with increasing concentration, as shown in (Fig. 4). It was found that the plot of I_p versus concentration showed linearity over the drug concentration range of 1.0×10^{-5} to 6.0×10^{-4} M. The linear equation was I_p (μA) = 0.202 + 0.363 C (R^2 = 0.969, C is in μM). Deviation from linearity was observed for more concentrated solutions, due to the adsorption of oxidation products of ERT on the electrode surface [16,17]. Related statistical data of the calibration curves were obtained from the five different calibration curves. Limit of detection (LOD) and quantification (LOQ) were calculated [18] based on the peak current using the following equations shown below.

$$LOD = 3\ S/m;\ LOQ = 10\ S/m$$

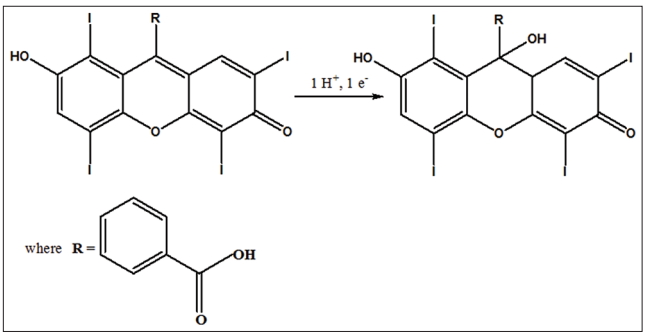
Where S is the standard deviation of the peak currents of the blank (five replicates), and m is the slope of the calibration curve. The LOD and LOQ values were calculated to be 1.9×10^{-7} M and 6.6×10^{-7} M, respectively. The LOD and LOQ values calculated by the present method are better compared to the reported work [8-10]. Analyzing five replicates, for the process of the validation within-day variations and for intraday assay were studied. According to the obtained results, it was possible to apply this technique to the quantitative analysis of ERT.

Effect of interferents

For the analytical applications of the proposed method, the effects of potential interferents that are likely to be in biological samples were evaluated under the optimum experimental conditions. Differential pulse voltammetric experiments were carried out for 1.0 μM ERT dye in the presence of 1.0 mM of each of the interferents. The experimental results (Table 1) showed that thousand fold excess of glucose, starch, sucrose, dextrose, lactose, gum acacia, citric acid, and oxalic acid did not interfere with the voltammetric signal of ERT. Therefore, the proposed method can be used as a selective method.

Detection of ERT in urine samples

The applicability of the DPV to the determination of ERT dye in spiked urine was investigated. The recoveries from urine were measured by spiking dye free urine with known amounts of ERT. The urine samples were diluted 100 times with the phosphate buffer solution before analysis without further pretreatments. A quantitative determination can be carried out by adding the standard solution of ERT into the detect system of urine sample. The calibration curve was used for the determination of spiked ERT in urine samples. The detection results



Scheme 2: Possible electrode reaction mechanism of erythrosine

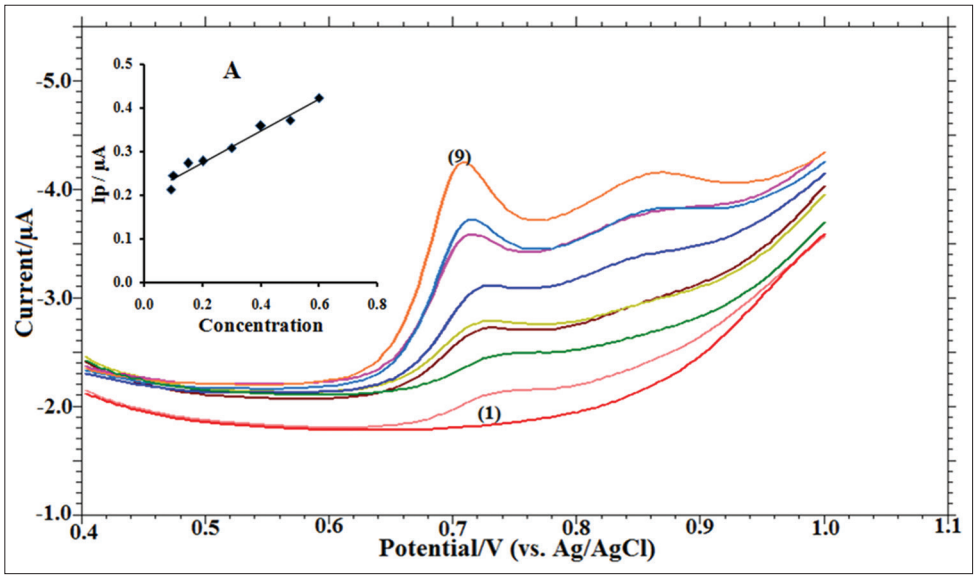


Fig. 4: Differential pulse voltammograms with increasing concentrations of erythrosine in pH 5.0 phosphate buffer solution on glassy carbon electrode: (1) blank; (2) 1×10^{-5}; (3) 2×10^{-5}; (4) 3×10^{-5}; (5) 4×10^{-5}; (6) 5×10^{-5}; (7) 6×10^{-5}; (8) 1×10^{-4}; (9) 2×10^{-4}

Table 1: Influence of potential interferents on the voltammetric response of 1.0×10^{-3} M ERT at GCE by DPV

Interferents	Concentration/mM	Signal change (%)
Oxalic acid	1.0	−0.25
Citric acid	1.0	−0.12
Lactose	1.0	−0.23
Sucrose	1.0	−0.22
Starch	1.0	−0.27
Gum acacia	1.0	−0.21

*Average five determinations, DPV: Differential pulse voltammetry, GCE: Glassy carbon electrode, ERT: Erythrosine

Table 2: Determination of ERT in urine samples by DPV at GCE

Sample	Declared (mol/L)	Detected (mol/L)	Recovery (%)
Urine sample 1	0.1×10^{-4}	0.099×10^{-4}	99.0
Urine sample 2	0.3×10^{-4}	0.284×10^{-4}	94.6
Urine sample 3	0.5×10^{-4}	0.498×10^{-4}	99.7

DPV: Differential pulse voltammetry, GCE: Glassy carbon electrode, ERT: Erythrosine

of four urine samples obtained are listed in (Table 2). The recovery determined was in the range from 97.5% to 101.0% and the relative standard deviation was 1.47%. Thus, satisfactory recoveries of the analyte from the real samples and a good agreement between the concentration ranges studied and the real ranges encountered in the urine samples when treated with the drug make the developed method applicable in clinical analysis.

CONCLUSIONS

A GCE was used first time for the oxidation of red dye ERT in phosphate buffer solution of pH=5.0. The oxidation of ERT was found to be an irreversible, diffusion controlled and one electron-one proton process at GCE. Under the selected conditions, the peak current was linear with dye concentrations over a certain range. A suitable mechanism was proposed. The proposed method offered the advantages of accuracy and time saving as well as the simplicity of reagents and apparatus. In addition, the results obtained in the analysis of ERT in spiked human urine samples demonstrated the applicability of the method for real sample analysis.

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