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Full Paper

Electro-oxidation of a Food Dye Fast Green FCF and Its Analytical Applications

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Abstract- The electrochemical oxidation of a food dye, fast green FCF has been investigated at a glassy carbon electrode using voltammetric techniques. The dependence of current on pH, concentration, scan rate and excipients was investigated to optimize the experimental conditions. According to the linear relation between peak current and the fast green concentration, differential pulse voltammetric method for the quantitative determination in spiked urine was developed. The linear response was obtained in the range of 30 μM to 0.2 mM with a detection limit of 0.8 μM. The proposed method was applied for the detection of fast green in food and urine as a real sample.

Keywords- Fast green FCF, Voltammetry, Electrochemical studies, Glassy carbon electrode

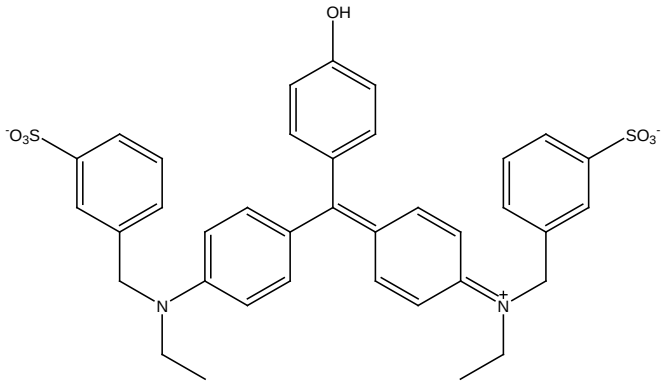
1. INTRODUCTION

Synthetic food dyes are important class of organic compounds originally synthesized from coal tar and from petroleum. These food dyes are commonly used as additives in food, medicines and soft drinks to get suitable and natural looking colors. Because of their high brightness, stability, low cost and wide ranges of shades. Consequently, they have become common industrial and environmental pollutants during their synthesis and applications.

Some of the artificial coloring ingredients are toxic to our health and environmentally hazardous.

However, some synthetic colorants can be pathogenic, particularly if they are excessively consumed [1]. Therefore the selection of proper colorants as an additive is an important factor for food and drink manufacturers which may determine the acceptability of the product. Although the number of permitted food colorants was reduced for food safety, in past years, many kinds of synthetic food colorants are still widely used all over the world, because of their low price, effectiveness and stability.

Fast Green FCF (FGF) (Scheme 1) is a triarylmethane food dye, which is widely used as food colorant in tinned green peas and other vegetables, jellies, sauces, fishes, desserts and dry bakery items (In the name of the dye, FCF refers to For Coloring Food). It is a substitute of Light Green SF Yellowish in histology, as its color is less likely to fade [2]. It is used as a protein stain in the electrophoresis. The extensive use of this dye induces carcinogenic effects [3] as it produces sarcomas at the site of repeated subcutaneous injection and causes eye, skin and upper respiratory tract irritation. It is an immuno toxic agent and inhibits the release of neurotransmitter [4,5]. The applications and proven toxicity of FGF render necessaries, to develop a sensitive, fast and reliable approach to determine FGF in pharmaceutical, food and biological samples. Owing to concerns over the analytical determination of the dye, several methods have been developed for the analysis of FGF employing variable techniques. A review of the literature reveals that the detection of this FGF dye by using high performance liquid chromatography [6,7], liquid chromatography-electro spray tandem mass spectroscopy [8], CPE scanometry [9], capillary electrophoresis [10], spectrofluorimetry [11] were reported. But most of these techniques often suffer from some unfavorable conditions regarding cost, complex sample preparation due to tedious analytical steps and expensive. Moreover, long analysis time is required which make these techniques practically not helpful in routine analysis. However voltammetric methods have attracted the attention of researchers towards their sensitivity for the determination of organic molecules [12-16], rapidity of analysis, inexpensive instrumentation and no complex sample pretreatment. The use of carbon based electrodes, especially glassy carbon electrode, for electroanalytical measurements has increased in past years because of their applicability to the determination of substances that undergo oxidation reactions, a matter of great importance in the field of clinical and pharmaceutical analysis. Redox properties of drugs can give insights into its metabolic fate or their in vivo redox processes or pharmaceutical activity [17,18].



Scheme 1. Chemical structure of FGF (Fast Green FCF)

In view of the pharmaceutical importance of fast green and the lack of literature on its electrochemical determination, a quick and sensitive method was developed. The aim of the present study is to establish the suitable experimental conditions, to investigate the oxidation mechanism of fast green dye and to analyze the dye in spiked urine samples using voltammetric techniques.

2. EXPERIMENTAL

2.1. Materials and Reagents

Analytical reagent grade chemicals and double distilled water were used throughout the experiment. Pure fast green dye in powdered form was obtained from Sigma Aldrich and used without any purification. Double distilled water was used to prepare stock solution of the dye of 1.0 mM concentration. The phosphate buffers of 0.2 M ionic strength were prepared to study the effect of supporting electrolyte pH [19].

2.2. Instrumentation

Electrochemical measurements were carried out using CHI D630 electrochemical analyzer (CH Instruments Inc., USA). A three-electrode system was used for the voltammetric measurements with Ag/AgCl as a reference electrode, a platinum wire as counter electrode and a glassy carbon electrode (GCE) as the working electrode in a 10 ml single compartment glass cell. The pH measurements were performed with Elico LI120 pH meter (Elico Ltd., India). All experiments were carried out at an ambient temperature of 25˚C±0.1˚C.

1.0 mM $K_3[Fe(CN)_6]$ in 1 M KCl was used to obtain the area of the electrode by cyclic voltammetric technique by recording the current voltage at different scan rates (Fig. 1). For a reversible process, the Randles-Sevcik formula has been used [20,21].

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

Where I_p refers to the anodic peak current, n is the number of electrons transferred during the electrode reaction i.e. 1. A is the surface area of the electrode, D_0 is the diffusion coefficient i.e. 7.6×10^{-6} cm^2 s^{-1}, ν is the sweep rate (0.1 Vs^{-1}) and C_0^* is the concentration of electro active species (1 mM). From the slope of the plot of I_p versus $\nu^{1/2}$ the electrode surface was calculated, and found to be 0.04 cm^2.

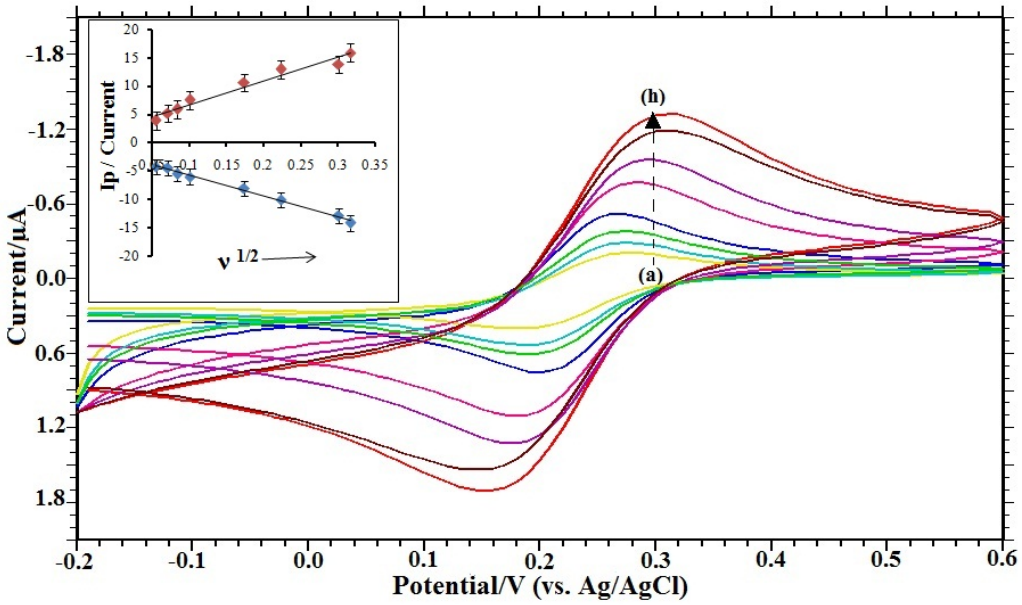


Fig. 1. Cyclic voltammograms of 1.0 mM $K_3[Fe(CN)_6]$ in 1 M KCl at different scan rates: (a) 0.003; (b) 0.005; (c) 0.007; (d) 0.01; (e) 0.03; (f) 0.05; (g) 0.09; (h) 0.1 V s^{-1}.**(A)** Dependence of peak current I_p / µA on the square root of scan rate $\upsilon^{1/2}$ / Vs^{-1}

2.3. Analytical procedure

The GCE was carefully polished using 0.3 micron Al_2O_3 slurry on a polishing cloth before performing each experiment. After polishing, the electrode was rinsed thoroughly with double distilled water. 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 −2.0 to and 2.0 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 fast green dye.

2.4. 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.

3. RESULTS AND DISCUSSION

3.1. Electrochemical behavior of dye

In order to understand the electrochemical process occurring at the glassy carbon electrode, cyclic voltammetry was carried out. The electrochemical behavior of 1.0 mM FGF at glassy carbon electrode was investigated at pH=5.0 (Fig. 2). The potential range is 0.0 to 1.4 V, with a sweep rate of 0.05 mVs^{-1}. In cyclic voltammogram, FGF exhibited one anodic peak at 0.907 mV. No peak was observed in the reverse scan suggests that the oxidation process is an irreversible one.

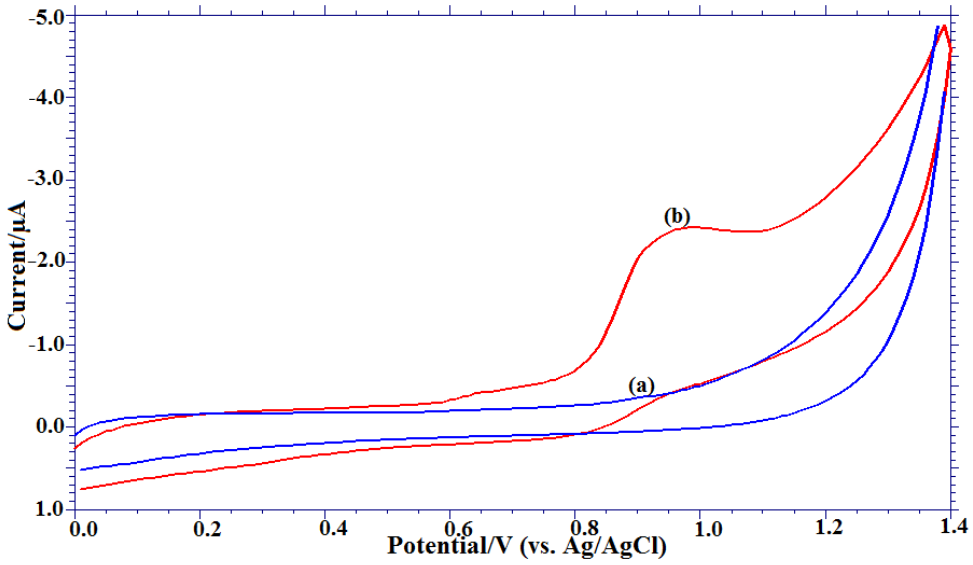


Fig. 2. Cyclic voltammograms of 1.0×10^{-4} M FGF on glassy carbon electrode in pH 5.0, phosphate buffer (I=0.2 M) (a) blank, (b) FGF run at 0.05 Vs^{-1}

3.2. Effect of Surfactant

Surfactants even in trace quantities can exert a strong effect on the electrode process. Adsorption of such substances at the electrode may inhibit the electrolytic process, brings out the irregularity in the voltammograms, and shift in the wave to more negative potentials [22,23]. Surface-active substances have the common tendency of accumulation at interfaces. The lack of affinity between the hydrophobic portion of the surfactant and water leads to the repulsion of these substances from the water phase. Experimental results showed that the addition of cationic surfactant, cetyltrimethylammonium bromide, as anionic surfactant, sodium dodecyl sulfate and the non-ionic surfactant, TtritonX-100 have no, much influence on the peak current and peak potential (Fig. 3).

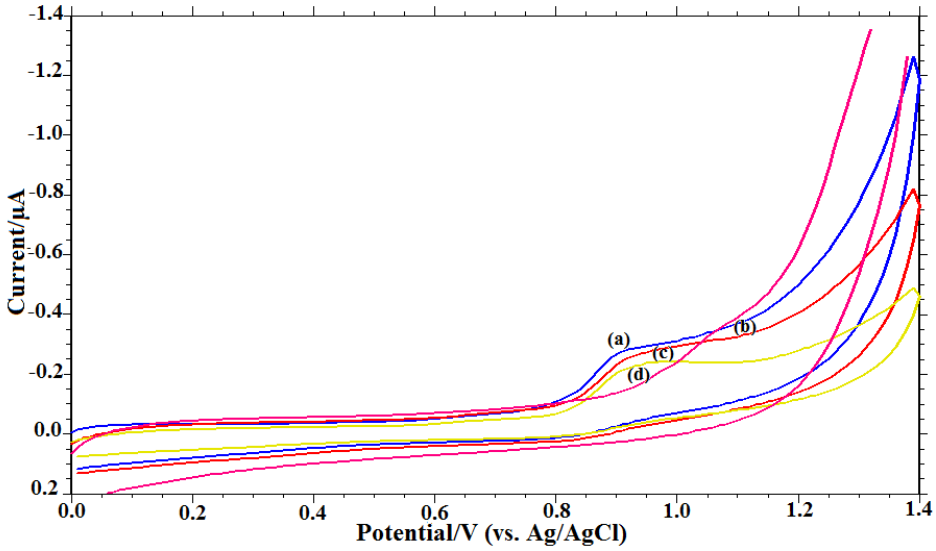


Fig. 3. Surfactant behavior on FGF**:** Cyclic voltammograms of FGF (a) without surfactant; (b) with sodium dodecyl sulfate (cationic surfactant); (c) with cetyltrimethylammonium bromide (anionic surfactant); (d) with Triton-X (non-ionic surfactant)

3.3. Influence of pH

The influence of pH on the electrochemical oxidation of fast green dye was studied in 0.2 M phosphate buffer as supporting electrolyte ranging from 3.0–11.2. Fast green was oxidized on glassy carbon electrode between pH 3.0 and 7.0, a well-defined oxidation peak thereafter no peaks were observed. It may be explained on the basis that at low pH, the anionic dye was attracted by positively charged surface of the electrode, but after a certain limit, further increase in pH turned surface as negatively charged [24]. Due to presence of lone pairs on two nitrogen atoms, fast green seems to face a force of repulsion from negatively charged surface, which results into a decrease in rate of oxidation. With increasing the pH of the buffer solution, the peak potential shifted to less positive values as shown in (Fig. 4).

The plot of E_p versus pH (Fig. 4(A)) shows that the peak potential was pH dependent. The variation of peak current with pH was as shown in (Fig. 4(B)).

$$E_p\ (V)=0.023\ pH+1.027;\ R^2=0.991$$

The slopes of 0.023 mV per pH, was not close to expected 0.059 mV per pH indicates that the number of protons and electrons involved in the oxidation of FGF were not equal [25]. From the experimental results, the highest peak current and better shape of the voltammogram was observed at pH 5.0, suggesting this pH is optimal pH value; hence it was selected for further experiments.

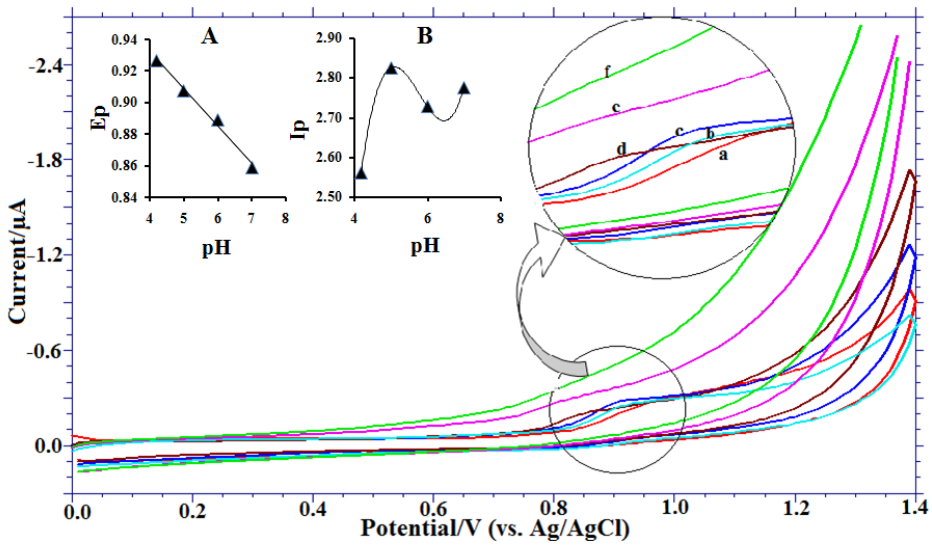


Fig. 4. Cyclic voltammograms obtained for 1.0×10^{-4} FGF in buffer solution at (a) pH 3.0; (b) pH 4.2; (c) pH 5.0; (d) pH 6.0; (e) pH 7.0; (f) pH 8.0. **(A)** Influence of pH on the peak potential E_p / V of FGF. **(B)** Variation of peak currents I_p / μA of FGF with pH.

3.4. Influence of scan rate

From the scan rate variation one can acquire the helpful information regarding the electrochemical mechanism and the type of electrode process. Therefore, the electrochemical behavior of fast green at different scan rates (Fig. 5) were studied. We observed that, with increase in scan rate, the peak potential was shifted to more positive values. Simultaneously, the width of peak was increased. This corresponds to the oxidation product formed at the GCE surface. There was a good linear relationship between peak current and scan rate (Fig. 5(A)).

$$I_p=20.10\ \upsilon+1.591;\ R^2=0.977$$

In addition, there was a linear relationship between log I_p and log υ (Fig. 5(B)), corresponding to the following equation: log I_p=0.483 log υ+1.094; R^2=0.985. The slope of 0.483 was close to the theoretically expected value of 0.5 for a diffusion controlled process, where the electro active species, FGF diffuses from the bulk solution to a planar electrode surface [26]. 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. 5(C)) can be expressed as: E_p=0.048 log υ+0.971; R^2=0.979.

For an irreversible electrode process, according to Laviron [27], E_p is defined by the following equation

$$E_P = E^0\left(\frac{2.303RT}{\alpha nF}\right)log\left(\frac{RTk^0}{\alpha nF}\right)+\left(\frac{2.303RT}{\alpha nF}\right)logv \qquad (2)$$

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 0.048, then αn calculated to be 1.232, taking T=298 K, R=8.314 $JK^{-1}mol^{-1}$ and F=96480 C mol^{-1}. For an irreversible process, $dE_p/dpH=0.059X/\alpha n$, where X is the number of protons transferred during the reaction. Accordingly, αn=1.232X was obtained. From αn value we got the value of X to be 1. According to Bard and Faulkner, [28] α can be given as,

$$\alpha = \frac{47.7}{E_P - E_{P/2}} mV \qquad (3)$$

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 electro oxidation of FGF was calculated to be 2.01≈2. 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 Eq. (2) can be obtained from the intercept of E_p versus υ curve by extrapolating to the vertical axis at υ=0 [29]. In our system the intercept for E_p versus log υ plot was 0.971 and E_0 was obtained to be 0.888, the k^0 was calculated to be 3.41×10^3 s^{-1}.

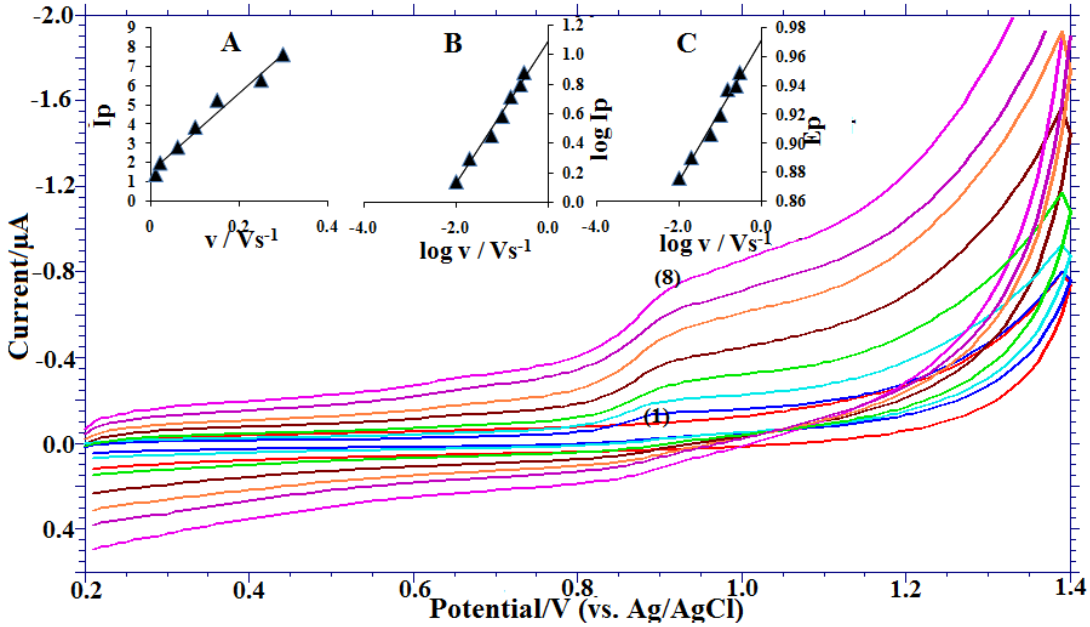
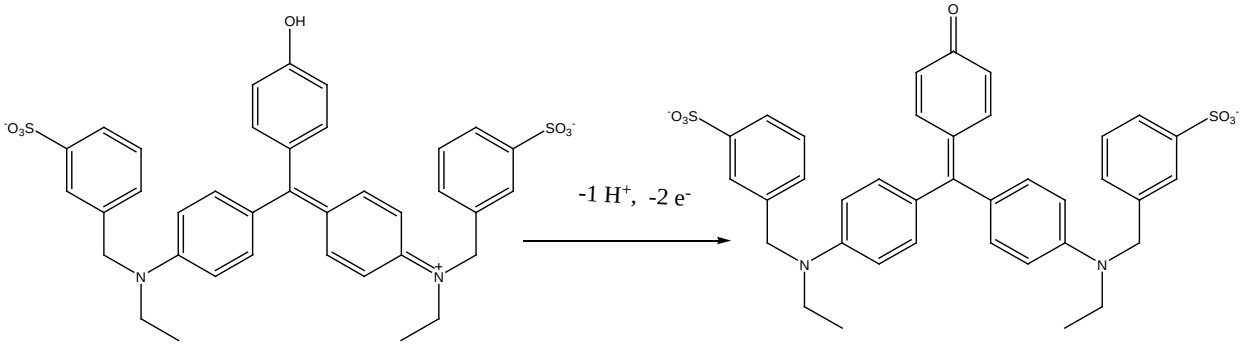


Fig. 5. Cyclic voltammograms of 1.0×10^{-4} M FGF in buffer solution of pH 5.0 (I=0.2 M) at scan rate of:(1) blank; (2) 0.01; (3) 0.02; (4) 0.06; (5) 0.1; (6) 0.15; (7) 0.25; (8) 0.3 V s^{-1}. **(A)** Dependence of peak current I_p / µA on the scan rate υ / Vs^{-1}. **(B)** Plot of logarithm of peak current log I_p / µA versus logarithm of scan rate log υ / Vs^{-1}. **(C)** Plot of variation of peak potential E_p / V with logarithm of scan rate log υ / Vs^{-1}

3.5. Oxidation mechanism

Fast green dye showed one well-resolved anodic signal in a limited pH range (i.e. 3.0-7.0). In acidic media the oxidation of fast green at glassy carbon electrode follows a proton-dependent mechanism. Based on the results obtained, we postulated the mechanism as shown in (Scheme 2).



Scheme 2. Possible electrode reaction mechanism of FGF (Fast Green FCF)

4. ANALYTICAL APPLICATIONS

4.1. Calibration curve and detection limit

As the DPV technique is considered to be a more sensitive technique in comparison to cyclic voltammetry, hence it was used for the further determination of the dye. The phosphate buffer solution of pH 5.0 was selected as the supporting electrolyte for the quantification of dye as it gave maximum peak current. Differential pulse voltammograms of fast green indicates that the peak current increased linearly with increasing concentration (Fig. 6). Using the optimum conditions described above, linear calibration curves were obtained for fast green dye in the range of 30 μM to 0.2 mM. The linear equation was I_p (μA)=1.402+1.456 (R^2=0.976, C is in μM). Deviation from linearity was observed for more concentrated solutions, due to the adsorption of oxidation product on the electrode surface [30]. Limit of detection (LOD) and quantification (LOQ) were calculated [31,32] 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 and m is the slope of the calibration curve. The limit of detection (LOD) and quantification (LOQ) were 0.8 μM and 2.6 μM respectively. Comparing to other analytical methods (Table 1) this voltammetric method is proved to be more sensitive and selective (6-10).

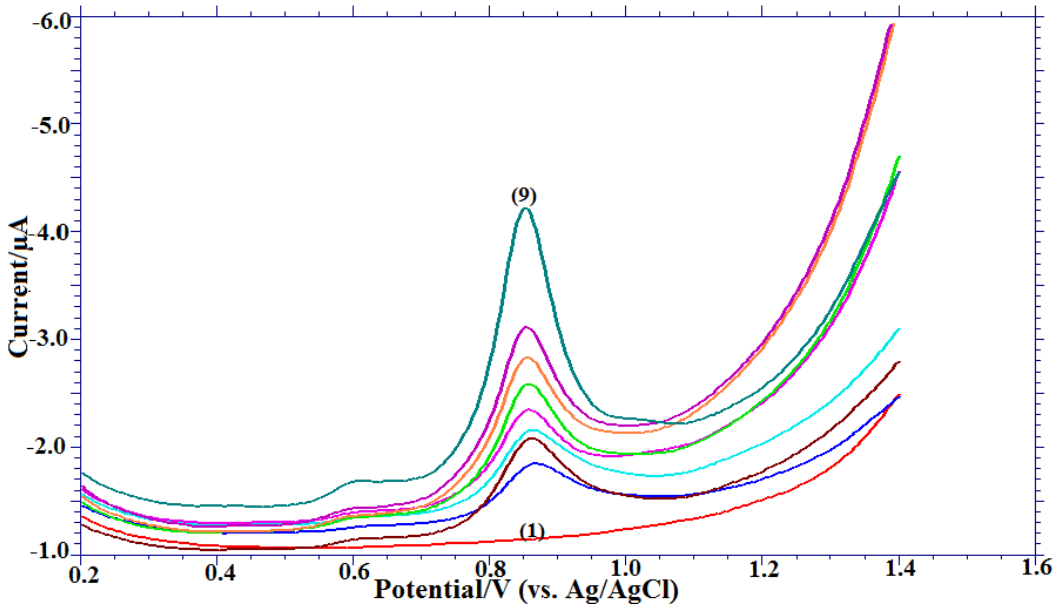


Fig. 6. Differential pulse voltammograms with increasing concentrations of FGF in pH 5.0 phosphate buffer solution on glassy carbon electrode: (1) blank; (2) 3×10^{-5}; (3) 5×10^{-5}; (4) 6×10^{-5}; (5) 7×10^{-5}; (6) 8×10^{-5}; (7) 9×10^{-5}; (8) 1×10^{-4}; (9) 2×10^{-4}

Table 1. Comparison of detection limits of FGF by different methods

Method	**LOD ($\mu g/L^{-1}$)**	**Reference**
High performance liquid chromatography	1.23	[6]
HPLC Photodiode array	5.0	[7]
Liquid chromatography-electro spray tandem mass spectrometry	2.0	[8]
CPE Scanometry	22.0	[9]
CPE UV- Visible	7.0	[9]
CE-CCD Instrument	300	[10]
Differential Pulse Voltammetry	0.8	[Present work]

LOD=Limit of Detection

4.2. 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 fast green dye in the presence of 1.0 mM of each of the interferents. The experimental results showed that hundred fold of glucose, starch, sucrose, dextrose, lactose, gum acacia, citric

acid and oxalic acid did not interfere with the voltammetric signal of fast green. Therefore, the proposed method can be used as a selective method.

4.3. Detection of fast green in urine samples

The applicability of the DPV to the determination of fast green in spiked urine was investigated. The recoveries from urine were measured by spiking dye free urine with known amounts of fast green dye. 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 fast green into the detect system of urine sample. The calibration curve was used for the determination of spiked fast green in urine samples. The detection results 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 R.S.D. was 1.47%.

Table 2. Determination of FGF in urine samples by DPV at glassy carbon electrode

Sample	Declared (mol/L)	Detected (mol/L)	Recovery (%)
Urine sample 1	0.1×10^{-4}	0.097×10^{-4}	97.5
Urine sample 2	0.2×10^{-4}	0.198×10^{-4}	99.0
Urine sample 3	0.5×10^{-4}	0.505×10^{-4}	101.0
Urine sample 4	0.8×10^{-4}	0.798×10^{-4}	99.7

*Average five determinations

5. CONCLUSION

The utilization of glassy carbon electrode has been observed to be agreeable for the determination fast green dye. The electrochemical oxidation of fast green dye was found to be an irreversible, diffusion controlled and two electron-one proton electrode process. Based on the results obtained, a suitable electrode mechanism was proposed. Under the optimized condition the peak current was linear with dye concentrations. A suitable electrode mechanism was proposed. In addition, the results obtained from the analysis of dye in spiked human urine samples demonstrated the applicability of the method for real sample analysis.

NOMENCLATURE

FGF=Fast Green FCF
GCE=Glassy Carbon Electrode
DPV=Differential Pulse Voltammetry
CV=Cyclic Voltammetry

I_p=Peak Current (μA)
E_p=Peak potential (V)
A=Surface area of the electrode (cm^2)
D_0=Diffusion coefficient (cm^2s^{-1})
υ=Scan rate ($mV\ s^{-1}$)
C_0^*=Concentration ($mol\ dm^{-3}$)
n=Number of electrons transferred
α=Transfer coefficient
E_0=Formal Redox Potential
k^0=Standard Heterogeneous Rate Constant (s^{-1})
F=Faraday constant ($C\ mol^{-1}$)
R=Gas constant ($J\ K^{-1}mol^{-1}$)
T=Temperature (K)
LOD=Limit of Detection ($mol\ dm^{-3}$)
LOQ=Limit of Quantification ($mol\ dm^{-3}$)
S=Standard deviation of the peak currents
m=Slope of the calibration curve
RSD=Relative Standard Deviation

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