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Voltammetric Determination of Antipsychotic Drug Flupentixol HCl in Human Serum at a Boron-Doped Diamond Electrode

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Abstract: This study aims to offer a voltammetric method for determining the antipsychotic drug flupentixol from serum samples. According to pH and scan rate studies, the oxidation behavior of flupentixol at boron-doped diamond electrode was found as irreversible and diffusion-controlled. To determine flupentixol from bulk form and serum samples, differential pulse voltammetry was preferred as the working method because of the repeatability. Linear responses were obtained in the range of 6.0×10^{-7} – 8.0×10^{-6} M and 8.0×10^{-7} – 1.0×10^{-5} M for bulk form and serum samples in pH 2.0 Britton-Robinson buffer solution, with detection limit values of 1.09×10^{-7} M and 1.08×10^{-7} M, respectively. Required validation parameters were also studied and according to recovery from serum samples (99.91%) and precision studies, it can be inferred that the developed method was accurate and precise.

Keywords: Flupentixol, voltammetry, boron-doped diamond electrode, serum, validation.

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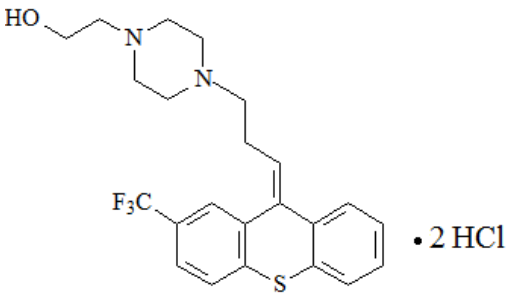
INTRODUCTION

Depression is one of the most common and schizophrenia is one of the most serious psychiatric illnesses worldwide. According to the World Health Organization (WHO), around 280 million people have been diagnosed with depression (1) and nearly 20 million people with schizophrenia (2). Because of the severity and breadth of these two diseases, the pharmaceutical industry is encouraged to develop new and more effective medications. One of such drugs is flupentixol dihydrochloride (FLP, Scheme 1), which is a thioxanthene derivative antipsychotic drug used for the relief of psychotic symptoms such as schizophrenia and depression. Its oral bioavailability is approximately 55%, and the peak serum concentration occurs between 3 and 8 hours (3).



Scheme 1: Chemical structure of FLP.

Electrochemical methods, particularly voltammetry, are frequently preferred because they are simple, cheap, sensitive and reliable; also, their short analysis time makes them more popular (4). Carbon-based materials are commonly used as electrode materials in voltammetric studies because they match the specifications of an ideal electrode material, which is expected to be stable over time, have low residual current and a wide potential range (5). Even though all allotropes of carbon have diverse chemical, electrical, and physical properties, diamond stands out for its exceptional thermal conductivity and mechanical strength. However, diamond has very low conductivity that

can be overcome by doping it with boron (6). Diamond behaves like an insulator, a metal-like conductor, or even a low-temperature superconductor, depending on the level of doping. A relatively high boron doped diamond (10^3 - 10^4 ppm) can be used as an electrode material and it demonstrates metal-like conductivity. Highly conductive boron-doped diamond (BDD) electrodes are quite popular in various electrochemical assays (7).

When compared to traditional electrodes, BDD working electrode offers numerous benefits. In both alkaline and acidic conditions, these electrodes are exceptionally robust, corrosion resistant, chemically inert, and electrochemically stable. Moreover, they have high thermal conductivity and resist passivation of the electrode surface. In addition, they are less sensitive to dissolved oxygen and have a very low and stable background, allowing them to operate over a wide range of potentials (7,8).

Antipsychotics are typically given at oral doses of only a few milligrams per day and are extensively metabolized in the body and plasma concentrations of these medications are quite low. Furthermore, therapeutic drug monitoring (TDM) of antipsychotic medicines has proven to be useful in identifying patients' poor compliance and resolving the problems associated with significant genetic variability in their metabolism. Highly sensitive, selective, and accurate bioanalytical methods are required to undertake pharmacological and toxicological investigations and clinical TDM of antipsychotics, as well as to address the problems associated with polypharmacy and drug metabolism (9). When the literature studies are examined, it has been seen that there are voltammetric (10), potentiometric (11), spectrofluorimetric (12), second-order spectrophotometric (13), reversed-phase high performance liquid chromatographic (HPLC) (14), LC-MS/MS (3) and LC-ESI-MS (15) methods designed to determine FLP from the pharmaceutical preparations and biological samples in an individual manner.

In this study, voltammetric behavior of FLP was investigated utilizing a boron-doped diamond electrode and employing DP voltammetric method for its determination from the serum. The absence of a voltammetric study for FLP with BDDE in the literature shows the originality of the study.

EXPERIMENTAL SECTION

Chemicals and Reagents

1×10^{-3} M FLP (kindly supplied from Lundbeck, Istanbul) stock solution was prepared in methanol and kept in a refrigerator. Analytical grade reagents were used for preparing required solutions. Measurement solutions were prepared before use by adding 20% methanol and supporting electrolyte onto the required amount of FLP solutions. H_2SO_4 solutions (0.1 and 0.5 M) and Britton-Robinson (BR) buffer solutions (0.04 M, pH 2.0 – 10.0) were preferred as supporting electrolytes and prepared in distilled water. Sigma-Aldrich provided the synthetic serum samples.

Apparatus and Measurements

AUTOLAB 204 PGSTAT device and NOVA 2.1 software were used for electrochemical measurements. A conventional three-electrode cell was used, BDDE (Windsor Scientific Ltd.; 3 mm diameter) working electrode, Ag/AgCl (BAS, 3 M NaCl) reference electrode and platinum wire auxiliary electrodes were employed. Baseline correction was applied (except cyclic voltammograms) to the measured voltammograms using moving average. Alumina powder and a polishing cloth were used to clean the electrode surface before each measurement. Cyclic voltammetry (CV), DPV and square wave voltammetry (SWV) were employed for investigating the oxidation behavior of FLP. DPV was selected for the determination of FLP with the following parameters: step potential: 10 mV; modulation amplitude: 50 mV; modulation time: 50 ms; interval time: 500 ms. The pH of the buffer solutions was measured using a SevenCompact™ pH/Ion S220 model pH meter (Mettler Toledo, Switzerland).

Preparation of Serum Samples

Serum stock solution containing 1×10^{-3} M FLP was prepared by mixing required volume of 1×10^{-2} M FLP, methanol and acetonitrile that was used to precipitate serum proteins. The mixture was ultrasonicated for 15 minutes and centrifuged at 5000 rpm for 15 minutes to collect the precipitate at the bottom of the tube. Supernatant was carefully transferred to a clean tube and measurement solutions were prepared from this supernatant with the addition of 20% methanol and pH 2.0 BR buffer solution. Recovery studies were carried out by adding standard solution onto the known amount of serum solution. Results were analyzed using calibration curve obtained from serum studies.

RESULTS AND DISCUSSION

pH and Scan Rate Effect on FLP Signal at BDDE

To examine the voltammetric behavior of FLP at BDDE, pH scanning was first performed. For this purpose, the response of FLP in BR buffer solutions with pH values between 2.0 and 10.0 and 0.5 M and 0.1 M H_2SO_4 solutions were studied by using CV, DPV and SWV methods.

Figure 1 shows repetitive cyclic voltammograms of 8×10^{-5} M FLP in 0.5 M H_2SO_4 solution (A), pH 2.0 BR buffer (B), and pH 6.0 BR (C) buffer solution at a scan rate of 100 mV/s. FLP showed two well-defined oxidation peaks and a very weak wave in 0.5 M H_2SO_4 solution; a well-defined oxidation peak and a wave in pH 2.0 BR buffer (optimum pH value) and one well-defined oxidation peak in pH 6.0 BR buffer solutions. As can be observed from the figure, in the first scan, the intensity of peaks was higher compared to the second and third scans. The decrease in the peak intensity could be explained via electrode surface fouling. In the cathodic direction, no peak was observed which showed the irreversible electrode reaction.

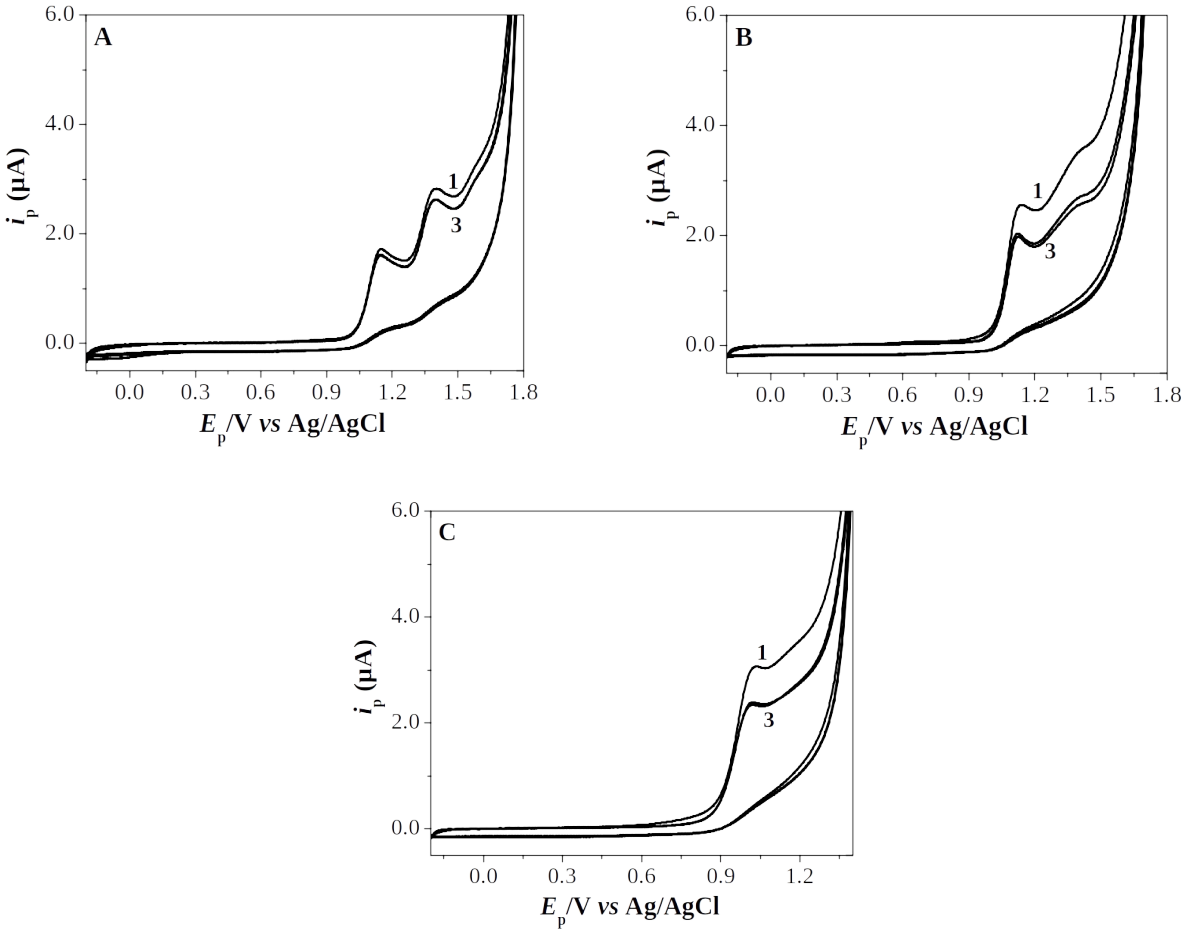


Figure 1: Repetitive cyclic voltammograms of 8×10^{-5} M FLP in 0.5 M H_2SO_4 (A), pH 2.0 BR buffer (B), pH 6.0 BR buffer (C) solutions. Scan rate: 100 mV/s. "1" means first scan and "3" means third scan of voltammograms.

The increase in the pH value resulted in a slight shift towards less positive potential values in the peak potential until pH 8.0 (using CV, DPV, SWV). The reason for this shift may probably be stemmed from the changes in protonation of the acid-base functions in the FLP. After this pH (pH 8.0), peak potential was nearly constant meaning that there were no proton transfer steps before the electron transfer rate-determining step at these pH values (Figure 2). The breakup at about pH 4.0 (for SWV pH 3.0) and pH 8.0 may be attributed to the pK_a values of FLP which are 4.64 (pK_{a1}) and 8.16 (pK_{a2}) (10). The shift in the Ep values is given by the following equations:

E_p (mV) = –34.5 pH + 1192.5; r = 0.966 (pH 1.0 – 4.0 with CV)
E_p (mV) = –15.0 pH + 1119.4; r = 0.983 (pH 4.0 – 8.0 with CV)
E_p (mV) = –34.5 pH + 1115.9; r = 0.988 (pH 0.3 – 4.0 with DPV)
E_p (mV) = –18.0 pH + 1054.0; r = 0.976 (pH 4.0 – 8.0 with DPV)
E_p (mV) = –30.8 pH + 1131.1; r = 0.970 (pH 0.3 – 3.0 with SWV)
E_p (mV) = –20.1 pH + 1104.8; r = 0.976 (pH 4.0 – 8.0 with SWV)

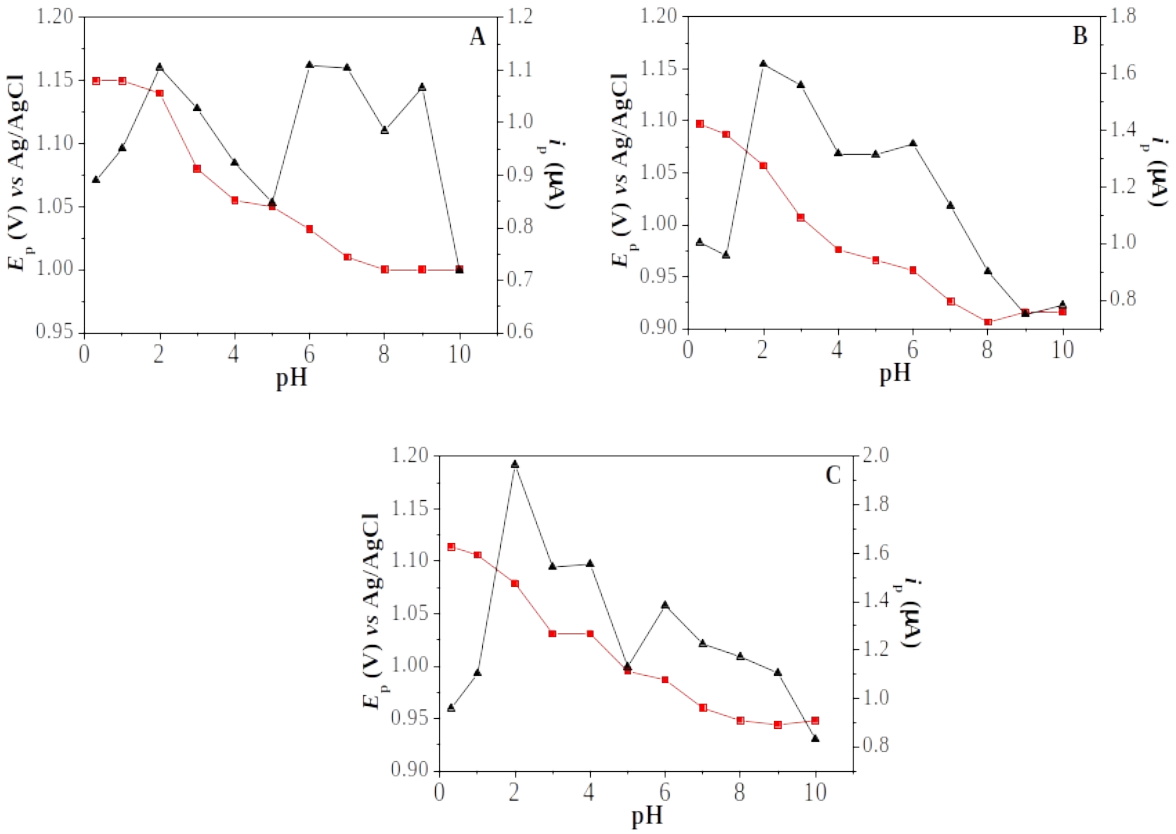


Figure 2: E_p – pH (red) and i_p – pH (black) graphs of 8.0×10^{-5} M FLP using CV (A), DPV (B) and SWV (C).

The graph of the pH values against the measured current values in this working pH range was shown in Figure 2. As can be seen from the graph, pH 2.0 BR buffer solution with the highest peak current and the best peak shape was selected as the most suitable medium for further studies.

The scanning rate studies were performed via CV by using 1.0×10^{-4} M FLP in pH 2.0 BR buffer solution at the range of 5 – 1000 mV/s. The result of the linear Randles-Sevcik plot (i_p *vs* $v^{1/2}$) with a correlation coefficient of 0.9944 showed that the mass transport to the electrode surface was diffusion controlled. This result was also confirmed by plotting log i_p against log v; a straight line with a slope of 0.5207 was obtained (r = 0.9981) (Figure 3). Related equations were given below:

$\log i_p = 0.5207 \log v - 0.9973$, n = 10, r = 0.9981
$i_p = 0.1189\ v^{1/2} - 0.0696$, n = 10, r = 0.9944

Increase in the scan rate values from 5 mV/s to 1000 mV/s resulted in 66 mV shift towards the positive potential values which was related to the electrode reaction irreversibility (16).

Calibration and Validation

Quantitative assessment is based on the linear relationship between current and concentration. Therefore, DPV was selected because of the repeatability. The studies were performed in pH 2.0 BR buffer solution (the methanol ratio was kept constant at 20%) where the best peak shape and the highest peak current were found. Linearity was obtained at the concentration range of 6.0×10^{-7} – 8.0×10^{-6} M (Figure 4). The related equation between peak current and concentration was as follows:

i_p (μA) = 25525.44 C (M) – 0.00969 (r = 0.999)

The regression data of the calibration is shown in Table 1. The precision of the method is demonstrated by repeated measurements of the peak potential and peak current of the FLP, within and between days. LOD and limit of quantification (LOQ) values were calculated with the formulas of 3 s/m and 10 s/m, respectively; where "s" represents the standard deviation of the response and "m" represents the slope of the calibration curve. LOD and LOQ values showing the sensitivity of the proposed method were presented in Table 1.

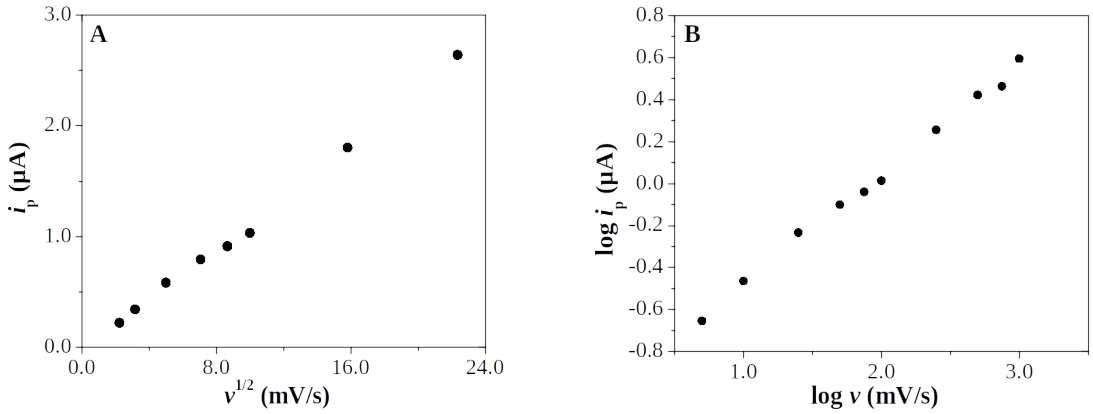
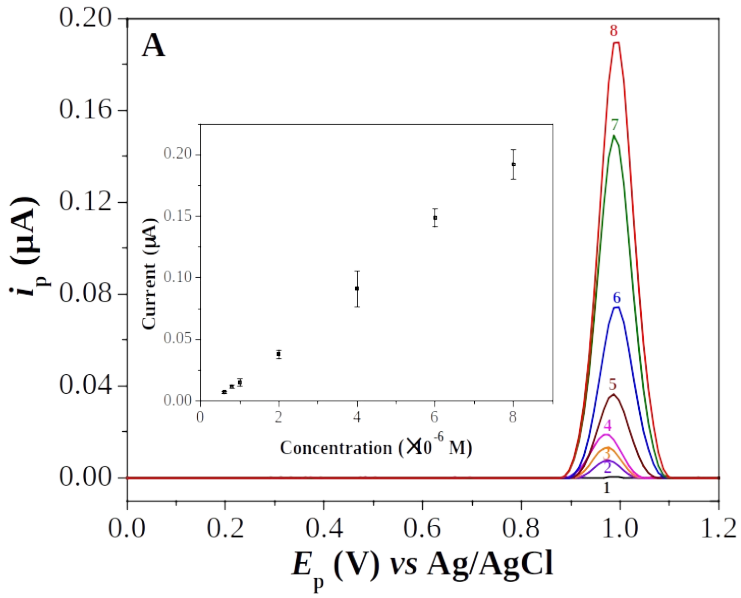


Figure 3: i_p *vs* $v^{1/2}$ (A) and log i_p *vs* log v (B) graphs of 1.0×10^{-4} M FLP in pH 2.0 BR buffer solution obtained in the range of 5 – 1000 mV/s.



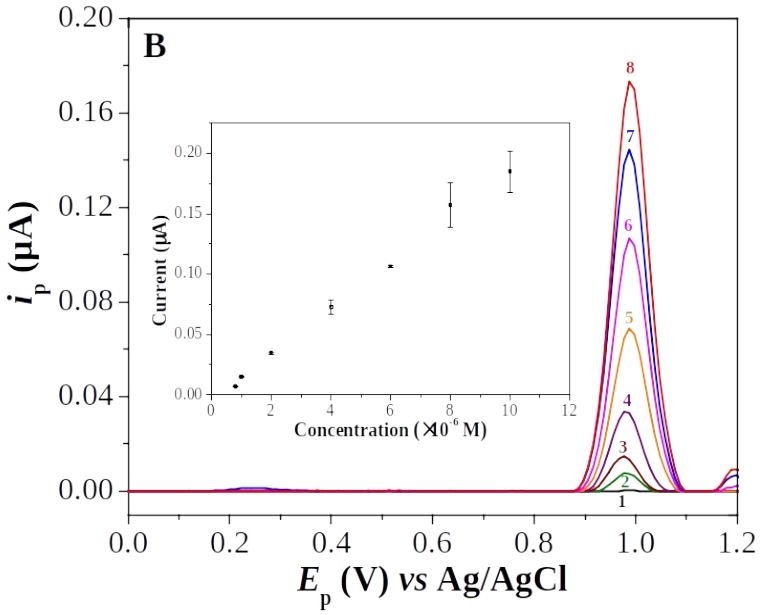


Figure 4: DP voltammograms for FLP (bulk solution) in different concentrations between 6.0×10^{-7} – 8.0×10^{-6} M (A) and DP voltammograms of serum samples in different concentrations between 8.0×10^{-7} – 1.0×10^{-5} M (B) in pH 2.0 BR buffer solution. Inset: Calibration curves.

Table 1: Regression data for calibration of FLP obtained from bulk solution and serum samples using DPV at BDDE.

	Bulk form	**Serum**
Measured potential (mV)	986	986
Linearity range (M)	6.0×10^{-7}–8.0×10^{-6}	8.0×10^{-7}–1.0×10^{-5}
Slope (µA M^{-1})	25525.44	19435.34
Intercept (µA)	–0.00969	–0.00571
Correlation coefficient (*r*)	0.999	0.998
LOD (M)	1.09×10^{-7}	1.08×10^{-7}
LOQ (M)	3.64×10^{-7}	3.61×10^{-7}
Intra-day precision of peak current (RSD %)*	1.98	1.86
Inter-day precision of peak current (RSD %)*	2.42	2.83

*Obtained from five measurements.

Serum Sample Analysis

Quantitative determination of FLP from synthetic serum samples was realized in pH 2.0 BR buffer solutions and linear relationship between concentration and peak current was obtained as 8.0×10^{-7} – 1.0×10^{-5} M (Figure 4) with the equation below:

i_p (µA) = 19435.34 *C* (M) – 0.00571 (*r* = 0.998)

LOD and LOQ values obtained from spiked serum samples were calculated as 1.08×10^{-7} M and 3.61×10^{-7} M, respectively. Recovery studies were also made, and the average recovered % result was obtained as 99.91% using five repeatable measurements. Regression results for the calibration of serum samples were summarized in Table 1 and recovery results were shown in Table 2.

Table 2: Recovery studies obtained from serum samples using DPV at BDDE.

	DPV
Added concentration (M)	2.00×10^{-6}
Found concentration (M)	1.99×10^{-6}
Average recovered %	99.91
Number of experiments	5
RSD % of recovery	3.34
Bias %	0.09

Comparison of Published Analysis Methods

Table 3 showed the comparison of literature methods for FLP determination according to linearity range, LOD and LOQ values. Potentiometry (11), LC-MS/MS (3) and LC-ESI-MS (15) studies gave lower LOD or LOQ values when compared to this study. When comparing LC-MS/MS and LC-ESI-MS, it can be said that in the voltammetric method the requirement for organic solvent and the cost of instrumentation were minimum, also there was no need for separation and pre-treatment steps. In the potentiometric method, multi-walled carbon nanotube and copper nanoparticles modified carbon paste electrode was used for the determination of FLP which was time-consuming and costly. The method developed in this study was compared to the previously offered voltammetric methods (10), in which a glassy carbon electrode (GCE) was used. The parameters of comparison were linearity range and limit of detection (LOD) values. In this study linearity range and LOD value were obtained as 6.0×10^{-7} – 8.0×10^{-6} M and 1.09×10^{-7} M, respectively which were comparable to the literature study (8.0×10^{-7} – 1.0×10^{-4} M and 1.17×10^{-7} M using differential pulse voltammetry-DPV). The serum analysis results via DPV revealed that the LOD value (1.08×10^{-7} M) obtained by using BDDE was lower than that obtained by using GCE (5.06×10^{-7} M). These results demonstrate that BDDE can be preferred as an alternative electrode to GCE for better detection and determination of FLP from bulk form and especially from serum.

Table 3: Comparison of developed method with literature studies about FLP.

Method	Linearity range (M)	LOD (M)	LOQ (M)	Reference
DPV	8.0×10^{-7} – 1.0×10^{-4}	1.17×10^{-7}	3.89×10^{-7}	(10)
SWV	1.0×10^{-6} – 1.0×10^{-4}	2.86×10^{-7}	4.29×10^{-7}	
Potentiometry	1.0×10^{-10} – 1.0×10^{-2}	2.5×10^{-11}	-	(11)
Spectrofluorimetry	9.85×10^{-7} – 4.92×10^{-6}	1.77×10^{-7}	5.71×10^{-7}	(12)
Second-order spectrophotometry	5.91×10^{-6} – 2.95×10^{-5}	7.09×10^{-7}	2.18×10^{-6}	(13)
LC-MS/MS	1.97×10^{-11} – 3.94×10^{-9}	-	1.97×10^{-11}	(3)
LC-ESI-MS	7.68×10^{-11} – 4.92×10^{-9}	-	7.68×10^{-11}	(15)
DPV	6.0×10^{-7} – 8.0×10^{-6}	1.09×10^{-7}	3.64×10^{-7}	This study

CONCLUSION

The voltammetric oxidation behavior of FLP was investigated by using H_2SO_4 supporting electrolytes and BR buffer solutions with pH values of 0.3 – 10.00 at BDDE. In all pH media, FLP was irreversibly oxidized, and the electrode transfer process onto the electrode surface was found to be diffusion-controlled. DPV method was used for the determination of FLP from bulk form and synthetic serum samples combined with BDDE. When the developed method was compared to DP voltammetric method in the literature (10) it was shown that the LOD values were lower in the present study. It can be said that simple, low cost, relatively fast, environmentally friendly, accurate and precise electrochemical method was developed for FLP detection and determination.

CONFLICT OF INTEREST

There is no conflict of interest.

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