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Electrochemical Determination of Gemifloxacin Mesylate in Commercial Tablets and Biological Fluids By Differential Pulse Polarography

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An ultrasenstive electrochemical probe was suggested for the estimation and quantification of gemifloxacin mesylate (GFX) in its pharmaceutical products and biological media. Differential pulse (DPP), Cyclic voltammtery (CV) and Alternative current AC_t were exploited to investigate the electrochemical nature of GFX. Over the pH range of 2.6-10, the investigated drug demonstrated significant cathodic peaks. The best polarographic response was achieved in acetate buffer (pH 5), scan rate 15 mV s^{-1} and pulse amplitude -90 mV. The outcome linearity was 1.59×10^{-6} – 2.70×10^{-5} mol L^{-1} (0.77 – 13.1 µg mL^{-1}). The limits of detection and quantification were determined as 2.89×10^{-7} and 8.76×10^{-7} mol L^{-1}, respectively. The proposed electrochemical approach was validated and successfully used to quantify the selected drug in different media. The outcome results were compared with others obtained from reference method and displayed good accuracy and precision agreement.

Keywords: Gemifloxacin mesylate; Differential pulse polarography; Commercial products; Biosamples

1. INTRODUCTION

Polarography is an electrochemistry technique used to analyze liquid solutions containing oxidizing or reducing substances. It is the most extensively used voltammetric technique. In this technique, the measurements are conducted by only diffusion mass transport [1]. To determine the reducible substances, it is believed that mercury electrodes are the most simple electrodes with smooth surface and the high cathodic potential window [2].

Gemifloxacin mesylate (GFX) (Figure 1) is a novel member belongs to fluoroquinolones. It is a broad spectrum third generation antimicrobial agent. GFX is active against many pathogens involved in the urinary tract and respiratory infections [3]. Its mode of action based on the inhibition of DNA synthesis [4]. GFX was determined by different separation, electrochemical and spectroscopic techniques such as liquid chromatography coupled with spectroscopic detectors [5,6] and mass spectrometer detector [7], capillary zone electrophoresis [8] ion selective electrodes [9] voltammetry [10] spectrophotometry [11-13], spectrofluorimetry [14, 15] and chemiluminescence [16].

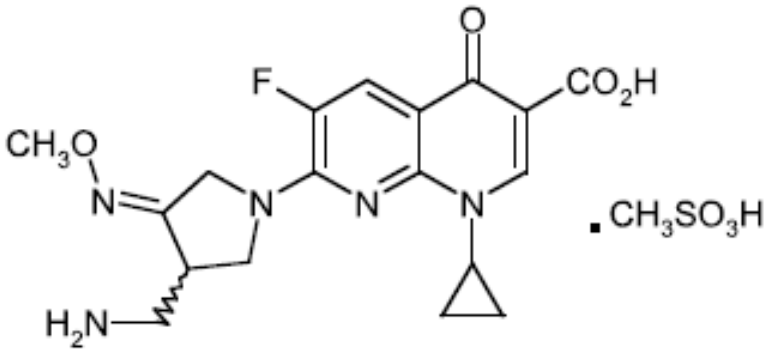

Figure 1. Structural formula of gemifloxacin mesylate

The previously addressed voltammetric method for quantification of GFX in pharmaceutical tablets displayed linear concentration range of 2.47-15.5 µg mL^{-1}. It was conducted using cyclic, square wave and differential pulse voltammetric techniques. These methods require the preparation and generation of a glassy electrode modified with carbon nanotube in the presence of cetyltrimethyl ammonium bromide.

Herein, the objective of this study is to propose a sensitive polarographic probe for the estimation of GFX by reducing the carbonyl group on the surface of dropping mercury electrode (DME).

2. EXPERIMENTAL

2.1. Chemicals and solvents

Pure sample of (GFX) and its tablets (Factive® 320 mg/tablet) were kindly gifted by Tabuk Pharmaceuticals. MFG. CO., Saudi Arabia). In distilled water, a standard GFX solution of 1.0×10^{-3} mol L^{-1} was prepared and stored at 4°C. This solution was stable for two weeks when cooled in the refrigerator. Acetate buffers (0.2 mol L^{-1}) in the pH range (2.6- 6), Britton-Robinson buffers (0.08 mol L^{-1}) in the pH range 3.0-12.0, phosphate buffer (0.05 mol L^{-1}) in the pH range 5.0-8.0 and borate buffers in the pH range 7-12 were used as mediating electrolytes when studying the effect of pH and supporting electrolyte [17].

2.2. Instrumentation

Metrohm of model-797 VA Computrace (Metrohm, Switzerland) was used to measure all electrochemical analysis. The electrochemical system consists of a working electrode (mercury electrode, 4 mm), an auxiliary electrode (platinum wire) and a reference electrode (saturated Ag/AgCl electrode). Phase–selective AC_T polarograms of 2.7×10^{-5} $molL^{-1}$ were recorded using (AC) 15 mV voltage, 75 Hz frequency and 90º a phase angle. Cyclic voltammogram of 1.57×10^{-5} $molL^{-1}$ solution was recorded by graduating the scan rate from 20 to 500 mVs^{-1}. The pH was adjusted using a HANNA instrument pH-211 microprocessor pH-meter (Romania).

2.3. General analytical procedure

The polarographic cell was filled with about 25 mL of supporting electrolyte (acetate buffer pH 5). Then the tested solutions of GFX were transferred to the same cell. Before analysis, nitrogen gas was used to purge the investigated solutions for 5 min. The investigated solutions were cathodic scanned using the range from 0.0 to -2.0 V *vs*. standard Ag/AgCl electrode. The calibration graph was constructed by plotting of current (μA) *vs*. GFX concentrations (mol L^{-1}). All electrochemical measurements were performed at ambient temperature.

2.3.1. Estimation of GFX in tablets

Standard GFX tablet solution corresponding to 1.0×10^{-3} mol L^{-1} was obtained by weighting and pulverizing ten tablets into fine powder. Then accurate amount equivalent to the required solution was dissolved in a 100-mL volumetric flask under 30 min sonication using 50 mL distilled water. The prepared solution was filtered and the same solvent was used to reach the mark. The required standard solutions of GFX tablets were added to 25 mL of supporting electrolyte in the polarographic cell and the analysis was carried out using the same above previously mentioned steps.

2.3.2. Estimation of GFX in biosamples

For Spiked serum: Accurately measured amounts of GFX aqueous solution were added into a number of centrifuged tubes containing 0.1 mL serum. 1.0 mL of acetonitrile was dropped to each tube and centrifuged at 1500 rpm for 10 min. Approximately, 0.1 mL volume of the clear supernatant was injected into polarographic cell containing a 25 mL of acetate buffer pH 5 to prepare a concentration range of 1.59×10^{-6}– 2.70×10^{-5} mol L^{-1}. Then GFX was determined by applying the proposed differential pulse polarography (DPP) procedure as previously described above.

For spiked urine: Accurately measured amounts of GFX aqueous solution were added into a number of 25 mL measured flask containing 1.0 mL urine, then they were shacked well and diluted to the mark with distilled water. 0.1 mL volume was transferred to the polarographic cell that contains a

25 mL of acetate buffer pH 5, to obtain a concentration range of 1.59×10^{-6}–2.70×10^{-5} mol L^{-1}. Then GFX was determined by using the proposed DPP procedure as described above.

3. RESULTS AND DISCUSSION

3.1. Voltammeteric behavior of GFX

The cyclic voltammetric behavior of 1.57×10^{-5} mol L^{-1} GFX in acetate buffer of pH 5 at the hanging mercury dropping electrode (HMDE) monitored in the cathodic direction yielded a single well-defined peak at -1.64 V which could be due to the cathodic reduction of the C=O group in the tested molecule (Figure 2). The irreversible characteristic of the electrode was observed due to the absence of oxidation peak in the positive half cycle.

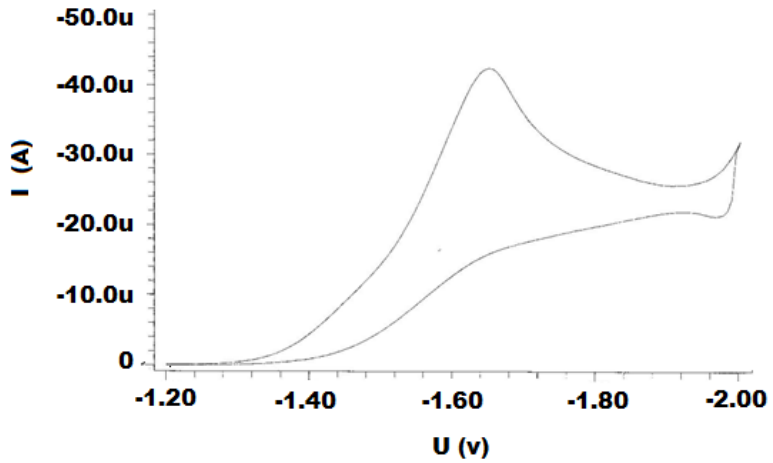

Figure 2. Cyclic voltammogram of GFX (1.57 Mol L^{-1}) in acetate buffer pH 5 and scan rate 50 MV s^{-1}

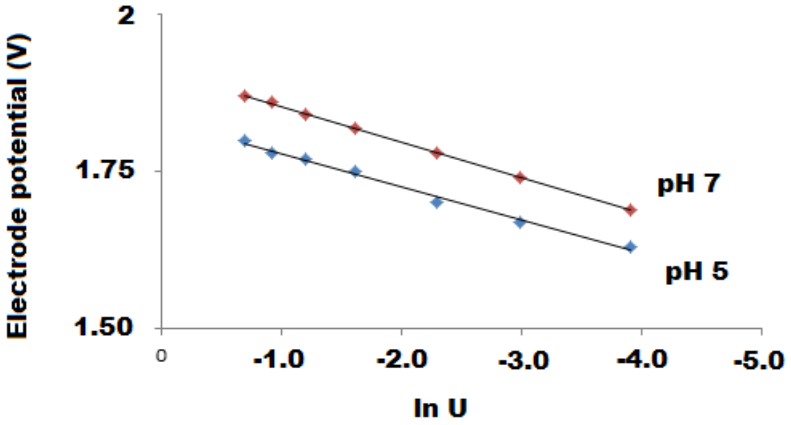

Figure 3. Plot of peak potential versus lnU (cyclic voltammetry of GFX 1.57×10^{-5} mol L^{-1})

The cyclic voltammograms of GFX (1.57×10^{-5} mol L^{-1}) were recorded to elevate the scan rate values over the range 20-500 mV s^{-1} at pH 5 and 7. By increasing the scan rate, the peak potentials showed a cathodic shift, indicating the irreversible feature of the reduction process [18]. On graphing

peak potential (Ep) versus ln scan rate (ln ν) at different pH values a linear relationship was obtained (Figure 3). The gradients of the slopes are proportional to αn_a (where α is charge transfer coefficient, n_a number of electrons in the rate determining step). The gradient of the slope increased as the pH is increased. Thus, the value of αn_a increased, so the degree of reversibility increased as the pH is increased.

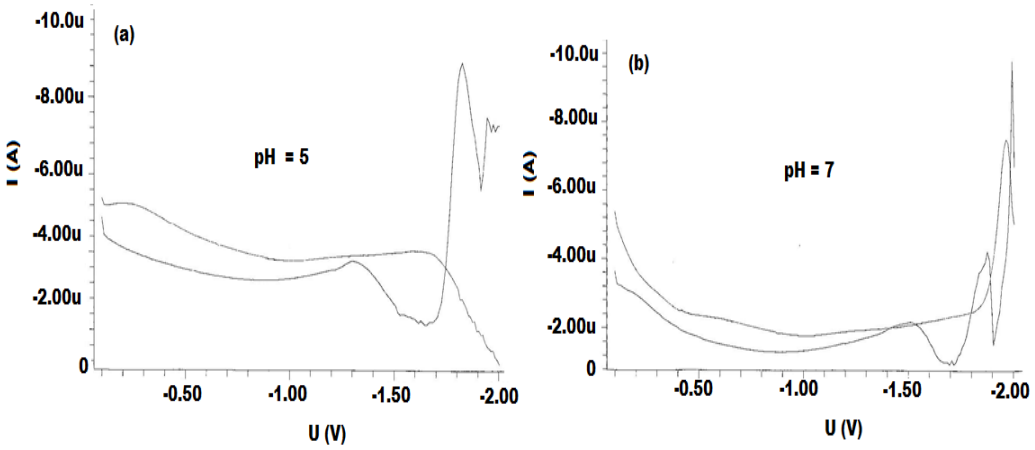

Figure 4. Alternating current behavior of GFX (2.7×10^{-5} mol L^{-1}) in acetate buffer of pH 5 and 7 values. Superimposed alternating voltage: 15 mV

The alternating current behavior (AC_T) of 2.70×10^{-5} mol L^{-1} GFX solution was studied using a phase-selective angle of 90º. A solution of pH 5 and 7, the summit potentials (E_s) were shifted to a more negative value of 180 and 280 mV, respectively. Figure 4 showed that at pH 7 adsorption of depolarizer and its reduction product occurs, while at pH 5 a slight adsorption of depolarizer may occur.

3.2. Adjusting the analytical conditions

3.2.1. Effect of pH and assisting electrolyte

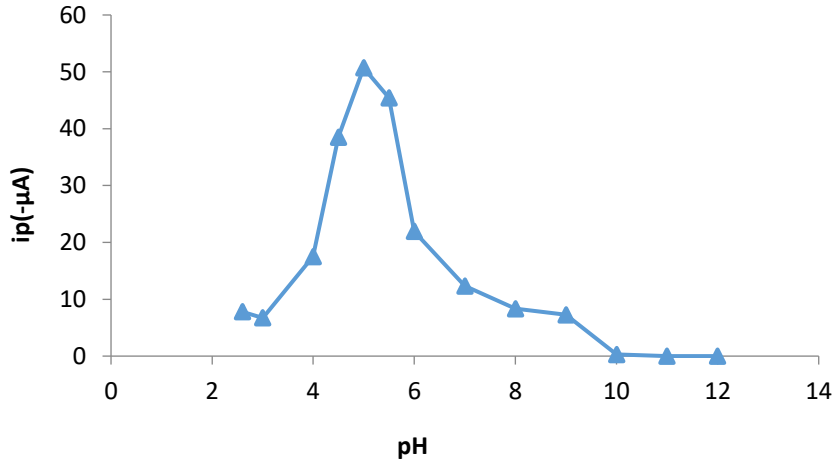

Figure 5. Effect of pH on DPP peak current of GFX (1.96×10^{-5} mol L^{-1}), pulse amplitude -90 mV, Es 6 mV

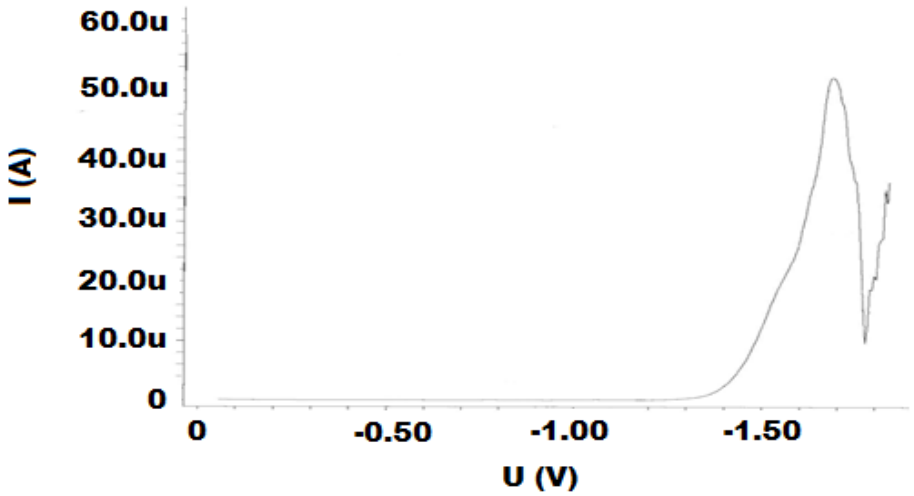

Figure 6. Typical polarogram of GFX (1.96×10^{-5} mol L^{-1}) in acetate buffer pH 5

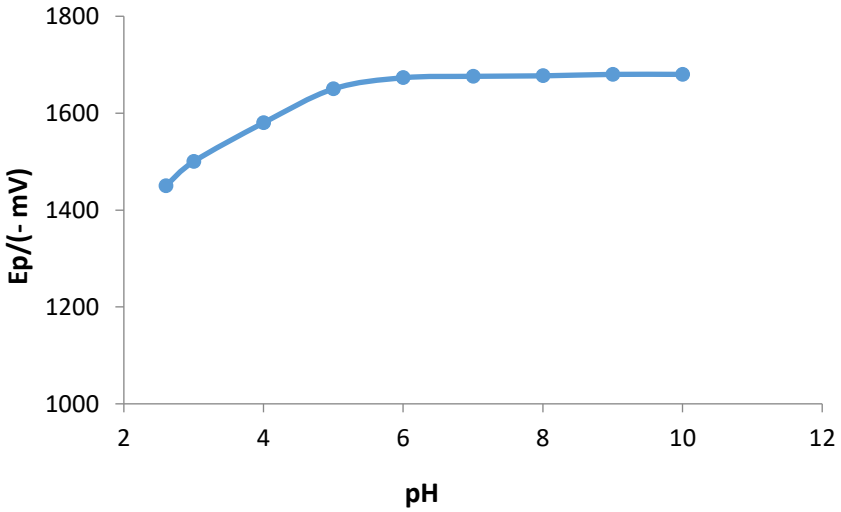

Figure 7. Effect of pH value on peak potential Ep of GFX (1.96×10^{-5} mol L^{-1}), pulse amplitude -90 mV, Es 6 mV

The effect of several assisting electrolytes (acetate, borate, Britton Robinson and phosphate) on the electrochemical signal was tested using DPP. A cathodic peak was obtained over the pH range 2.6-10. Figure 5, demonstrated the pH effect on DPP peak current. The peak current was elevated to maximize value at pH 5 and this value was selected for subsequent investigations. Figure 6, showed a typical polarogram of GFX in acetate buffer of pH 5.

The plotted curve of Ep vs. pH exerted two linear intervals with a significant break at pH 5.5. The linearity was dependent on the pH in the range from 2.6 to 5.0 and from 5.0 to 9.0. The calculated slope was 81.55 per unit and 4.97 mV per pH unit, respectively. The observed break at pH 5.5 could be due to GFX acid-base constant (Figure 7). This value was agreed with the pK_a value reported by GFX [19].

3.2.2. Effect of volume of electrolyte

The influence of the volume of electrolyte on the GFX peak current was studied using different volumes of acetate buffer (5-25 mL). The peak current elevated by increasing the buffer concentration. Therefore, further studies were performed using 25 mL acetate buffer as a supporting electrolyte.

3.2.3. Effect of pulse amplitude

The influence of pulse amplitude on DPP polarograms of GFX in acetate buffer of pH 5 was studied in the range (-90 to +90 mV). When the pulse amplitude negative polarity up to 90 mV value, an increase in a peak current (Ip) was appeared.

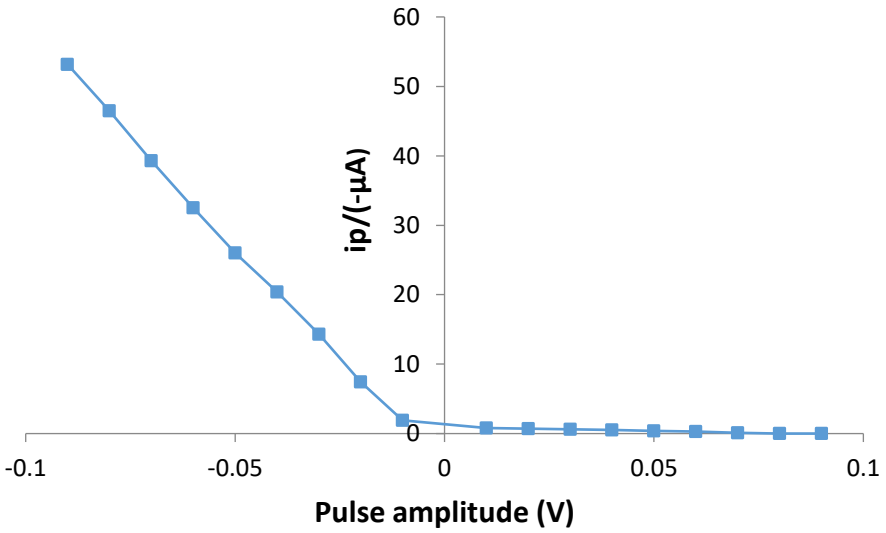

Figure 8. Effect of pulse amplitude on DPP peak current of GFX (1.96×10^{-5} mol L^{-1}), Es 6 mV

Therefore, this value of pulse amplitude was chosen as an optimum value (Figure 8). Meanwhile, the increase of pulse amplitude positive polarity displayed no increase in the peak current.

3.3. Validation study

Method validity was performed by following the validation criteria such as linearity, selectivity, intermediate precision, accuracy and reproducibility.

Under optimized conditions, a linear relationship was obtained between DPP peak intensity and the drug concentration over the range of 1.59×10^{-6} to 2.70×10^{-5} mol L^{-1}. The regression equation was calculated by least-square method and it has the form:

I_p (-µA) = 2.17×10^6 C (molL^{-1}) + 10.37 r = 0.9999 (n = 8)

Where I_p is the DDP peak current in µ-amperes, C is GFX concentration and r is the correlation coefficient.

The limits of detection (LOD) and quantification (LOQ) were determined using $3.3S_a/b$ and $10S_a/b$ respectively, where S_a is the standard deviation of the intercept, and b is the slope. It was found that LOD = 2.89×10^{-7} mol L^{-1} and LOQ = 8.76×10^{-7} mol L^{-1}.

The repeatability was performed by analyzing three different concentrations of GFX (2.39, 7.94 and 2.3) $\times 10^{-6}$ mol L^{-1} in triplicates within the same day. The mean recoveries of 100.28±0.64, 99.54±0.19 and 99.71±1.09, were achieved, which indicated good precision of the electrochemical system.

The intermediate precision was studied by repeating analysis of GFX in pure form, for a period of three successive days using the concentrations of GFX (2.4, 7.9 and 2.3) $\times 10^{-6}$ mol L^{-1}. The mean recoveries of 100.14±0.49, 100.29 ± 0.84 and 99.71±0.66, were achieved, which confirmed high precision of this method.

Table 1. Results obtained from the analysis of GFX using DPP in pure form and its pharmaceutical tablets (Factive®Tablets) using the proposed DPP polarographic method

	Proposed DPP method Pure bulk powder			Reference Method [11]	Proposed DPP method Factive® Tablets 320 mg GFX/Tablet			Reference Method [11]
	Taken	Found	% Recovery		Taken	Found	% Recovery	
	1.6×10^{-6}	1.58×10^{-6}	99.4	98.00	1.59	1.57	99.4	99.3
	4.0×10^{-6}	3.96×10^{-6}	99.0	99.94	5.96	5.92	99.0	99.7
	7.9×10^{-6}	8.07×10^{-6}	101.6	99.75	7.94	7.9	101.6	98.8
	1.2×10^{-5}	1.20×10^{-5}	100.8	99.91	11.9	11.8	100.8	99.1
	1.6×10^{-5}	1.55×10^{-5}	98.7	99.6	15.7	15.8	98.7	99.6
	2.7×10^{-5}	2.69×10^{-5}	99.6	99.82	27	26.9	99.6	99.8
$\bar{X}$±SD	99.87±1.14			99.50±0.75	99.50±0.64			99.38±0.39
F-test	2.30(5.05)*				2.73(5.05)*			
t-test	0.66(2.23)*				0.39(2.23)*			

*Theoretical values for t and F at 95% confidence limit (n=6) were 2.23 and 5.05 respectively

To confirm the validity of the current probe, different concentrations of GFX as cited in Table 1 were examined. The percentage recovery obtained was 99.87±1.14 for the quantification of GFX in pure form. The good agreement of the % recovery with others obtained using a reference UV spectrophotometric method which is based on a simple determination of GFX at absorption 263.8 nm [11]. No significant difference was noticed after using the statistical analysis (Student's t-test and Variance ratio F-test) [20], revealing excellent accuracy and precision.

The selectivity of the current system was investigated towards the optimized assay of GFX in the presence of some additives usually present in pharmaceutical products [21]. No observed difference in the assay in the presence or absence of those occupants revealing excellent selectivity of the current system for the determination of the chosen analyte.

3.4. Analytical employments

3.4.1. Assay of GFX in tablets

The suggested DPP system was employed to estimate the GFX in its commercial tablets (Factive 320 GFX/tablet). Mean percentage recoveries of GFX using three replicate determinations were summarized in Table 1. The outcome result was 99.50±0.64 which showed good acceptance with those of spectrophotometric reference method [11]. The calculation of t-test and F-test [20] values also revealed good accuracy and precision due to the calculated values did not exceed the theoretical values.

3.4.2. Analysis of spiked urine and serum

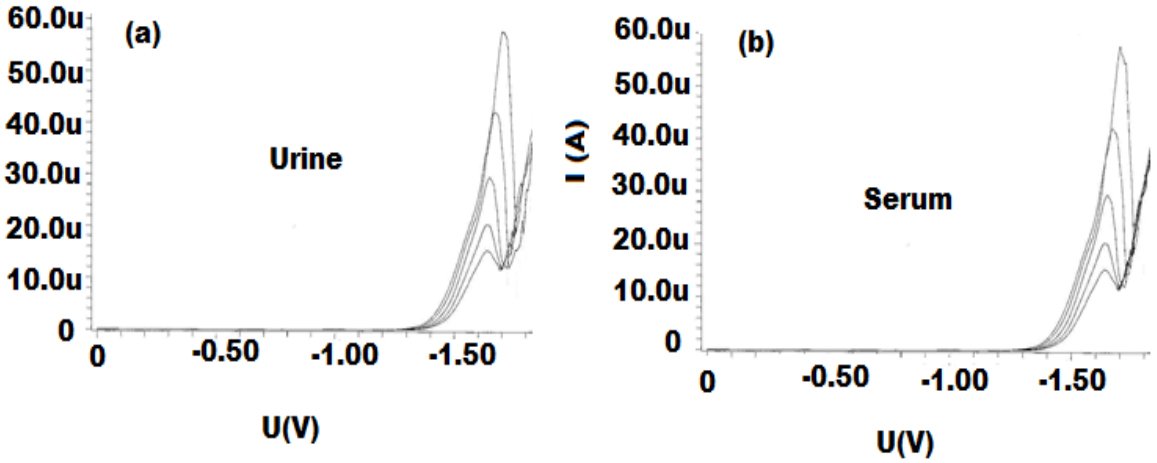

Figure 9. Differential pulse polarography of different concentrations of GFX in urine and serum samples

Table 2. Results obtained from the analysis of GFX using DPP in spiked serum and urine using the proposed DPP polarographic method

Proposed DPP method			Proposed DPP method		
Serum samples			Urine samples		
Taken	Found	% Recovery	Taken	Found	% Recovery
1.59×10^{-6}	1.58×10^{-6}	99.4	1.59×10^{-6}	1.57×10^{-6}	98.7
3.19×10^{-6}	3.23×10^{-6}	101.3	3.19×10^{-6}	3.16×10^{-6}	99.1
4.00×10^{-6}	3.97×10^{-6}	99.3	4.00×10^{-6}	3.98×10^{-6}	99.5
7.94×10^{-6}	8.12×10^{-6}	102.3	7.94×10^{-6}	7.87×10^{-6}	99.1
1.57×10^{-5}	1.58×10^{-5}	100.6	1.57×10^{-5}	1.55×10^{-5}	98.7
2.30×10^{-5}	2.29×10^{-5}	99.6	2.30×10^{-5}	2.28×10^{-5}	99.1
$\bar{X}$±SD	100.39±1.22		$\bar{X}$±SD	99.04±0.29	

Allen et al [22] studied the absorption of GFX after administered, and determined the linear relation between the maximum concentrations of the drug substance (C_{max}) and the dose taken in the plasma. After 1 h of 320 mg GFX single dosing, it was noticed that C_{max} was achieved with mean C_{max} value 1.48±0.39 μgmL^{-1}. This concentration is much higher than the LOQ of the proposed method

(0.43 μgmL^{-1}). The simplicity and high sensitivity of the described probe encouraged the determination of GFX in bio-fluids. Serum protein was removed from the serum samples using acetonitrile, however a twenty-five times dilution of urine sample was necessary. Figure 9, illustrated that the DP polarographic response of different concentrations of spiked serum and urine. The percentage recoveries obtained from the assay of GFX in biosamples were represented in Table 2.

Table 3. Comparative studies between the determination of GFX using proposed DPP method and previously reported techniques

Method	Linear range	LOD	LOQ	Reference
The proposed method	0.77-1.1 μgmL^{-1}	0.14 μgmL^{-1}	0.43 μgmL^{-1}	----
RP-HPLC/UV	0.25-20 μgmL^{-1}	0.004 μgmL^{-1}	0.013 μgmL^{-1}	[6]
HPLC/Fluorescence	20-5000 $n$gmL^{-1}	---	20 $n$gmL^{-1}	[7]
Capillary electrophoresis	0.1-200 μgmL^{-1}	0.1 μgmL^{-1}	1.0 μgmL^{-1}	[8]
Spectrophotometry	2-12 μgmL^{-1}	0.12 μgmL^{-1}	0.37 μgmL^{-1}	[11]
Spectrofluorimetry	0.01-0.50 μgmL^{-1}	1.19 μgmL^{-1}	3.6 μgmL^{-1}	[14]
Chemiluminescence	0.009-0.3 μgmL^{-1}	0.037 μgmL^{-1}	-----	[16]
Voltammetry	2.47-15.5 μgmL^{-1}	0.9 $n$gmL^{-1}	3.0 $n$gmL^{-1}	[18]

To ensure the efficacy of this new method the obtained data were compared with other results previously reported (Table 3). All methods showed good sensitivity towards the assay of GFX, indicating their accuracy and precision. In spite of the proposed method showed less sensitivity than some addressed techniques, it exhibited more simplicity and rapidity in the analysis. However, the previously reported techniques such as chromatography and capillary zone electrophoresis have certain drawbacks, including the use of large quantity of solvent, required high technical skills and consuming long time for detection. On the other hand the proposed electrochemical methods showed higher sensitivity more than other reported methods [7, 18].

4. CONCLUSIONS

Herein, a simple polarographic probe was described for the quantification of GFX in its pharmaceutical products and bio-fluids. The recent study showed significant advantages rather than the previously reported techniques including, high sensitivity, short time consuming and does not require a pretreatment of samples prior to analysis. The proposed polarographic probe displayed a rectilinear relationship between the current and GFX concentrations in the range from 1.59×10^{-6} to 2.70×10^{-5} mol L^{-1}. The procedure of the developed polarographic method represents a good alternative for routine laboratory analysis as well as sensitive enough for analysis of the investigated drug in very low limits of detection and quantification of 2.89×10^{-7} and 8.76×10^{-7} mol L^{-1}, respectively.

References

1. C. Zoski, Handbook of Electrochemistry. Elsevier Science (2007).
2. J. Gajdar, E. Horakova, J. Barek, J. Fischer and V. Vyskocil, *Electroanalysis,* 28 (2016) 2659.

3. S. M. Bhavnani, *Pharmacotheraphy*, 25 (2005) 717.
4. C. A. Fief, K. G. Hoang, S. D. Phipps, J. L. Wallace and J. E. Deweese, *ACS Omega*, 4 (2019) 4049.
5. M. S. Baig and M. Dehghan, *World J. Pharm. Pharm. Sci.,* 4 (2015) 1965.
6. M. Gumustas and S. A. Ozkan, *Turk. J. Pharm. Sci.,* 9 (2012)161.
7. M. Kaiser, L. D. Grunspan, T. D. Costa and L. Tasso, *J. Chromatogr. B,* 879 (2011) 3639. doi: 10.1016/j.jchromb.2011.09.011. Epub 2011 Sep 10.
8. R. N. Rao, Ch. G. Naidu, K. G. Prasad and R. Narasimha, *Biomed. Chromatogr.,* 25 (2011) 1222.
9. A. M. Al-Mohaimeed, S. A. Al-Tamimi, N. A. Alarfaj and F. A. Aly, *Int. J. Electrochem. Sci.,* 7 (2012) 12518.
10. R. Jain and J. A. Rather, *Coll. Surface B,* 83 (2011) 340.
11. R. R. Ambadas, P. P. Sunita, *E-J. Chem.,* 7 (2010), S344.
12. A. A. Gouda, A. S. Amin, R. El-heikh and A. G. Yousef, *J. Anal. Methods Chem.,* 2014 (2014) 286379. doi: 10.1155/2014/286379
13. S. T. Veeraiah and V. R. Reddy, *IOSR J. Pharm.,* 8 (2018) 01.
14. S. A. Al-Tamimi, N. A. Alarfaj, F. A. Aly and A. M. Al-Mohaimeed, *Luminescence*, 29 (2014) 127.
15. P. Nagaraju, P. Ravisankar, G. D. Rao and Y. R. Prasad, *Int. J. Chem. Sci.,* 12 (2014) 1382.
16. F. Zhao, W. Zhao and W. Xiong, *Luminescence,* 25 (2013)108.
17. J. Heyrovsky and P. Zuman, in practical polarography, Academic, New York (1968) p. 179, 163.
18. E. M. Espinoza, J. A. Clark, J. Soliman, J. B. Derr, M. Morales and V.I. Vullev, *J. Electrochem. Soc.,* 166 (2019) H3175.
19. H. A. Wagdy, M. Tarek, A. Amer, M. Gamal and M. Elmazar, *Turk. J. Pharm. Sci.,* 16 (2019) 8. DOI: 10.4274/tjps.04934
20. J. C. Miller, Statistics for Analytical Chemistry, third ed., Ellis Horwood-Prentice Hall, Chichester, (1993).
21. C. S. Paim, F. Fuhr, A. B. Barth, C. E. Gonçalves, N. Nardi, M. Steppe and E. E. Schapoval, *Talanta,* 83 (2011) 1774.
22. A. Allen, E. Bygate, S. Oliver, M. Johnson, C. Ward, A. J. Cheon, Y. S. Choo and I. C. Kim, *Antimicrob. Agents Chemother*., 44 (2000)1604.