Differential Pulse Polarographic Behavior and Quantification of the Flucloxacillin in Pure and Pharmaceutical Dosage Forms Using a Static Mercury Drop Electrode

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ABSTRACT

Differential pulse polarographic analysis (DPPA) by using static mercury drop electrode (SMDE) for quantification of flucloxacillin (FLUX) in pure and pharmaceutical dosage forms was studied. The optimum conditions for the polarographic signal of the different parameters affecting the electrochemical process were determined. The best definition of the analytical signals was found in Britton Robinson buffer (0.06 M) at pH 4.0. Under the optimum conditions, liner calibration graph, $I_p=f(C_{FLUX})$, was obtained in the concentration ranges of 0.1 µM (0.0494 µg.mL^{-1}) to 26 µM (12.8414 µg.mL^{-1}) at -940 to -1000 mV (versus Ag/AgCl) with relative standard deviations (RSD) did not exceed 2.4% for the concentrations of FLUX (0.0494 µg.mL^{-1}). Regression analysis showed a good correlation coefficient (R^2=9998) between I_p and concentration over the mentioned range. The limit of detection (LOD) and the limit of quantification (LOQ) were to be 0.0040 and 0.0120 µg.mL^{-1}, respectively. The proposed method was validated for linearity, precision and accuracy, repeatability, sensitivity (LOD and LOQ), robustness and specificity. The developed method is applicable for the determination of FLUX in pure and different dosage forms in presence a same amount of amoxicillin (AMOX) with average recovery of 99.4 to 102.2 % and the results are in good agreement with those obtained by the HPLC reference method.

Keywords: Differential pulse polarography; static mercury drop electrode, Flucloxacillin

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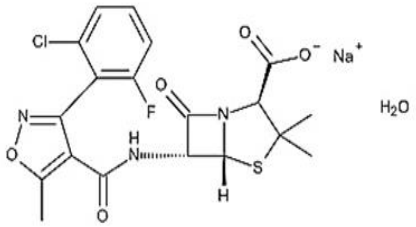
1. INTRODUCTION

Flucloxacillin [3-(2-chloro-6-fluorophenyl)-5-methylisoxazol-4-yl] carbonyl] amino]-3,3dimethyl -7-oxo-4-thia-1-azabicyclo [3. 2 .0] heptane 2-carboxylate] are the penicillinase-resistant penicillins. It is used as an antibiotic [1]. It is normally synthesized for use pharmaceutically usually as the sodium salt and less so, the magnesium salt [2 , 3]. Freely soluble in water and in methanol. The chemical formula of flucloxacillin sodium is $C_{19}H_{16}ClFN_3NaO_5S.H_2O$, its molecular weight is 493.9 $g.moL^{-1}$. The chemical structure of flucloxacillin sodium showed in scheme 1.



Scheme 1: Chemical structure of Flucloxacillin.

The hydrolysis of flucloxacillin at pH 4.9 yields a degradation product which is polarographically oxidizable. It gives a diffusion-controlled anodic polarographic wave with a half-wave potential at -0.24 V (versus Ag/AgCl) [4]. A potentiometric method for determination of flucloxacillin is developed. The method involves development of a flucloxacillin sensor with a membrane consisting of Aliquat flucloxacillin as an electro active material in poly vinyl chloride matrix membrane plasticized with ortho nitro phenyl-octyl ether or dioctylphthalate. The sensor shows fast, stable and reproducible response over the concentration range of 1.0×10^{-5} – 1.0×10^{-2} M flucloxacillin and pH ranges of 6-11 and 7-11 for o-nitro phenyl octyl ether and dioctylphthalate plasticized based membrane sensors, respectively [5].

The separation was made by a ZORBAX 300-SCX column using 0.025 M ammonium dihydrogen phosphate (adjusted to pH 2.6 with phosphoric acid)–acetonitrile (95:5) as mobile phase [6]. A simple, precise, fast and accurate HPLC method has been developed for the simultaneous estimation amoxicillin and flucloxacillin in capsules. The analytes were resolved, by using a mobile phase mixture of buffer (prepared from 0.001 M diammonium hydrogen orthophosphate and 0.04 M tetra butyl ammonium bromide pH adjusted to 7.0±0.1 with ortho phosphoric acid) and acetonitrile in the ratio (90:10, v/v), on a strong cation exchange column, (LUNA SCX, 250mm x 4.6mm I.D. 5 µm particles).The retention times for amoxicillin and flucloxacillin were found to be 3.828 and 5.89 min, respectively [7].

On the other hand, there are several methods available in the literature for the quantification of flucloxacillin including high performance liquid chromatography [6-12], spectrophotometry [13-16] and nuclear magnetic resonance spectrometry [17].

The SMDE used successfully in polarographic analysis. The SMDE combines the features of the dropping mercury electrode (DME) and hanging mercury drop electrode (HMDE): As with the DME, the drops are constantly renewed, but during the measurement the drop area is constant as in the HMDE case. In a subsequent voltage (U) sweep, the Hg drops are knocked off by the tapping mechanism after the time t. step set in the measurement mode. The SMDE is primarily used for sensitive measurements in which the surface of the mercury drop must be renewed for every measurement [18].

In the present work, differential pulse polarographic behavior and quantification of the flucloxacillin in pure and pharmaceutical dosage forms using a static mercury drop electrode was applied. The method is an easy, fast and sensitive for the determination this compound in pure and in pharmaceuticals.

2. MATERIALS AND METHODS

2.1 Equipment and Materials

A Metrohm 746 VA processor, A Metrohm 747 VA stand with a static mercury drop electrode (SMDE) as a working electrode, an auxiliary platinum electrode and a reference electrode, double junction type, (Ag/AgCl) saturated with a 3.0 M KCl solution and the three-electrode cell were used. All measurements were done at room temperature 25±5°C. Highly pure nitrogen gas (99.999 %) was used for de-oxygenation. pH meter from Radiometer company model ion check was used for the studying and monitoring the pH effects. The diluter pipette model DIP-1 (Shimadzu), having 100 µL sample syringe and five continuously adjustable pipettes covering a volume range from 20 to 5000 µL (model PIPTMAN P, GILSON), were used for preparation of the experimental solutions. An ultrasonic processor model Powersonic 405 was used to sonicate the sample solutions. Electronic balance (Sartorius-2474; d=0.01 mg) was used for weighing the samples.

Working reference standard of flucloxacillin (99.2%) was supplied by D.K. Pharma chem. Pvt. Ltd INDIA, (Mfg.12-2017, Exp. 11-2020). Lithium perchlorate trihydrate, di-Sodium hydrogen phosphate dodecahydrate, Sodium chloride, Sodium hydroxid, Perchloric acid (70%), ortho-Phosphoric acid (85%), Acetic acid (100%), Boric acid (100%) were of GR for analysis purchased from MERCK.

Commercial formulations (as capsule) were used for the analysis of FLUX by using DPPA with SMDE. The pharmaceutical formulations were subjected to the analytical procedures:

(1) *Amoxipen* capsule, BARAKAT PHARMACEUTICAL, Aleppo–SYRIA, each capsule contains: 250 mg of FLUX and 250 mg AMOX (Exp. 03.2020).

(2) *Amoxam* capsule, IBN HAYYAN, Homs –SYRIA, each capsule contains: 250 mg of FLUX and 250 mg AMOX (Exp. 06.2020).

(3) *Penifloxam* capsule, APHAMEA, Hama–SYRIA, each capsule contains: 250 mg of FLUX and 250 mg AMOX (Exp. 04.2020).

(4) *Floxin* capsule, ALBALSAM PHARMA, Homs- SYRIA, each capsule contains: 250 mg of FLUX and 250 mg AMOX (Exp. 04.2020).

(5) *Maxipen* capsule, ASIA, Aleppo–SYRIA, each capsule contains: 250 mg of FLUX and 250 mg AMOX (Exp. 06.2020).

2.2 Standard stock solutions

2.2.1 A stock standard solution of flucloxacillin ($1x10^{-4}$ $mol.L^{-1}$)

This solution was prepared by dissolving 49.79 mg from flucloxacillin in 100 mL double distilled deionized water ($1x10^{-3}$ $mol.L^{-1}$), then dilute 10.000 mL from this solution to 100 mL ($1x10^{-4}$ $mol.L^{-1}$).

2.2.2 Supporting electrolyte

Britton Robinson, H_3PO_4-Na_2HPO_4, lithium perchlorate, sodium chloride as supporting electrolytes (buffers) 0.200 $mol.L^{-1}$ at pH (2.5-9.0) were used.

2.3 Recommended Procedure

The stock solutions were further diluted to obtain working solutions daily just before use in the ranges of FLUX: 0.100, 0.200, 0.300, 0.400, 0.800, 1.000, 2.000, 4.000, 8.000, 12.000 , 16.000, 20.000, 22.000, 24.000 and 26.000 µmol.L^{-1} (0.0494, 0.0988, 0.1482, 0.1976, 0.3951, 0.4939, 0.9878, 1.9756, 3.9512, 5.9268, 7.9024, 9.8780, 10.8780, 11.8658 and 12.8414 µg.mL^{-1}) by dilution of the volumes: 0.025, 0.050, 0.075, 0.100, 0.200, 0.250, 0.500, 1.000, 2.000, 3.000, 4.000, 5.000, 5.500, 6.000 and 6.500 mL from stock standard solutions were transferred into 25 mL volumetric flask. 7.5 mL of supporting electrolyte were added, and diluted with double distilled deionized water to the mark. Ultrapure mercury from Metrohm Company was used throughout the experiments.

2.4 Procedure for pharmaceutical formulations

Contents of 20 capsules of each studied pharmaceutical formulations were weighted accurately, crushed to a fine powder and mixed well. Equivalent weight of contents of one capsule, was solved in 50 mL double distilled deionized water by using ultrasonic, filtered over a 100 mL flask and diluting to 100 mL with double distilled deionized water, which content as the follows: 2500 µg.mL^{-1} for all studied pharmaceutical formulations content 250 mg/cap.

These solutions were prepared daily by diluting 100 µL (0.100 mL) from stock solutions of pharmaceutical formulations, adding 30 mL from supporting electrolyte, then diluting to 100 mL with double distilled deionized water (each solution contents 2.5 µg.mL^{-1} of FLUX).

2.5 Analytical procedure

25 mL of working standard of flucloxacillin or working solutions of pharmaceuticals was transferred to the cell. The solution was deoxygenated with N_2 gas for 300 s. The potential range studied was from –400 to –1400 mV (versus Ag/AgCl) with differential pulse polarographic analysis using static mercury drop electrode in the optimum conditions were applied.

3. RESULTS AND DISCUSSION

3.1 Differential pulse polarographic behavior

The polarograms for concentration 0.10-26.0 µmol.L^{-1} (0.0494-12.8414 µg.mL^{-1}) of FLUX in the optimal conditions (supporting electrolytes, pH, scan rate, initial potential, final potential, ...etc.) using DPPA at SMDE were studied. The best definition of the analytical signals was found in Britton Robinson (0.06 M) buffer (pH 4.0) at -940 to -1000 mV (versus Ag/AgCl).

3.2 The effect of supporting electrolytes (buffer)

The electrochemical behavior of flucloxacillin was studied in various supporting electrolytes such as (Britton Robinson, di-sodium hydrogen phosphate dodecahydrate, lithium perchlorate trihydrate, sodium chloride) was studied at pH (2.5-9.0). The best definition of the analytical signals was found in Britton- Robinson buffer (pH 4.0) at concentration 0.06 M. The effect of supporting electrolytes (buffer) on the I_p and E_p was studied. The values of E_p were -968, -965, -893 and -890 mV for the mentioned buffers, respectively, see Figure 1. The effect of the concentration of Britton Robinson was tested over the 4, 8, 10, 20, 30, 35, 40, 50, 60, 70, 80, 90 and 100 mM. The DPPA at SMDE of 8.0 µM of FLUX with the varying mentioned concentrations of supporting electrolyte was studied. The values of I_p increase with increasing concentration of supporting electrolyte of 4 to 50 mM, then become semi-fixed until concentration of supporting electrolyte 100 mM, while E_p remains quasi-static.

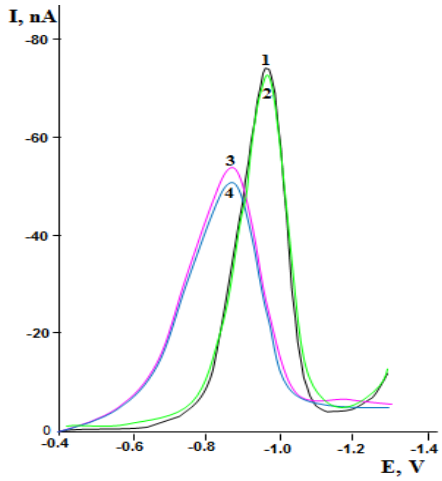


Fig.1: The effect of buffer solutions on polarograms of FLUX (8.00 µM) using DPPA at SMDE buffers (0.06 M) at pH 4.0: 1- Britton Robinson, 2- $Na_2HPO_4.12H_2O$, 3- NaCl, 4- $LiClO_4.3H_2O$ (Purge gas N_2, purge time 300 s, sweep rate 4 mV/s, U. amplitude -100 mV, t. meas 32 ms, t. pulse 35 ms, t. step 2 s, U. step 8 mV, drop size 9, temperature 25°± 5°C).

3.3 The effect of pH

The influence of pH from 2.5 to 9.0 using Britton Robinson (0.06 M) buffer on I_p and E_p was studied. The values of I_p increase with increasing pH value of 2.5 to 4.0, then decrease until pH 6.0 and finally become semi-fixed until pH 9.0 . While E_p values are growing a positive value from -1075 mV (when pH 2.5) to -910 mV (when pH 5.0) then become semi-fixed until pH 9.0 , see Figures 2,3.

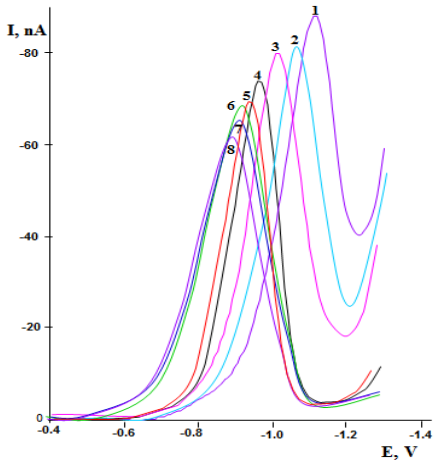


Fig.2: The effect of pH solution on polarograms of FLUX (8.00 µM) using DPPA at SMDE at pH: 1- 2.5; 2- 3.0; 3- 3.5; 4- 4.0; 5- 4.5; 6- 5.0; 7- 6.0 and 8- 9.0 using Britton Robinson buffer (0.06 M). (Purge gas N_2, purge time 300 s, sweep rate 4 mV/s, U. amplitude -100 mV, drop size 9, t. meas 32 ms, t. pulse 35 ms, t. step 2 s, U. step 8 mV, temperature 25°± 5°C).

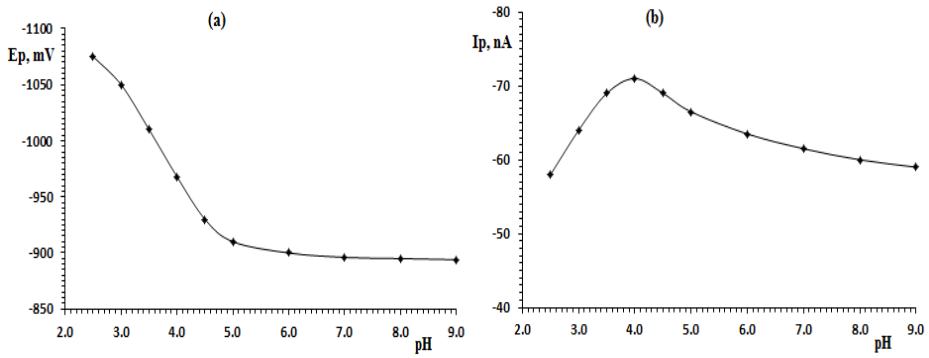


Fig.3: The effect of pH solution on E_p (a) and I_p (b) of FLUX (8.00 µM) using DPPA at SMDE containing buffer Britton Robinson (0.06 M) (Purge gas N_2, purge time 300 s, sweep rate 4 mV/s, U. amplitude -100 mV, drop size 9, t. mesa 32 ms, t. pulse 35 ms, t. step 2 s, U. step 8 mV, temperature 25°± 5°C).

3.4 The effect of negative pulse amplitude (U. ampl)

The effect of negative pulse amplitude (U. ampl) between -10 to -100 mV on I_p and E_p was studied. I_p linearly increases with increasing amplitude value until -100 mV. While E_p stay semi-fixed. The value -100 mV was better than another's.

3.5 The effect of initial and final potential

The effect of initial and final potential on the I_p and E_p was studied. It was found that the best initial potential was -400 mV and the best final potential was -1400 mV.

3.6 The effect of temperature and time

The effect of temperature and time on the electrochemical behavior of FLUX was studied at different values (15-35°C and 5-60 min) by continuous monitoring of the I_p. It was found that, the value of I_p was not affected by temperature between 20 to 30°C (the temperature at 25±5°C was used). The effect of waiting time was determined at laboratory ambient temperature (25±5°C). It was found that, the value of I_p was not affected by time between 5 to 60 min.

3.7 The effect of time pulse (t. pulse)

The effect of time pulse (35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95 and 100 ms) on polarograms was as the follows: I_p decreases with increasing time pulse and E_p has become increasingly positive value (-968 to -944 mV) with increasing t. pulse. The peak was more symmetrical and I_p was the highest when the t. pulse value of 35 ms.

3.8 The effect of time interval for voltage step (t. step)

It found that the I_p linearly increases with increasing taste (0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.5, 2.0 and 2.5 s), while E_p has become increasingly positive value (-1006 to -975 mV) with increasing taste. The value of the preferred t. step was 2 s.

3.9 The effect of measurement time (t. meas)

It found that the I_p increases with increasing t. meas. (2, 4, 6, 8, 10, 12, 16, 20, 24, 28, 30, and 32 ms), while E_p remains quasi-static. The value of the preferred t. meas was 32 ms.

3.10 The effect of drop size

It found that the I_p increases slightly with increasing drop size from 1 to 9 size, while E_p stays semi-fixed with increasing drop size. The value of the preferred drop size was 9.
The optimum parameters established for determination of FLUX using DPPA on SMDE showed in Table 1.

Table 1: The optimum parameters established for determination of FLUX using DPPA on SMDE.

Parameters	Operating modes
Working electrode	Static Mercury Drop Electrode (SMDE)
Supporting electrolytes (buffer)	Britton Robinson 0.06 M
pH	4.0
Solvent of flucloxacillin	double distilled deionized water
Purge gas	Pure N_2
Purge time	300 s
Initial potential	-400 mV
Final potential	-1400 mV
Scan rate	4 mV/s
t. meas	32 ms
U. ampl	-100 mV
t. pulse	35 ms
t. step	2 s
Drop size	9
Temperature of solution	25°± 5°C

4. Calibration curves

Calibration curves for the determination of FLUX using differential pulse polarographic analysis on SMDE with negative amplitude in Britton Robinson (0.06 M) buffer at pH 4.0 were applied. One peak was observed in the range -940 to -1000 mV (E_p). The peak current (I_p) was proportional to the concentration of FLUX over the range 0.0494-12.8414 µg.mL^{-1} (0.100-26.000 µmol.L^{-1}). The polarograms in the optimum conditions using DPPA at SMDE of FLUX at different concentrations are showed in Figure 4. The regression equation was as the follows: y=-18.191x-0.5094, R^2=0.9998 (y: I_p, nA and x: C_{FLUX}, µg.mL^{-1}), see Figure 5.

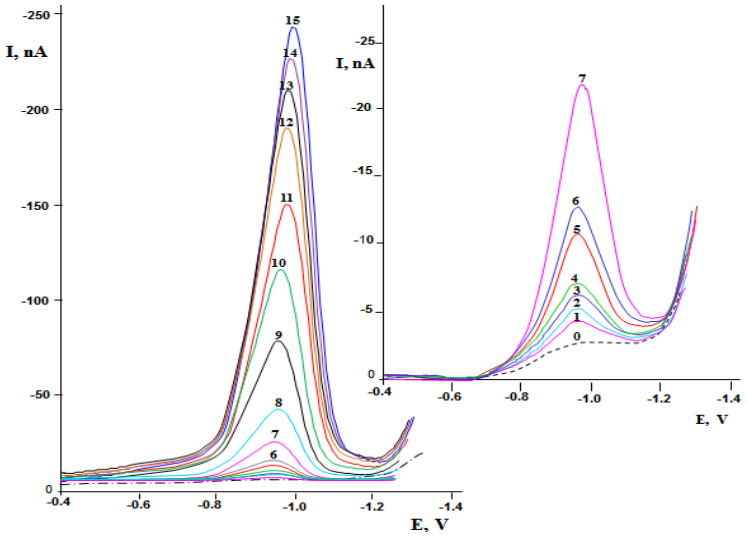


Fig.4: The polarograms of FLUX on SMDE using DPPA in Britton Robinson buffer (0.06 M) at pH 4.0 at concentrations: 1- 0.0494, 2- 0.0988, 3- 0.1482, 4-0.1976, 5- 0.3951, 6- 0.4939, 7- 0.9878, 8- 1.9756, 9- 3.9512, 10- 5.9268, 11- 7.9024, 12- 9.8780, 13- 10.8780, 14- 11.8658 and 15- 12.8414 µg.mL^{-1} (Purge gas N_2, purge time 300 s, sweep rate 4 mV/s, U. ampl -100 mV, t. meas 32 ms, t. pulse 35 ms, t. step 2 s, U. step 8 mV, drop size 9, temperature 25°±5°C).

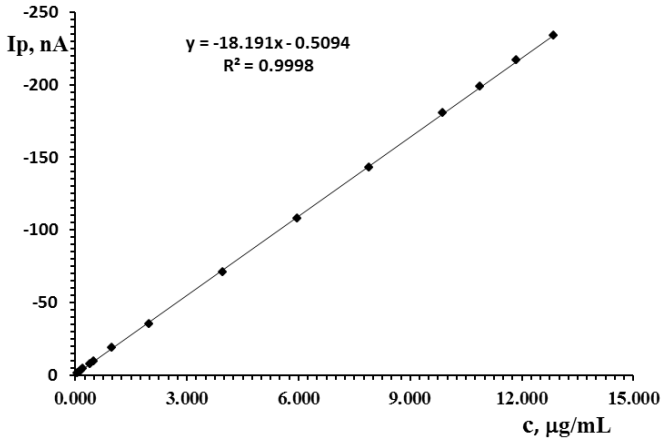


Fig.5: Calibration curves for the determination of FLUX using DPPA on SMDE in the optimum conditions ($I_p = I_{p,total} - I_{elect}$).

5. Analytical results

Determination of FLUX using DPPA on SMDE in the optimum conditions using analytical curves, $I_p=f(C_{FLUX})$, showed that the accuracy was over the range of FLUX concentration between (0.0494-12.8414 µg.mL^{-1}). The relative standard deviation (RSD) not more than 2.4%, see Table 2. Limit of detection (LOD) and limit of quantitation (LOQ) for the determination of FLUX by this method were as the follows: 0.0040 and 0.0120 µg.mL^{-1}, respectively.

Table 2: Determination of flucloxacillin using differential pulse polarographic analysis on SMDE with negative amplitude in Britton Robinson (0.06M) buffer at pH 4.0.

Taken x_i		**Found**	**SD, µg.mL^{-1}**	**$\frac{SD}{\sqrt{n}}$, µg.mL^{-1}**	**$\bar{x} \pm \frac{t.SD}{\sqrt{n}}$, µg.mL^{-1}**	**RSD%**
µM	**µg.mL^{-1}**	***$\bar{x}$, µg.mL^{-1}**				
0.1	0.0494	0.0501	0.00120	0.00054	0.0501±0.00148	2.4
0.2	0.0988	0.1094	0.00252	0.00113	0.1094±0.00313	2.3
0.3	0.1482	0.1534	0.00337	0.00151	0.1534±0.00418	2.2
0.4	0.1976	0.2138	0.00449	0.00201	0.2138±0.00557	2.1
0.8	0.3951	0.4008	0.00842	0.00376	0.4008±0.01045	2.1
1.0	0.4939	0.5162	0.01032	0.00462	0.5162±0.01282	2.0
2.0	0.9878	1.0252	0.02050	0.00917	1.0252±0.02545	2.0
4.0	1.9756	1.9509	0.03707	0.01658	1.9509±0.04760	1.9
8.0	3.9512	3.8750	0.06975	0.03119	3.8750±0.08659	1.8
12	5.9268	5.9089	0.09454	0.04228	5.9089±0.11737	1.6
16	7.9024	7.8605	0.12576	0.05625	7.8605±0.15614	1.6
20	9.8780	9.8944	0.13852	0.06195	9.8944±0.17197	1.4
22	10.8658	10.9115	0.14185	0.06344	10.9115±0.17610	1.3
24	11.8536	11.9009	0.15471	0.06918	11.9009±0.19207	1.3
26	12.8414	12.8355	0.15402	0.06888	12.8355±0.19122	1.2

* n=5 t=2.776.

6. APPLICATIONS

Many applications for the determination of flucloxacillin in some Syrian pharmaceutical preparations (in presence a same amount of amoxicillin) using differential pulse polarographic analysis on static mercury drop electrode with negative amplitude in Britton Robinson 0.06 M buffer at pH 4.0 according to the optimal conditions were studied. The amount (m) of FLUX in one capsule was calculated from the following relationship: m=h. m', where: m' is the amount of FLUX in capsule calculated according to the regression equation of calibration curve, h conversion factors are equal to 100 for all pharmaceuticals content 250 mg/cap. The results of quantitative analysis for FLUX in pharmaceutical preparations were summarized in Tables 3. The proposed method was simple, direct and successfully applied to the determination of FLUX in pharmaceuticals without any interference from amoxicillin and excipients. Average assay ranged between 99.4 to 102.2%. The results obtained by this method agree well with the contents stated on the labels and were validated by HPLC method [7]. Therefore, the presented method can be recommended for routine analysis of FLUX in pharmaceutical formulations.

Table 3: Determination of FLUX in some Syrian pharmaceutical preparations using DPPA on SMDE with negative amplitude in Britton Robinson 0.06 M buffer at pH 4.0 according to the optimal conditions.

Commercial name	**Label Claim of FLUX & AMOX, mg/cap.**	***Mean ±SD (as FLUX), mg/ cap.**	**RSD%**	**Assay %**	***** (Assay%), by HPLC[7]**
Amoxipen capsule, BARAKAT pharmaceutical	250	249.40	2.7	99.8	99.5
Amoxam capsule, IBN HAYYAN	250	250.90	2.6	100.4	100.6
Penifloxam capsule, APHAMEA	250	255.40	2.6	102.2	102.3
Floxin capsule, ALBALSAM pharma	250	248.40	2.7	99.4	99.4
Maxipen capsule, ASIA	250	253.00	2.6	101.2	101.1

***** n=5, Assay= (found mean/label claim) x100.

7. Method validation

The developed method for simultaneous estimation of FLUX has been validated in accordance with the International Conference on Harmonization guidelines (ICH) [19].

7.1 Selectivity

Several other components were examined under the conditions that had been optimized for flucloxacillin determination. The results appeared that amoxicillin and ampicillin did not interfere when they present at the same amount with flucloxacillin. While cloxacillin is interfere.

7.2 Linearity

Several aliquots of standard stock solution of FLUX were taken in different 25 mL volumetric flasks such that their final concentrations were 0.0494-12.8414 µg.mL^{-1} (0.100-26.000 µmol.L^{-1}) for FLUX using DPPA at SMDE in Britton Robinson 0.06 M buffer at pH 4.0. Linearity equation obtained was: y=-18.191x-0.5094, for the mentioned range (R^2=0.9998).

7.3 Precision and Accuracy

The precision and accuracy of proposed method were checked by recovery study by addition of standard drug solution to pre-analyzed sample solution at three different concentration levels (80%, 100% and 120%) within the range of linearity for FLUX. The basic concentration level of sample solution selected for spiking of the FLUX standard solution was 3.951 µg.mL^{-1}. The proposed method was validated statistically and through recovery studies, and was successfully applied for the determination of FLUX in pure and dosage forms with percent recoveries ranged from 99.9% to 101.6 %, see Table 4.

Table 4: Results of recovery studies **(n=5)**

Level	Recovery%
80%	100.5
100%	99.9
120%	101.6

7.4 Repeatability

The repeatability was evaluated by performing 10 repeat measurements for 3.951 µg.mL^{-1}of FLUX using the studied DPPA at SMDE Britton Robinson 0.06 M buffer at pH 4.0 under the optimum conditions. The found amount of FLUX ($\bar{\mathbf{x}}$ ± SD) was 3.971± 0.067 µg.mL^{-1} and the percentage recovery was found to be 100.5±1.7 with RSD of 0.017. These values indicate that the proposed method has high repeatability for FLUX analysis.

7.5 Sensitivity (limit of detection [LOD] and limit of quantitation [LOQ])

The sensitivity of the presented method was evaluated by determining the LOD and LOQ. The values of LOD and LOQ for FLUX are 0.0040 and 0.0120 µg.mL^{-1}, respectively.

7.6 Robustness

The robustness of the method adopted is demonstrated by the constancy of the current peak (I_P) with the deliberated minor change in the experimental parameters such as the change in the concentration of excipients, temperature (25±5ºC), pH (4.0±0.20), and C_{elect} (0.06±10% mol.L^{-1}) and reaction waiting time (10 min), see Table 5. This table indicates that the robustness of the proposed method was good (I_p was measured and assay was calculated for five times).

7.7 Specificity

The specificity of the method was ascertained by analyzing standard FLUX in presence of excipients. These findings prove that the suggested methods are specific for determination of the investigated drugs without interference from the co-formulated adjuvants.

Table5: Robustness of the proposed DPPA method at SMDE for determination of flucloxacillin.

Experimental parameter variation	**Average recovery (%) ***
	C_{FLUX} =3.951 µg.mL^{-1}
Temperature	
20°C	99.8
25°C	100.5
30 °C	100.6
pH	
3.8	99.8
4.2	100.2
$C_{Britton\ Robinson}$	
0.054 mol/L	99.4
0.066 mol/L	100.3
reaction time	
10 min	99.7
30 min	100.4
60 min	101.6

*** n=5.**

7.8 The homogenization of capsule

The homogenization of capsule in terms of the weight and the amount of drug was studied. It found that the mean weight capsule was 0.6965 ± 0.015 g (i.e. ±2.2%), 0.6625 ± 0.011 g (i.e. ±1.7%), 0.7044± 0.021 g (i.e. ±3.0%), 0.6687 ± 0.015 g (i.e. ±2.2%) and 0.7116 ± 0.0098 g (i.e. ±1.4%) for *Amoxipen* capsule, *Amoxam* capsule, *Penifloxam* capsule, *Floxin* capsule and *Maxipen* capsule (250 mg/cap). And amount of drug in the capsule was 249.00 ± 5.2 mg (i.e. ±2.1%), 250.70 ± 5.2 mg (i.e. ±2.1%), 253.00± 5.5 mg (i.e. ±2.2%), 250.00 ± 4.0 mg (i.e. ±1.6%) and 254.00 ± 3.0mg (i.e. ±1.2%) for *Amoxipen* capsule, *Amoxam* capsule, *Penifloxam* capsule, *Floxin* capsule and *Maxipen* capsule (250 mg/cap); which shows that homogeneity of capsule is acceptable.

8. CONCLUSION

DPPA of FLUX in pure form and in pharmaceutical preparations using SMDE with Britton Robinson 0.06 M buffer at pH 4.0 according to the optimal conditions was applied. One peak was observed. I_p is linear over the range 0.0494-12.8414 µg.mL^{-1} (0.100-26.000 µmol.L^{-1}). The relative standard deviation did not exceed 2.4% for the concentration 0.0494 µg.mL^{-1} of FLUX. Regression analysis showed a good correlation coefficient (R^2=0.9998) between I_p and concentration over the mentioned range. The proposed method was successfully applied to the direct analysis of FLUX in pharmaceutical formulations without any interference from excipients and the co-formulated adjuvants with adequate accuracy and sensitivity without any pre-separation such as extraction.

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