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Voltammetric Determination of Flucloxacillin in Pure Form and Pharmaceutical Formulations Using Hanging Mercury Drop Electrode

Abdul Aziz Ramadan[1*], Hasna Mandil[2], Reham Abu-Saleh[3]

Department of Chemistry, Faculty of Science, University of Aleppo, Syria.

*[1]E-mail: dramadan@scs-net.org or dramadan1946@gmail.com;
[2] E-mail: promandil955@gmail.com [3]E-mail: reham a-s@hotmail.com

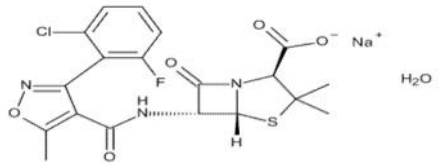
Abstract

Electroreduction and adsorption of Flucloxacillin (FLUX) using differential pulse voltammetry (DPV) and differential pulse adsorptive stripping voltammetry (DPAdSV) at hanging mercury drop electrode (HMDE) has been studied. The reduction peak potential (E_p) of FLUX using DPV was between the range -965 to -1000 mV, liner calibration graph were the concentration ranges of 24.695-740.850 ng.mL^{-1}.Determination of FLUX using DPAdSV were studied. E_p was between - 250 to - 270 mV and -145 to -170 mV at pH 4.5 and pH 1.35, respectively. Liner calibration graphs at E_{acc} +150 mV, t_{acc} 120 s and 160 s and at pH 4.5 and 1.35, were of 4.939-493.900 ng.mL^{-1} and 0.494–19.756 ng.mL^{-1}, respectively. It was found that the use of pH 1.35 has increased sensitivity 10 times. These methods give good results for the determination of FLUX in pure and different dosage forms.

Keywords: Differential pulse voltammetry, Differential pulse adsorptive stripping voltammetry, hanging mercury drop electrode, Flucloxacillin.

Introduction

Flucloxacillin,(2S,5R,6R)-6-[[[3-(2-chloro-6-fluorophenyl)-5-methylisoxazol-4-yl]carbonyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo heptane-2-carboxylate, is a bactericidal antibiotic drug. It is the best of the anti-staphylococcal penicillins. The chemical formula of flucloxacillin sodium (FLUX)is $C_{19}H_{16}ClFN_3NaO_5S.H_2O$, its molecular weight is 493.9 g/moL. The chemical structure of FLUX[1], see scheme 1.



Scheme 1: Chemical structure of Flucloxacillin

The literatures for the quantification of flucloxacillin were including high performance liquid chromatography[1-7], spectrophotometry[8-10], potentiometric method [11] and polarography[12-14].

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) [12]. Electrochemical behavior and differential pulse polarographic determination of FLUX in pure and pharmaceutical dosage forms using dropping mercury electrode (DME) and static mercury drop electrode (SMDE) have been studied. Different buffer solutions were used over a wide pH range (2.5-10.0). The best definition of the analytical signals was found in Britton–Robinson buffer at pH 4.0. Under the optimum conditions, liner calibration graph was obtained in the concentration ranges of $1x10^{-7}$-$2.6x10^{-5}$ $mol.L^{-1}$ (0.049-12.8414 $\mu g.mL^{-1}$) and $1x10^{-7}$-$2x10^{-5}$ $mol.L^{-1}$ (0.0494 - 9.8780 $\mu g.mL^{-1}$) with RSD did not exceed 2.4% and 2.1% on SMDE and DME respectively. The developed method is applicable for the determination of FLUX in pure and different dosage forms in presence a same amount of amoxicillin (AMOX) [13, 14].

In the present work, DPV and DPAdSV analyses for determination of flucloxacillin in pure form and pharmaceutical formulations using a HMDE were applied.

Experimental

Reagents

Working reference standard of flucloxacillin (99.2%) was supplied by D.K. Pharmachem Pvt. Ltd INDIA, (Mfg.12-2017, Exp. 11-2020). Lithium perchlorate trihydrate, sodium hydroxid, perchloric acid (70%), were of GR for analysis purchased from MERCK. Ultrapure mercury from Metrohm Company was used throughout the experiments.

Instruments and apparatus

A Metrohm 746 VA processor, a Metrohm 747 VA stand with a hanging mercury drop electrode (HMDE) 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 ^{o}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 10 to 5000 µL (model PIPTMAN P, GILSON), were used for preparation of the experimental solutions. A ultrasonic processor model Powersonic 405 was used to sonicate the sample solutions. Electronic balance (Sartorius-2474; d=0.01 mg) was used.

Preparation supporting electrolyte

Lithium perchlorate buffer 0.1000 $mol.L^{-1}$and 0.02 $mol.L^{-1}$at pH (1.0-9.0) were used.

A stock standard solution of flucloxacillin

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 1.000 mL and 0.100 mL from this solution to 100 mL ($1x10^{-5}$ $mol.L^{-1}$and $1x10^{-6}$ $mol.L^{-1}$).

Working solutions

The stock solutions were further diluted to obtain working solutions daily just before use in the ranges of FLUX: 0.001, 0.002, 0.004, 0.008, 0.010, 0.020, 0.030, 0.040, 0.050, 0.060, 0.080, 0.100, 0.200, 0.400, 0.600, 0.800, 1.000 and 1.500 $\mu mol.L^{-1}$ (0.494, 0.988, 1.976, 3.951, 4.939, 9.878, 14.817, 19.756, 24.695, 29.634, 39.512, 49.390, 98.780, 197.560, 296.340, 395.120, 493.900 and 740.850 $ng.mL^{-1}$) by dilution of the volumes: 0.025, 0.050, 0.100, 0.200, 0.250 mL from stock standard solutions $1x10^{-6}$ $mol.L^{-1}$ and 0.050, 0.075, 0.100, 0.125, 0.150, 0.200, 0.250, 0.500, 1.000, 2.000, 2.500, and 3.750 mL from stock standard solutions $1x10^{-5}$ $mol.L^{-1}$ were transferred into 25 mL volumetric flask, diluted with Lithium perchlorate buffer 0.02 M to the mark.

Samples

Commercial formulations (as capsule) were used for the determination of FLUX by using DPV and DPAdSV analysis using HMDE. 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).

Stock solutions of pharmaceutical formulations

Contents of 20 capsules of each studied pharmaceutical formulations were weighted accurately and mixed well. An amount of the powder equivalent to the weight of tenth content of capsule of FLUX was solved in 25 mL double distilled deionized water by using ultrasonic, filtered over a 100 mL flask and diluting to 100 mL with double distilled deionized water, this solution contents 250 $\mu g.mL^{-1}$ of FLUX for all studied pharmaceutical formulations.

Working solutions of pharmaceuticals

These solutions were prepared daily by diluting 20μL (0.02 mL) from stock solutions of pharmaceutical formulations into 100 mL volumetric flask, diluted with Lithium perchlorate buffer 0.02 M (pH 4.5 or 1.35) to the mark (each solution contents 0.05 $\mu g.mL^{-1}$ of FLUX).

Analytical procedure

Differential pulse voltammetry (DPV)

A 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) using DPV with HMDE in the optimum conditions were applied. The peak height was measured at -965 to -1000 mV in 0.02M $LiClO_4$ at pH 4.5.

Differential pulse adsorptive stripping voltammetry (DPAdSV)

A 25 mL volume of working solution containing an appropriate concentration of FLUX was transferred into an electrochemical cell. The solution was deoxygenated with N_2 gas for 300 s. The accumulation potential (E_{acc}) +150 mV, accumulation time (t_{acc}) 80, 120 and 160s were applied. The potential scanned from +100 to -1000 mV (versus Ag/AgCl) using DPAdSV with HMDE in the optimum conditions were applied. The peak height (I_p) was measured at -250 to -270 mV in 0.02M $LiClO_4$ at pH 4.5 and -145 to -170 mV at pH 1.35.

Results and Discussion

Voltammetric behavior of FLUX on HMDE

The reduction peak current (I_P) appear at potential-965 to -1000 mV using DPV method. The values of Ip were increase with increase the concentration of FLUX from $5x10^{-8}$ – $1.5x10^{-6}$ $mol.L^{-1}$, see Fig. 1, (a).

In using DPAdSV method, I_p appear at potential -250 to -270 mV at pH 4.5 and -145 to -170 mV at pH 1.35 and I_p were increase with increase the concentration of FLUX from $1x10^{-8}$ – $1.0x10^{-6}$ $mol.L^{-1}$ at pH 4.5, see Fig. 1, (b) and at $1x10^{-9}$ – $4.0x10^{-8}$ $mol.L^{-1}$ at pH 1.35, see Fig. 1, (c).

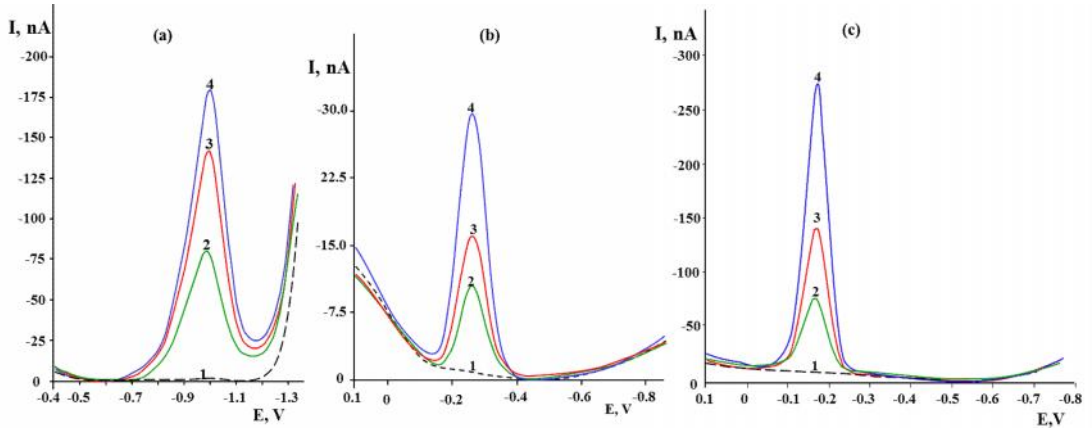


Fig.1: The polarograms using DPV for determination of FLUX in presence of 0.02 M $LiClO_4$ buffer at pH 4.5, (a); and DPAdSV in presence of 0.02 M $LiClO_4$ buffer at pH 4.5 (b) and 1.35 (c).
(a): 1- electrolyte, 2- 0.4 μM,3- 0.8 μM and 4- 1.0 μM of FLUX.
(b) and (c): The polarograms using DPAdSV for determination of FLUX in presence of 0.02 M $LiClO_4$ buffer at pH 4.5, t_{acc} 120 s, E_{acc} +150 mV (b); and at pH 1.35, t_{acc} 160 s, E_{acc} +150 mV (c):
1- electrolyte, 2- 0.01 μM, 3- 0.02 μM 4 - 0.04 μM of FLUX. (Purge gas N_2, purge time 300 s, sweep rate 120 mV/s, U.amplitude -100 mV, drop size 9 mm^2, t.step 0.1 s, t.meas 32 ms, t.pulse 35 ms, U.step 12 mV, temperature 25°± 5°C).

The effect of pH

The influence of pH from 1.0-9.0 using 0.02M $LiClO_4$ buffer on I_p and E_p were studied by DPV and DPAdSV. It was found that the best pH solution was 4.5 using DPV, see Fig. 2(a). But in using DPAdSV the best pH solutions were 4.5 and 1.35, see Fig. 2(b).

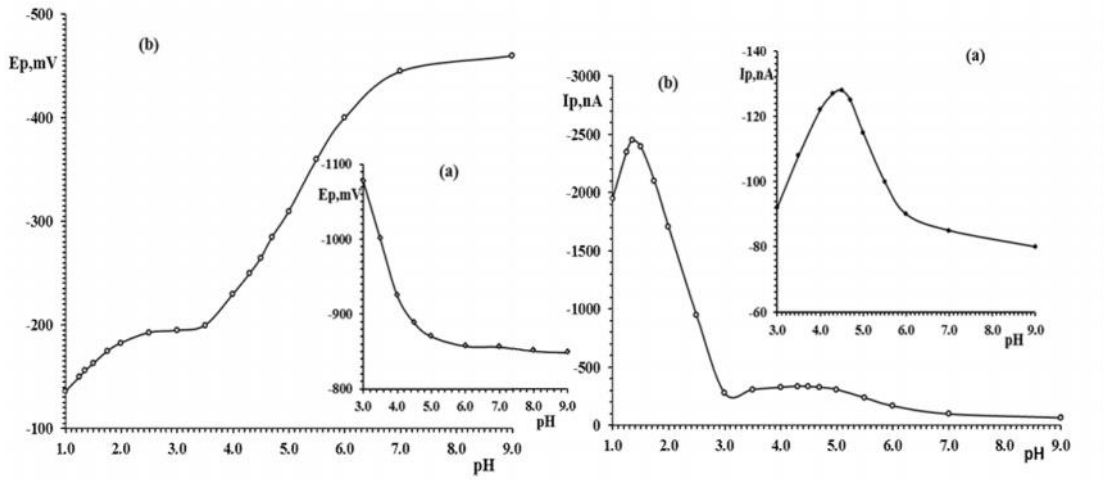


Fig.2: (a) The effect of pH solution on I_p and E_p using DPV (C_{FLUX} 0.8 μM) at HMDE containing 0.02 M $LiClO_4$. (b) The effect of pH solution on I_p and E_p using DPAdSV (C_{FLUX} 0.8 μM) at HMDE containing 0.02 M $LiClO_4$, at t_{acc} 80 s, E_{acc} +150 mV. (Purge gas N_2, purge time 300 s, sweep rate 120 mV/s, U.amplitude -100 mV, drop size 9 mm^2, t.mesa 32 ms, t.pulse 35 ms, t.step 0.1 s, U.step 12 mV, temperature 25°± 5°C).

The effect of negative pulse amplitude (U.ampl)

The effect of negative pulse amplitude, U.ampl (Pulse amplitude of the voltage pulse superimposed on the dIpect voltage) between -10 to -100 mV on I_p and E_p by DPV and DPAdSV. 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.

The effect of time pulse (t.pulse)

The effect of time pulse, t.pulse (Time interval during which a voltage pulse is superimposed on the dIpect voltage) between 35 -100 ms on polarograms was as the follows: I_p decreases with increasing time pulse, the peak was more symmetrical and I_p was the highest when the t.pulse value of 35 ms by DPV and DPAdSV.

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

I_p linearly decreases with increasing t.step (Time interval after which the voltage in the sweep is increased or decreased by the amount U.step) between 0.1 - 2.5 s, at using DPV and DPAdSV the value of the preferred t.step was 0.1 s.

The effect of measurement time (t.meas)

I_p increases with increasing t.meas (Time during which the current is measured. Measurement is performed at the end of the time interval t.step immediately before the pulse start and at the end of the pulse) between 2 - 32 ms, while E_p remains quasi-static. The value of the preferred t.meas was 32 ms at using DPV and DPAdSV.

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 at pH 4.5 and 35-60min at pH 1.35.

The effect of the accumulation potential (E_{acc})

The dependence of the differential pulse adsorptive stripping peak current on the accumulation potential (E_{acc}) +230 to -300 mV was examined. It was found that the maximum response for FLUX occurs with E_{acc} equal to +150 mV on HMDE electrode.

Effect of accumulation time (t_{acc})

The peak current depended on the accumulation time (t_{acc}) for FLUX concentrations were studied. The peak current increases with increasing t_{acc}. The best t_{acc} was 120 s for FLUX concentrations $1x10^{-8}$ -1 $x10^{-6}$ M at pH 4.5 on HMDE electrode. But at pH 1.35, the best t_{acc} was 80 s for FLUX concentrations $4x10^{-8}$ -$1x10^{-6}$ M, while t_{acc} was160 s for FLUX concentrations$1x10^{-9}$ - $4x10^{-8}$ M on HMDE electrode. The optimum parameters for DPV and DPAdSV determination of FLUX were selected and presented in the (Table 1).

Table 1: The optimum parameters established for DPV and DPAdSV determination of FLUX.

Parameters	Operating modes		
	DPV	DPAdSV	DPAdSV
Working electrode	hanging mercury drop electrode (HMDE)		
Supporting electrolyte	0.02 M $LiClO_4$		
Solvent flucloxacillin	double distilled deionized water		
Purge gas	Pure N_2 for 300 s		
Value of pulse amplitude	-100 mV		
t. pulse	35 ms		
Drop modified size	9 mm^2		
Temperature of solution	25°± 5°C		
t.meas	32 ms		
Stlpring speed	2000 rpm		
pH	4.5	4.5	1.35
Waiting time	5 min		35 min
t.step	0.1 s		
u.step	8 mV	12 mV	
Scan rate	80 mV/s	120 mV/s	
Initial potential	-400 mV	+100 mV	
Final potential	-1400 mV	-1000 mV	
Accumulation potential	-	+150 mV	
Accumulation time	-	120 s	80 and 160 s
u.meas	-500 mV	-	-
Peak potential	-965 to -1000 mV	-250 to -270 mV	-145 to -170 mV

Analytical results

The analytical curves, I_p=f (C_{FLUX}) for the determination of FLUX at pH 4.5 in presence of 0.02 M $LiClO_4$ on HMDE by DPV and DPAdSV with E_{acc}+150 mV and t_{acc}120 s showed good linear ($5x10^{-8}$-$1.5x10^{-6}$ and $1x10^{-8}$-$1x10^{-6}$ $mol.L^{-1}$), see (Figures 3 and 4), but at pH 1.35 and t_{acc} 80 and 160 s showed too good linear ($4x10^{-8}$-1.0 x 10^{-6} and $1x10^{-9}$-4 x 10^{-8} $mol.L^{-1}$), see (Figures 5 and 6). Regression equations and correlation coefficient were as in tables (2-4). This method showed very sensitive results for the determination of FLUX by DPAdSV at pH 1.35 more than that obtained using DPV or by DPAdSV at pH 4.5.

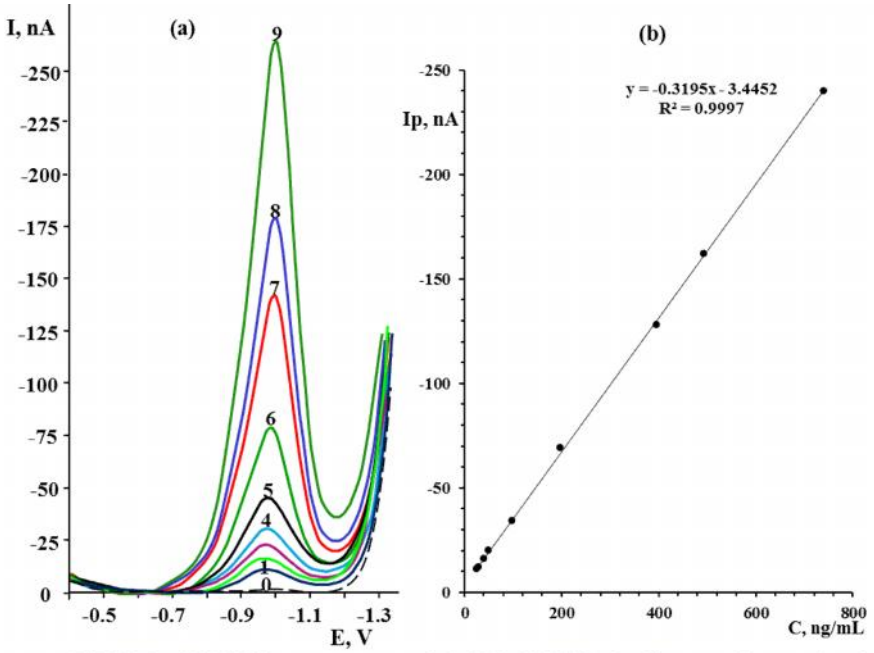


Fig.3: (a) The DPV Curves on HMDE of FLUX in presence of 0.02 M $LiClO_4$ buffer at pH 4.5: 0- electrolyte, 1- 24.695, 2- 29.634, 3- 39.512, 4- 49.390, 5- 98.780, 6- 197.560, 7- 395.120,8- 493.900 and 9- 740.850 $ng.mL^{-1}$. (b) Calibration curves for the determination of FLUX (Purge gas N_2, purge time 300 s, sweep rate 80 mV/s, U.amplitude -100 mV, drop size 9 mm^2, t.step 0.1 s, t. meas 32 ms,u.meas -500 mV, t.pulse 35 ms, U.step 8 mV, temperature 25°± 5°C).

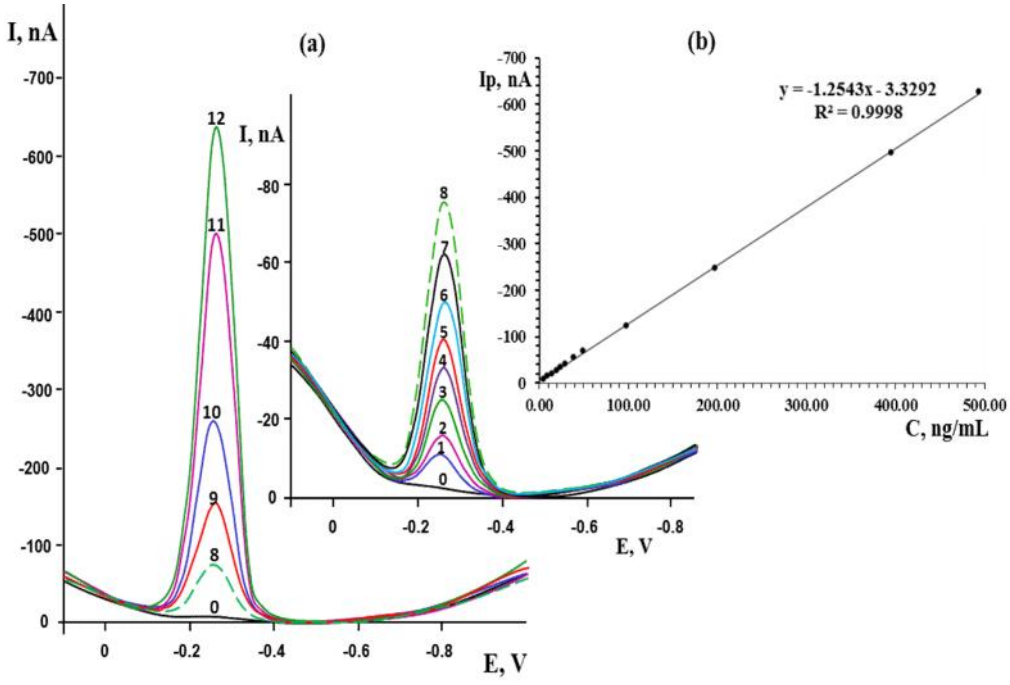


Fig.4: (a) The DPASdV Curves on HMDE of FLUX in presence of 0.02 M $LiClO_4$ buffer at pH 4.5, E_{acc} +150 mV and t_{acc} 120 s: 0- electrolyte, 1- 4.939, 2- 9.878, 3- 14.817, 4- 19.756, 5- 24.695, 6- 29.634, 7- 39.512, 8- 49.390, 9- 98.780, 10- 197.560, 11- 395.120 and 12- 493.900 $ng.mL^{-1}$. (b) Calibration curves for the determination of FLUX (Purge gas N_2, purge time 300 s, sweep rate 120 mV/s, U.amplitude -100 mV, drop size 9 mm^2, t.step 0.1 s, t.meas 32 ms, t.pulse 35 ms, U.step 12 mV, temperature 25°± 5°C).

Table 2: Analytical parameters for determination of FLUX using DPV and by DPAdSV methods

Parameter	DPV	DPAdSV, pH 4.5, t_{acc} 120 s	DPAdSV, pH 1.35,	
			t_{acc} , 80 s	t_{acc} ,160 s
Regression equations	y = -0.3195x -3.4452	y = -1.2543x - 3.3292	y = -6.2664x - 6.5015	y = -12.948x - 1.3082
concentration range,ng.mL^{-1}	24.965-740.850	4.939- 493.900	19.756-493.900	0.494-19.756
concentration range, mol.L^{-1}	5×10^{-8} to 1.5×10^{-6}	1×10^{-8} to 1×10^{-6}	4×10^{-8} to 1×10^{-6}	1×10^{-9} to 4×10^{-8}
R^2	0.9997	0.9998	0.9998	0.9999
Low concentration ng.mL^{-1}	24.965	4.939	19.756	0.494
RSD%	3.0	3.6	2.6	4.5
LOD,ng.mL^{-1}	2.403	0.585	1.691	0.071
LOQ, ng.mL^{-1}	7.282	1.773	5.124	0.215

y: I_p, nA and x: C_{FLUX}, ng.mL^{-1}

Table 3: Determination of flucloxacillin on HMDE in 0.02 M $LiClO_4$ buffer at pH 4.5 (n=5, t=2.776.)

Taken x_i		DPV				DPASdV			
μM	ng.mL^{-1}	Found, $\bar{x}$ ng.mL^{-1}	SD, ng.mL^{-1}	$\bar{x} \pm \frac{t.SD}{\sqrt{n}}$, ng.mL^{-1}	RSD%	Found, $\bar{x}$ ng.mL^{-1}	SD, ng.mL^{-1}	$\bar{x} \pm \frac{t.SD}{\sqrt{n}}$, ng.mL^{-1}	RSD%
0.010	4.939	-	-	-	-	4.926	0.1773	4.926± 0.2202	3.6
0.020	9.878	-	-	-	-	9.703	0.3105	9.703± 0.3855	3.2
0.030	14.817	-	-	-	-	14.687	0.4406	14.687± 0.5470	3.0
0.040	19.756	-	-	-	-	20.068	0.5619	20.068± 0.6976	2.8
0.050	24.695	24.272	0.7282	24.272± 0.9040	3.0	24.692	0.6420	24.692± 0.7970	2.6
0.060	29.634	28.928	0.8100	28.928± 1.0056	2.8	30.130	0.7231	30.130± 0.8977	2.4
0.080	39.512	39.295	1.0217	39.295± 1.2684	2.6	40.195	0.8843	40.195± 1.0978	2.2
0.100	49.390	50.415	1.1595	50.415± 1.4396	2.3	50.126	1.0025	50.126± 1.2446	2.0
0.200	98.780	95.633	1.9127	95.633± 2.3746	2.0	96.836	1.7430	96.836± 2.1640	1.8
0.400	197.560	203.180	3.4541	203.180±4.2881	1.7	195.460	2.9319	195.460± 3.6398	1.5
0.800	395.120	392.850	5.8928	392.850± 7.3156	1.5	393.870	5.1203	393.870± 6.3567	1.3
1.000	493.900	496.260	6.4514	496.260± 8.0092	1.3	497.323	5.4706	497.323± 6.7915	1.1
1.500	740.850	740.390	8.8847	740.390±11.0300	1.2	-	-	-	-

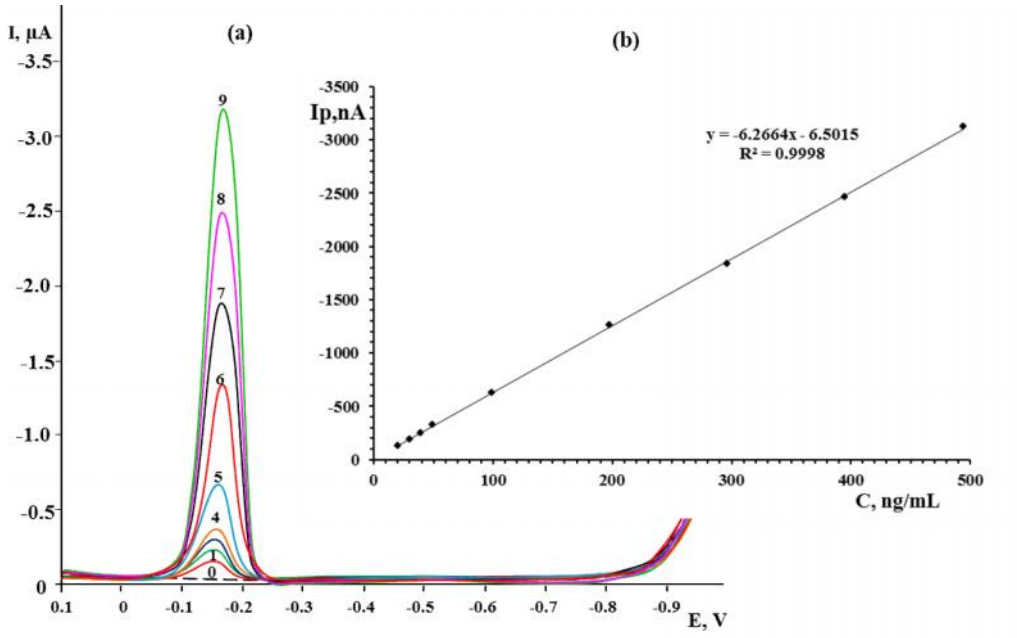


Fig.5: (a) The DPASdV Curves on HMDE of FLUX in presence of 0.02 M $LiClO_4$ buffer at pH 1.35, E_{acc} +150 mV , t_{acc} 80 s: 0- electrolyte, 1- 19.756, 2- 29.634, 3- 39.512, 4- 49.390, 5- 98.780, 6- 197.560, 7- 296.340,8- 395.120 and 9- 493.900 ng.mL^{-1}, (b) Calibration curves for the determination of FLUX (Purge gas N_2, purge time 300 s, sweep rate 120 mV/s, U.amplitude -100 mV, drop size 9 mm^2, t.step 0.1 s,t.meas 32 ms, t.pulse 35 ms, U.step 12 mV, temperature 25°± 5°C).

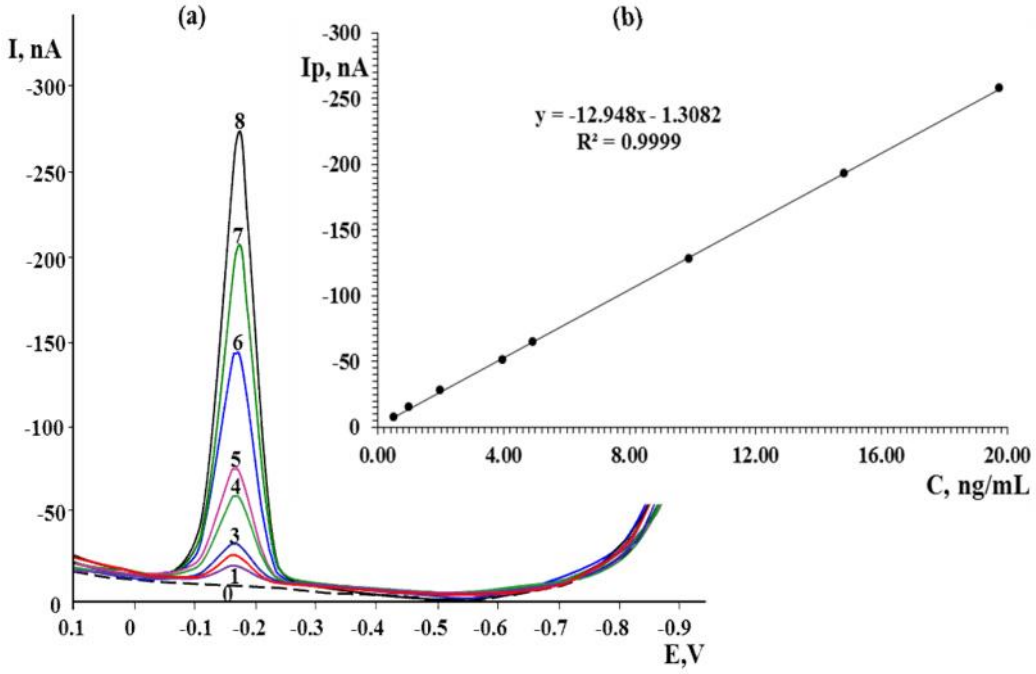


Fig.6: (a) The DPASdV Curves on HMDE of FLUX in presence of 0.02 M $LiClO_4$ buffer at pH 1.35,E_{acc} +150 mV and t_{acc} 160 s: 0- electrolyte, 1- 0.494, 2- 0.988, 3- 1.976, 4- 3.951, 5- 4.939, 6- 9.878, 7- 14.817 and 8- 19.756 ng.mL^{-1}. (b) Calibration curves for the determination of FLUX (Purge gas N_2, purge time 300 s, sweep rate 120 mV/s, U.amplitude -100 mV, drop size 9 mm^2, t.step 0.1 s, t.meas 32 ms, t.pulse 35 ms, U.step 12 mV, temperature 25°± 5°C).

Table 4: Determination of flucloxacillin using DPAdSV on HMDE with negative amplitude in 0.02M $LiClO_4$ buffer at pH 1.35, t_{acc} 80, 160 s,E_{acc} +150 mV (n=5, t=2.776.)

Accumulation time, s	Taken x_i		Found, $\bar{x}$, ng.mL^{-1}	SD, ng.mL^{-1}	$\bar{x} \pm \frac{t.SD}{\sqrt{n}}$, ng.mL^{-1}	RSD%
	μM	ng.mL^{-1}				
160	0.001	0.494	0.478	0.0215	0.478 ± 0.0267	4.5
	0.002	0.988	1.057	0.0444	1.057± 0.0551	4.2
	0.004	1.976	2.092	0.0837	2.092± 0.1039	4.0
	0.008	3.951	3.837	0.1419	3.837± 0.1763	3.7
	0.010	4.939	4.919	0.1673	4.919± 0.2076	3.4
	0.020	9.878	9.785	0.2935	9.785± 0.3644	3.0
	0.030	14.817	14.805	0.3849	14.805± 0.4779	2.6
	0.040	19.756	19.825	0.4758	19.825 ± 0.5907	2.4
80	0.040	19.756	19.708	0.5124	19.708 ± 0.6362	2.6
	0.060	29.634	29.483	0.7076	29.483±0.8785	2.4
	0.080	39.512	39.158	0.8223	39.158± 1.0209	2.1
	0.100	49.390	50.226	0.9041	50.226± 1.1224	1.8
	0.200	98.780	99.500	1.5920	99.500± 1.9764	1.6
	0.400	197.560	200.035	3.0005	200.035± 3.7251	1.5
	0.600	296.340	295.856	4.1420	295.856 ± 5.1423	1.4
	0.800	395.120	394.330	4.7320	394.330 ± 5.8747	1.2
	1.000	493.900	497.654	4.9765	497.654± 6.1782	1.0

The proposed mechanism of flucloxacillin on HMDE

Because there are different functional groups on FLUX that are available to be reduced according to similar mechanisms [15-21], several reduction mechanisms may be proposed. The influence of electrochemical parameters known to affect the DPV, it is postulated that the isoxazole ring is the site of reduction (1).On the basis of the experimental results obtained DPAdSV at pH 4.5, the mechanism could be suggested for the voltammetric reduction of flucloxacilin, which corresponds to the usual reduction mechanism for the >C=O group (2).The mechanism by DPAdSV at pH 1.35 could be suggested for the voltammetric reduction C=N group in isoxazole ring (3).The electrochemical reaction is suggested to proceed as follows:

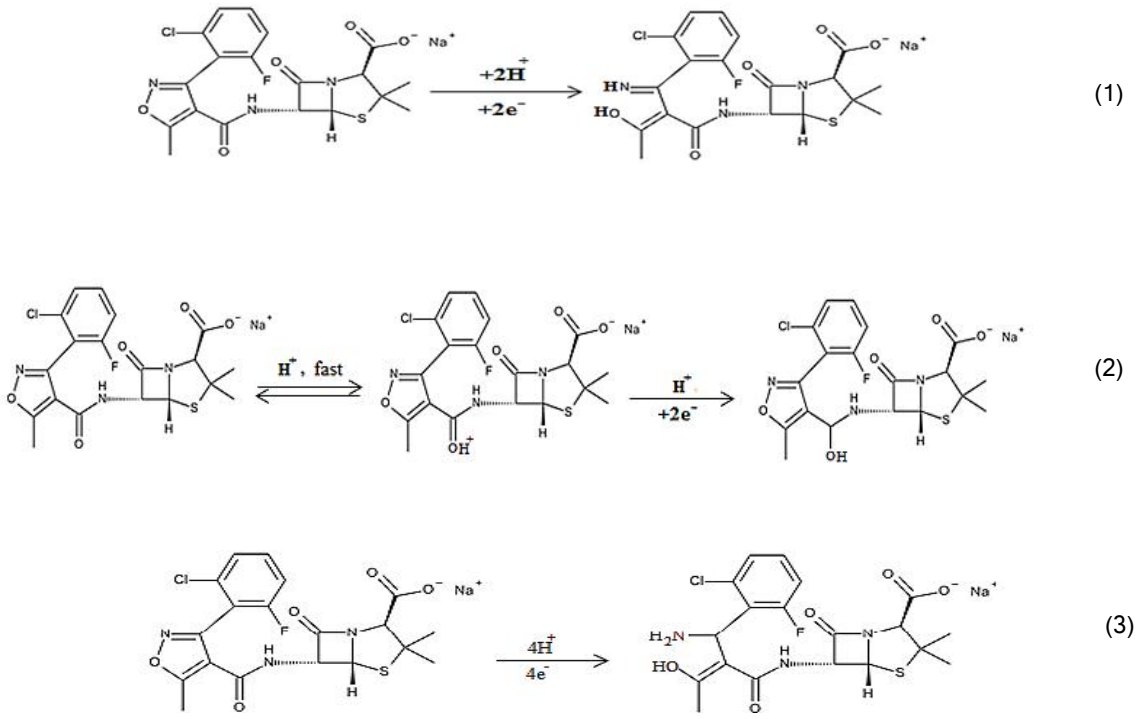


Figure 9: Electrochemical mechanisms of flucloxacillin.

Applications

Many applications for the determination of FLUX in some Syrian pharmaceutical preparations (in presence a same amount of amoxicillin) on HMDE with negative amplitude in 0.02 M $LiClO_4$ buffer using DPV(at pH 4.5) and DPAdSV (at pH 4.5 and 1.35, t_{acc}120 and 80 s, and E_{acc} +150 mV) 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 5000 for all pharmaceuticals content 250 mg/cap.The results of quantitative analysis for FLUX in pharmaceutical preparations were summarized in Table 5. 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 99.5 to 101.9% using DPV, and by using DPAdSV were 99.6 to 102.1% and 99.8 to 102.3% at pH 4.5 and 1.35 respectively. The results obtained by this method agree well with the contents stated on the labels and were validated by HPLC method [1]. Therefore, the presented method can be recommended for routine analysis of FLUX in pharmaceutical formulations.

Table 5: Determination of FLUX in some Syrian pharmaceutical preparations using DPV and DPAdSV

Commercial name	method	Label Claim of FLUX & AMOX, mg/cap.	Mean ±SD (as FLUX), mg/ cap.	RSD%	Assay %	(Assay %), by HPLC [1]
Amoxipen capsule, BARAKAT	**DPV**	250	248.70±6.944	2.8	99.5	100.1
	DPAdSV at pH 4.5 , t_{acc} 120 s		249.40±5.986	2.4	99.8	
	DPAdSV at pH 1.35 , t_{acc} 80 s		250.00± 5.000	2.0	100.0	
Amoxam capsule, IBN HAYYAN	**DPV**	250	251.90±6.494	2.6	100.8	100.6
	DPAdSV at pH 4.5 , t_{acc} 120 s		252.50± 5.808	2.3	101.0	
	DPAdSV at pH 1.35 , t_{acc} 80 s		252.50± 4.978	1.9	101.0	
Penifloxam capsule, APHAMEA	**DPV**	250	254.74± 6.878	2.7	101.9	102.0
	DPAdSV at pH 4.5 , t_{acc} 120 s		255.22±6.381	2.5	102.1	
	DPAdSV at pH 1.35 , t_{acc} 80 s		255.75± 5.371	2.1	102.3	
Floxin capsule, ALBALSAM pharma	**DPV**	250	248.65± 7.211	2.9	99.5	99.7
	DPAdSV at pH 4.5 , t_{acc} 120 s		249.00± 5.976	2.4	99.6	
	DPAdSV at pH 1.35 , t_{acc} 80 s		249.50± 5.489	2.2	99.8	
Maxipen capsule, ASIA	**DPV**	250	253.25± 7.091	2.8	101.3	101.5
	DPAdSV at pH 4.5 , t_{acc} 120 s		253.00± 6.072	2.4	101.2	
	DPAdSV at pH 1.35 , t_{acc} 80 s		254.25± 4.831	1.9	101.7	

Method validation

The developed method for simultaneous estimation of FLUX has been validated in accordance with the International Conference on Harmonization guidelines (ICH) [22].

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 using DPV at pH 4.5 and DPAdSV at pH 1.35,while cloxacillin is interfere. But using DPAdSV at pH 4.5 amoxicillin, ampicillin and cloxacillin were interfere.

Linearity

In the proposed methods, linear plots (n=5) with good correlation coefficients were obtained in the concentration ranges of y = -0.3195x-3.4452 (R^2=0.9997) by DPV for the concentration from 24.695-740.850 $ng.mL^{-1}$ and y=-1.2543 x-3.3292 (R^2=0.9998) on HMDE electrode at t_{acc} 120 s by DPAdSV for the concentration from 4.939 - 493.900 $ng.mL^{-1}$ by DPAdSV at pH 4.5. In this method a very low concentration 24.695 $ng.mL^{-1}$ (5×10^{-8} $mol.L^{-1}$) and 4.939 $ng.mL^{-1}$(1×10^{-8} $mol.L^{-1}$) of FLUX, respectively. Using DPAdSV at pH 1.35 correlation coefficients were y=-6.2664x-6.5015 (R^2=0.9998) for the concentration from 19.756-493.900 $ng.mL^{-1}$ at t_{acc} 80 s and y=-12.948x-1.3082 (R^2=0.9999) at t_{acc} 160 s for the concentration from 0.494-19.756 $ng.mL^{-1}$.In these methods a very low concentration 19.756 ng. mL^{-1} (4×10^{-8} $mol.L^{-1}$) and 0.494 $ng.mL^{-1}$ (1×10^{-9} mol. L^{-1}) of FLUX were determined by DPAdSV at t_{acc} 80 and 160 s, respectively.

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% and120%) within the range of linearity for FLUX. The basic concentration level of sample solution selected for spiking of the FLUX standard solution was 49.390 $ng.mL^{-1}$. The proposed method was validated statistically and through recovery studies, and was successfully applied for the determination of FLUX in pure and dosage forms, table 6.

Table 6: Results of recovery studies (n=5).

Level	Recovery%		
	pH 4.5		pH 1.35
	DPV	DPAdSV	DPAdSV
80%	100.5	99.9	99.7
100%	99.8	100.8	101.2
120%	100.8	101.2	101.5

Repeatability

The repeatability was evaluated by performing 10 repeat measurements for 29.634 $ng.mL^{-1}$ of FLUX using the studied methods under the optimum conditions. The found amount of FLUX ($\overline{x}$ ±SD) were 29.485±0.710 $ng.mL^{-1}$, 29.515±0.620 $ng.mL^{-1}$ and 29.600 ±0.533 $ng.mL^{-1}$, the percentage recovery were found to be 99.5±0.24 with RSD of 0.024,99.5± 0.21with RSD of 0.021 and 99.9 ±0.18 with RSD of 0.018 using DPV at pH 4.5 and DPAdSV at pH 4.5and pH 1.35, respectively. These values indicate that the proposed method has high repeatability for FLUX analysis.

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 for FLUX were 2.403, 0.585 and 0.071 $ng.mL^{-1}$, and LOQ were 7.282, 1.773 and 0.215 $ng.mL^{-1}$using DPV and DPAdSV at pH 4.5 (t_{acc} 120 s and E_{acc} +150 mV), and DPAdSV at pH 1.35 (t_{acc}160s and E_{acc} +150 mV), respectively.

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±5°C), pH (4.5±0.20, 1.35±0.20), reaction waiting time (10 min) and accumulation potential (+150±5 mV). This table indicates that the robustness of the proposed methods was good (I_p was measured and assay was calculated for five times).

Table 7: Robustness of the proposed DPV and DPAdSV methods at HMDE for determination of flucloxacillin (n=5 calculated for five times).

Experimental parameter variation	Average recovery (%)C_{FLUX} = 29.634 $ng.mL^{-1}$		
	pH 4.5		pH 1.35
	DPV	DPAdSV	DPAdSV
Temperature			
20°C	99.6	99.8	99.4
25°C	100.3	100.1	100.0
30°C	100.5	100.6	101.2
pH			
4.2	99.7	99.9	-
4.7	100.2	100.5	-
1.33	-	-	100.3
1.37	-	-	100.8
Reaction time			
25 min	99.6	100.2	99.5
35 min	100.2	100.6	101.5
60 min	100.8	101.4	101.8
Accumulation potential			
145 mV	-	100.4	99.6
155 mV	-	100.7	101.5

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.

Conclusion

Electroreduction and adsorption of Flucloxacillin (FLUX) using DPV and DPAdSV at HMDE has been studied. The reduction peak potential (E_p) of FLUX using DPV was between the range -965 to -1000 mV, liner calibration graph were the concentration ranges of 24.695-740.850 ng.mL^{-1}. Determination of FLUX using DPAdSV were studied. E_p was between -250 to -270 mV and -145 to -170 mV at pH 4.5 and pH 1.35, respectively. Liner calibration graphs at E_{acc} +150 mV, t_{acc}120 s and 160 s and at pH 4.5 and 1.35, were of 4.939-493.900 ng.mL^{-1} and 0.494–19.756 ng.mL^{-1}, respectively. These methods give good results for the determination of FLUX in pure and different dosage forms.

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