الدراسات الكهركيميائية والتحليل الفولط امبيرومتري بالموجة المربعة لتحديد فلوكلوكساسيلين بشكله النقي وبالمستحضرات الصيدلانية باستخدام مسرى قطرة الزئبق المعلقة

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الملخص

طورنا طرائق تحليلية فولط أمبيرومترية (SWV) و فولط أمبيرومترية تراكمية (SWAdSVA) بالموجة المربعة باستخدام مسرى قطرة الزئبق المعلقة (HMDE) للتحديد الكمي لمركب فلوكلوكساسيلين؛ درسنا عوامل مختلفة كنوع الكهرليت و pH الوسط وزمن التراكم وسعة النبضة والتردد وغيرها من العوامل المؤثرة في تحديد المركب المدروس وفقاً للطرائق التحليلية المستخدمة, وحصلنا على أفضل النتائج في وسط مائي يحتوي على كهرليت فوق كلورات الليثيوم $LiClO_4$ بتركيز 0.04 M عند قيمة pH تساوي 4.5 و 1.38 لكلا الطريقتين SWV و SWAdSVA على التسلسل. تظهر بطريقة SWV القمة الفولط أمبيرومترية عند كمون يتراوح من 9.68V- حتى 9.88V- باستخدام مسرى مقارن من Ag/AgCl عند قيمة pH تساوي 4.5 وتردد 160Hz وتراوح المنحني العياري ضمن مجال للتراكيز (20.00 – 1000) nM . وتظهر القمة بطريقة SWAdSVA مابين كمون 1.55 V- حتى 1.72 V- باستخدام مسرى مقارن من Ag/AgCl عند قيمة pH تساوي 1.38 وكمون تراكم 150 mV وزمن تراكم 80 s و 160 s وتردد 120 Hz وتراوح المنحني العياري ضمن مجال للتراكيز مابين (5.00-500) nM و (0.60-10.00) nM على التسلسل.

الكلمات المفتاحية: فلوكلوكساسيلين , التحليل الفولط أمبيرومتري بالموجة المربعة , التحليل الفولط أمبيرومتري التراكمي بالموجة المربعة , مسرى قطرة الزئبق المعلقة.

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Electrochemical studies and square-wave voltammetric determination of flucloxacillin in pure form and pharmaceutical formulations using hanging mercury drop electrode

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Abstract

An analytical methods using square wave voltammetry (SWV) and square-wave adsorptive stripping voltammetry analysis (SWAdSVA) at a hanging mercury dropping electrode (HMDE) surface was developed for the quantitative determination of flucloxacillin (FLUX); Various parameters (supporting electrolyte, medium of pH (1.0 - 9.0), deposition time, accumulation potential, frequency, and scan increment, etc.) affecting the FLUX determination were examined. The best results were obtained in an aqueous $LiClO_4$ buffer 0.04 M at values pH 4.5 and 1.38. In the technique SWV a voltammetric peak is obtained at -9.68 V to -9.88 V vs. Ag/AgCl at pH 4.5, frequency 160 Hz. Linear calibration graph were the concentration ranges of 20.00 – 1000 nM. In the technique SWAdSVA voltammetric peak is obtained at -1.55 V to -1.72 V vs. Ag/AgCl at pH 1.38, accumulation potential of +150 mV and accumulation time 80 s and 160 s, frequency 120 Hz. Analytical curves were obtained for application in the range of 5.00-500 nM and 0.60-10.00 nM respectively.

Keywords: Square wave voltammetry, Square-wave adsorptive stripping voltammetry, Hanging mercury drop electrode, Flucloxacillin.

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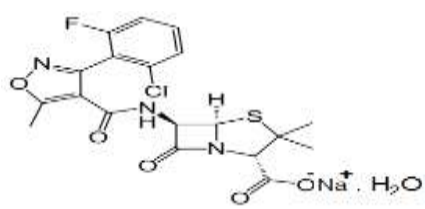
1-Introduction

Square wave voltammetry (SWV) is a form of linear potential sweep voltammetry that uses a combined square wave and staircase potential applied to a stationary electrode [1]. It has found numerous applications in various fields, including within medicinal and various sensing communities.

In a square wave voltammetric experiment, the current at a (usually stationary) working electrode is measured while the potential between the working electrode and a reference electrode is swept linearly in time. The potential waveform can be viewed as a superposition of a regular square wave onto an underlying staircase in this sense, SWV can be considered a modification of staircase voltammetry [2].

Despite both the forward and reverse current waveforms having diagnostic worth, it is almost always the case in SWV for the potentiostat software to plot a differential current waveform derived by subtracting the reverse current waveform from the forward current waveform. This differential curve is then plotted against the applied potential. Peaks in the differential current vs. applied potential plot are indicative of redox processes, and the magnitudes of the peaks in this plot are proportional to the concentrations of the various redox active species [1- 4].

Chemically, Flucloxacillin is, (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, its molecular weight is 493.9 g/moL. Flucloxacillin (FLUX) is used in the treatment of severe essential. It is the best of the anti-staphylococcal penicillins is a bactericidal antibiotic drug [5].



2- Previous Research

Several analytical methods have been reported for as-say of FLUX including spectrophotometry [6,7], performance liquid chromatography [8 -12], potentiometric method [13] and polarography [14-17].

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) [14].

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 $1x10^{-7}$ - $2.6x10^{-5}$ $mol.L^{-1}$ and $1x10^{-7}$ - $2x10^{-5}$ $mol.L^{-1}$ 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) [15, 16]. Electro reduction and adsorption of 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 (Ep) 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. Ep was between -250 to -270 mV and -145 to -170 mV at pH 4.5 and pH 1.35, respectively. Liner calibration graphs at Eacc +150 mV, tacc 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 [17].

The aim of this study was to identify the characteristics of the electrode reaction of FLUX at a hanging mercury drop electrode in an aqueous lithium perchlorate buffer (pH 1.0 – 9.0), and consequently to develop a direct, simple, rapid, sensitive, precise and inexpensive square-wave adsorptive stripping voltammetric procedure for its quantification in bulk form, pharmaceutical formulation.

3. Experimental

3.1. Instruments and apparatus

A Metrohm 746 VA processor, a Metrohm 747 VA stand with a hanging mercury drop electrode (HMDE) 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 ^{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.

3.2. Reagents

Working reference standard of flucloxacillin (99.2%) was supplied by D.K. Pharmachem Pvt. Ltd INDIA, 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.

3.3. Preparation supporting electrolyte

Lithium perchlorate buffer 0.1000 mol.L^{-1} and 0.04 mol.L^{-1} at pH (1.0 - 9.0) were used.

3.4. A stock standard solution of flucloxacillin

This solution was prepared by dissolving 49.79 mg from FLUX (99.2%) in 100 mL double distilled deionized water (1x10^{-3} mol.L^{-1}), then dilute 1.000 mL, 0.100 and 0.01mL from this solution to 100 mL (1x10^{-5} mol.L^{-1}, and 1x10^{-6} mol.L^{-1}).

3.5. Working solutions

The stock solutions were further diluted to obtain working solutions daily just before use in the ranges of FLUX: 0.0006, 0.0008, 0.0010, 0.0016, 0.002, 0.004, 0.005, 0.0060, 0.008, 0.010, 0.020, 0.030, 0.060, 0.080, 0.100, 0.200, 0.300, 0.400, 0.500, 0.800 and 1.000 µM by dilution of the volumes: 0.015, 0.020, 0.025, 0.040, 0.050, 0.100, 0.125, 0.150, 0.200, 0.250, 0.500 and 0.750 mL from stock standard solutions 1x10^{-6} mol.L^{-1} and 0.150, 0.200, 0.250, 0.500, 0.750, 1.000, 1.2500, 2.000, and 2.500 mL from stock standard solutions 1x10^{-5} mol.L^{-1} were transferred into 25 mL volumetric flask, diluted with Lithium perchlorate buffer 0.04 M to the mark.

3.6. Samples

Commercial formulations (as capsule) were used for the determination of FLUX by using SWV and SWAdSVA analysis using HMDE. The pharmaceutical formulations were subjected to the analytical procedures:

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

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

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

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

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

3.6.1 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.

3.6.2 Working solutions of pharmaceuticals

These solutions were prepared daily by diluting 10 µL (0.01 mL) from stock solutions of pharmaceutical formulations into 100 mL volumetric flask, diluted with Lithium perchlorate buffer 0.04 M (pH 4.5 or 1.38) to the mark (each solution contents 0.025 µg.mL-1 of FLUX)

3.7. Analytical procedure

Square wave voltammetry (SWV)

Transfers 25 mL of working standard of flucloxacillin or working solutions of pharmaceuticals to the cell. deoxygenat with N_2 gas for 300 s for the solution. In the optimum conditions were applied the potential range studied was from -500 to -1400 mV (versus Ag/AgCl) using SWV at frequency 160 Hz, with HMDE. The peak height was measured at -9.68 V to -9.88 V in 0.04 M $LiClO_4$ at pH 4.5, see Fig.1,(a).

Square-wave adsorptive stripping voltammetry analysis (SWAdSVA)

Transfers into an electrochemical cell 25 mL volume of working solution containing an appropriate concentration of FLUX.

The solution was deoxygenated with N_2 gas for 300 s. The accumulation potential (Eacc) +150 mV, accumulation time (tacc) 80 s and 160 s were applied. In the optimum conditions were applied the potential scanned from +100 to -1000 mV (versus Ag/AgCl) using SWAdSVA at frequency 120 Hz, with HMDE. The peak height (Ip) was measured at -1.55 V to -1.72V in 0.04 M $LiClO_4$ at pH 1.38, see Fig.1,(b).

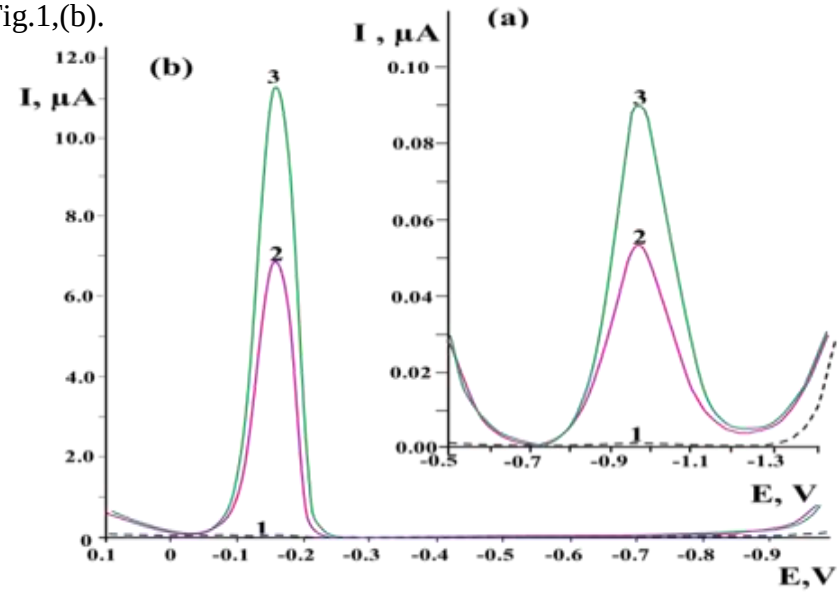


Fig.1: The polarograms using SWV and SWAdSVA for determination of FLUX in presence of 0.04 M $LiClO_4$ buffer: 1- electrolyte, 2- 0.060 µM, 3- 0.100 µM.
(a) The polarograms using SWV at pH 4.5, sweep rate 80 mV/s , frequency 160 Hz.
(b) The polarograms using SWAdSVA at pH 1.38, tacc 80 s, Eacc +150 mV, sweep rate 120 mV/s frequency 120 Hz. (Purge gas N_2, purge time 300 s, U.amplitude +50 mV, drop size 9, t.step 0.1 s, t.meas 2 ms, temperature 25°± 5°C).

4. Results And Discussion

4.1. Square-wave operational parameters

4.1.1. The effect of pH

The influence of pH from 2.5 to 9.0 using lithium perchlorate (0.04 M) buffer on Ip and Ep was studied, the values 4.5 was selected.

4.1.2. The effect of pulse amplitude (U.ampl)

The effect of pulse amplitude, U.ampl between 10 to 50 mV on Ip and Ep by SWV. Ip linearly increases with increasing amplitude value until 50 mV. While Ep stay semi-fixed. The value 50 mV was better than another's.

4.1.3. Influence of frequency (f, Hz)

The SWV peak current is linearly dependent on the frequency (60 -250 Hz), while a well-defined peak was observed at 160 Hz. A relationship was observed between the stripping peak current and frequency. The response of flucloxacillin is increased with frequency, above 160 Hz the peak current was obscured by a large residual current then the peak current decreases. Thus the best peak was recorded using 160 Hz frequency, see Fig. 2.

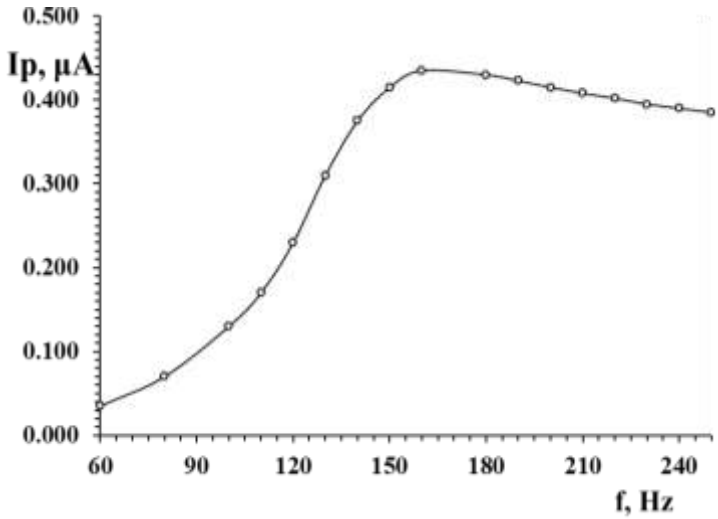


Fig.2: Effect of frequency on square-wave voltammetry analysis of FLUX 0.10 µM at HMDE, using 0.04 M $LiClO_4$ at pH 4.5 (Purge gas N_2, purge time 300 s, U. amplitude +50 mV, drop size 9 sweep rate 80 mV/s, t. meas 2 ms, t. step 0.1 s, U. step 8 mV, temperature 25°± 5°C).

4.2. Square-wave adsorptive stripping voltammetry operational parameters

4.2.1. The effect of pH

The influence of pH from 1.0 - 9.0 using 0.04 M $LiClO_4$ buffer on Ip and Ep were studied by SWAdSVA. It was found that the best pH solution was 1.38.

4.2.2. The effect of the accumulation potential (Eacc)

The dependence of the square wave adsorptive stripping peak current on the accumulation potential (Eacc) +200 mV to -300 mV was examined. It was found that the maximum response for FLUX occurs with Eacc equal to +150 mV on HMDE electrode, see Fig. 3.

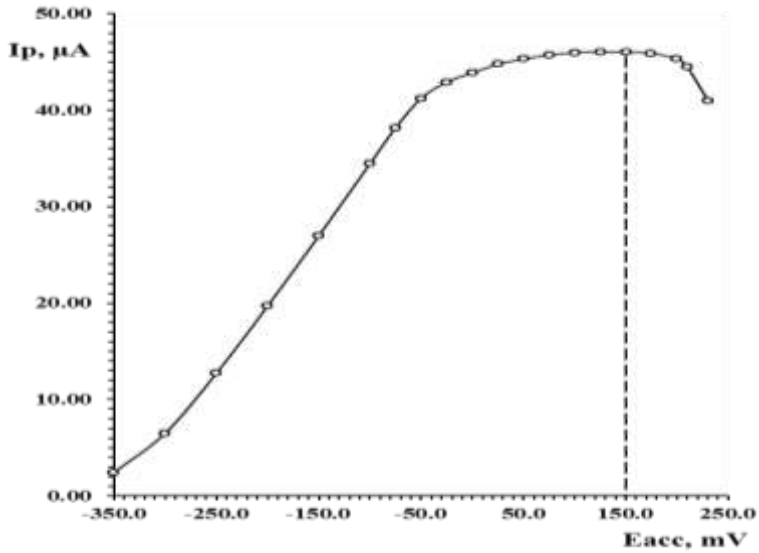


Fig.3: Effect of accumulation potential on square-wave adsorptive stripping voltammetry analysis of FLUX (0.100 µM) at HMDE, using 0.04 M $LiClO_4$ at pH 1.38, accumulation time 80 s , U. amplitude 50 mV (Purge gas N_2, purge time 300 s, sweep rate 120 mV/s, t. meas 2 ms, t. step 0.1 s, U. step 12 mV, temperature 25°± 5°C).

4.2.3. Effect of accumulation time (tacc)

The peak current depended on the accumulation time (tacc) for FLUX concentrations were studied. The peak current increases with increasing tacc. The best tacc was 80 s for FLUX concentrations 5.00 - 500 nM, while tacc was 160 s for FLUX concentrations 0.60 -10.00 nM on HMDE electrode.

4.2.4. Influence of frequency (f, Hz)

The SWAdSVA peak current is linearly dependent on the frequency (60 -160 Hz), while a well-defined peak was observed at 120 Hz. A relationship was observed between the stripping peak current and frequency. The response of FLUX is increased with frequency, above 120 Hz the peak current was obscured by a large residual current then the peak current decreases. Thus the best peak was recorded using 120 Hz frequency, Fig. 4.

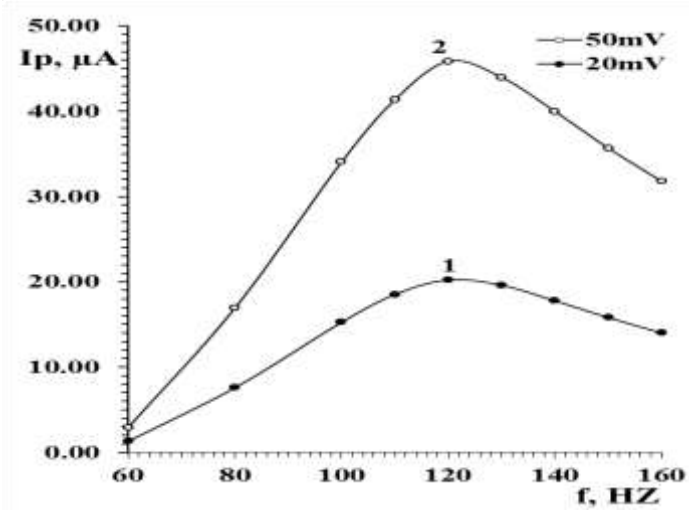


Fig.4: Effect of frequency on square wave adsorptive stripping voltammetry analysis of FLUX 0.100 µM at HMDE, using 0.04 M $LiClO_4$ at pH 1.38, accumulation potential +150 mV, accumulation time 80 s, 1- U. amplitude 20 mV , 2- U. amplitude 50 mV (Purge gas N_2, purge time 300 s, sweep rate 120 mV/s, t. meas 2 ms, t. step 0.1 s, U. step 12 mV, temperature 25°± 5°C).

The optimum parameters for SWV and SWAdSVA determination of FLUX were selected and presented in the (Table 1).

Table 1: The optimum parameters established for SWV and SWAdSVA determination of FLUX.

parameters	Operating modes	
	SWV	**SWAdSVA**
Working electrode	hanging mercury drop electrode (HMDE)	
Supporting electrolyte	0.04 M $LiClO_4$	
Solvent flucloxacillin	double distilled deionized water	
Purge gas	Pure N_2 for 300 s	
Value of pulse amplitude	50 mV	
Drop modified size	9 (0.6 mm^2)	
Temperature of solution	25°± 5°C	
t.meas	2 ms	
Rot. speed	2000 rmp	
pH	4.5	1.38
frequency (f, Hz)	160Hz	120 Hz
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	-500 mV	+100

Final potential	-1400 mV	-1000
Accumulation potential	-	+150 mV
Accumulation time	-	80 and 160 s
Peak potential	-9.68 V to -9.88 V	-1.55 V to -1.72 V

5. Analytical Results

The analytical curves, Ip =f (CFLUX) for the determination of FLUX at pH 4.5 in presence of 0.04 M $LiClO_4$, frequency 160 Hz, on HMDE by SWV showed excellent linear 0.020-1.000 µM (20.00-1000 nM), see (Figure 5), and SWAdSVA at pH 1.38 and tacc 80 and 160 s, frequency 120 Hz, showed too excellent linear 0.005-0.500 µM (5.00-500.00 nM) and 0.0006-0.010 µM (0.600-10.00 nM), see (Figures 6 and 7). Linearity equations obtained and correlation coefficient were as in tables (2-4). This method showed very sensitive results for the determination of FLUX by SWAdSVA at pH 1.38 more than that obtained using SWV at pH 4.5.

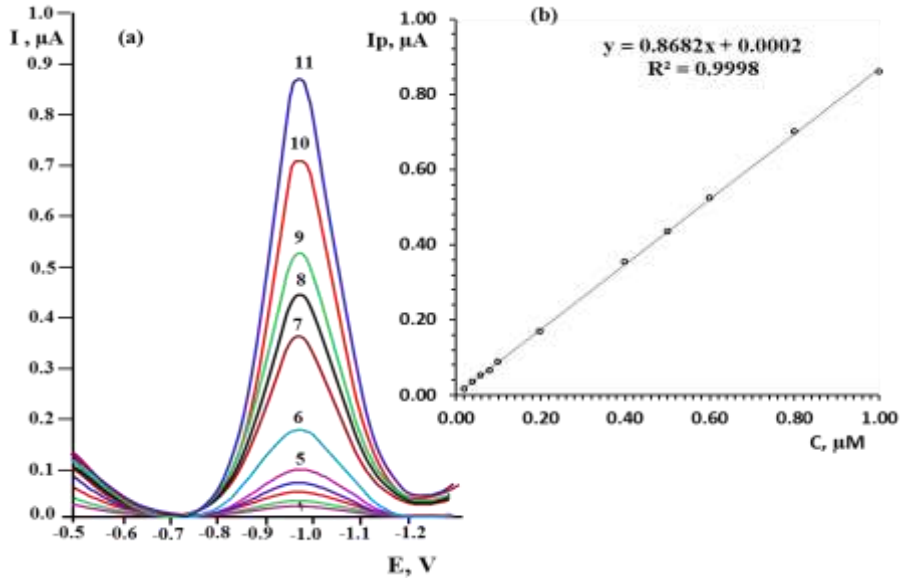


Fig.5: (a) The SWV Curves on HMDE of FLUX in presence of 0.02 M $LiClO_4$ buffer at pH 4.5:
1- 0.020 µM, 2- 0.040 µM, 3- 0.060 µM, 4- 0.080 µM, 5- 0.100 µM, 6- 0.200 µM, 7- 0.400 µM, 8- 0.500 µM, 9- 0.600 µM, 10- 0.800 µM and 11- 1.000 µM. (b) Calibration curves for the determination of FLUX (Purge gas N_2, purge time 300 s, sweep rate 80 mV/s, U.amplitude 50 mV, frequency 160Hz, drop size 9, t.step 0.1 s, t. meas 2 ms, U.step 8 mV, temperature 25°± 5°C).

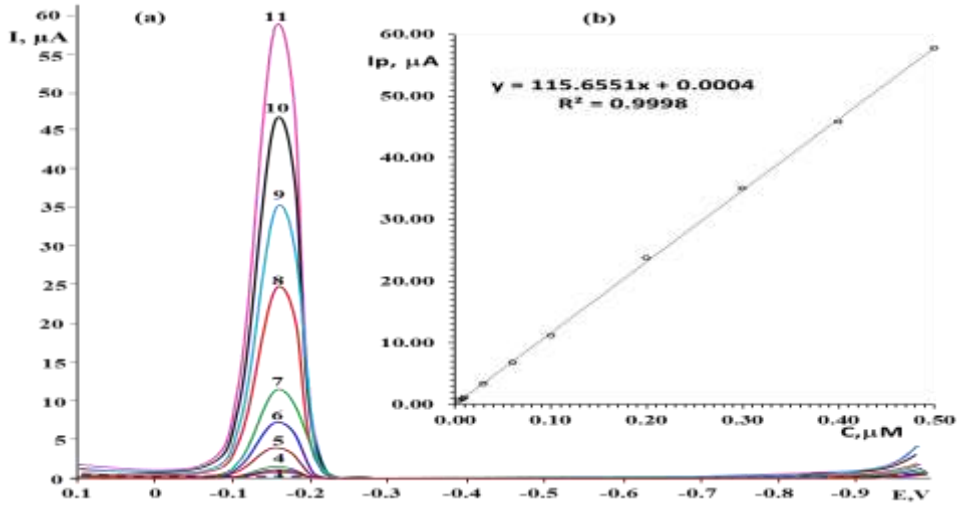


Fig.6: (a) The SWAdSVA Curves on HMDE of FLUX in presence of 0.04 M $LiClO_4$ buffer at pH 1.38 tacc, 80 s, Eacc +150 mV: 1- electrolyte, 2- 0.005 µM, 3- 0.008 µM, 4- 0.010 µM, 5- 0.030 µM, 6- 0.060 µM, 7- 0.100 µM, 8- 0.200 µM, 9- 0.300 µM, 10- 0.400 µM and 11- 0.500 µM. (b) Calibration curves for the determination of FLUX (Purge gas N_2, purge time 300 s, sweep rate 120 mV/s, U.amplitude 50 mV, frequency 120 Hz, drop size 9, t.step 0.1 s, t. meas 2 ms, , U.step 12 mV, temperature 25°± 5°C).

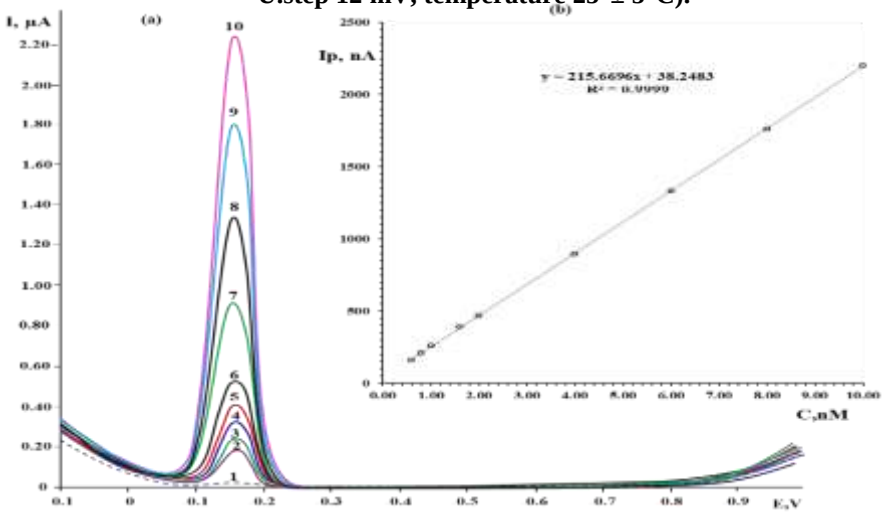


Fig.7: (a) The SWAdSVA Curves on HMDE of FLUX in presence of 0.04 M $LiClO_4$ buffer at pH 1.38 , tacc 160 s, Eacc +150 mV: 1- electrolyte, 2- 0.0006 µM, 3- 0.0008 µM, 4- 0.0010 µM, 5- 0.0016 µM, 6- 0.0020 µM, 7- 0.0040 µM, 8- 0.0060 µM , 9- 0.0080 µM and 10- 0.0100 µM (b) Calibration curves for the determination of FLUX (Purge gas N_2, purge time 300 s, sweep rate 120 mV/s, U.amplitude 50 mV, frequency 120 Hz, drop size 9, t.step 0.1 s, t. meas 2 ms, , U.step 12 mV, temperature 25°± 5°C).

Table 2: Determination of flucloxacillin using SWV at frequency 160 Hz, on HMDE in 0.04 M LiClO4 buffer at pH 4.5 (n=5, t=2.776).

Taken x_i			Found, $\bar{x}$ µM	SD, µM	$\bar{x} \pm \frac{t.SD}{\sqrt{n}}$, µM	RSD%
µM	nM	ng.mL^{-1}				
0.0200	20.00	9.878	0.0190	0.00076	0.0190±0.00094	4.0
0.0400	40.00	19.756	0.0400	0.00136	0.0400±0.00169	3.4
0.0600	60.00	29.634	0.0611	0.00183	0.0611±0.00228	3.0
0.0800	80.00	39.512	0.0790	0.00221	0.0790±0.00275	2.8
0.1000	100.00	49.390	0.1020	0.00286	0.1020±0.00355	2.8
0.2000	200.00	98.780	0.1980	0.00515	0.1980±0.00639	2.6
0.4000	400.00	197.560	0.4060	0.00974	0.4060±0.01210	2.4
0.5000	500.00	246.950	0.5015	0.01103	0.5010±0.01370	2.2
0.6000	600.00	296.340	0.6032	0.01206	0.6032±0.01498	2.0
0.8000	800.00	395.120	0.8073	0.01534	0.8073±0.01903	1.9
1.0000	1000.0	493.900	0.9940	0.01690	0.9940±0.02098	1.7

Table 3: Determination of flucloxacillin using SWAdSVA at frequency 120 Hz, on HMDE in 0.04 M LiClO4 buffer at pH 1.38, tacc 80, Eacc +150 mV (n=5, t=2.776.)

Taken x_i			Found, $\bar{x}$,µM	SD, µM	$\bar{x} \pm \frac{t.SD}{\sqrt{n}}$, µM	RSD%
µM	nM	ng.mL^{-1}				
0.0050	5.000	2.469	0.0052	0.00017	0.0052±0.00027	3.2
0.0080	8.000	3.951	0.0080	0.00022	0.0080±0.00028	2.8
0.0100	10.000	4.939	0.0098	0.00025	0.0098±0.00031	2.5
0.0300	30.000	14.817	0.0297	0.00071	0.0297±0.00089	2.4
0.0600	60.000	29.634	0.0596	0.00131	0.0596±0.00162	2.2
0.1000	100.000	49.390	0.0988	0.00217	0.0988±0.00270	2.2
0.2000	200.000	98.780	0.2028	0.00426	0.2028±0.00530	2.1
0.3000	300.000	148.170	0.3022	0.00604	0.3022±0.00751	2.0
0.4000	400.000	197.560	0.3978	0.00716	0.3978±0.00889	1.8
0.5000	500.000	246.950	0.4997	0.00750	0.4997±0.00931	1.5

Table 4: Determination of flucloxacillin using SWAdSVA at frequency 120 Hz, on HMDE in 0.04 M LiClO4 buffer at pH 1.38, tacc 160, Eacc +150 mV (n=5, t=2.776.)

Taken x_i			Found, $\bar{x}$,nM	SD, nM	$\bar{x} \pm \frac{t.SD}{\sqrt{n}}$, nM	RSD%
µM	nM	ng.mL^{-1}				
0.0006	0.600	0.296	0.594	0.02257	0.594±0.02280	3.8
0.0008	0.800	0.395	0.796	0.02786	0.796±0.03459	3.5
0.0010	1.000	0.494	1.028	0.03290	1.028±0.04084	3.2
0.0016	1.600	0.790	1.640	0.04920	1.640±0.06108	3.0
0.0020	2.000	0.988	2.000	0.05400	2.000±0.06704	2.7
0.0040	4.000	1.976	3.975	0.09938	3.975±0.12337	2.5
0.0060	6.000	2.963	5.990	0.13777	5.990±0.17104	2.3
0.0080	8.000	3.951	7.984	0.16766	7.984±0.20816	2.1
0.0100	10.000	4.939	10.023	0.20046	10.023±0.24887	2.0

The limits of detection (LOD) and quantitation (LOQ) were 2.40 nM and 7.20 nM by SWV and LOD 0.62 and 1.86 nM , LOQ 0.073 and 0.22 nM at accumulation time 80 and 160 s respectively.

6. The proposed mechanism of flucloxacillin on HMDE

FLUX contains different functional groups obtained by electrochemical reactions, several reduction mechanisms, several reduction mechanisms may be proposed. The influence of electrochemical parameters known to affect the SWV, it is postulated that the isoxazole ring is the site of reduction (1) at pH 4.5. The mechanism by SWAdSVA at pH 1.35 could be suggested for the voltammetric reduction C=N group in isoxazole ring (2). The electrochemical reaction is suggested to proceed as follows:

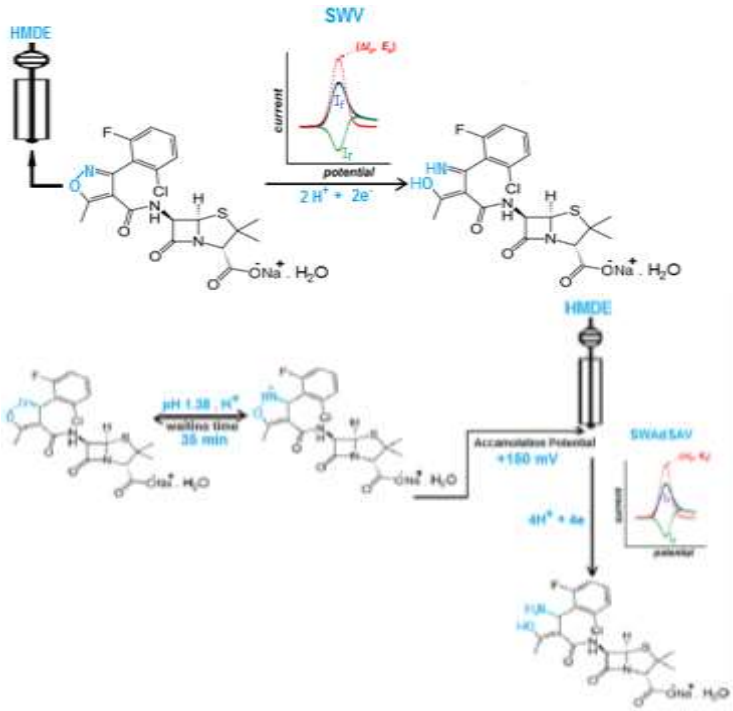


Figure 8: Electrochemical mechanisms of flucloxacillin by SWA and SWAdSVA.

7. APPLICATIONS

The proposed SWV and SWAdSVA voltammetric procedure was successfully applied to direct determination of FLUX in the commercial Syrian pharmaceutical preparations (in presence a same amount of amoxicillin) on HMDE in 0.04 M $LiClO_4$ buffer using SWV at pH 4.5, frequency 160 Hz and SWAdSVA at pH 1.38, frequency 120 Hz tacc 80 s, and Eacc +150 mV (as labeling to contain 250 mg

FLUX per capsule as an individual drug) without the necessity for samples pretreatment and/or time-consuming extraction steps prior to the analysis. 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 10000 for all pharmaceuticals content 250 mg/cap. The mean percent-age recovery of FLUX, based on the average of five replicate measurements was found to be 99.6 to 102.2% and 99.8 to 102.6% using SWV and SWAdSVA respectively using the calibration curve method. The results of quantitative analysis for FLUX in pharmaceutical preparations were summarized in Table 5.

Table 5: Determination of FLUX in some Syrian pharmaceutical preparations using SWV(at pH 4.5, frequency 160 Hz) and SWAdSVA(at pH 1.38, frequency 120 Hz tacc 80 s, and Eacc +150 mV) on HMDE according to the optimal conditions(n=5).

Commercial name	method	Label Claim of FLUX & AMOX, mg/cap.	Mean ±SD (as FLUX), mg/ cap.	RSD%	Assay %
Amoxipen capsule,	SWV	250	250.00 ± 6.500	2.6	100.0
	SWAdSVA		250.50 ± 5.761	2.3	100.2
Amoxam capsule, IBN	SWV	250	251.25 ± 7.035	2.8	100.5
	SWAdSVA		253.25 ± 6.078	2.4	101.3
Penifloxam capsule, APHAMEA	SWV	250	255.50 ± 7.154	2.8	102.2
	SWAdSVA		256.50 ± 5.643	2.2	102.6
Floxin capsule, ALBALSAM	SWV	250	249.00 ± 6.723	2.7	99.6
	SWAdSVA		249.50 ± 5.489	2.2	99.8
Maxipen capsule, ASIA	SWV	250	253.50 ± 7.098	2.8	101.4
	SWAdSVA		254.00 ± 5.334	2.1	101.6

8. Method validation [18]

8.1. Linearity and Sensitivity (limit of detection [LOD] and limit of quantitation [LOQ])

In the proposed methods, linear plots (n=5) with good correlation coefficients were obtained on HMDE electrode vs. Ag/AgCl in an aqueous 0.04 M $LiClO_4$ buffer at values pH 4.5 and 1.38. In the technique SWV at pH 4.5, frequency 160 Hz. Linear calibration graph were the concentration ranges of 20.00 – 1000 nM. In

the technique SWAdSVA at pH 1.38, accumulation potential of +150 mV and accumulation time 80 s and 160 s, frequency 120 Hz. Analytical curves were obtained for application in the range of 5.00-500 nM and 0.60-10.00 nM respectively, The limits of detection (LOD) and quantitation (LOQ) were 2.40 nM and 7.20 nM by SWV and LOD 0.62 and 1.86 nM , LOQ 0.073 and 0.22 nM at accumulation time 80 and 160 s respectively, see table 6.

Table 6: Analytical parameters for determination of FLUX using SWV and by SWAdSVA methods

Parameter	SWV	SWAdSVA	
		tacc , 80 s	tacc ,160 s
Regression equations (y, μA , x, μM)	y = 0.8682x +0.0002	y = 115.6551x +0.0004	y = 215.4304x +0.038248 or y = 215.6696x +38.2483 y, nA , x, nM
concentration range, M	2×10^{-8} to 1×10^{-6}	5×10^{-9} to 5×10^{-7}	6×10^{-10} to 1×10^{-8}
concentration range, $ng.mL^{-1}$	9.878- 493.900	2.469- 246.950	0.296 - 4.939
R^2	0.9998	0.9998	0.9999
Low concentration, nM	20.00	5.00	0.60
RSD%	4.0	3.2	3.8
LOD, nM	2.400	0.620	0.073
LOQ, nM	7.200	1.860	0.220

8.2. 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 0.050 μM (50.00 nm) .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 7.

Table 7 : Results of recovery studies (n=5).

Level	Recovery%	
	SWV	SWAdSVA
80%	100.1	100.7
100%	99.2	99.8
120%	101.2	101.5

8.3. Repeatability

The repeatability was evaluated by performing 10 repeat measurements for 0.100 µM of FLUX using the studied methods under the optimum conditions. These values indicate that the proposed methods have high repeatability for FLUX analysis, see Table 8.

Table 8: Results of Repeatability studies

method	$\bar{x}$ µg.mL^{-1}	SD, µg.mL^{-1}	Recovery %	RSD %
SWV	0.098	0.0028	98.0	2.9
SWAdSVA at (80 s)	0.102	0.0025	102.0	2.4

8.4. Robustness

The robustness of the method adopted is demonstrated by the constancy of the current peak (Ip) with the deliberated minor change in the experimental parameters such as the change in the concentration of excipients, temperature (25 ±5^{o}C), pH (4.5 ±0.30, 1.38 ±0.05), reaction waiting time (10 min) and accumulation potential (+150 ±15 mV), frequency (160 ±5 Hz, 120 ±5 Hz). This table indicates that the robustness of the proposed methods was good (Ip was measured and assay was calculated for five times) see Table 9.

Table 9: Robustness of the proposed SWV and SWAdSVA methods at HMDE for determination of flucloxacillin (n=5 calculated for five times).

Experimental parameter variation	Average recovery (%) C_{FLUX} = 0.050 µM	
	SWV	**SWAdSVA**
Temperature		
20^{o}C	99.7	99.8
25^{o}C	100.4	100.3
30 ^{o}C	100.3	100.5
pH		
4.2	99.9	-
4.8	100.1	-
1.33	-	99.7
1.43	-	100.2
Reaction time		
25 min	98.8	99.2
35 min	100.1	100.3
60 min	100.8	101.6
Accumulation potential		
145 mV	-	100.4
155 mV	-	101.3
frequency		
155 Hz	99.5	-
165 Hz	99.8	-
115 Hz	-	100.2
125 Hz	-	99.9

8.5. 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.

9. Conclusion

The electrochemical behavior of flucloxacillin in pure form and in pharmaceutical preparations at hanging mercury drop electrode was examined in 0.04 M $LiClO_4$ buffer at pH 4.5 and 1.38 by SWV and SWAdSVA Both the techniques gave good results but SWAdSVA is more sensitive than SWV. In the technique SWV a voltammeric peak is obtained at -9.68 V to -9.88 V vs. Ag/AgCl at pH 4.5, frequency 160 Hz.. In the technique SWAdSVA voltammeric peak is obtained at -1.55 V to -1.72 V vs. Ag/AgCl at pH 1.38, accumulation potential of +150 mV and an accumulation time 80 s and 160 s, frequency 120 Hz. Ip =f (C_{FLUX}) for the determination of FLUX by SWV showed excellent linear 0.020 -1.000 μM and SWAdSVA at tacc 80 and 160 s, showed too excellent linear 0.005-0.500 μM (5.00-500 nM) and 0.0006-0.010 μM respectively. These methods give a good results for the determination of FLUX in pure and different dosage forms.

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