Indirect Determination of Amitriptyline Hydrochloride by Square Wave Voltammetry of $SnCl_2.2H_2O$

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Abstract
A simple, fast and sensitive electroanalytical method consist studying the square wave of $SnCl_2.2H_2O$ at hanging mercury dropping electrode in aqueous solution for the indirect determination of (AMI) drug. $SnCl_2.2H_2O$ has revealed a major reduction potential at (-0.450) Volt against the reference electrode (Ag/AgCl/Sat.KCl). The calibration curve in the Phosphate buffer as the supporting electrolyte at pH=2 was constracted. The range concentrations up to ($0.99X10^{-5}$–$9.91X10^{-5}$) M, the correlation coefficient (r= 0.9989) and then studied the decreased of peak current of the drug by the (HMDE) behavior in the presence of $SnCl_2.2H_2O$. Results were as follow: The calibration curves were ($0.316\ X10^{-6}$ -$3.136\ X10^{-6}$) M and thismethods was successfully applied to determination of AMI in apharmaceutical formulations.
***Keywords**: Amitriptyline hydrochloride; Square wave voltammetry.*

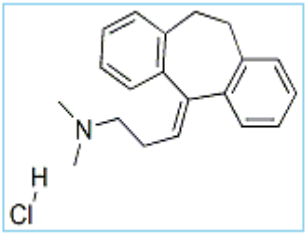
التقدير غير المباشر لعقار هيدروكلوريد الامتربتلين بوساطة فولتامتري مربعة الموجة لكلوريد القصديروز المائي

سهام توفيق امين علي ابراهيم خليل أسماء أحمد محمد

الخلاصة
طريقة كهروكيميائية بسيطة وحساسة وسريعة تتضمن دراسة لفولتامتري الموجة المربعة لتقدير كلوريد القصديروز المائي عند قطب قطرة الزئبق المعلقة في محلول مائي للتقدير غير المباشر لعقار هيدروكلوريد الامتربتلين وقد اعطى المركب قمة اختزال عند (-0.450) فولت مقابل قطب فضة /كلوريد الفضة كقطب مرجع.وكان مدى التراكيز في المحلول المنظم للفوسفات عند دالة حامضية (2) ($0.99\ X10^{-5}$- $9.91X10^{-5}$) مولاري وبمعامل ارتباط 0.9989 ثم درس التناقص في قمة الاختزال بوجود العقار وكان مدى التركيز($0.316\ X10^{-6}$-$3.136\ X10^{-6}$) مولاري وطبقت الطريقة بنجاح على المستحضرات الصيدلانية مع قيم عالية للاستردادية لم تقل عن 99.72.

Introduction

Amitriptyline hydrochloride (AMI). 3-(10, 11- dihydro-5H-dibenzo-[a,d]-cyclohepten-5-ylidene)-N,N-dimethylpropan-1-amine hydrochloride , (Brand Name: Elavil, Endep, and Vanatrip) [1]. Its structure is:



And its structural formal is C_{20} H_{24}ClN: molecular weight 313.86, melting point: 196 -197 ° C, which is a white powder or colorless crystals soluble in water, alcohol and methylene chloride. This drug is one of the group of tricyclic drugs .It is used in the treatment of depression or anxiety or chronic insomnia and helps in adjusting the levels of the chemical compounds in the brain such as noradenalin which are responsible for the psychological state. It is also used for the treatment of nocturnal urination especially in children over the age of six years and to prevent migraine headaches [2] ,in addition to treatment pain and numbness and severe twitching resulting from the pain of spine [3].This drug is determined in different analytical methods, GC/MS within the range of 80 – 5000 ng/L and RSD% was found to be not more than 5.5 % [4].Amitriptyline HCl and perphenazine were determined simultaneously the isoabsorptive point for both drugs was 253.20 nm in tablet dosage form[5].Eriochrome cyanine R(ECR) and pyrocatechol violet (PCV) have been tested as reagent for the determination of amitriptyline (AMI) [6]. Liquid- membrane ion-selective electrodes that respond to the cationic forms of chlorpromazine to the quaternary ammonium compound propantheline [7].Isocratic RP-HPLC method was developed by T.Reddy *et. al.* [8] for the determination of amitriptyline HCl in pure and dosage forms method [9] based on high performance liquid chromatography with electrospray ionization mass spectrometry (HPLC-MS/ESI) has been developed for the simultaneous determination of amitriptyline and nortriptyline in plasma of rat. The construction and performance characteristics of potentiometric amitriptyline–plastic membrane sensors, based on ion-pair complexes with triphenylstibenyl borate and tetra(2-chlorophenyl)borate, respectively[10].A novel low-cost amperometric method of sensor based on thick-film technique is presented and used to determine tricyclic antidepressant drug AMI[11].A simple spectrophotometric method for the estimation of chloride ion in AMI based on reaction between chloride ion and mercuric thiocyanate, forming a coloured complex absorbs at 460nm [12]. The present work aim to develop indirect method for determination of AMI through the square wave voltammetric behavior of $SnCl_2.2H_2O$.

Experimental

Apparatus

Voltammetric measurements were carried out using a metrohm instrument, model 797VA, computerized HMDE (hanging mercury dropping electrod) as a working electrode, and (Ag/AgCl-Sat KCl) as

reference electrode and a platinum wire as an auxiliary electrode. The pH of the solutions was controlled with a (Jenway 3310 UK) pH meter**.**

Reagents

All of the chemicals used were of analytical-reagent grade. The supporting electrolyte used for all experiments was phosphate buffer which was prepared by mixing (1:1) a certain volumes of (0.2) M of each of $Na_2HPO_4.2H_2O$, $NaH_2PO_4.2H_2O$. Phosphate buffer (pH=2) was prepared by dissolved (0.78) g of $NaH_2PO_4 .2H_2O$ in 0.34 ml of phosphoric acid (85%) and the volume was completed to100ml by distilled water.

Hydrous Tin Chloride ($SnCl_2.2H_2O$) 0.01 M

was prepared by dissolving 0.0225 g in small amount of 0.1M HCl and the volume was completed to 10 ml by distilled water .

Amitriptyline HCl (AMI) standard solustion 0.00318M.

Prepared by dissolved 0.1000 g of (AMI) in a small amount of distilled water and the volume was completed by distilled water in a 100 ml volumetric flask, the least concentrations ranged were prepared by appropriate dilution.

Preparation of pharmaceutical form solution0.0079 M.

A homogenized powder was prepared from ten accurately weighed Amitriptyline HCl tablets (Amitriptyline Tablets 25 mg (actavis, Barnstaple, EX32 8NS, UK). An appropriate amount (0.10339gm) equivalent to one tablet was dissolved in distilled water. Dissolution of the drug was assisted by means of a magnetic stirring and an ultrasonic bath .the mixture was then filtered and made up to the mark with distilled water in 100ml volumetric flask to obtain solution (0.0079M) and the least concentrations were prepared by sequential dilution .

Procedure

Standardization of instrument:

A square wave voltagram (SWV) has been recorded for10ml in acetate buffer solution at pH=4.5 in a clean and dry cell after degassing for 300 second by nitrogen gas under experimental conditions as shown in table (2),then 50 µl of (250 ppm) of Cu(II) ,Pb(II) ,Cd(II) and Zn(II) solution is added and SWV have been recorded for these ions, after being degassed for 15sec.by N_2 gas and compared with values of standard voltammetric diagram. (Figure1).

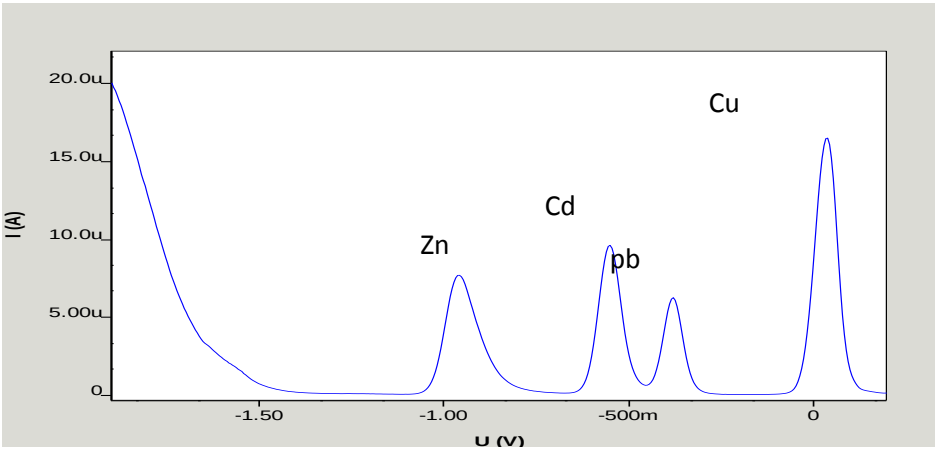


Fig. (1):- SWV voltammogram of 250 ppm of (Cu ,Cd ,Pb and Zn)(II)

Results and discussion

Preliminary investigations.

The SWV(HMDE) of (4.98 $X10^{-5}$) M $SnCl_2.2H_2O$ in different phosphate buffers

(2-10) were recorded for the sample using a dry cell containing 10 ml of each buffer solution after degassing for 300 sec. The optimum pH for determination the sample is pH=2 which gave the heights diffusion current (Ip) at potential -0.450[13]v (Ep) (table 1 and Figure 2).

Table (1):- Effect of different phosphate buffers on SWV of $SnCl_2.2H_2O$.

pH	Potential (Ep) V	(Ip) nA
2	**-0.450**	**410**
3	-0.466	350
4	-0.483	203
5	-	-
6	-0.585	161
7	-0.602	152
8	-0.632	110
9	-	-
10	-0.68	98

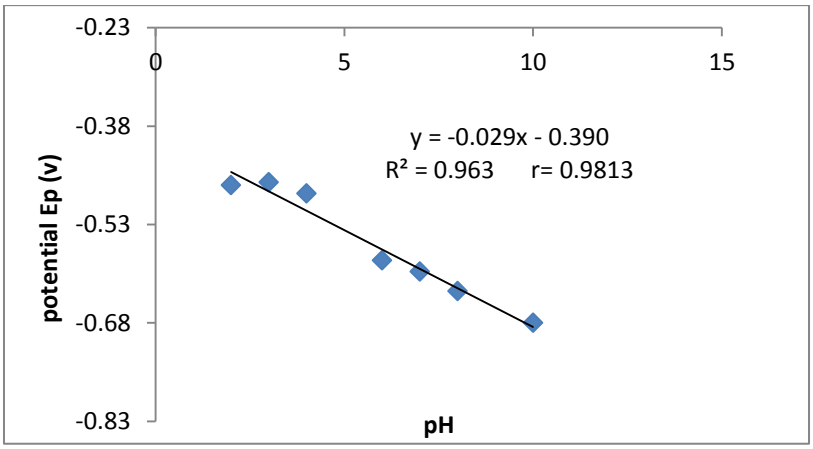
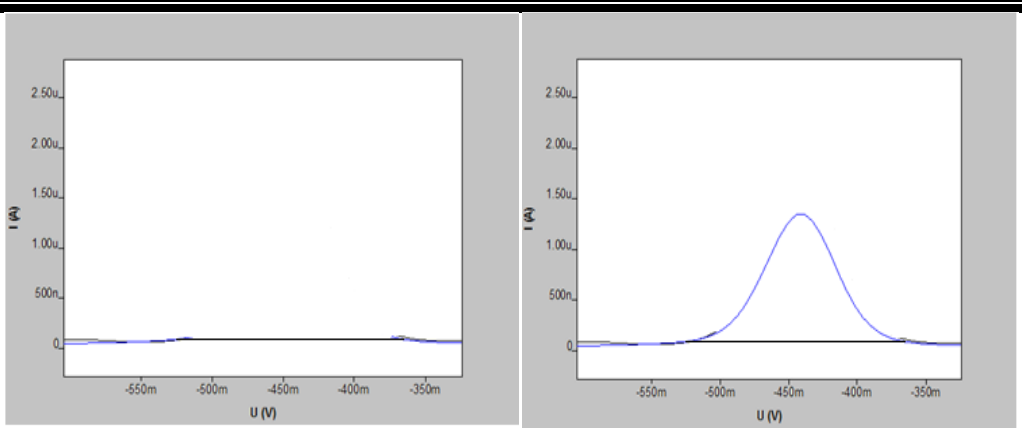


Fig. (2):- Effect of different phosphate buffers on SWV of $SnCl_2.2H_2O$.

The result shown in table (1) and (Figure 2) indicates that pH=2 phosphate buffer ave maximum current values and have been used for determination. The slope close is to value of Nernest equation for divalent species. Depend on this result recorded solution of ($4.98X10^{-5}$) M $SnCl_2.2H_2O$ in 10 ml phosphate buffer pH=2 at Instrumental parameter after degassing the solution by N_2 gas for 300 sec., table (2) shows that

Table (2):- Instrumental parameter

Parameter	Values
Start potential	-1.99v
End potential	+ 0.199v
Drop size	$4mm^2$
Voltage step	0.005v
Pulse amplitude	0.02v
Frequency	50.000Hz
Sweep rate	0.252(v/s)
Equilibrium time	3.000 sec.
Deposition time	60.000sec.
Deposition potential	-0.9v



A B

Fig (3):- A- SWV voltammogram for the buffer solution (pH=2).
B- SWV voltammogram of $SnCl_2.2H_2O$ in buffer solution (pH=2).

Study of optimum conditions

A set of SWV experiments were carried out for deareated solution of ($2.998X10^{-5}$) M of $SnCl_2.2H_2O$ in buffer solution pH=2.The measurements were performed by changing one of the operating conditions and fixing the parameters. The summary of optimum values that gives the highest peak current for ($2.998X10^{-5}$) M of $SnCl_2.2H_2O$ are shown in Table (3).

Table (3):- The optimum values which give the highest peak current for $SnCl_2.2H_2O$.

Conditions	Values
Voltage step V	0.0060v
Pulse amplitude V	0.0700v
Frequency Hz	60.0000Hz
Sweep rate (v/s)	0. 3570 (v/s)
Equilibrium time sec.	6.0000 sec.
Deposition potential V	-0.6000v
Deposition time sec.	60.0000sec.
Drop size mm^2	4.0000mm^2

Calibration curve of $SnCl_2.2H_2O$ solutions.

The SWV of different concentrations of (0.01) M $SnCl_2.2H_2O$ between (10 -100 µl) equivalent to ($0.99X10^{-5}$ – $9.91X10^{-5}$) M have been recorded at optimum conditions at Ep = - 0.45 as in (table 3) in 10 ml phosphate buffer pH=2 after degassing for 15 sec. For each sample, (Figure 4) the current plotted against the $SnCl_2.2H_2O$ concentration.

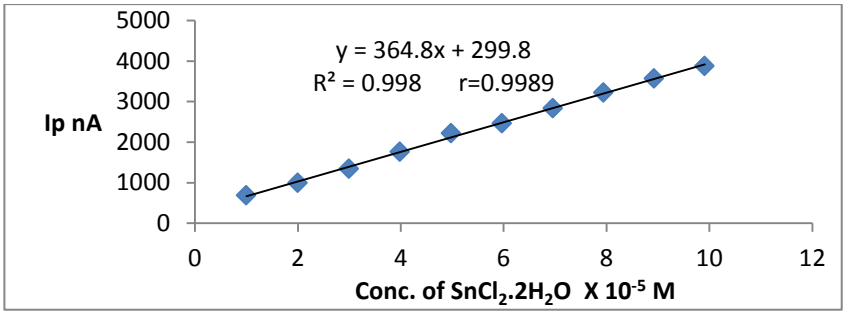


Fig.(4):- Calibration curve of ($0.99X10^{-5}$ – $9.91X10^{-5}$) M of $SnCl_2.2H_2O$.

The figure shows a linear relationship between Ip and concentration with correlation coefficient r = 0.9989, (Figure 5) shows the voltammogram of different concentrations of $SnCl_2.2H_2O$.

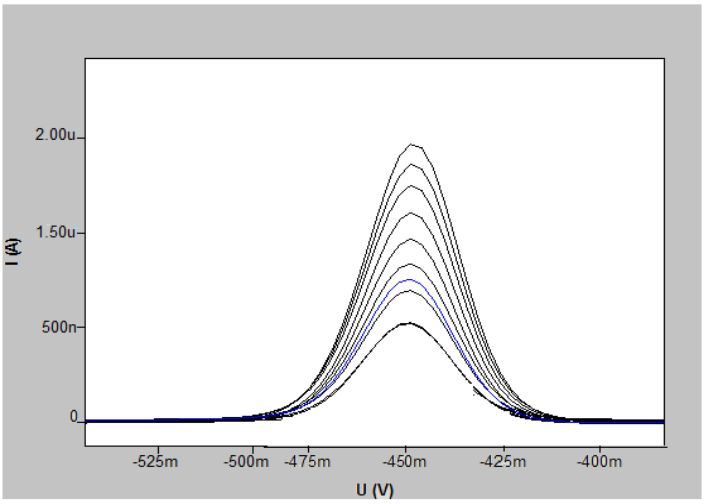


Fig. (5):- Voltammograms of different concentrations X $10^{-5}$of $SnCl_2.2H_2O$ solution.

The value (0.9989) of correlation coefficient and 0.4360X10^{-7}M for detection limit indicating a good linearity and sensitivity. Statistical treatment of the calibration curve results showed that values of RSD%, limit of confidence at 95% and relative error does not exceed 0.1543, 3.795 and (-4.639–0.0965) respectively. The recovery was ranged between (95.364-103.990). These values (Table 4 and 5) indicates a good precision and accuracy.

Table (4):- Statistical treatment results of the calibration curve of $SnCl_2.2H_2O$.

Conc. ($SnCl_2.2H_2O$) X10^{-5} M	*Measured response (nA)	(S)	(RSD%)	confidence limits %95
0.99	688.00	1.000	0.1453	688.00 ± 2.480
1.98	989.33	1.527	0.1543	989.33 ± 3.795
2.99	1340.00	1.000	0.0746	1340.00 ± 2.479
3.98	1756.66	0.7018	0.0399	1756.66 ± 1.743
4.98	2217.67	0.7018	0.0316	2217.67 ± 1.743
5.97	2461.00	1.0000	0.0406	2461.00 ± 2.479
6.95	2835.34	1.5270	0.0538	2835.34 ± 3.795
7.94	3218.67	1.0000	0.03107	3218.67 ± 2.48
8.92	3567.67	0.7018	0.0196	3567.67 ± 1.743
9.91	3874.34	1.5270	0.0394	3874.34 ± 3.795

*n=3

Table (5):- Accuracy and precision for the $SnCl_2.2H_2O$.

Conc. ($SnCl_2.2H_2O$) X10^{-5} M	*Measured response (nA)	Calculated value	Rec%	RE%
0.99	688.00	664.6.000	96.5980	-3.4011
1.98	989.33	1025.3872	103.5486	3.5486
2.99	1340.00	1393.4704	103.9903	3.9903
3.98	1756.66	1754.6224	99.8840	-0.1159
4.98	2217.67	2119.4224	95.3644	-4.6355
5.97	2461.00	2480.9392	100.8102	0.8102
6.95	2835.34	2838.0784	100.0965	0.0965

7.94	3218.67	3197.7712	99.3507	-0.6492
8.92	3567.67	3556.7344	99.6953	-0.3065
9.91	3874.34	3917.8864	101.1239	1.1239

*n=3

Stability of $SnCl_2.2H_2O$.

SWV using ($2.998X10^{-5}$) M of $SnCl_2.2H_2O$ in 10ml phosphate buffer pH=2 after degassing by N_2 gas for 300sec. were recorded under optimum conditions and at different times. The results obtained are shown in Table (6).

Table (6):- Stability of $SnCl_2.2H_2O$ on Ip

Time (min)	Ip (nA)
0	1340
5	1344
10	1343
15	1342
20	1340
25	1342
30	1343
35	1330
40	1329
45	1330
50	1317
55	1315
60	1309

Table (6) shows that $SnCl_2.2H_2O$ is stable for at least (45) min. which is quite enough to perform all the voltammetric measurements.

Indirect SWV determination of Amitriptyline hydrochloride (AMI) drug.

Applying the optimum conditions , the SWV has been recorded on degassed solution of 4.988 $X10^{-5}$ $SnCl_2.2H_2O$ in 10 ml phosphate buffer pH=2 which gives peak current $Ip_{\circ}$=2259nA. A decrease to 1221 nA of the peak is observed when $1.575X10^{-6}$ M of (AMI) was added. so,

$$\Delta Ip = Ip° - Ip$$

$$2259 - 1221 = 1038$$

This behavior is due to interaction of $SnCl_2.2H_2O$ after reduced to Sn^{+4} with AMI, (Figure 6)

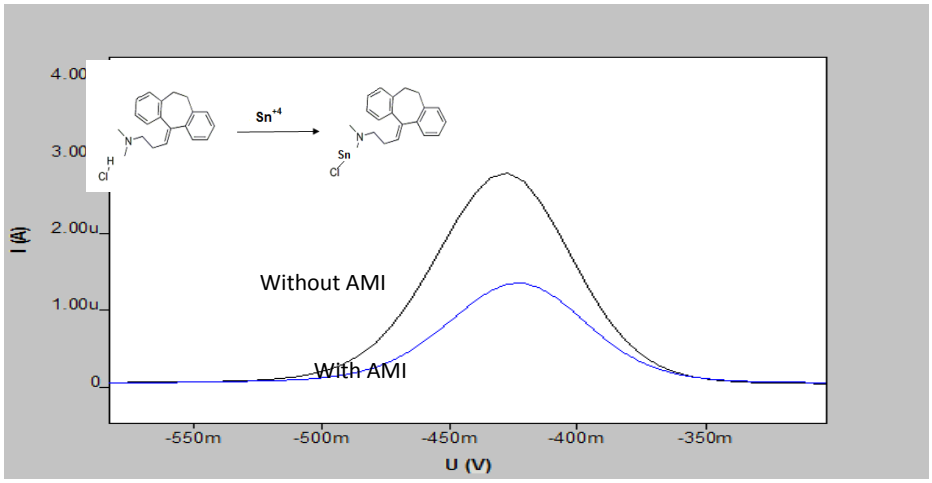


Fig. (6):- Voltammogram of $SnCl_2.2H_2O$ with and without AMI drug.

The calibration curve of AMI in the presence of $SnCl_2.2H_2O$

The SWV has been recorded on degassed solution of 4.988 $X10^{-5}$ M $SnCl_2.2H_2O$ in 10ml phosphate buffer at pH=2, Successive amounts of AMI ($0.318X10^{-3}$) M between (10-100) µl were then added and the SWV was recorded after each addition .The peak current Ip of $SnCl_2.2H_2O$ was found to be decreased gradually with the addition of AMI.The plot of (ΔIp) versus concentration of AMI drug added is shown in (Figure 7).

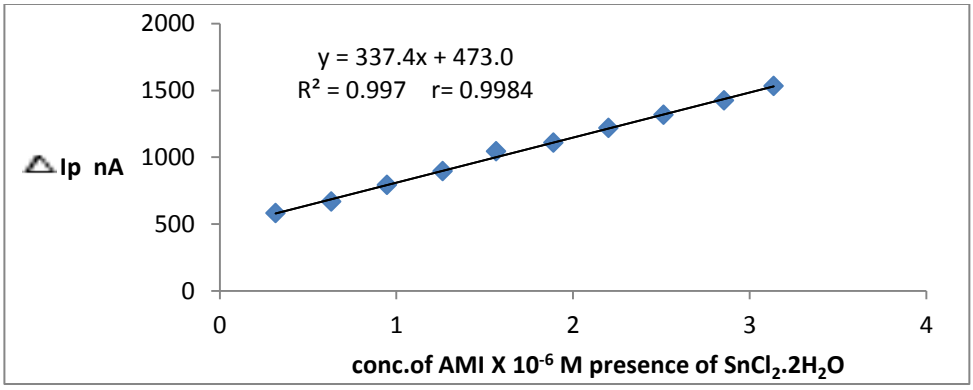


Fig. (7):- Calibration curve of ($0.316X10^{-6}$ – $3.136X10^{-6}$) M of AMI in the presence of $SnCl_2.2H_2O$.

Table (7):- Statistical treatment of the results of the calibration curve of AMI in the presence of $SnCl_2.2H_2O$.

Conc. (AMI) $X10^{-6}$ M	*(ΔIp) (nA)	(S)	(RSD%)	confidence limits %95
0.316	581.00	1.000	0.0595	581.00 ± 2.4844
0.632	666.34	1.527	0.0959	666.34 ± 3.7936
0.947	791.00	1.000	0.0681	791.00± 2.4844
1.261	895.00	1.000	0.0733	895.00± 2.4844

1.575	1042.00	2.000	0.1643	1042.00 ± 4.968
1.889	1108.67	1.680	0.1460	1108.67±4.1743
2.201	1219.00	2.000	0.1923	1219.00± 4.968
2.513	1315.67	1.680	0.1781	1315.67 ± 4.174
2.825	1423.00	2.000	0.2392	1423.00 ± 4.968
3.136	1532.00	2.000	0.2715	1532.00 ± 4.968

*n=3

Table (8):- Results of accuracy for the determination of AMI in the presence of $SnCl_2.2H_2O$.

Conc. (AMI) $X10^{-6}$ M	*(ΔIp) (nA)	Calculated value	Rec%	RE%
0.316	581.00	579.7533	99.7854	-0.2145
0.632	666.34	686.3042	102.9961	2.9961
0.947	791.00	792.6190	100.2046	0.2046
1.261	895.00	898.5288	100.3942	0.3942
1.575	1042.00	1004.4387	96.3952	-3.6047
1.889	1108.67	1110.3486	100.1514	0.1514
2.201	1219.00	1215.6174	99.7225	-0.2774
2.513	1315.67	1320.8862	100.3964	0.3964
2.825	1423.00	1426.3237	100.2335	0.2335
3.136	1532.00	1531.0864	99.9403	-0.0596

*n=3

Stability of AMI in the presence of $SnCl_2.2H_2O$.

SWV of $SnCl_2.2H_2O$ ($2.998X10^{-5}$) M + (AMI) 1.5751 $X10^{-6}$ M in 10ml phosphate buffer pH= 2 after degassing by N_2 gas for 300sec. were recorded under optimum conditions and at different times. The results obtained are shown in table (9)

Table (9):- Effect of time on SWV of AMI in the presence of $SnCl_2.2H_2O$.

Time (min)	ΔIp (nA)
0	1045
5	1043
10	1040
15	1038
20	1035
25	1031
30	1028
35	1025
40	1021
45	1020
50	1017

55	1014
60	1010

As it is clear from table (9) there is a gradual decrease in the Ip value and it is possible to record the measurement within (20) min.

Application of the method for the determination of (AMI) drug in pharmaceutical formulations.

AMI drug is determined in tablet pharmaceutical preparation after preparing a series of solutions. The measurements was achieved by direct method of the calibration curve and an average of three readings for each concentration Table (10) shows the concentrations of the drug in the formulation and the relative error and recovery.

Table (10):- Application the method for the determination of pharmaceutical preparation (Amitriptyline Tablets) by direct method in the presence of $SnCl_2.2H_2O$.

Conc. (Amitriptyline Ta.) taken $X10^{-6}$ M	*(ΔIp) (nA)	Conc. (Amitriptyline Ta.) found $X10^{-6}$ M	Rec.%	RE%
0.316	583.00	0.3260	103.0412	3.0412
1.261	897.34	1.2576	99.7206	-0.2793
2.513	1313.00	2.4896	99.0698	-0.9301

* n=3

The results of table (10) shows a good accuracy where the relative error does not exceed 3.0412 and the recovery is not less than 99%.

Conclusion

A simple, fast and sensitive electroanalytical method for the indirect determination of AMI drug based on decreased of peak current for reduction of $SnCl_2.2H_2O$ using SWV technique at HMDE in the presences of Phosphate buffer as the supporting electrolyte at pH=2. A proposed method has been observed a accuracy and precision from the values of relative error and recovery and this method has been applied to determination the drugs in the pharmaceutical formulations with satisfactory results.

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