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SQUARE-WAVE VOLTAMMETRIC TRACE DETERMINATION OF AMINOPHYLLINE IN URINE – APPLICATION FOR PHARMACEUTICAL FORMULATION

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The voltammetric properties of pure aminophylline have been studied by a direct method in the aqueous solution. The substance has revealed a clear and major reduction peak at potential –0.622 V against the reference electrode (Ag/AgCl/sat. KCl).The calibration curve of aminophylline in the ammonium buffer (NH_3/NH_4Cl, pH = 10.0) has been studied under optimum conditions. The relationship has been linearized within the scope of molar concentration (1.09×10^{-6}–2.107×10^{-5}). The correlation coefficient is R = 0.9903. The calibration curve has been also studied in the presence of human urine. The standard addition method was used successfully to determine the drug in urine of the patients as well as for the analysis of drug in the tablets, and compared with standard method.

Keywords: *aminophylline, square wave voltammetry, human urine, drug*

INTRODUCTION

Aminophylline is a chemical compound with the formula $C_{16}H_{28}N_{10}O_5$. It forms water soluble white or slightly yellowish granules or powder [1]. Aminophylline is a component of the broncho-dilator Theophylline with ethylenediamine in 2:1 ratio. Ethylenediamine improves solubility and aminophylline is usually found as a dihydrate [2]. Aminophylline is most common use in the treatment of airway obstruction from asthma or chronic obstructive pulmonary disease (COPD). It is used off-label as a reversal agent during nuclear stress testing. Aminophylline is a nonselective adenosine receptor antagonist and phospho-diesterase inhibitor [3]. Aminophylline has been found to decrease the sedative effects of propofol [4] and to decrease topiramate antiseizure action [5]. Aminophylline relaxes muscles in lungs and chest to allow more air in, decreases the sensitivity of lungs to allergens and other substances that cause inflammation [6]. Aminophylline was originally used in the treatment of bronchial asthma, aminophylline cream have shown to be very capable in cellulite removal and to minimize body fat those areas, it is used as a topical cream [7]. Several methods have been described for its determination. A simple and rapid procedure is described for determination of aminophylline, amobarbital, and ephedrine hydrochloride in a capsule preparation. Aminophylline and amobarbital are determined simultaneously using differential UV spectrophotometry [8]. There is a novel method to determine aminophylline (Ami) with boric acid (BA) by spectrophotometry. The study indicates that at pH 12.0 the absorbance of Ami decreases when BA is added. A simple, rapid, sensitive, and reliable method based on the product of Ami and BA is obtained. Beer's law is obeyed in the range of Ami concentration of (0.20–200 μg/ml). The equation of linear regression is $A = -2.57309\times10^{-4}-0.00355\ C$ (μg/ml), with a linear correlation coefficient of 0.9969 and relative standard deviation (RSD) 0.28 %. The method is successfully applied to the determination of Ami in pharmaceutical samples and mixed serum samples, and average recoveries are in the range of 97.1-105.9 % [9]. Electrochemical behavior of some alkaloids, namely, caffeine, aminophylline, codeine phosphate and papaverine hydrochloride, that are in solution in various combinations or in the presence of other compounds contained in pharmaceuticals or in real samples (urine) has been examined using cyclic voltammetry (CV), square-wave voltammetry (SWV), and differential pulse voltammetry (DPV) on electrochemically activated glassy carbon electrode [10]. There is

another method used for determination of aminophylline by cathodic stripping voltammetry (CSV) in Britton–Robinson (BR) buffer, pH 7.5 at a hanging mercury drop electrode. The detection limit was 3×10^{-8} M for 60 s accumulation at –0.6 V versus Ag/AgCl reference electrode. A linear range was demonstrated up to 5×10^{-7} M using CSV. The electrochemical behavior of aminophylline on glassy carbon electrodes, carbon paste electrode, and on electrodeposited platinum electrodes was investigated in BR buffer (pH 2.59 and 8.08), phosphate buffer (pH 2.12 and 6.06), acetate buffer, and aqueous medium containing supporting electrolyte. The effects of factors such as deposition material, time, and concentration of platinum on the precision of the analysis have been explored. A comparison is made between the detection limit of glassy carbon, carbon paste and thin–film modified electrode. The electrooxidation process is applied to the quantitative determination of thigh creams and in the treatment of asthma products. Comparision with the results obtained from high performance liquid chromatography shows good agreement [11].

This paper describes a novel method using square wave voltammetry (SWV) successfully applied to trace determination of aminophylline in both human urine and pharmaceutical formulations.

EXPERIMENTAL

Apparatus. All the experiments were performed using a 797 VA-Computrace from Metrohm company (Switzerland). A three electrode systems were used. The working electrode was HMDE; the reference electrode was Ag/AgCl, saturated KCl electrode and the counter electrode was a Pt-wire one. The pH measurements were made using a pw 9421-Philips pH-meter.

Reagents. All the chemical reagents used were of analytical grade, aminophylline was obtained from Fluka, solutions of 5.47×10^{-3} M were prepared of 0.250 g of aminophylline dissolved in deionised distilled water, completing the final volume to 10 ml using a volumetric flask. All the solutions were prepared with deionised distilled water. NH_3/NH_4Cl buffer (pH = 7.8) solution [12] was prepared by mixing 0.5 ml of 2 M ammonia solution with 9.5 ml of 2 M ammonium chloride solution and then the volume was completed until 100 ml, the pH of buffer will be 7.8. Eufilin tablets from Samara (Iraq).

Procedure. The square wave voltammetry mode was used with deposition time 100 s; condition time 20 s; equilibrium time 5 s; frequency 120 Hz; Scan increament 2 mV/s; conducting potential 0.000 V; Pulse height 0.04 mV. The solution was deaerated by passing through it a slow stream of purified nitrogen gas for 240 s to remove the dissolved oxygen. The square wave voltammogram was recorded on a degassed phosphate buffer solution at (pH = 7.0) (5 ml). The back current was recorded, appropriate amount of aminophylline stock solution were added to this solution to yield the desired concentration and the current – voltage - current was recorded again. A calibration curve was then constructed.

RESULTS AND DISCUSSION

Structure of aminophylline is shown in Fig. 1.

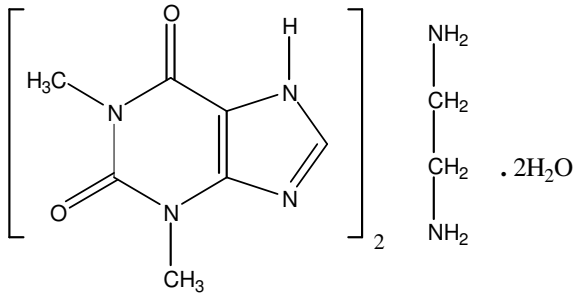

Fig. 1. Chemical structure of aminophylline; M.wt=456.5 g/mol; m.p. = 270–274 °C

The electrochemical behavior of aminophylline gives a typical square wave voltammogram of (8.624×10^{-4}) M aminophylline in NH_3/NH_4Cl buffer at pH=10, as shown in Fig. 2.

It can be seen from Fig. 2 that a well-defined SWV peak appeared at –0.622 V versus Ag/AgCl, saturated KCl electrode.

Optimum conditions. The SWV voltammogram of 8.624×10^{-4} M of aminophylline was investigated in NH_3/NH_4Cl buffer at pH = 10, with variation of all the parameters dependent on the measurement; the optimum values obtained are tabulated in Table 1.

Effect of pH. The square wave voltammograms of 8.624×10^{-6} M of aminophylline were investigated at different pH values of NH_3/NH_4Cl buffer (7–11) using the optimum conditions shown in Table 1. The peak current (Ip) and peak potential (Ep) obtained are shown in Table 2.

The peak current (Ip) is clearly dependent on the pH, maximum current response was found at pH = 10 chosen for the present study; on the other hand, the peak potential (Ep) is found to be greatly dependent on pH and moves to more negative value with increasing pH values. Linear plots of Ep versus pH were obtained as shown in Fig. 3, with correlation coefficient R = 0.9988. The slope (–0.0498 $V \cdot pH^{-1}$) is very close to the theoretical value (0.059) obtained by Hammett [13].

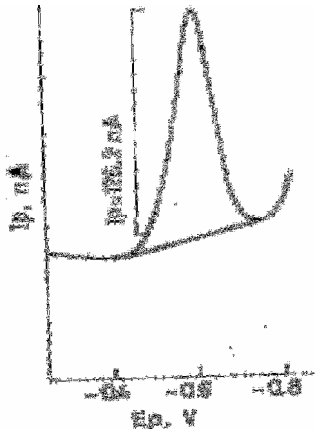

Fig. 2. Square wave voltammogram of (8.624×10^{-4}) M aminophylline

Table 1. The optimum values obtained which give either the highest peak current or the best resolution of 8.624×10^{-4} M of aminophylline

Condition	Value	Condition	Value
initial potential	–0.350 V	Frequency	120 Hz
final potential	–0.820 V	Scan increament	2 mV/s
deposition time	100 seconds	Cond. Potential	0.00 mV
condition time	20 seconds	Pulse height	0.04 mV
equilibrium time	5 seconds		

Table 2. Effect of pH on SWV peak and peak current of 8.624×10^{-6} M of aminophylline

pH	Ep, V	Ip, nA
7	–0.470	30
8	–0.514	80
9	–0.572	137
10	–0.622	229.7
11	–0.665	199.4
	R	0.9988
	R^2	0.9976
	slope	–0.0498
	intercept	–0.1204

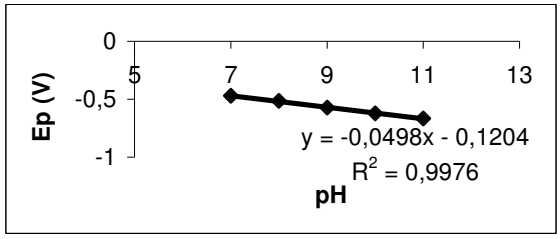

Fig. 3. A relation between Ep and pH of 8.624×10^{-6} M of aminophylline

Stability of aminophylline in aqueous ammonium buffer. The square wave voltammograms of 2.1816×10^{-6} M of aminophylline were recorded at different times in 5 ml of NH_3/NH_4Cl buffer at pH = 10 (Table 3).

Table 3. Effect of time on SWV peak of 2.1816×10^{-6} M of aminophylline at pH = 10 in aqueous solution

Time, min	Ip, nA
15	86.4
18	90.9
21	94.9
24	99.2
27	85.4
30	82.6
33	88.0
36	89.6
39	88.4

It can be seen from the Table 3 that aminophylline is stable for more than 39 min what is quite enough for voltammetric measurement.

Analytical Consideration. Using the optimum condition shown in Table 1, a calibration curve was constructed using 5.476×10^{-4} M of a standard aminophylline in 5 ml of aqueous ammonium buffer (pH = 10). Some typical results are listed in Table 4. These solutions were prepared by adding appropriate aliquots of standard aminophylline to the NH_3/NH_4Cl buffer.

From the results in Table 4 we can see that the values of Ip increase with increasing concentration of aminophylline. Drawing the relation between diffusion current Ip versus concentration of aminophylline gave a straight line with (R = 0.9903) as is explained in Fig. 4.

The plot of peak current (Ip) versus molar concentration of aminophylline is shown in Fig. 4. Regression analysis on standard indicated a straight line. The lowest experimental detection limit was 1.09×10^{-6} M.

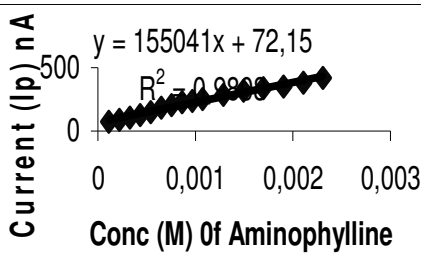

Fig. 4. The relation between peak current (Ip) and concentration of 1.09×10^{-6}–2.107×10^{-5} M of aminophylline at pH = 10 in aqueous ammonium buffer

Effect of concentration (calibration curve of aminophylline) with human urine. Using the optimum condition shown in Table 1, a calibration curve was constructed using a serial dilution of a standard aminophylline in the presence of 50 µl of human urine. Some typical results are listed in Table 5. These solutions were prepared by adding appropriate aliquots of standard aminophylline to the ammonium NH_3/NH_4Cl buffer (5 ml) at pH = 10.

From the results in Table 5 we can see that the values of Ip increase with increasing concentration of aminophylline. Drawing the relation between diffusion current (Ip) versus concentration gave a straight line with (R = 0.9903) as is explained in Fig. 5.

Table 4. Effect of concentration on peak current of 1.09×10^{-6}–2.107×10^{-5} M of aminophylline at pH = 10 in aqueous ammonium buffer at Ep= –0.606 V

Addition × 10^{-6} M	Ip (nA)
1.09	74.1
2.18	89.3
3.26	108
4.34	128.1
5.42	149.9
6.49	184.4
7.56	204.6
8.62	225.4
9.68	240
10.73	252
12.83	285
14.91	320
16.97	337.5
19.02	350
21.06	380
R	0.9903
R^2	0.9808
intercept	72.15

The plot peak current (Ip) versus molar concentration of aminophylline is shown in Fig. 5. Regression analysis on standard indicated a straight line. The lowest experimental detection limit was 6.429×10^{-6} M.

Measurement of aminophylline concentration in human urine from patients using this drug. Using the optimum condition shown in Table 1, the square wave voltammograms were recorded for 10 µl of urine for patients applying aminophylline, after 3–5 h, of each sample in 5 ml of NH_3/NH_4Cl buffer through measuring diffusion current, the concentration of aminophylline calculated using the standard addition method. The results are tabulated in Table 6.

Table 5. Effect of concentration on peak current of 6.429×10^{-6}–2.5830×10^{-5} M of aminophylline at pH=10 in the presence of human urine at Ep= –0.546 V

Concentration (M)×10^{-6}	Ip, nA
6.429	195.0
7.486	208.0
8.539	220.0
9.588	253.0
10.633	272.2
11.673	295.3
12.710	300.0
13.742	320.0
15.796	339.4
17.833	369.3
19.855	390.3
21.862	404.8
23.853	437.0
25.830	451.0
R	0.9903
R^2	0.9809
Intercept	123.3

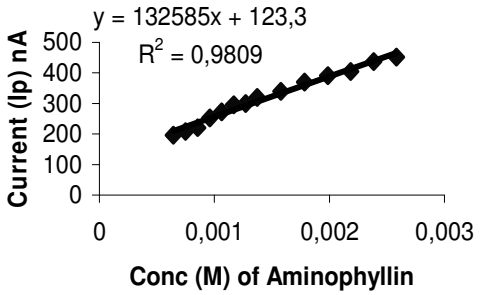

Fig. 5. A relation between peak current (Ip) and concentration of 6.429×10^{-6}–2.5830×10^{-5} M of aminophylline at pH=10 in the presence of human urine at Ep = –0.546 V

Measurement of aminophylline concentration in Eufilin tablet. Using the optimum condition shown in Table 1, the square wave voltammograms

were recorded for 1×10^{-4} M of solution containing Eufilin in 5 ml of NH_3/NH_4Cl buffer (pH=10) through measuring diffusion current, the measuring was taken concentration in order to calculate the recovery ratio, and compared the result with standard method, data the obtained are tabulated in Table 7.

The results in Table 7 showed no significant difference between two methods (electrical and atomic absorption methods), (t = 0.924) at the level of propability (0.01). Finally, the study has shown no significant difference in found concentration and taken one of eufilin.

Table 6. The results of urine samples for patients who apply aminophylline tablets, in 5 ml of NH_3/NH_4Cl buffer (pH = 10)

No	Ip, nA	Conc., (M)×10^{-6}	Conc., mg/L
1	36.5	1.8886	0.8621
2	42.0	2.1732	0.9921
3	23.0	1.1901	0.5433
4	38.0	1.9662	0.8976
5	34.0	1.7592	0.8031
6	28.0	1.4488	0.6614
7	34.0	1.7592	0.8031
8	37.0	1.9145	0.8740
9	39.0	2.0180	0.9212
10	39.0	2.0180	0.9212
11	31.5	1.6299	0.7440

Table 7. The data of calibration curve of solution containing 2.181×10^{-6}–2.3078×10^{-5} M of Eufilin

By SWV					By colorimetric method		T-test
Taken Concentration, (M)×10^{-6}	Found Concentration, (M)×10^{-6}	Ip, nA	% Recovery	% Error	Found Concentration (M)×10^{-6}	% Recovery	
2.18167	2.5380	111.5	116.3341	+16.3341	2.3944	109.75	0.924
3.2660	3.4281	125.3	104.9637	+4.9637	3.5842	109.74	–
4.3460	3.9634	133.6	91.1972	–8.8027	4.7700	109.75	–
7.5605	8.8912	210.0	117.5998	+17.5998	8.2978	109.75	–
8.6236	10.5037	235.0	121.8012	+21.8012	9.4646	109.75	–
9.6825	11.7936	255.0	121.8037	+21.8037	10.6268	109.76	–
19.0286	17.5986	345.0	92.4850	–7.5149	20.8848	109.76	–
21.0615	19.2111	370.0	91.2139	–8.7860	23.1162	109.76	–
23.0789	21.1460	400.0	91.6248	–8.3751	25.3304	109.79	–

Визначення слідових кількостей амінофілліну у людській сечі методом прямокутної вольтамперометрії та його застосування у фармацевтиці

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Вольтамперометричні властивості чистого амінофілліну досліджено прямим методом у водному розчині. Речовині відповідає чіткий головний пік відновлення при потенціалі –0.622 В відносно референсного електрода (Ag/AgCl/насичений KCl). Калібрувальна крива амінофілліну в амоніачному буфері (NH_3/NH_4Cl, pH = 10.0) вивчена за оптимальних умов. Співвідношення було лінеаризовано в рамках молярної концентрації 1.09×10^{-6}–2.107×10^{-5}. Коефіцієнт кореляції R = 0.9903. Калібрувальну криву вивчено також у присутності людської сечі. Стандартний метод добавок успішно використано для визначення медикаменту в сечовині пацієнтів, а також для його аналізу в таблетках, і порівняно зі стандартним методом.

Ключові слова: *амінофіллін, прямокутна вольтамперометрія, людська сеча, медикамент*

Определение следовых количеств аминофиллина в человеческой моче методом прямоугольной вольтамперометрии и его применение в фармацевтике

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Вольтамперометрические свойства чистого аминофиллина исследованы прямым методом в водном растворе. Веществу соответствует четкий главный пик восстановления при потенциале –0.622 В относительно электрода сравнения (Ag/AgCl/насыщенный KCl). Калибровочная кривая аминофиллина в аммиачном буфере (NH_3/NH_4Cl, pH = 10.0) изучена при оптимальных условиях. Соотношение было линеаризовано в рамках молярной концентрации 1.09×10^{-6}–2.107×10^{-5}. Коэффициент корреляции R = 0.9903. Калибровочная кривая изучена также в присутствии человеческой мочи. Стандартный метод добавок успешно использован для определения медикамента в моче пациентов, а также для его анализа в таблетках и сравнен со стандартным методом.

Ключевые слова: *аминофиллин, прямоугольная вольтамперометрия, человеческая моча, медикамент*

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