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Indirect Electrochemical Determination of Carvedilol through its Interaction with Nitrous Acid

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ABSTRACT

An indirect electrochemical determination of carvedilol (CAR) as pure and in its pharmaceutical tablets through its interaction with nitrous acid has been studied using square wave voltammetry (SWV). Nitrous acid which is prepared instantaneously in a voltametric cell contains a three-electrodes detection system, a glassy carbon electrode (GCE) as the working electrode, Pt-wire as an auxiliary electrode and silver/silver chloride/ saturated potassium chloride (Ag/AgCl. sat. KCl) as a reference electrode, gives a well-defined oxidation peak at 1.35 V vs. Ag/AgCl. saturation KCl. The effect of different pH (buffer types), time and concentration were tested. The calibration curve was constructed under measured optimum conditions by following the decrease in nitrous acid peak as a result of the addition of carvedilol solution. The plot of carvedilol concentration added and the differences in current gives two straight lines, first at a low concentration range (0.499 -5.99) × 10^{-8} M by a calibration equation Y=93.893x-4.9093 and second, at a high concentration range (6.99 - 11.98) ×10^{-8} M by a calibration equation Y=9.2363x+484.69 with correlation coefficient R^2 equal 0.9937 and 0.9574 respectively. The limit of detection (LOD) was (0.10048×10^{-8}) M and the limit of quantitation (LOQ) was (0.33493 ×10^{-8}) M.

Keywords: Indirect method, nitrous acid, GC electrode, carvedilol, square wave voltammetry.

INTRODUCTION

Carvedilol (CAR), (±)-1-(carbazol-4-yloxy)-3-[(2-(O-methoxy phenoxy) ethyl) amino]-2-proanol, is a non-cardio selective Beta-blocker Fig. (1).

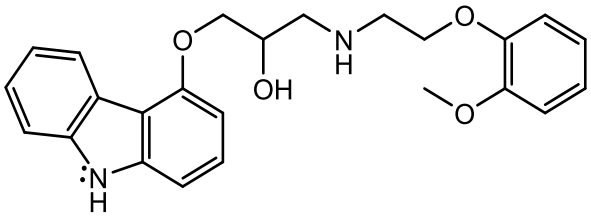

Fig. 1:Carvedilol Structure , $C_{24}H_{26}N_2O_4$, 406.474 g/mol

It is used in the management of hypertension and angina pectoris and as an adjunct to standard therapy in symptomatic heart failure, it is also used to reduce mortality in patients with left ventricular dysfunction after myocardial infarction (Sweetmns, 2009). Carvedilol has been studied and determined by several analytical methods, such as reverse phase high-performance liquid chromatographic (RP-HPLC) (Angeles *et al.,* 2021), and spectroscopic methods via Schiff's base reaction with 4-hydroxybenzaldehyde (Alallaf *et al.,* 2022), through the ion-pair complex with Eriochrome Black-T in an acidic media (pH=2) (Mzban *et al.*, 2020), by using charge transfer complex formation (Ahmed *et al.,* 2022) and oxidative coupling with phenothiazine in the present of potassium dichromate (Mohammed *et al.,* 2022). Direct voltametric oxidation of carvedilol using Pt-electrode, acetonitrile solution containing 0.1 M tetra-butyl ammonium perchlorate ($TBAClO_4$) was studied (Yilmaz and Kabane, 2014), the indirect method by an interaction with two amino acids using the dropping mercury electrode (DME) as the working electrode was tested (Adnan *et al.*, 2016), 0.25 M of Britton-Robinson Buffer (BRB) (pH8.5), 0.1M potassium chloride as supporting electrolyte and glassy carbon electrode as the working electrode was used for the determination of carvedilol (Coelho *et al.*, 2016).

The present work describes an indirect determination of CAR through its interaction with nitrous acid as its sensitive method compared with other method used in literature (Coelho *et al.,* 2016), also the suggested method improved the concentration range for CAR determination.

EXPERIMENTAL

Apparatus

All voltametric measurements were performed using the 797 VA computerize (Metroham company. Switzerland), connected to a PC and run by the control software (version 1.1). A three electrodes system consisting of 2mm diameter glassy carbon electrode (GCE) as the working electrode, 1.5 mm diameter Pt-wire as an auxiliary electrode, and silver/silver chloride/saturated potassium chloride (Ag/AgCl. sat. KCl) as a reference electrode has been used, all the three electrodes positioned in a glass voltametric cell (its volume 5-10 ml). Hanna pH-meter model 211 (Romania) was used for pH adjustment.

Chemicals and prepared solutions

All materials in this work were used without any further purification.

CAR solution with a concentration of

1×10^{-4} M solution was prepared by dissolving 0.0040 g of pure CAR kindly given from Samarra Drugs Industry (S.D.I) (Samarra, IRAQ) in 100 ml methanol.

$NaNO_2$ solution at a concentration of 1.0% w/v

1.0% w/v solution was prepared by dissolving 1.00 g of sodium nitrite (supplied by the English company (BDH) in a 100ml of distilled water.

HCl solution

1.0 M solution was prepared by diluting 25 ml of the concentrated hydrochloric acid solution prepared in the form of an ampoule (supplied by the German company Merck) with 100 ml distilled water.

Britton-Robinson buffer solution

pH2 B.R.B was prepared by mixing Equal volumes of 0.2 M for each of boric acid, phosphoric acid, and acetic acid, then the volume was completed to 100 ml in a 100 ml with distilled water, and the required pH of the buffer solution was adjusted using a pH meter. (Perrine and Dempsey 1974).

Carve pharmaceutical solution

1×10^{-4}M of CAR solution from the Carve tablet, which is produced by the German company Pharma GmbH, as each tablet contains 12.5 mg of CAR, and the solution was prepared with a weight of 10 tablets, (the average weight was 122.6 mg), crushed and mixed well, and a weighed quantity equivalent to 0.004 g of pure CAR was dissolved in 100 ml of methanol, and ultrasonic was used to ensure complete of the dissolution, and the solution was filtered with filter paper.

Alphabeta pharmaceutical solution

1×10^{-4}M of CAR solution from the Ahphabeta tablets, which is produced by the Swiss company (Hoffmann-La Roche), each tablet contains 6.25 mg of CAR, and the solution was prepared with a weight of 10 tablets, (the average weight was 121.1 mg), crushed and mixed well, and a weighed quantity equivalent to 0.0040 g of pure CAR was dissolved in 100 ml of methanol, and ultrasonic was used to ensure completion of the dissolution, and the solution was filtered with filter paper.

RESULTS AND DISCUSSION

Principal of the suggested method

The suggested method depends on the indirect determination of CAR by following the decrease in nitrous acid peak which is appeared at E_p=1.35V and is proportional to the concentration of CAR added according to the equations in Fig. (2). The voltammogram was recorded by scanning the potential between (0.2-1.6) V versus Ag/AgCl.sat.KCl in acidic media (pH= 2-2.2) using GCE electrode and square wave voltammetry (SWV).

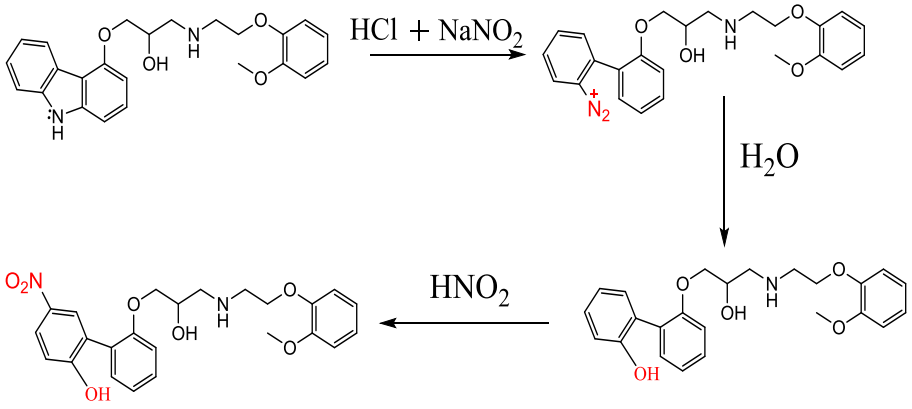

Fig. 2: Proposed reaction mechanism between carvedilol and nitrous acid (Verma *et al.*,1988).

Electrochemical behavior of nitrous acid

The electrochemical behavior of nitrous acid was studied by mixing 1 ml of 1% sodium nitrite with 1.0 ml 1.0M hydrochloric acid in a glass cell containing B.R.B (pH=2) as a supporting electrolyte.

The voltammograms were recorded under the default instrument conditions (Table 1) for B.R.B. and nitrous acid as depicted in Fig. (3) one peak was observed (E_p = 1.35 V) for nitrous acid.

Table 1: The default instrument conditions for nitrous acid voltammogram recorded at pH2

Parameters	Values
Start potential (V)	0.2
End potential (V)	1.6
Voltage step (V)	0.01
Frequency (Hz)	50
Amplitude (V)	0.0199
Deposition potential (V)	0.9
Deposition time (s)	60
Equilibration time (s)	5.0
No. of cycles	1
Cleaning potential (V)	-0.1
Cleaning time (s)	0.0
Sweep rate V/s	0.500

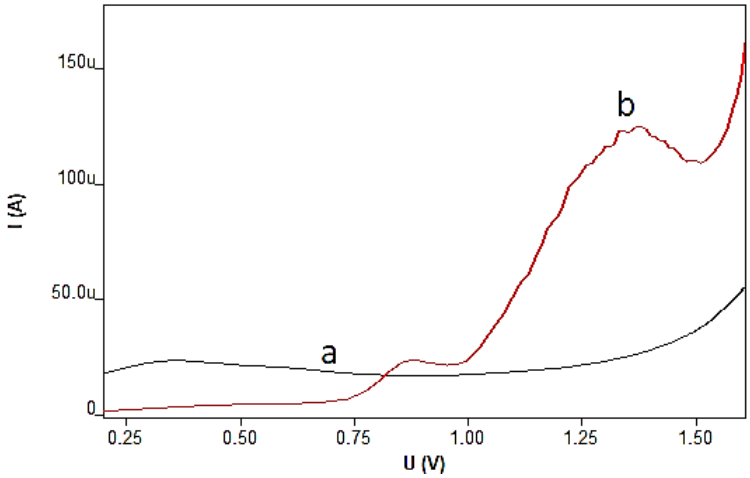

Fig. 3: a: SW-voltammogram of B.R.B at pH=2
b: SW-voltammogram of nitrous acid at pH=2

The pH impacts

The effect of pH was examined using 1.0 ml of 1% sodium nitrite with 1.0 ml 1.0 M hydrochloric acid in a glass cell, the results (Table 2) show that the optimum pH was 2 due to the highest current obtained.

Table 2: Effect of pH on the current of nitrous acid peak

pH	Ep(V)		Ip(µA)
1.0	1.34		46.4
2.0	**1.35**		**58.1**
3.0	1.38		53.1
4.0	1.42		43.5
5.0	1.74		21.6

The effect of sodium nitrite and hydrochloric acid ratio

The effect of the $NaNO_2$/HCl ratio was optimized using 1 ml of hydrochloric acid with different amounts of sodium nitrite, the results obtained are shown in (Table 3), the optimum ratio was 1/2 (HCl/$NaNO_2$) whereas more than this ratio cause noise in voltammogram shape and gave rise to bubble gas NO liberated. (Binnewies *et al.*, 2010):

$$3HNO_2 \longrightarrow HNO_3 + 2NO + H_2O$$

Table 3: Effect of sodium nitrite amount on nitrous acid peak

Ip (µA) of HNO_2 at 1.35 V	**$NaNO_2$ 1.0 %, ml**			
	0.5	1.0	2.0	3.0
	4.81	8.93	39.18	53.47*

*A distortion in shape peak.

The Optimum conditions

Various instrumental and experimental variables were tested and optimized in order to obtain maximum current and/or better voltammogram shape Fig. (4). The results obtained are summarized in (Table 4).

Table 4: The measured optimum conditions

Parameters	**Values**
Start Potential (V)	0.2
End potential (V)	1.6
Voltage Step (V)	0.006
Frequency (Hz)	50
Pulse Amplitude	0.08
Deposition Potential (V)	-0.6
Deposition Time (s)	40
Equilibration Time (s)	4
No. of Cycles	4
Cleaning Potential (V)	-0.1
Cleaning Time (V)	1.0
Sweep Rate V/s	0.300

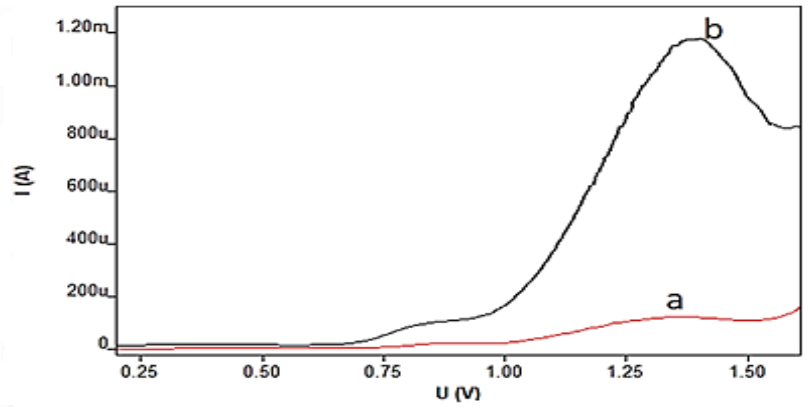

Fig. 4: a. Nitrous acid voltammogram at the instrument default conditions, b. nitrous acid voltammogram at the measured optimum conditions.

The stability of nitrous peak

The stability of the nitrous acid peak was tested in B.R.B. All measurements were performed at ambient temperature (24±2) ºC. The results demonstrated that the nitrous acid peak is time-independent, and it is stable for the studied time (60 min), as shown in (Table 5).

Table 5: Effect of time on the stability of nitrous acid peak

Time (min)	Ep of (HNO_2)	Ip of HNO_2 (µA)
0	1.35	672
5	1.35	670
10	1.36	667
15	1.35	660
20	1.35	655
25	1.35	652
30	1.35	650
35	1.36	650
40	1.37	655
45	1.37	654
50	1.37	650
55	1.36	658
60	1.37	650

Calibration curve of CAR

The SW-Voltammograms were recorded at E_p= 1.33V for sequence additions of CAR (1x 10^{-4} M) to the voltametric cell containing 1 ml of 1M HCl and (2) ml of 1% $NaNO_2$, under the fixed optimum conditions. A plot of ΔI_p versus concentration of CAR added gives two straight lines, first at low concentration range (0.499 – 5.99) $\times 10^{-8}$M by a calibration equation Y=93.893x-4.9093 and second, at high concentration range (6.99 – 11.98) $\times 10^{-8}$M, Fig. (5) by calibration equations Y=9.2363x+484.69 with regression coefficient r^2=0.9937 and 0.9574 respectively, the limit of detection LOD (0.10048×10^{-8}) M, the limit of quantitation LOQ (0.33493×10^{-8}) M. Fig. (6).

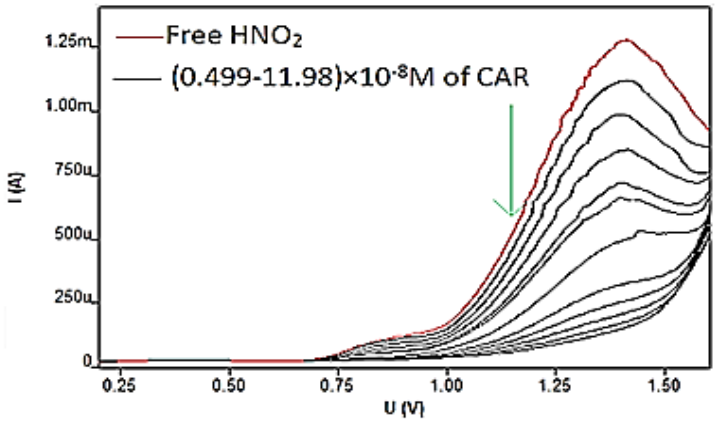

Fig. 5: Voltammograms of serial additions of CAR.

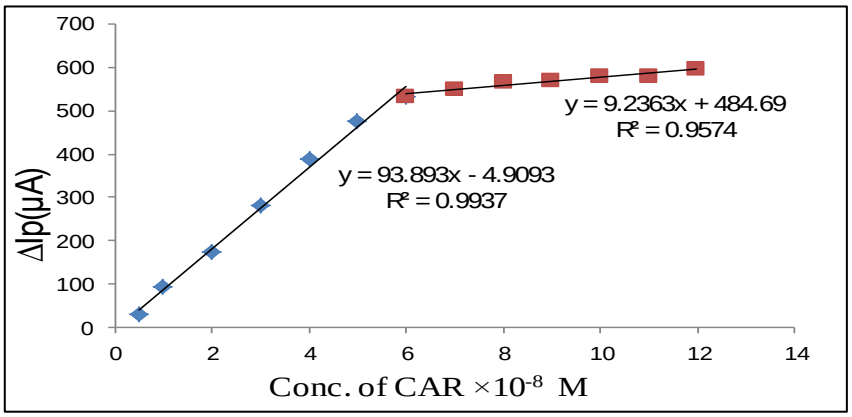

Fig. 6: Calibration curve of CAR under the measured optimum conditions.

Applications

The suggested method was successfully applied for the determination of CAR in two pharmaceuticals Alphabeta (6.25 mg /tab Hoffmann La-Roche) and Carve/ TDA (12.5 mg/ tab. from Pharma GmbH Germany). The recovery percentage was calculated for three different concentrations, the results are shown in (Table 6), and a good recovery was obtained for all the concentrations used.

Table 6: Recovery values for CAR in pharmaceuticals used

Tablet	Taken conc.× 10^{-8} (M)	ΔIp (μA) tab.	ΔIp (μA) pure	Recovery %	Found conc.× 10^{-8} (M)
Alphabeta 6.25 mg / tab Hoffmann La-Roche	0.99	90.0	93.8	95.94	0.949
	2.99	190.0	181.3	104.79	3.133
	6.99	527.1	534.0	98.70	6.899
Carve/ TDA 12.5 mg/ tab. Pharma GmbH Germany	0.99	68.0	71.0	95.77	0.956
	2.99	183.0	181.3	100.93	3.018
	6.99	507.0	534.0	94.94	6.636

Accuracy and precision

The accuracy and precision of the suggested method represented by relative standard deviation and relative error were tested through the calculation of three different concentrations of CAR ($1x10^{-4}$ M) in Alphabeta tablets for five reads of each concentration under the measured optimum conditions. The results obtained (Table 7) show that the suggested method has good accuracy and precision.

Table 7: The relative standard deviation and relative error for the suggested method

Drug	Amount of CAR Taken ×10^{-8}M	Recovery %	Amount found of CAR ×10^{-8}M	RE%	RSD%
Alphabeta 6.25 mg / tab, Hoffmann La Roche	0.999	98.78	0.9868	-1.221	0.0199
	2.990	96.99	2.898	-3.076	1.6383
	3.99	97.61	3.894	-2.388	1.0179

The comparison of the suggested method

The suggested method was compared with the standard method (RP-HPLC, with mobile phase methanol: orthophosphoric acid) (50:50) and the stationary phase was C_{18}, detected at 285nm) in

the British pharmacopeia (British pharmacopeia, 2022) by application of a t-test and through the measurement of five sample recovery percentages of Alphabeta tablets containing (6.25 mg/ tablet) of CAR the results are summarized in (Table 8).

The obtained results show there are no significant differences between the two methods and there is the applicability of the suggested method for the determination of CAR in pharmaceutical preparations.

Table 8: Comparison of the suggested method

Drug	Recovery %		± t.exp
	Present method	(British pharmacopeia, 2022)	
Alphabeta 6.25 mg / tab Hoffmann La Roche	97.82	99.42	1.2363

CONCLUSION

CAR can be determined indirectly through its interaction with the nitrous acid oxidation peak. As it is an indirect method so it is less interferences, by using square wave voltammetry (SWV). Nitrous acid which is prepared instantaneously in a voltametric cell contains a three-electrodes detection system, a glassy carbon electrode (GCE) as the working electrode, Pt-wire as an auxiliary electrode and silver/silver chloride/ saturated potassium chloride (Ag/AgCl. sat. KCl) as a reference electrode, gives a well-defined oxidation peak at 1.35 V vs. Ag/AgCl. sat. KCl. The plot of concentration versus current gives two straight lines, first at a low concentration range (0.499 -5.99) × 10^{-8} M and second, at a high concentration range (6.99 - 11.98) ×10^{-8} M with determination coefficient R^2 equal 0.9937 and 0.9574 respectively. The limit of detection (LOD) was (0.10048×10^{-8}) M and the limit of quantitation (LOQ) was (0.33493 ×10^{-8}) M. The suggested method has good accuracy, precision, wide concentration range, and can be applied for estimation of CAR in different pharmaceutical preparations.

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التقدير غير المباشر للكارفيديلول من خلال تداخله مع حامض النتروز

اخلاص ذنون العلاف عامر ذنون الطائي نبيل صبيح عثمان

قسم الكيمياء/ كلية العلوم/ جامعة الموصل

الملخص

قدر الكارفيديلول النقي وفي مستحضراته الصيدلانية بطريقة غير المباشرة من خلال تداخله مع حامض النتروز باستخدام تقنية فولتاموتري الموجة المربعة. حامض النتروز المحضر آنياً في خلية فولتامترية تحوي على نظام كشف ثلاثي – الاقطاب، الكاربون الزجاجي (GC) بوصفه قطب عامل، سلك البلاتين (Pt) بوصفه قطب مساعد و قطب الفضة / كلوريد الفضة / كلوريد البوتاسيوم المشبع (Ag/AgCl/ sat.KCl) بوصفه قطب مرجع حيث يعطي قمة اكسدة واضحة عند 1.35 فولت ضد قطب المرجع Ag/AgCl/ sat.KCl. درس تأثير كل من الدالة الحامضية (محاليل منظمة مختلفة) والزمن والتركيز. تم متابعة التناقص الحاصل في قمة حامض النتروز مع الاضافات المتعاقبة من محلول الكارفيديلول تحت الظروف المثلى المثبتة مسبقا و عند رسم العلاقة بين تركيز الكارفيديلول المضاف و التغير في قيمة التيار(ΔIp) اعطت علاقتين خطيتين، الاولى عند مدى التراكيز الواطئة (5.99-0.499) × 10^{-8} مولاري و بمعادلة خط المستقيم Y=93.893x-4.9093 و الثانية في مدى التراكيز العالية (11.98 - 6.99) × 10^{-8} مولاري و بمعادلة خط المستقيم Y=9.2363x+484.69 وبمعاملي تقدير (R^2= 0.9937) و(R^2=0.9574) على التوالي، وكان حد الكشف والحد الكمي LOD و LOQ للطريقة المقترحة هي (0.10048 و 0.33493)×10^{-8} مولاري على التوالي.

الكلمات الدالة: طريقة غير مباشرة، حامض النيتروزو، قطب الكاربون الزجاجي، كارفيديلول، قياس جهد الموجة المربعة.