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Research Article

STABILITY-INDICATING POTENTIOMETRIC METHOD FOR THE DETERMINATION OF CYCLOBENZAPRINE HCL USING TWO CARBON PASTE ION-SELECTIVE ELECTRODES

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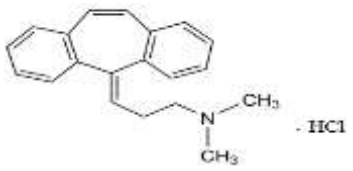
ABSTRACT

Objective: Two sensitive and selective carbon paste electrodes were developed and investigated for determination of cyclobenzaprine hydrochloride. **Methods:** Sensor I was developed using ammomium renikate as an anion exchanger and dibutyl phthalate as a plasticizer while sensor II was developed using phosphotungestic acid as an anion exchanger and dibutyl phthalate as a plasticizer. **Results:** The proposed electrodes showed a near-nernsation response in the range from 1.00×10-4-1.00×10-2 M and 5.00×10-5-1.00×10-2 M for sensor I and sensor II, respectively. The selectivity of the proposed electrodes to a number of interferences was investigated. **Conclusion:** Cyclobenzaprine hydrochloride was successfully determined in pure form, pharmaceutical formulation and in the presence of anthraquinone (its oxidative degradation product) using the two proposed sensors.

Keywords: Cyclobenzaprine hydrochloride (CB), Ion-Selective Electrodes (ISEs), Carbon Paste, Potentiometry.

INTRODUCTION

Cyclobenzaprine hydrochloride is a white crystalline powder [1], its M.p. 215 to 219°C. It is freely soluble in water and ethanol, sparingly soluble in isopropanol, slightly soluble in methylene chloride and chloroform; practically insoluble in hydrocarbons. CB is related to tricyclic antidepressant as centrally acting muscle relaxant [2,3]. It is in the symptomatic treatment of condition associated with painful muscle spasms. Cyclobenzaprine hydrochloride is an official drug. Several methods were reported in the literature review for its determination.



Chemical Structure of Cyclobenzaprine Hydrochloride

CB was determined in U.S.P. using non-aqueous titration with 0.1 M Perchloric acid, the end point was determined potentiometrically [4].The Spectrophotometric determination of CB is described in Clarke in Aqueous acid at 290 nm [1]. Spectrophotometric determination of CB was determined using several reagents [5-7]. Different GC methods were developed [8-18], Liquid chromatography [19], HPTLC methods for analysis CB in pharmaceutical formulation [20-21] and different HPLC methods with variable mobile phases and different detectors [22-38]. There was one stability indicating potentiometric method for its determination using PVC [39].

The most common type of potentiometric ISE comprises a sensing membrane, usually a PVC membrane. This membrane sandwiched between two solutions, the sample and the inner filling solutions [40]. This configuration requires large samples volumes for analysis and good deal of practice and patience. The carbon paste ISEs have no inner filling solution comparing to PVC membrane ISEs [41-43]; hence, they have the advantage of simplicity and ease of preparation. In addition, the main advantage of the carbon paste ISE is the ease of regeneration; simply, a new active surface can be obtained by rubbing the electrode surface against a filter paper.

EXPERIMENTAL

MATERIALS

Pure sample (CB): Batch No. mt4431014, was supplied by Multi-Apex Pharma, Badr City, Cairo, Egypt. According to the pharmacopeia method [4] its purity was found to be 99.50 ± 0.328

Pharmaceutical formulation (Multi-Relax tablets): Batch No CBP/1506004-M, labeled to contain 10.00 mg CB, manufactured by Multi-Apex Pharma Company for Pharmaceutical Industries, purchased from a local market.

Degraded sample: Anthraquinone (AQ) [3], a degradation product of CB was purchased from Sigma-Aldrich (St. Louis, MO).

Chemicals and reagents

All reagents and chemicals used were of analytical grade.

- Graphite powder and Ammonium renikate (AR) (Sigma Aldrich).
- Dibutylphthalate (DBP) (Merck, Newgersy USA).
- Phosphotungestic acid (PTA) (BDH Ltd, Poole, UK).
- Tetrahydrofurane (THF) (Lab-Scan Analytical Science)
- •1 M Sodium hydroxide, 1 M Hydrochloric acid, aqueous solution (Prolabo VWR International, West Chester, PA).
- Bi-distilled water

Apparatus

- pH meter Jenway 3510 (Braloworld Scientific Ltd, Dunmous, Essex CM63LB. UK), serial no. 04487 with Ag/AgCl double junction reference electrode no. 924072-BO3-Q11C.
- pH glass electrode (Jenway, UK) no. 924072-BO3-Q11C.
- Hot plate stirrer, CB302 (Biocote, UK assemble by PRC).

Standard and degraded solutions

CB stock standard solution: 1.00×10^{-2}M (3.12 mg/mL) in bi-distilled water.

CB working standard solution

(1.00×10^{-6} -1.00×10^{-3}M) in bi-distilled water.

AQ stock solution

1.00×10^{-3}M (0.21 mg/mL) in bi-distilled water.

Laboratory prepared mixtures containing different ratios of CB and AQ

A 10 mL volume of CB solution (1.00×10^{-3} M) was quantitatively transferred into a series of 100-mL volumetric flasks. Aliquots (1–9 mL) of AQ solution (1.00×10^{-3} M) were added, and the volume was completed with bi-distilled water to prepare mixtures containing10:1, 10:2, 10:3, 10:4, 10:5, 10:6, 10:7,10:8 and 10:9 CB: AQ ratios. Then 25 mL from the prepared solutions were transferred separately into a series of 50-mL beakers.

PROCEDURE

CB-AR and CB-PTA ion pairs preparation

In two different beakers, CB-AR and CB-PTA ion-pairs were prepared by adding 100-mL of 1.00×10^{-2} M CB to 100 mL 1.00×10^{-2} M AR and PTA respectively. Then the solutions were stirred for 10 min, the pink and buff precipitates were filtered, washed with bi-distilled water and left to dry overnight at room temperature.

Fabrication of sensor I and II

Sensor I was prepared by dissolving in a glass mortar 50.00 mg of CB-AR ion pair with the aid of 2 mL of THF in 475.00 mg of DBP. About 475.00 mg of carbon powder was added to the mixture and homogenized with glass rod.

While sensor II was prepared by dissolving in a glass mortar 32.00 mg of CB-PTA ion pair in 484.00 mg of DBP with the aid of 2 mL of THF. About 484.00 mg of carbon powder was added to the mixture and homogenized with glass rod. The THF in the two sensors was allowed to evaporate at room temperature.

Assembly of electrode

The electrode body was made using an empty 50 µl plastic tip, its tip was completely filled with each paste. The back connection was made by inserting a copper wire into the paste through the apex of the tip. The wide open of the tip served as the electrode surface. To confirm of complete and compact packing of the paste, we pressed the tip (at the wide open) into the paste several times until it had a shiny appearance with no cracks. The electrode was used directly for measurements. Using double junction Ag/AgCl electrode as an external reference electrode, the electrochemical system for each electrode is represented as follows:

Cu wire/carbon paste/test solution//KCl salt bridge/Ag/AgCl.

Direct potentiometric determination of CB in pure samples using sensors I and II (linearity)

Aliquots of 25 mL from (1.00×10^{-6} –1.00×10^{-2} M) standard solutions of CB were transferred separately into a series of 50-mL beaker. Each sensor separately was conjugated with double junction Ag/AgCl reference electrode, calibrated by being immersed in its respective drug solutions and allowed to equilibrate while stirring until the constant reading of the potentiometer. Then (e.m.f) were recorded within ± 1 mV. Calibration graphs were plotted relating the recorded electrode potentials obtained by the two proposed sensors versus -log molar concentrations of CB. The fabricated sensors were washed with bi-distilled water before and after each run till reaching a constant potential. This calibration graph or the computed regression equation was used for subsequent measurements of unknown concentration of CB.

Accuracy

The accuracy of the results was checked by applying the proposed method for the determination of different samples of CB ($1.00x10^{-4}$ - $1.00x10^{-2}$ M). The concentrations were obtained from the corresponding regression equations.

Precision

Repeatability

Three concentrations of CB (5.00×10^{-3}, 1.00×10^{-3} and 5.00×10^{-4} M) were analyzed three times, each intra-daily using the previously mentioned procedures under **(linearity)**. The standard deviation for the studied drug was calculated from the corresponding regression equations.

Intermediate precision

The above mentioned CB samples were analyzed three times on three successive days using the previously mentioned procedures under **(linearity).**The standard deviation for the studied drug was calculated from the corresponding regression equations.

Direct potentiometric determination of laboratory prepared mixtures of CB and AQ (Specificity)

The prepared electrodes (sensors I and II) were immersed separately in the prepared mixtures **(linearity)** in conjunction with the double junction Ag/AgCl reference electrode, the resulting potential was recorded for each solution at constant stirring at room temperature and then the respective concentration was calculated from the corresponding regression equations.

Direct potentiometric determination of CB in pharmaceutical formulation

Ten tablets were accurately weighed, an amount equivalent to 78.00 mg CB was accurately weighed and transferred into a 25-mL volumetric flask, about 15 mL of bi-distilled water was added, sonicated for 30 min and finally diluted to the mark with bi-distilled water to obtain a solution of concentration 1.00×10^{-2} M of CB. Suitable dilution was made to prepare solution containing 1.00×10^{-3} M. The prepared electrodes (sensors I and II) in conjunction with the double junction Ag/AgCl reference electrode were immersed separately in 25 mL of the prepared solution, the resulting potential was recorded and then the respective concentration was calculated from the corresponding regression equations. applying the standard addition technique assesses the validity of the proposed method .

Study of the experimental conditions

Determination of the slope, response time and operative life of the studied electrodes

The electrochemical performance of the two proposed sensors was evaluated according to the IUPAC recommendations data [44].

Effect of pH

The effect of pH on the potential values of the two sensors was studied over pH ranges of 2-10 using 1.00×10^{-3} M solutions of CB. The potential obtained at each pH value was recorded.

Effect of interfering substances on the electrode selectivity

The potential response of the proposed sensors in the presence of CB and a number of related substances was studied and the potentiometric selectivity coefficient were calculated by the separate solutions method (SSM) [45], where potentials were measured for 1.00×10^{-3} M drug and interferent solution, separately, then potentiometric selectivity coefficients were calculated using the following equation:

$$-\log (K^{pot}_{\text{primary ion interferent}}) = E_1-E_2 / S$$

Where E_1 is the potential measured in 1.00×10^{-3} M solution of 1^{ry} ion solution, E_2 the potential measured in 1.00×10^{-3}M solution of interferent and S is the slope of the investigated sensor.

RESULTS AND DISCUSSION

In this proposed work carbon paste electrodes were introduced for determination of CB in pure form, in pharmaceutical formulation and also in the presence of AQ.

OPTIMIZATION OF THE METHOD

Effect of composition (Sensor fabrication)

Different carbon paste electrodes were prepared with 2.50, 5.00, 7.50 and 10.00 % (w/w) of CB-AR and 3.20, 6.40, 9.60 and 12.80% of CB-PTA. The best composition was that containing 5.00% and 3.20% for CB-AR and CB-PTA respectively (Table 1). The electrode showed a Nernestian slope of 59.151 mV/decade over the concentration range from $1.00x10^{-4}$ to $1.00x10^{-2}$ M for CB-AR and 59.714 mV/decade from $5.00x10^{-5}$ to $1.00x10^{-2}$ M for CB-PTA, Figures (1,2)

Lifetime of the electrodes

The carbon paste electrode possesses the advantage of ease of surface regeneration. The slope of the carbon paste electrode before and after regeneration was about 59 mV/decade.

Selectivity of the electrodes

The selectivity of the proposed electrodes to CB in presence of tablets excipients, organic and inorganic related substances, was assessed using the separate solution method (SSM) [45]. Table 2 summarizes the selectivity coefficient of the two electrodes for some common cations, sugars and amino acids.

Sensors calibration and response time

The slopes of the calibration plots were 59.151 and 59.714 mV/decade for sensor I and II, respectively. The dynamic response time of the proposed electrodes was studied by measuring the time required to achieve a steady state potential (within ±1 mV) after successive immersion of the electrodes in a series of stirring CB solutions (1.00×10^{-6} to 1.00×10^{-2} M). The required time was found to be 10 seconds for both sensors, Table 3.

Effect of pH

The effect of pH on the potential of electrodes was investigated by recording the variation in the cell potential when small volumes of HCl and/or NaOH (0.1-1.0 M of each) were added to 1.00×10^{-3} M CB (Figures 3,4). The electrodes do not respond to pH changes in the range from 5-8 and 4-9 for electrodes I and II respectively. The decrease in the cell potential at pH values higher than 9 is most probably due to the formation of nonprotonated drug or the free CB base in the test solution, Table 3.

The proposed electrodes were successfully applied for the determination of CB in pure solution from 1.00×10^{-4}-1.00×10^{-2} M and 5.00×10^{-5}-1.00×10^{-2} M for electrode I and II respectively, Figures (1-2). The regression equations were found to be:

E_I= 59.151 C + 433.155 r: 0.9995

E_{II}= 59.714 C + 486.571 r: 0.9995

Where, E = the recorded electrode potentials obtained by sensor I and II, respectively, C = the concentration of CB in M and r =the correlation coefficient.

Validation of the proposed method was assessed according to ICH guidelines [46] by measuring linearity range, accuracy, precision, specificity, robustness and LOD. The results obtained are depicted in Table (3).

The potentiometric measurement was valid in the presence of up to 90 % of AQ for both electrodes, (Tables 3,4).To study the method robustness, three different concentrations (5.00×10^{-3},1.00×10^{-3} and 5.00×10^{-4} M) solution of CB were analyzed with change pH (±1) and temperature (25±5ºC), they did not have a significant effect on the potentiometric measurement, illustrating the robustness of the method (Table 3).

According to IUPAC recommendations data [44] Limit of detections (LOD) defined as drug concentration obtained at the intersection of the extrapolated high concentration (linear segment) with a low concentration (zero slope segment) of the calibration plot, (Table 3).

The proposed method has been successfully applied to assay CB in Multirelax tablets. The validity of the proposed method was further assessed by applying the standard addition technique for the analysis of Multirelax tablet, (Table 5).

The results obtained by applying the proposed method for the analysis of the studied drug in pure form were statistically compared with those obtained by applying the official method [4] for CB. The values of the calculated t and F were less than the tabulated ones which reveal that there was no significant difference with respect to accuracy and precision as shown in Table (6).

CONCLUSION

The proposed potentiometric method using the two suggested electrodes were sufficiently precise, accurate and prove the greater selectivity of the sensors for the quantitative determination of CB in pure form, in pharmaceutical formulations and in the presence of its degradation product. Moreover, the use of the proposed sensors compromises the great advantage of eliminating any need for drug pretreatment or separation steps. They can therefore be used for routine analysis of CB in quality control laboratories.

Table 1: Effect of composition on the response of the two carbon paste CB -selective electrodes.

Carbon paste I	CB-AR (mg)	Graphite (mg)	DBP (mg)	Slope (mV/decade)	Linear range (M)	LOD (M)
I	25	487.5	487.5	48.007	-	-
II	50	475	475	59.151	1.00×10^{-4} - 1.00×10^{-2}	1.513×10^{-6}
III	75	462.5	462.5	55.521	1.00×10^{-4} - 1.00×10^{-2}	4.155×10^{-5}
IV	100	450	450	48.342	-	-
Carbon paste II	CB-PTA (mg)	Graphite (mg)	DBP (mg)	Slope (mV/decade)	Linear range (M)	LOD (M)
I	32	484	484	59.714	5.00×10^{-5} - 1.00×10^{-2}	1.110×10^{-6}
II	64	468	468	50.034	1.00×10^{-4} - 1.00×10^{-2}	3.211×10^{-5}
III	96	452	452	46.205	-	-
IV	128	436	436	38.63	-	-

Table 2: Potentiometric selectivity coefficients of the two proposed sensors using the separate solutions method (SSM)

Interferent *	Sensor I	Sensor II
Anthraquinone (AQ)	1.010×10^{-3}	1.341×10^{-3}
NaCl	0.981×10^{-3}	1.381×10^{-5}
KCl	1.620×10^{-3}	1.409×10^{-3}
$CaCl_2$	1.764×10^{-4}	1.317×10^{-4}
Glucose	1.254×10^{-3}	1.311×10^{-4}
Lactose	1.307×10^{-3}	1.112×10^{-4}
Starch	1.212×10^{-3}	2.604×10^{-5}

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Talc	1.041×10^{-3}	1.866×10^{-4}
Mg Stearate	1.229×10^{-3}	1.374×10^{-3}

*** Average of three determinations.All interferents are in the form of 1.00 × 10−3 M solution**

Table 3: Results of assay validation obtained by applying the two proposed ion selective electrode method for the determination of CB in its pure powdered form.

Parameters	Sensor I	Sensor II
Validation of response		
Concentration range (M)	1.00×10^{-4}-1.00×10^{-2}	5.00×10^{-5}-1.00×10^{-2}
LOD (M)	1.513×10^{-6}	1.110×10^{-6}
Accuracy*(Mean± S.D)	99.80±0.377	99.77±0.395
Precision (R.S.D%)		
Repeatability**	0.435	0.456
Intermediate precision **	0.123	0.334
Specificity*** (Mean± S.D)	100.29±0.259	100.26±0.398
Robustness ***(R.S.D%)	0.173	0.278
Change in pH	0.286	0.567
Change in tempreture		
Working pH range	05-Aug	04-Sep
Response time (sec.)	10	10
Stability (weeks)	Surface regenration	Surface regenration
Validation of regression equation		
Slope (mV/decade)	59.151	59.714
SE of slope	0.585	0.639
Confidence limit of the slope ****	57.5271–60.7550	57.4882–61.4882
Intercept (mV)	433.155	486.571
SE of intercept	1.772	2.147
Confidence limit of the intercept ****	428.2352–438.0861	480.6123–492.5320
Correlation coefficient	0.9995	0.9995
SE of estimation	0.935	1.336

*** n = 6. ** n = 3×3. *** n = 6. **** 95% confidence limit.**

Limit of detections (LOD) defined as drug concentration obtained at the intersection of the extrapolated high concentration (linear segment) with low concentration (zero slope segment) of the calibration plot

Table 4: Specificity of the proposed potentiometric method for the determination of CB in laboratory prepared mixtures containing different concentration of CB and AQ using sensor I and II

Ratio (AQ:CB)	Recovery* % Sensor I	Sensor II
01:10	100.54	100.43
02:10	100.34	100.56
03:10	100.13	100.76
04:10	100.37	100.19
05:10	100.56	100.38
06:10	100.54	100.59
07:10	99.89	99.78
08:10	100.32	99.53
09:10	99.91	100.16
Mean ±S.D	100.29±0.259	100.26±0.398

***Average of three determinations.**

Table 5: Determination of CB in pharmaceutical formulation by the proposed potentiometric method using sensor I and II and application of standard addition technique

Pharmaceutical formulation	Standard added (M)	Sensor I Recovery % ** of Standard added	Sensor II Recovery % ** of Standard added
Multi-Relax tablets(CB), BN: (10.00mg/tablet) CBP/1506004-M	5.00×10^{-3} (1.55 mg/mL)	99.34	100.73
	1.00×10^{-3} (0.31 mg/mL)	99.19	99.54
	5.00×10^{-4} (0.16 mg/mL)	100.37	99.48
Mean ± S.D		99.63±0.642	99.92 ± 0.705
Found %* ± S.D		99.85±0.439	99.82±0.612

*** Average of five determinations, claimed amount taken (1.00 ×10-3M=0.31 mg/mL).**Average of three determinations**

Table 6: Statistical comparison between results of the proposed potentiometric method using sensor I and II and the official method for determination of CB in pure powdered form

Item	Sensor I	Sensor II	Official Method[4]*
Mean	99.8	99.77	99.5
S.D.	0.377	0.395	0.328
Variance	0.142	0.156	0.108
n	6	6	6
t-test (2.228)**	1.042	0.915	
F-test (5.050)**	1.315	1.444	

*** Non aqueous titration with 0.1 M perchloric acid, the end point was determined potentiometrically. ** Figures in parentheses are the corresponding tabulated values at p = 0.05.**

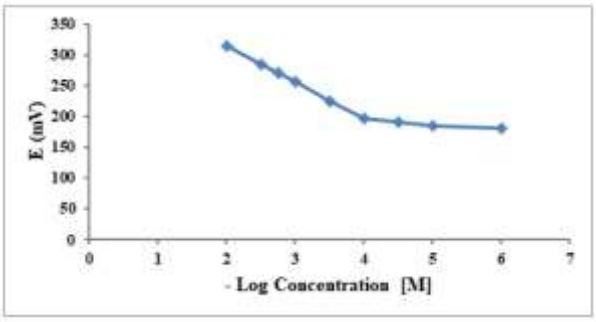

Fig.1: Profile of the Potential in mV/-Log CB Molar Concentration Using Sensor I CB-AR/DBP/Carbon [1×10-4 M –1×10-2 M].

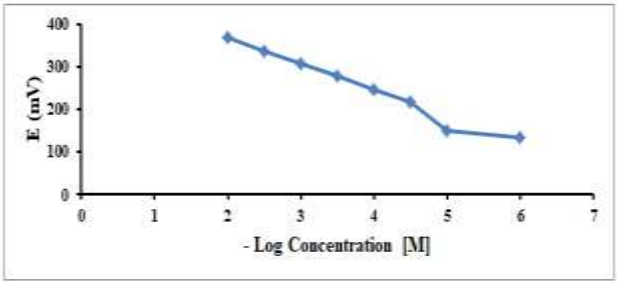

Fig.2: Profile of the Potential in mV/-Log CB Molar Concentration Using Sensor II CB-PTA/DBP/Carbon [5×10-5 M –1×10-2 M].

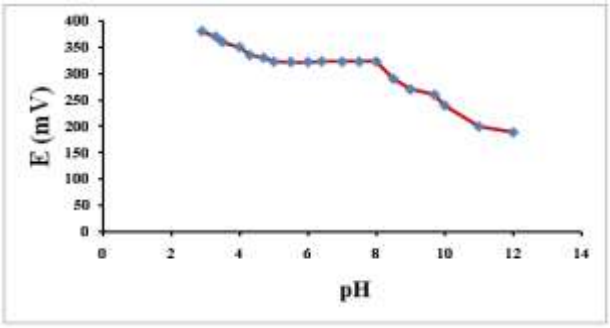

Fig. 3: Effect of pH on the Response of Sensor I CB-AR/DBP/Carbon Using 1.00×10-3 M [Working pH Range: 5-8].

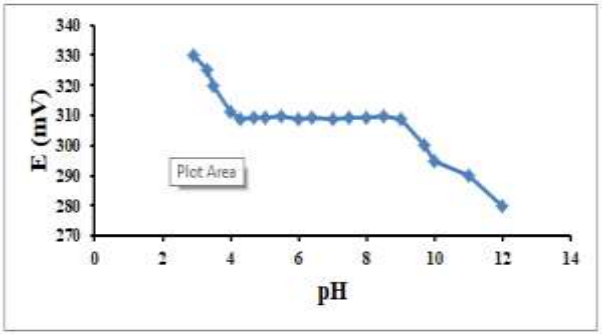

Fig. 4: Effect of pH on the Response of Sensor II CB-PTA/DBP/Carbon Using 1.00×10-3 M [Working pH Range: 4-9].

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