Anal. Bioanal. Electrochem., Vol. 4, No. 5, 2012, 507 - 517

Analytical & Bioanalytical Electrochemistry

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Full Paper

Novel Ion Selective Electrode for Determination of Pregabalin in Pharmaceutical Dosage Form and Plasma

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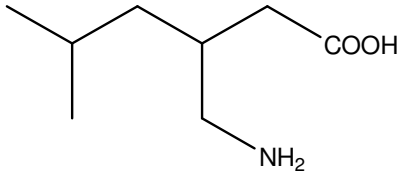
Received: 16 September 2012 / Accepted: 8 October 2012 / Published online: 30 October 2012

Abstract-Ion selective electrode technique was developed for determination of pregabalin. The key to construct such an electrode is to produce a sensitive and selective membrane that responds to a particular ionic species. Such membrane is usually prepared by in corporating an appropriate ion exchanger and solvent mediator into a poly (vinyl chloride) or (PVC) membrane matrix. The present work originates from the fact that pregabalin behaves as a cation in 0.1 N HCl solution and forms a precipitate with anionic potassium tetrakis *p*-chlorophenyl borate which used in fabrication of the membrane sensor. The potentiometric response was linear with constant slope over a drug concentration range of 10^{-6}–10^{-3} M with slope of 53±1 mV/decade .The developed method was applied successfully for the determination of pregabalin in the pure powder form, pharmaceutical formulation and in spiked human plasma without any interference.

Keywords- Pregabalin, Ion Selective Electrode, PVC, Potassium Tetrakis *P*-Chlorophenyl Borate, Pharmaceutical Formulation, Spiked Plasma

1. INTRODUCTION

Pregabalin is a new anti-epileptic drug which is a structurally related to the inhibitory neurotransmitter GABA. It has the following scheme 1:



Scheme 1. Structural formula of (S)-3-(aminomethyl)-5-methylhexanoic acid

Pregabalin reduces the calcium dependent neurotransmitters, possibly by modulation of calcium channel function [1,2].

Pregablin was analyzed by HPLC [3-6] and by colorimetry [7,8]. This work suggests simple and accurate method of analysis of pregabalin using ion selective electrode. Application of the suggested method to the routine analysis of pregabalin in pharmaceutical formulations as well as spiked plasma is also important.

2. EXPERIMENTAL

2. 1. Apparatus

1) Potentiometric measurements were carried out using Jenway, U.K (model 3505) pH/mV meter. A single junction Ag/AgCl reference electrode was used in conjugation with the drug sensor.
2) Bandelin sonorex, RK 510 S, magnetic stirrer.
3) Silver wire (3 mm diameter) immersed in the internal reference solution.

2. 2. Materials and reagents

1) Standard pure pregabalin: was kindly supplied by Pfizer Company, Cairo, Egypt. The purity was found to be 99.93±1.45 according to the reference method [7].
2) Lyrica ® capsule: manufactured by Pfizer Company, Cairo, Egypt (batch number 0458037 and 0366128). Each capsule is claimed to contain 150 mg pregabalin.
3) Potassium tetrakis *p*-chlorophenyl borate (Sigma, Germany): was prepared as saturated alcoholic solution.
4) Tetrahyrdofuran (THF) 99% (Sigma, Germany).
5) Nitrophenyloctyl ether (NPOE) (Sigma, Germany).
6) Poly (vinyl chloride) (PVC) powder (Sigma, Germany).
7) Britton–Robinson (BR) buffer: prepared by mixing the acid mixture containing phosphoric acid (0.04 M), acetic acid (0.04 M) and boric acid (0.04 M). Buffer solutions of different pH values were adjusted by the necessary amount of 0.2 M NaOH [9].
8) NaCl solution (10^{-2} M) for the internal reference solution.

9) Plasma sample (collected from healthy volunteer): obtained kindly from Vacsera, Egypt.

2.3. Standard solution

Stock standard solution (10^{-2} M) of the drug was prepared in 0.1 N HCl by transferring 0.159 g of pregabalin powder into 100 mL volumetric flask. 50 mL of 0.1 N HCl was added, shaked for few minutes and the volume was completed to the mark with the same solvent. Working solutions of lower concentrations (10^{-7}–10^{-3} M) were prepared by serial dilution.

2. 4. General procedure

2.4.1. Precipitation –Based technique for the preparation of PVC membrane sensor

10 mL of 10^{-2} M solution of the drug was mixed with 10 mL of saturated alcoholic solution of potassium tetrakis *p*-chlorophenyl borate. The resulting precipitate was filtered, washed with water, allowed to dry at room temperature and grinded to fine powder.

In a glass Petri dish (5 cm diameter), 10 mg of the previously prepared ion association complex was mixed with 0.35 mL of nitrophenyloctyl ether and 0.19 g of PVC. This mixture was dissolved in 5 mL of THF, covered with a filter paper and left to stand overnight to allow slow evaporation of the solvent at room temperature forming the master membrane with 0.1 mm thickness.

2.4.2. Electrode assembly

A disk of appropriate diameter was cut from the master membrane and cemented to the flat end of PVC tubing with THF. A mixed solution consisting of equal volumes of 10^{-2} M pregabalin and 10^{-2} M NaCl was used as an internal reference solution. Ag/AgCl coated wire was employed as an internal reference electrode. The sensor was conditioned by soaking for 24 h in 10^{-2} M solution of the drug and stored in the same solution when not in use.

2.4.3. Application to pharmaceutical dosage form

The content of five pregabalin capsules was emptied and mixed. An amount equivalent to 0.159 g of pregabalin was transferred into 100 mL volumetric flask. 50 mL of 0.1 N HCl was added, shaken for few minutes and completed to volume with the same solvent and tenfold dilution was done using the same solvent. The e.m.f produced by immersing the prepared electrode in conjugation with the reference electrode in the prepared solution was recorded then the concentration of pregabalin was calculated from the regression equation.

2.4.4. Application to spiked human plasma samples

4.5 mL of plasma samples were placed in 2 stoppard shaking tubes, then 0.5 mL of 10^{-3} and 10^{-4} M of pregabalin solutions were separately added and shaken. The membrane sensor was immersed in conjugation with the reference electrode in the prepared solutions. The e.m.f

produced for each solution was recorded and the concentration of pregabalin was calculated from the regression equation.

2. 5. Study of the experimental conditions

2.5.1. Identification of slope, response time and lifetime of the studied electrode

The electrochemical performance characteristics of the studied electrode were evaluated according to IUPAC standards [10].

The dynamic response time of the electrode was tested for the concentrations 10^{-6}–10^{-3} M of pregabalin solutions. Sensor life span was examined by repeated monitoring of the slope of the drug calibration curve periodically.

2.5.2. Effect of pH on the electrode response

The effect of pH on the potential values of the electrode system was studied over pH range of 4–11 at one pH interval by immersing the electrode in 10^{-4} and 10^{-5} M solutions of the drug. The pH was gradually increased by adding aliquots of Britton–Robinson buffers of different pH values. The potential obtained at each pH was recorded.

2.5.3. Effect of foreign compounds

The performance of the electrode in presence of interfering substance such as pharmaceutical additives and diluents commonly used in drug formulation was assessed. Selectivity coefficient was calculated by the separate solutions method where 10^{-3} M solutions of KCl, NaCl, NH_4Cl, $CaCl_2$, glucose, lactose and magnesium stearate were used.

3. RESULTS AND DISCUSSION

The rapid growth in the analytical chemistry techniques is necessary to match the development of a wide variety of science and technology approaches. In the last three decades, being commercially and not expensive, ion selective electrode techniques (ISE) have become an item of general equipment for analytical work and used for selective determination of many drugs [11-21].

In this work, ion selective electrode was developed for the determination of pregabalin. The method of Egorov and Bolotin [22] was applied for the preparation of the membrane. In the proposed PVC sensor, pregabalin acts as a cation in 0.1 N HCl which suggests the use of ion exchanger of the anionic type. Potassium tetrakis *p*-chlorophenyl borate was found to be optimum anion exchanger for the studied drug and it has the following advantages over other anionic exchangers like sodium tetraphenyl borate [23].

1- Provides better selectivity coefficients.

2- The hydrophobic property prohibits the water transfer within the membrane.

The resulting precipitate had low solubility product and suitable grain size. Acidic pregabalin solution reacted with potassium tetrakis *p*-chlorophenyl borate to form stable 1:1, water insoluble ion association complex which is confirmed by the elemental analysis data as shown in Table 1 and by the Nernest response of the suggested sensor which was 53±1 mV/decade.

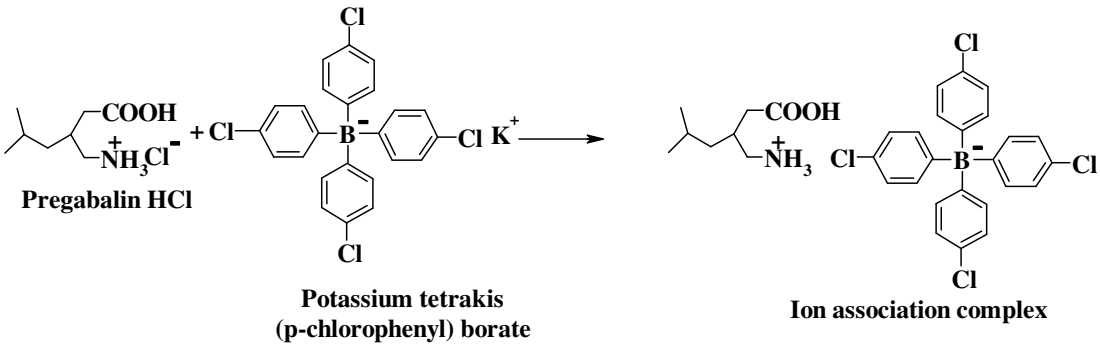
Table 1. Elemental analysis of pregabalin–potassium tetrakis *p*-chlorophenyl borate complex

Parameters	**Analysis %**		
	Carbon	**Hydrogen**	**Nitrogen**
Calculated %	62.43	5.52	2.27
Found%	61.76	5.83	2.68

The electrochemical cell of the suggested membrane for the determination of pregabalin can be illustrated as follow:

Ag/AgCl reference electrode	Test solution	Association complex incorporated in PVC mb	Internal reference solution	Ag/AgCl internal reference wire

The reaction is represented as follow [22]:



It has been reported that the PVC matrix is a regular support and reproducible trap for ion association complexes in ion selective electrodes. In the present study, NPOE plasticizer was used in the fabrication of the proposed sensor. It plasticized the membrane and adjusted both the permittivity of the final membrane and the mobility of the exchanger sites.

Electrochemical performance characteristics of the proposed sensor were evaluated according to the IUPAC recommendation data [24] in Table 2. It was found that the calibration slope did not change by more than 2 mV per decade over a period of 3 weeks. The response time of the electrode was tested for concentrations of the drug from 10^{-7}–10^{-2} M. The measurement was characterized by a fast stable response within 30 –35 s.

Table 2. Electrochemical response characteristics of the proposed electrode used for the determination of pregabalin

Parameter	Suggested sensor
Slope (mV/decade)	53±1
Intercept (mV)	356
Response time (seconds)	30–35
Working pH range	6-9
Concentration range (M)	10^{-6}–10^{-3}
Stability (weeks)	3
Average recovery (%)	99.63
SD	0.96
Correlation coefficient	0.9997

The effect of pH on the electrode potential was investigated and it was found that electrode gave useful pH from 6-9 as shown in Fig. 1.

The potentiometric response at the optimum pH was linear with constant slope over a drug concentration range of 10^{-6}–10^{-3} M as shown in Fig. 2.

The performance of the electrode in presence of commonly used pharmaceutical additives used in drug formulations (e.g.: sodium chloride, potassium chloride, lactose ……) was assessed.

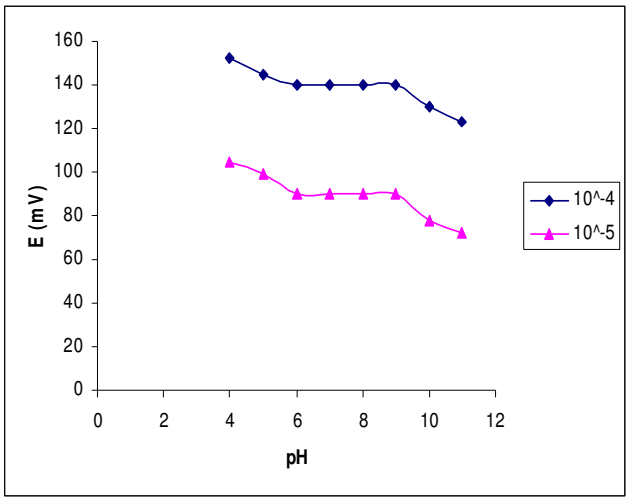


Fig. 1. Effect of pH on the response of proposed electrode

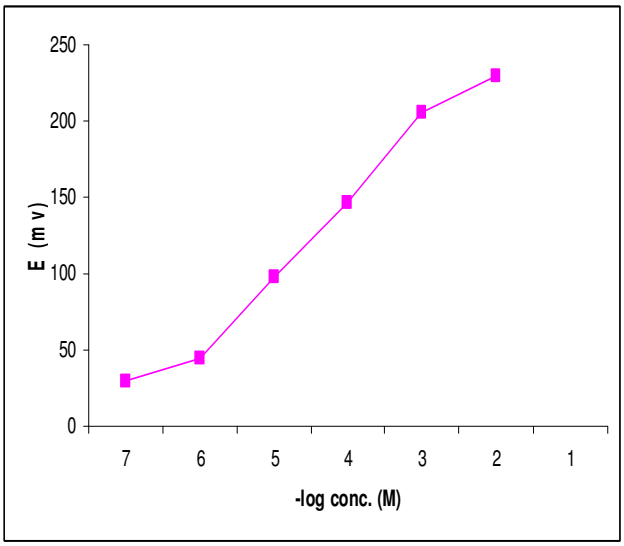


Fig. 2. Profile of the potential in mV to -log concentration of pregabalin using the proposed ion selective electrode method

Selectivity coefficient values ($K^{pot}_{A,B}$) was calculated by the separate solutions method where the potentials were measured for 10^{-3} M of the drug and then for 10^{-3} M of the interferent solution, separately. The selectivity coefficients were calculated as shown in Table 3 using the following equation [22]:

$$\log K^{pot}_{A,B} = \frac{(E_B - E_A)}{S} + \left(1 - \frac{Z_A}{Z_B}\right) \log a_A$$

Where $K^{pot}_{A,B}$ is the selectivity coefficient , E_A and E_B are the potentials of the drug and the interferent solutions respectively, S is the slope of the calibration plot , a_A is the activity of the drug , Z_A and Z_B are the charges on the drug and the interfering ions respectively

Table 3. Potentiometric selectivity coefficients for the proposed electrode used for the determination of pregabalin

Interferent	Selectivity coefficient
NaCl	1.22×10^{-4}
KCl	6.35×10^{-4}
$CaCl_2$	1.27×10^{-4}
Mg stearate	7.86×10^{-3}
NH_4Cl	4.85×10^{-2}
glucose	6.65×10^{-3}
lactose	1.2×10^{-3}

The accuracy of the proposed membrane sensor for the determination of blind samples of pregabalin was assessed. The results showed mean percent recovery of 99.63±0.96 as shown in the validation parameters in Table 4.

Table 4. Validation parameters for the proposed electrochemical method for the determination of pregabalin

Parameter	Suggested sensor
Range	10^{-6}–10^{-3} M
Detection limit	2.23×10^{-7} M
Slope	53±1 mV
Intercept	356 mV
Mean	99.63
SD	0.93
Variance	0.86
RSD	0.933 %
Correlation coefficient	0.9997

The proposed method was successfully applied for the determination of pregabalin in capsules without any interference from the additives as shown in Table 5.

Table 5. Determination of pregablin in its pharmaceutical dosage form

Pharmaceutical formulation	**Taken**	**Mean ± SD**[a]
Lyrica® capsule BN 0458025	10^{-3} M	100.65±0.83
		Reference method[b] 99.48±0.45

[a] Average of 3 determinations

[b] Reference method [7], colorimetric determination of pregabalin using NBD reagent

Table 6. Determination of pregablin in spiked human plasma by the proposed electrochemical method

Concentration (M)	**Recovery % ± SD**[a]
10^{-5}	98.86±0.61
10^{-4}	98.32±0.72

[a] Average of 3 determinations

On application to biological fluids, it has been found that the electrode gave stable results without any interference from the plasma electrolytes as revealed by the high accuracy and precision of the recovery results of the spiked plasma samples as shown in Table 6. The sensitivity of the method is more than satisfactory since Cmax of pregabalin (7.15 mg/L) [25] after oral administration of one capsule (150 mg) was covered by the linearity range of the proposed method.

Statistical analysis of the results of analysis of pregabalin by the proposed electrode and the reference method showed no significant difference as shown in Table 7.

Table 7. Statistical comparison between the results of the proposed electrochemical method for the determination of pregabalin and the reference method

Values	Proposed method	Reference method[a]
Mean	99.63	99.93
SD	0.93	1.45
RSD	0.933 %	1.451 %
Variance	0.86	2.10
n	5	6
Student's t-test (2.22)[b]	0.72	
F-value (5.05)[b]	2.44	

4. CONCLUSION

The use of the developed sensor offers the advantages of fast response, elimination of drug pretreatment or separation steps, selective, low detection limit and direct determination of the drug in turbid and colored solutions. The technique therefore can be used for routine analysis of pregabalin in quality control laboratories.

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