Full Paper

A Clidinium Potentiometric Membrane Sensor for Determination of Clidinium in Pharmaceutical Formulation

Anahita Karimi[1], Farnoush Faridbod [1,2*] and Leila Safaraliee[1]

[1]Center of Excellence in Electrochemistry, University of Tehran, Tehran, Iran
[2]Endocrinology and Metabolism Research Center, Tehran University of Medical Sciences, Tehran, Iran

* Corresponding Author, Tel: 021-61112788; Fax: 021-66495291

E-Mail: faridbodf@tums.ac.ir

Abstract- This work introduces a PVC membrane sensor for determination of clidinium active ingredient. For the membrane preparation, clidinium-tetraphenyl borate ion-pair was employed as a sensing material in the PVC membrane. Several plasticizers were studied in the membrane composition dibutyl phthalate (DBP), benzylacetat (BA), and nitrobenzene (NB). After a series of experiments, the best electrode response was obtained from a membrane composed of NB. The electrodes illustrated a fast, stable and Nernstian response over a wide clidinium bromide concentration range of 1×10^{-5} to 1×10^{-1} M, in the pH range of 4.0–10.0.

Keywords- Clidinium Bromide, Potentiometric Sensor, PVC membrane, Ion-Pair

1. INTRODUCTION

Clidinium bromide, 3-[(2-hydroxy-2,2-diphenylacetyl)oxy]-1-methyl-1-azabicyclo [2.2.2]octan-1-ium bromide, (Fig. 1), is an anticholinergic drug which blocks the neuro-transmitter acetylcholine in the central and the peripheral nervous system. It helps symptoms of cramping and abdominal/stomach pain by decreasing stomach acid. It is commonly

prescribed in combination with chlordiazepoxide using the brand name Librax [1]. It is a relatively polar hydrophilic compound with water solubility of 3.77×10^{-4} g/l at 25°C and a log partition coefficient (octanol/water) of −0.5 [2]. Several methods have been reported for the determination of clidinium including derivative spectroscopy [3], reverse-phase high performance liquid chromatographic [4], spectrophotometric methods [5,6], stability-Indicating HPLC Method [7]. However, most of these methods involve several manipulation steps before the final result of the analysis is obtained. The low cost and ease of operation of potentiometric instrumentation make the potentiometric determination of clidinum a highly desirable alternative.

Ion-selective membrane electrodes (ISEs) are now widely used for the direct potentiometric determination of ion activities in different samples. Solvent polymeric membranes have proved to be suited for clinical analysis since they can easily be manufactured in different sizes and shapes and are less affected by the response of biological substrate [8-10]. Their advantages are simple design, low cost, adequate selectivity, low detection limit, high accuracy, and wide concentration range.

In the present work, a plastic membrane electrode is prepared based on incorporation of an ion-pair complex of sodium tetraphenylborate with clidinum cation in a PVC matrix plasticized with nitrobenzene. The electrode was applied for analysis of clidinium in the pharmaceutical formulation using the Nernstain equation.
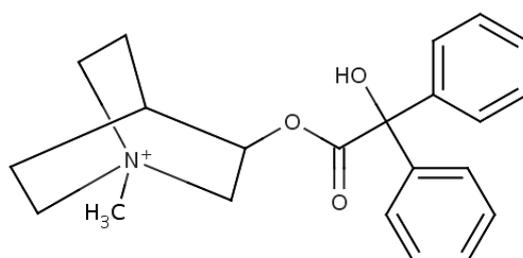

Fig. 1. Chemical structure of clidinum cation

2. EXPERIMENTAL SECTION

2.1. Apparatus

R684 model Analion Ag/AgCl double junction reference electrode was used as internal and external reference electrode. The reference and indicator electrodes were connected to a

Corning ion analyzer with a 250 pH/mV meter with ±0.1 mV precision in order to measurement the potentials. The cell chamber was filled with an ammonium nitrate solution and both electrodes were connected to a Corning ion analyzer with a 250 pH/mV meter with ±0.1 mV precision.

2.2. Materials and Reagents

The necessary chemicals (of analytical grade) were: high-molecular weight polyvinylchloride (PVC) (Fluka Co.), sodium tetraphenylborate (NaTPB), benzylacetat (BA), dibutyl phthalate (DBP), nitrobenzene (NB), and tetrahydrofuran (THF) (Merck Co.). All the materials were at the highest available purity and were used without further modification. Clidinium bromide and its pharmaceutical formulation were obtained from a local pharmaceutical manufacturer (Tehran, Iran).

2.3. Preparation of sensing element

Ion-pair compound of clidinium-tetraphenyl borate (CLD-TPB): About 10 mL of 0.01 M solution of clidinium bromide was mixed with 10 mL of 0.01 M solution of tetraphenylborate under stirring. The resulting precipitate was filtered off, washed with water and dried in room temperature [11-15].

2.4. Preparation of the Electrodes

The general procedure to prepare the PVC membrane was as follow: different amounts of the ion-pair along with appropriate amounts of PVC, plasticizer and additive were dissolved in tetrahydrofuran (THF), and the solution was mixed well. The resulting mixture was transferred into a glass dish of 2 cm diameter. The solvent was evaporated slowly until an oily concentrated mixture was obtained. A pyrex tube (3-5 mm o.d.) was dipped into the mixture for about 10 s so that a transparent membrane of about 0.3 mm thickness was formed. The tube was then pulled out from the mixture and kept at room temperature for about 10 h. The tube was then filled with an internal filling solution (1.0×10^{-3} M clidinium bromide). The electrode was finally conditioned for 24 h by soaking in a 1.0×10^{-3} M clidinium bromide solution [11-16].

2.5. Standard Clidinium Solutions

A stock standard solution of 0.1 M clidinium bromide was prepared by dissolving 4.32 g of pure drug in 100 ml distilled water. The working solutions (1×10^{-6} to 1×10^{-1} M) were prepared by appropriate dilution of the stock solution with water.

2.6. The emf Measurements

Following cell assembly for emf (electromotive force) measurements were used:

Ag-AgCl || internal solution, 1×10^{-3} M clidinium bromide | PVC membrane | sample solution || Ag-AgCl, KC1 (satd).

These measurements were preceded using calibration of the electrode with several standard solutions.

3. RESULT AND DISCUSSION

Since the sensitivity and selectivity degree of an ion-pair based electrode is greatly related to the membrane ingredients, the membrane composition influence on the potential response of the clidinium sensor was studied.

Actually, the operating characteristics of ISEs can be significantly modified by changing the relative proportions of the electrode membrane components. The main components of an electrode membrane of this type are PVC matrix, the plasticizer and the ion-pair. Each membrane component plays a special role in the membrane function [16-20].

3.1. PVC Membrane Composition Selection

The plasticizer mainly acts as a fluidizer, allowing homogeneous dissolution and diffusion mobility of the ion-pair inside the membrane. The nature and/or the amount of the plasticizer must be properly controlled in order to minimize the electrical asymmetry of the membrane and to limit fouling of the sensor.

The nature of the plasticizer has a marked influence on the responses of this kind of sensors and also on the selectivity of the PVC membrane electrodes. Here, many plasticizer types were tested, namely benzylacetat (BA), dibutyl phthalate (DBP), nitrobenzene (NB), as listed in Table 1.

After their evaluation, NB, having the highest dielectric constant than other plasticizers shows the best response. Thus, it was used in construction of sensor for further studies, because it provided an effective linear range and a lower detection limit which is due to the better extraction of the clidinium in the organic phase of the membrane [11-15].

As it can be seen from Table 1, the absence of the ion-pair in the membrane result a very poor response (membrane no. 7), which is shows the significance of the ion-pair. As a conclusion, the membrane no. 2 with the composition of 30% PVC, 5% ion-pair, and 65% NB was the optimum one for the sensor design.

3.3. Calibration Graph and Statistical Data

The measuring range of a potentiometric sensor is the linear part of the calibration graph as shown in Fig. 2. Measurements could be performed in this lower range, but noted that more closely spaced calibration points are required for more precise determinations. For many electrodes the measuring range can extend from 10^{-1} molar to 10^{-6} molar concentrations [8, 21-24]. Calibration graph slope for PVC membrane electrode is 57 mV per decade of the clidinium concentration and a standard deviation of ±0.3 mV after

eight replicate measurements (Fig. 2). A linear response towards the clidinium concentration was from 1.0×10^{-5}-1.0×10^{-1} M .

Table 1. Optimization of the membrane ingredients

Membrane no.	PVC (% wt.)	Plasticizer (% wt.)	Ion-pair (% wt.)	Slope (mV/decade)	Linear range (M)	Detection Limit (M)
1	30	NB, 67	3	41.6± 0.4	1.0×10^{-5}-1.0×10^{-1}	1.0×10^{-5}
2	30	NB, 65	5	57.0± 0.3	1.0×10^{-5}-1.0×10^{-1}	1.0×10^{-5}
3	30	NB,60	7	56.7± 0.3	5.0×10^{-5}-1.0×10^{-1}	1.0×10^{-5}
4	30	DBP, 65	5	20.8± 0.3	1.0×10^{-5}-1.0×10^{-1}	1.0×10^{-5}
5	30	DBP, 63	7	16.5± 0.2	5.0×10^{-4}-1.0×10^{-2}	5.0×10^{-4}
6	30	BA, 65	5	32.1± 0.4	1.0×10^{-4}-1.0×10^{-2}	1.5×10^{-4}
6	30	BA, 63	7	22.1± 0.4	1.0×10^{-4}-1.0×10^{-2}	1.5×10^{-4}
7	30	NB, 70	-	4.3± 0.2	5.0×10^{-4}-5.0×10^{-3}	5.5×10^{-4}

Detection limit was calculated from the intersection of two extrapolated segments of the calibration graph. In this work, detection limit of PVC membrane sensor was 1.0×10^{-5} M which was calculated by extrapolating the segments of the calibration curve.

3.4. Dynamic Response Time

Dynamic response time is the required time for the electrode to achieve values within ±1 mV of the final equilibrium potential, after successive immersions in the sample solutions [16-18]. Its calculation involved the variation and the recording of the clidinium concentration in a series of standard solutions. Sensor was able to quickly reach its equilibrium response in the whole concentration range. The response time for PVC membrane electrode in the diluted solution (10^{-6}-10^{-4}) was about 25 s and in concentrated solution (10^{-3}-10^{-1} was 20 s.

3.5. pH Effect on the Electrode Response

To examine the effect of pH on the electrode responses, the potential was measured at a specific concentrations of the clidinium solution (1.0×10^{-4} M) from the pH value of 2.0 up to 12.0 (concentrated NaOH or HCl solutions were employed for the pH adjustment). The results showed that the potential remained constant despite the pH change in the range of 4.0 to 10.0, which indicates the applicability of this electrode in the specified pH range (Fig. 3). The fluctuations above the pH value of 10.0 might be justified by removing the positive

charge on the drug molecule. Fluctuations below the pH value of 4.0 were caused by removal of the ion-pair in the membrane or analyte in the solution.
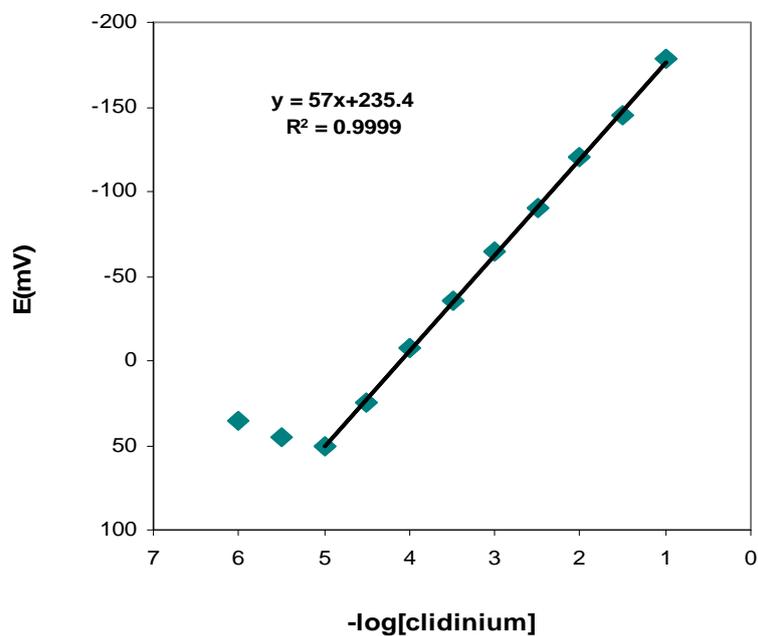

Fig. 2. Calibration curve of the clidinium PVC membrane electrode with the composition of the membrane no. 2. The results are based on 5 replicate measurements.
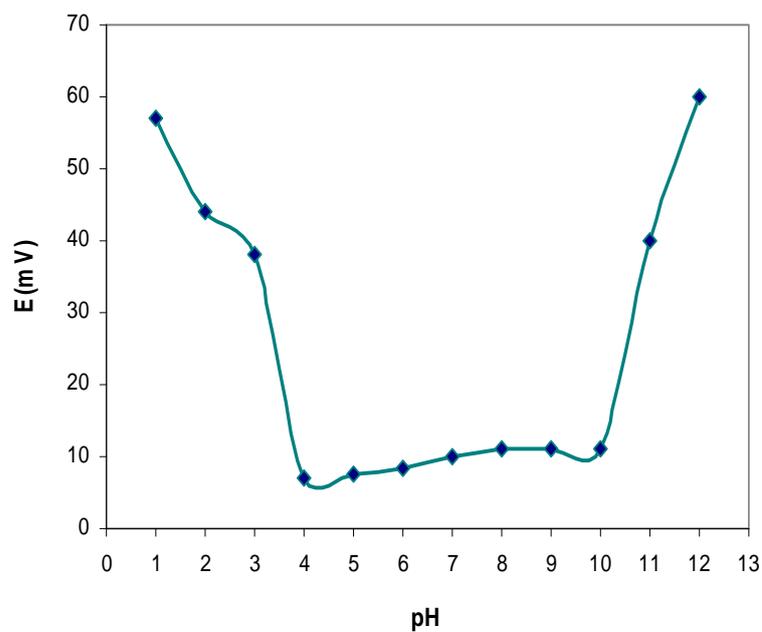

Fig. 3. pH effect of potential response of PVC membrane electrode

3.6. Life-time Study

Electrode lifetime was estimated with the calibration curve, periodical test of a standard solution and calculation of its response slope. For this estimation, four electrodes were employed extensively (1 h per day) for 10 weeks. After 6 weeks utilization of PVC membrane electrode, two changes were observed: a slight gradual decrease in the slope (from 57 to 47 mV/decade) and an increase in the detection limit (from 1.0×10^{-5} M to 5.0×10^{-4} M). In PVC membrane electrodes after several time of usage, the membrane ingredients leak from the organic layer and affect the membrane response.

3.7. Analytical Applications

Linearity, limit of detection, recovery test, selectivity, precision, and accuracy were the parameters used for the method validation. As mentioned before, the sensors were measured between 1×10^{-5} and 1×10^{-1} M for PVC membrane electrode. The calculated detection limit of the electrodes was 1.0×10^{-5} M (2.73 µg/mL).

3.7.1. Recovery Test from Tablet

The proposed sensor was evaluated by measuring the drug concentration in some pharmaceutical formulations (Table 2). Ten tablet of clidinium c (Sobhan Darou Co.) were carefully weighed. The mean weight of each tablet was calculated 0.0889 g. Each tablet contained 2.5 mg clidinium and 5 mg chlordiazepoxide. 15 tablets were gently grinded and powdered in a clean mortar. Then, as much as the weights of 10 tablets (10×0.0889) was weighed and transferred into a 50-mL volumetric flask, diluted with distilled water and adjust pH to 5. After centrifuge for 5 min (5000 rpm), the potential of this solution was measured. Clidinium content of each sample was calculated by the calibration method.

Table 2. Potentiometric determination of clidinium in some pharmaceutical formulations

Samples	Labeled amount (mg/tab.)	Found* (mg/tab.)
Sample I	2.5	2.81±0.23
Sample II	2.5	2.76.±0.16
Sample III	2.5	2.69±0.19

* The results are based on triplicate measurements

The drug concentration was determined using calibration method. The results are in satisfactory agreement with the labeled amounts. The corresponding recovery percentage value varied from 3.3-5.4%.

3.7.2. Release of Clidinium from Formulation

For the assessment of the sensor function, the clidinium release in its formulation was studied. The proposed sensor was employed as an indicator electrode in the titration of one clidinium tablet (2.5 mg active ingredient per tablet) in 100 ml of water solution (pH=5) having 200 mg NaCl. The profile of clidinium release is shown in Fig. 4.
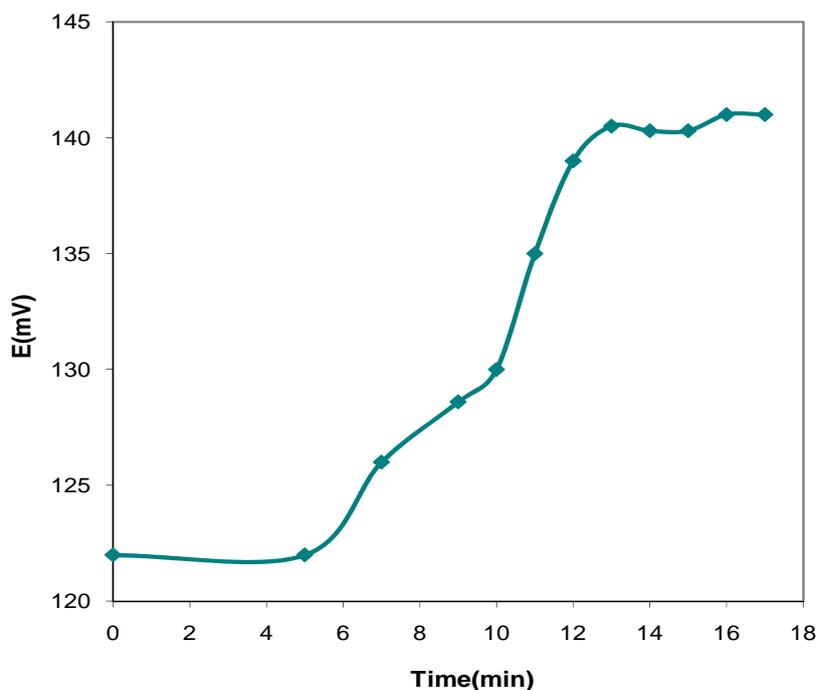

Fig. 4. Clidinium release from its formulation

3.7.3. Selectivity

Selectivity, which describes an ion-selective electrode's specificity toward the target ion in the presence of interfering ions, is the most important characteristic of these devices.

The potentiometric selectivity coefficients of the clidinium sensor were evaluated by the matched potential method (MPM) [25]. The resulting values of the selectivity coefficients are shown in Table 3. Note that all selectivity coefficients are between -4.5 and -3.4, suggesting were interferences negligible in the performance of the electrode assembly.

3.7.4. Precision and accuracy

For repeatability monitoring, 5 replicate standard samples of 5, 50, 500 µg/mL were measured. The respective RSD values obtained by PVC membrane were 4.1, 4.5, and 3.8%.

3.7.5. Ruggedness/Robustness

For ruggedness of the methods a comparison was performed between the intra- and inter-day assay results for clidinium obtained by two analysts.The RSD values for the intra- and

inter-day assays in the cited formulations performed in the same laboratory by the two analysts did not exceed 5.1%. On the other hand, the robustness was examined while the parameter values (pH of the solution and the laboratory temperature) changed slightly. Clidinium recovery percentages were good under most conditions, and not showing any significant change when the critical parameters were modified.

Table 3. Selectivity coefficients of various interfering compound for clidinium sensor

Interference	Log K_{MPM}
K^+	-3.8
Na^+	-3.5
NH_4^+	-3.4
Mg^{2+}	-4.1
Ca^{2+}	-4.0
NO_3^-	-4.5
Cl^-	-4.4
CO_3^{2-}	-3.8
Lactose	-4.1
Glucose	-4.2
Chlordiazepoxide	-3.6

4. CONCLUSION

Two kinds of potentiometric electrode were constructed for determination of clidinium bromide. The sensors demonstrated advanced performances with a fast response time, a lower detection limit of 1.0×10^{-5} M with potential responses across the range of 1.0×10^{-5}-1.0×10^{-1} M. The sensors enabled the clidinium determination in pharmaceutical formulations. Sensor respond based on ion-exchange mechanism. CLD-TPB pair was employed as a sensing element in construction of electrode. The best PVC membrane electrode performance was achieved by a membrane composition of 30% PVC, 65% NB and 5% ion-pair.

REFERENCES

[1]   [online] available: http://en.wikipedia.org/wiki/Clidinium_bromide

[2]   A. C. Moffat, M. D. Osselton, B. Widdop, J. Watts, Clarke's Analysis of Drugs and Poisons, Pharmaceutical Press, Third ed., 2004.

[3]   M. Inés Toral , P. Richter, N. Lara, P. Jaque, C. Soto, M. Saavedra, Int. J. Pharm. 189 (1999) 67.

[4]   I.M. Jalal, S.I. Sa'sa, A. Hussein, Anal. Lett., 20 (1987) 635.

[5]   A.S. Amin, H.A. Dessouki, M.M. Moustafa, M.S. Ghoname, Chem, Papers, 63 (2009) 716

[6]   M. R. Khoshayand, H. Abdollahi, A. Moeini, A. Shamsaie, A Ghaffar, S. Abbasian, Drug Testing and Analysis, 2 (2010) 430.

[7]   A. Pathak, P. Rai, S.J. Rajput, J. Chrom. Sci., 48 (2010) 235.

[8]   F. Faridbod, M. R. Ganjali, R. Dinarvand, and P. Norouzi, Sensors, 8 (2008) 2331.

[9]   C. Meier, D. Amman, W. E. Morf, and W. Simon, in: J. Koryta, ed., Applications of Electrochemical Devices in Medical and Biological, John Wiley and Sons, Chichester, New York, Brisbane, Toronto (1980) 13.

[10] D. R. Thomas, Anal. Chim. Acta 180 (1986) 289.

[11] M. R. Ganjali, T. Razavi, F. Faridbod, S. Riahi, and P. Norouzi, Curr. Pharm. Anal. 5 (2009) 28.

[12] M. R. Ganjali, A. Alipour, S. Riahi, B. Larijani and P. Norouzi, Int. J. Electrochem. Sci. 4 (2009)1262.

[13] F. Faridbod, M. R. Ganjali, R. Dinarvand, S. Riahi, P. Norouzi and M. B. A. Olia, J. Food Drug Anal. 17 (2009) 246.

[14] M. R. Ganjali, F. Aboufazeli, S. Riahi, R. Dinarvand, P. Norouzi, M. H. Ghasemi, R. Kiani-Anbuhi and S. Meftah, Int. J. Electrochem. Sci. 4 (2009) 1138.

[15] F. Faridbod, M. R. Ganjali, L. Safaraliee, S. Riahi, M. Hosseini and P. Norouzi, Int. J. Electrochem. Sci. 4 (2009) 1419.

[16] H. A. Zamani, G. Rajabzadeh, M. R. Ganjali and P. Norouzi, Anal. Chim. Acta 598 (2007) 51.

[17] V. K. Gupta, A. K. Jain, L. P. Singh and U. Khurana, Anal. Chim. Acta 355 (1997) 33.

[18] VA. K. Singh, V. K. Gupta and B. Gupta, Anal. Chim. Acta 585 (2007) 171.

[19] D. Ammann, W. E. Morf, P. Anker, P. C. Meier, E. Pretsch, and W. Simon, Ion-Sel. El. Rev. 5 (1983) 3.

[20] V. K. Gupta, R. Ludwig and S. Agarwal, Anal. Chim. Acta 538 (2005) 213.

[21] S. K. Srivastava, V. K. Gupta, S. Jain, Electroanalysis 8 (1996) 938.

[22] V. K. Gupta, S. Chandra, and H. Lang, Talanta 66 (2005) 575.

[23] H. A. Zamani, M. R. Ganjali, P. Norouzi, and S. Meghdadi, J. Appl. Electrochem. 37 (2007) 853.

[24] V. K. Gupta, A. K. Singh, and B. Gupta, Anal. Chim. Acta 575 (2006) 198.

[25] P. R. Buck, and E. Lindneri, Pure Appl. Chem. 66 (1994) 2527.