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Novel PVC Membrane Selective Electrode for the Determination of Clozapine in Pharmaceutical Preparations

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Novel clozapine ion selective electrode was fabricated, characterized and used for determination of clozapine in its pharmaceutical formulations. The proposed sensor incorporated clozapine phosphotungstate ion pair complex as electroactive material in PVC matrix membrane and dioctylsebacate as solvent mediator. The resulting electrode demonstrates near –Nernstain response over wide linear range (10^{-5}–10^{-2} M) clozapine with lower limit of detection of 3.7 x 10^{-6} M and slope of 57.46 ±0.1 mV/ decade. Fast and stable response, good reproducibility, long term stability (4 months) and applicability over a wide pH range (4.5-8). The sensor displayed a good selectivity for clozapine with respect to number of common foreign inorganic, organic species, excepients and the fillers added to the pharmaceutical preparation fortunately, such materials mostly do not interfere. The sensors were successfully applied for the determination of clozapine in its tablets.

Keywords: Clozapine , Clozapine phosphotungstate ion pair complex, Potentiometric determination.

1. INTRODUCTION

The quick determination of minute quantities of ionic species by simple methods has a great importance in analytical chemistry. potentiometric detection based on ion – selective electrodes (ISEs) is the simplest of all and offers unique advantages. Such as simple design and operation, reasonable selectivity, fast response applicability to colored and turbid solution and low cost. These electrodes can be prepared by incorporating any of the many ion –exchanger or neutral- sequestering agents within a plasticized PVC matrix, and used as very useful tools for clinical , chemical and environmental analysis [1-8]. Clozapine(CZ) (Figure1) is (8-Chloro-11(4-methyl piperazine-1-yl)- 5H- dibenzo [b,e] [1,2,9,10] diazepine) is an effective antipsychotic drug treating schizophrenia [9]. Owing to its importance, researches used many analytical methods to study it, including capillary zone

electrophoresis[10,11], chromatography [12-35], spectrophtomerty [36-40], conductimetry [37, 41] colorimetry [42] , mass spectrometry, [43]. Few electrochemical methods have been reported for clozapine determination as a drug substance or in commercial dosage forms [44-51].The use of ion selective electrode is simple and even allow in vivo measurements, horseradish peroxidase(HRP) modified carbon paste electrode, sepiolite modified carbon paste electrode and activated glassy carbon electrode by using wax-impregnated graphite electrode were used for constructing clozapine sensors [52] ,also the electrochemical behavior of clozapine at 16-mercaptohhexadecanoic acid self- assembled monolayer modified gold electrode was reported[53]. Recentily Farhadi et al.[54] developed an ISE based on triiodide – clozapine ion pair. Some of these methods involve several manipulation steps, which are not simple for routine analysis of pharmaceutical formulations and need sophisticated instruments. This work originates from the fact that clozapine behave as a cation, thus we prepare 1:1 ion pair complex between phosphotungstate as anionic exchanger and clozapine in order to develop a new potentiometric method for the drug determination. The high lipophilicity and remarkable stability of this complex suggest its selective use as electroactive material in PVC matrix membrane sensors. This facilitates its determination in pharmaceutical formulations in the presence of its degradation products without any interference.

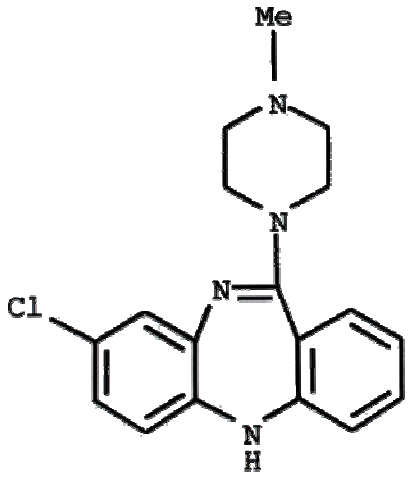

Figure 1 . The structure of clozapine

2. EXPERIMENTAL PART

2.1. Equipment

All potentiometric measurements were made at 25±1^{o}C with an Orion (Model 811) pH/mV meter. An Orion double junction Ag/AgCl reference electrode (Model 90-92) containing 10% (w/v) KNO_3 in the outer compartment was used in conjunction with PVC- Clozapine based sensor combined glass electrode (Orion HI 1332) was used for all pH adjustment.

2.2. Chemicals and solutions

Chemicals of analytical reagent grade were used. Doubly distilled water was used for all experiments. Clozapine provided by National Organization for Drug Control and Research.
Poly (vinyl chloride) powder (PVC), phosphotungstic acid (PTA),(THF), Dioctyl sebacate (DOS) were obtained from Fluka Alkali, alkaline earth, carbohydrate and transition metal salts in the form of chloride, sulphate or nitrate were obtained from Aldrich chemical company. Standard 10^{-2} M of solution was prepared with doubly distilled water. A standard solution of 10^{-2} M of clozapine was freshly prepared with doubly distilled water. Dilute solutions (10^{-2} -10^{-6} M) were prepared by successive dilutions of the respective stock solution using acetate buffer of pH 5.

2.3. Potentiometric determination of clozapine

2.3.1. Construction of the sensors

Clozapine phosphotungstate ion pair complexes were prepared by slow addition of twenty ml of 10^{-2}M aqueous solution of phosphotungstate and 10 ml of 10^{-2} M clozapine hydrochloride were mixed and stirred for 15 min. The yellow precipitate was filtered off with porosity sintered-glass crucible, washed with deionized distilled water, dried at room temperature and ground to fine powder. The elemental analysis agreed with the composition.

A 10 mg portion of clozapine ion pair complexes was thoroughly mixed with 0.19g PVC, 0.35g Dioctyl sebacate and 5ml of THF in glass petri dish (5-cm diameter) covered with a filter paper and left to stand over night to allow slow evaporation of the solvent at room temperature. A master PVC membrane approximately 0.1mm thick were cut with a cork borer and glued onto 7 mm PVC body using THF. The tube was then filled with internal filling solution consisted of equal volumes of 1x 10^{-2} M of both Clozapine hydrochloride and potassium chloride. An Ag/AgCl coated wire was employed as an internal reference electrode.

2.3.2. Sensor calibration

The sensor was pre-conditioned after preparation by soaking for at least 24 h in 10^{-2} M clozapine hydrochloride solution and was stored in the same solution when not in use**.** A solutions (25ml) of 10^{-6} - 10^{-2}M standard (CZ) were transferred into 50-ml beakers and PVC-CZ-PT membrane sensor in conjunction with the Ag/AgCl reference electrode was immersed in the solution. The solutions were stirred and the potential was recorded after stabilization and plotted as a function of (CZ) concentration, the graph was used for the subsequent determination of unknown concentration of CZ.

The potential readings were recorded after stabilization and the emf were plotted as a function of logarithm clozapine concentration. The lower detection limit was taken at the point of intersection of the extrapolated linear segments of the clozapine calibration curve.

2.3.3. Effect of dynamic response time

The response time of the sensor was measured in constantly stirred solutions of varying clozapine ions concentration. The stability of the potential was measured for different concentrations.

2.3.4. Effect of pH

A standard 10^{-2} M and 10^{-3} M of aqueous clozapine hydrochloride solutions were prepared and adjusted to various pH values in the range 2-12 with dilute hydrochloric acid and /or sodium hydroxide solutions. The change of potentials was examined by plotting the change of potential against pH values.

2.3.5. Determination of selectivity coefficients

Selectivity coefficients of the sensor were determined using the separate solution method (SSM) [55, 56] and calculated from the rearranged Nicolsky equation.

$$\log K^{pot}_{cloz.,M} = [E_X - E_{cloz.}/S] - \log a_X^{(Z_{cloz.M}/Z_X)} + \log a_{cloz}$$

Where

$E_{cloz.}$ is the potential measured in 10^{-2} M clozapine hydrochloride solution.

E_X is the potential measured in a 10^{-2} M solution of the interfering cations.

Z_{cloz} and Z_X are the charges of clozapine and interfering ions, respectively and S is the slope of the sensor calibration plot.

The selectivity was determined in the presence of buffer acetate. pH 5 prepared by dissolving 13.6 g of sodium acetate and 6ml of glacial acetic acid in sufficient distilled water to produce 1000 ml.

2.4. Determination of clozapine in its pharmaceutical preparation

Five tablets of the drug were weighed in a small dish, powdered and mixed well. A portion equivalent to one tablet (=100 mg of clozapine) was weighed and dissolved in 15 ml hydrochloric acid (1N) and completed to 1000 ml by distilled water, shaken well and filtered through glass crucible. One ml of this solution transferred to 25 ml volumetric flasks, followed by buffer acetate of pH 5. The EMF of the test solution was directly measured and compared with the calibration graph.

3. RESULTS AND DISCUSSION

3.1. The performance characteristics of clozapine tungstophosphate -PVC-membrane sensor

Clozapine cation reacts with phosphotungstate anion to form water insoluble ion association complex the prepared complex was identified and examined as ion exchange site in PVC membrane sensor responsive for clozapine cation.

The electrochemical performance characteristics of the sensor was evaluated according to IUPAC recommendation and the results are summarized in Table (1) and Figure 2. The proposed sensor showed near-Nernstian response for at four orders of magnitude of clozapine concentrations (10^{-5} -10^{-2} M) with calibration slope of 57.46 mV/decade and lower limit of detection of $3.7x10^{-6}$ M. The proposed sensor showed fast response time at various drug concentrations. By using the dipping method, the electrode is instantaneously immersed into a solution of known activity of the tested ion simultaneously, the response record is started. In order to examine the dependence of the response time on concentration and the operation repeated with a series of separate solutions **[57] .** At a concentration level of 10^{-3} M clozapine, their response times defined as the time required to attain 95 % of steady state potential , not exceed 5 s.

Table 1. Performance characteristics of clozapine membrane sensor

PARAMETER	Clozapine
Slope, (mV/decade)	57.46 ± 0.1
Linear concentration range,(M)	$1x10^{-5}$ -$1x10^{-2}$
Intercept, (mV)	303.16 ± 0.5
Life span, (month)	4 months
Lower limit of detection, (M)	$3.7x10^{-6}$
Working pH, (pH)	4.5 – 8
Response time, (s)	10
Correlation coefficient, (r)	0.99986

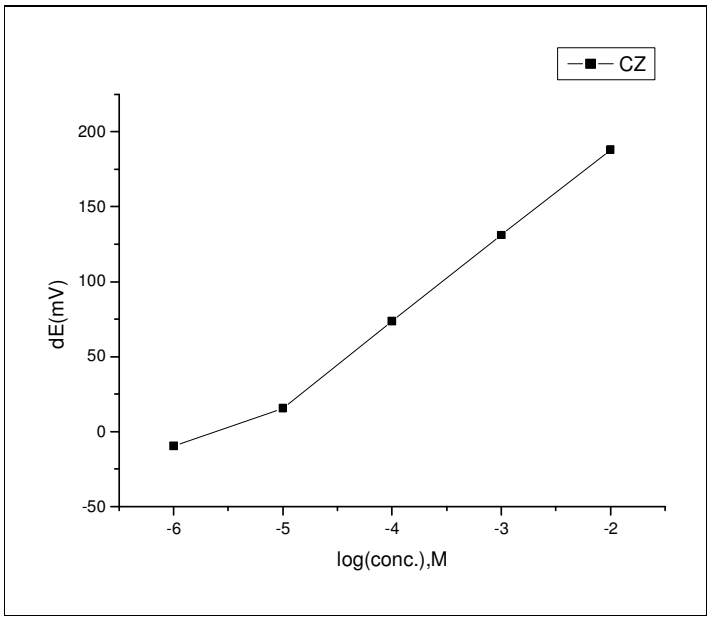

Figure 2. Calibration curve for clozapine sensor

At lower concentration (10^{-4} -10^{-5} M), the response time did not exceed 10 s. The response characteristics of the sensor did not change during 4 months of the continuous use. A study of the potential response of CZ – PVC – PT membrane sensor as a function of changes in the pH of the drug and no change occur over the pH range 4.5-8, see Figure 3. At lower pH the potential readings were decreased and at higher pH the disturbance happen in EMF reading due to interference of H^+ as shown in Figure 3.

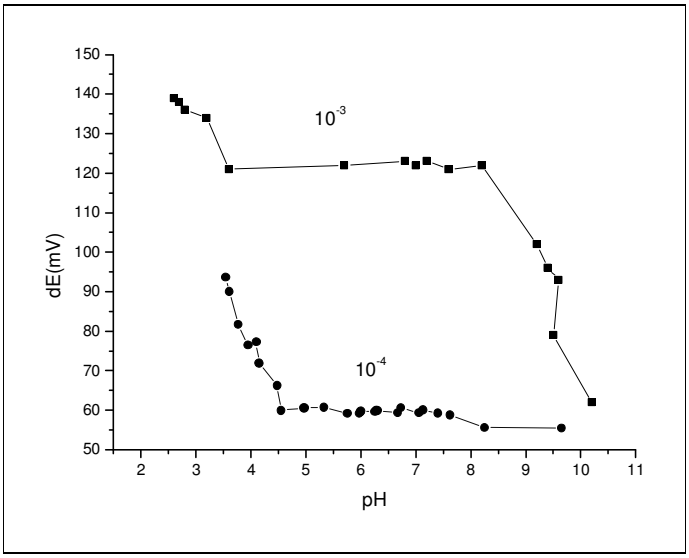

Figure 3. Effect of pH on the response of (CZ-PT) (DOS) based membrane sensor.

3.2 Selectivity of CZ-PT-PVC membrane sensor

The potentiometric selectivity coefficients $K^{pot}_{CZ,M}$ of clozapine sensor was evaluated using the separate solution **[**58**]** with 10^{-2} M concentration level of alkali, transition metal and some excipient, in the drug such as Tartarate, Fructose, Starch and others Figure 4. The bulk of the excipient in a pharmaceutical tablet does not show any interference. This result supports the fact that selectivity is determined primarily by the partition coefficient of the protonated amine between organic and aqueous phase **[**59**]**, and the inorganic cations do not interfere because of differences in ionic size and mobility [60]. The sensor showed a higher selectivity for clozapine ion than other interferents Table (2).

Table 2. Selectivity coefficients of sensors based on (CZ) ionophore.

Interferent (M)	$K^{pot}_{CZ,M}$
Tartarate	$2.5x10^{-3}$
Fructose	$1.6x10^{-3}$
Malonate	$1.7x10^{-3}$
Glucose	$0.7x10^{-3}$
Lactose	$1.0x10^{-3}$
Urea	$0.6x10^{-3}$
Starch	$0.7x10^{-3}$
Na^{+}	$0.1x10^{-3}$
Co^{++}	$1.4x10^{-3}$
Sr^{++}	$0.27x10^{-3}$

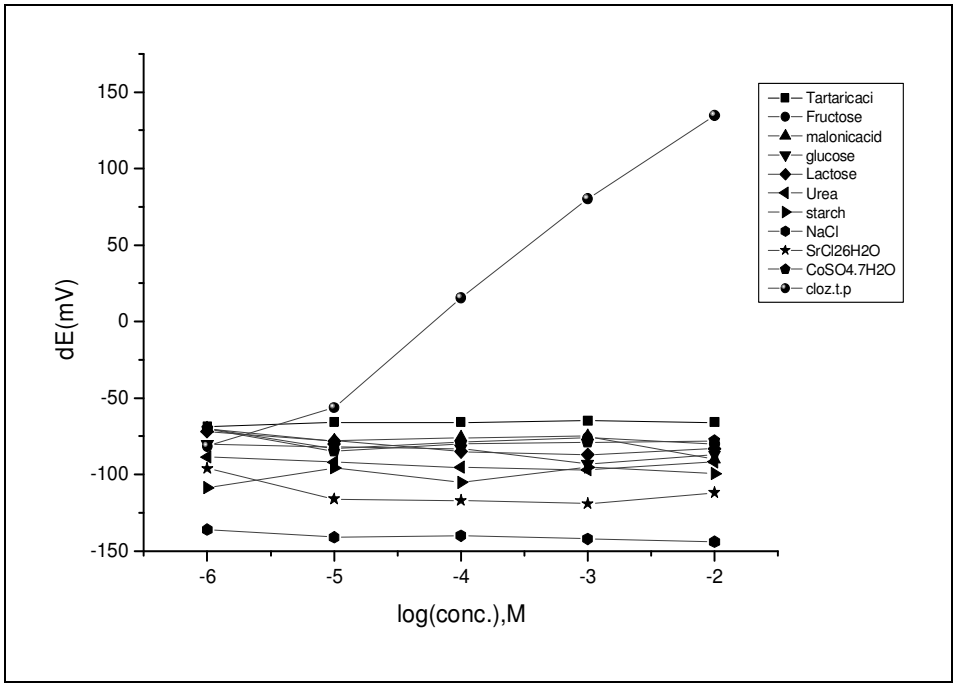

Figure 4. Selectivity characteristics of (CZ) sensors

3.3. Effect of ionic excluders

An effort was made to improve the response characteristics of clozapine sensor, by incorporating Nitron in the sensor cocktail to act as anion excluder, to improve selectivity, to reduce the activation barrier of the membrane/solution interface. The sensor gives a bad response and non Nernstian slope, and no improvement of the sensor occurs. The deviation from linear ship might be attributed to the fact that not enough potential was built on the electrode membrane surface [61**],** PVC

membrane used in this study contained enough anionic site even without addition of extrinsic additive[62]**.**

3.4 Analytical application

This membrane electrode can be successfully used for analysis of clozapine in pharmaceutical preparations. The results obtained for determining clozapine in its pharmaceutical preparation Leponex based on membrane sensor show average recoveries of 99.4%. In Table (3) no interferences occurred from excipients and diluents used in pharmaceutical preparations.

Table 3. Determination of clozapine in pharmaceutical preparation

Trade name and source	Nominal content (mg tablet^{-1})	Recovery
Leponex (Glaxo)	100 mg tablet^{-1}	99.4%

Table 4. Comparison between different methods for determination of clozapine

Method	Working Range	Lower Limit of detection	Accuracy	Reference
HPLC	50-1000 mg/1	15 mg/1	NR	15
HPLC	10-1000 ng/ml	5 ng/ml	93 - 109%	16
HPLC	50-400 mg/1	NR	90%	11
HPLC	1.5 – 30 mg/l	1.5 mg/ml	Error: ± 2.27%	10
HPLC – UV method	50-500 ng/ml	15 ng/ml	94.3 – 103.0%	8
Conductimetric	3.27 – 26.25 mg	NR	99.39 – 99.90%	31
Ion Selective Electrode	1x 10^{-5} – 1x 10^{-2} M	3.4 x 10^{-6} M	99.4%	This work

4. CONCLUSIONS

Although ion-selective electrodes have found many successful applications in pharmaceutical analysis mainly because of their low cost, ease of use, maintenance, the simplicity and speed of the assay procedures, it has not been applied yet to the determination of clozapine. It is usually possible to develop procedures for the determination of drugs in pharmaceutical preparations that need only a pre-

dilution step with a suitable buffer. Turbidity due to the matrix is not usually a problem. Usually, the potentiometric methods can be simple and fast for pharmaceutical analysis when suitable sensor is available. Table (4) reported a comparison between the present work and those of the previously done by other sophisticated methods. It was found that the proposed sensor has the advantages of high selectivity fast response and direct application of drug samples without prior separation or treatments. On the other hand the suggested method are simple and reproducible (RSD= 1.1-3.2 and 1.5 – 2.7 for pure and dosage forms respectively) The proposed method can analyze clozapine in its pharmaceutical forms without interferences from excipients.

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