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All-Solid-State Citalopram Sensor and its Application for the Analysis of Pharmaceutical Formulations

Mohammad Reza Ganjali, [1,2,*] and Bagher Larijani[3]

[1]*Center of Excellence in Electrochemistry, University of Tehran, Tehran, Iran*
[2]*Biosensor Research Center, Endocrinology & Metabolism Molecular-Cellular Sciences Institute, Tehran University of Medical Sciences, Tehran, Iran*
[3]*Endocrinology & Metabolism Research Center, Endocrinology & Metabolism Clinical Sciences Institute, Tehran University of Medical Sciences, Tehran, Iran*

* Corresponding Author, Tel.: +98-21-88001813
E-Mail: ganjali@khayam.ut.ac.ir

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Abstract- Citalopram (CP), which is a selective serotonin reuptake inhibitor (SSRI), is used for the treatment depression. Due to the importance of the analysis of this compound, an all-solid-state polymeric membrane electrode (ASS-PME) has been devised using an ion-pair agent as the sensing element of the device. The experimental results proved that the best sensor response is observed using membranes including 7%wt CP-tetraphenyl borate (the sensing element), 57%wt dibutyl phthalate as the solvent mediator, 33%wt poly(vinyl chloride) and 3%wt of an ionic liquid. The ASS element of the device is a conductive composite of graphite, multiwall carbon nanotubes (MWCNTs), and epoxy resin which is coated on a Cu wire, which is finally coated with a thin film of the PME. The response of the ASS-PME was linear in the range of 1.0×10^{-7} to 1.0×10^{-3} mol L^{-1} and reached the slope of 57.3±0.3 mV/decade under optimal conditions. The experiments revealed that the optimized Citalopram ASS-PME is applicable in the quality control analyses, which was validated by its application for measurement of Citalopram in its tablets.

Keywords- Citalopram, Sensor, All solid state, Potentiometry, Pharmaceutical formulation

1. INTRODUCTION

1-(3-dimethylaminopropyl)-1-(4-fluorophenyl)-5-phthalan carbonitrile or in short Citalopram (Figure 1), is a selective serotonin reuptake inhibitor (SSRI) known as an antidepressant which is used in the treatment of anxiety, depression, body dysmorphic disorder, as well as social anxiety, panic, obsessive-compulsive, and premenstrual dysphoric disorders and Huntington's disease.

Neurotransmitter actually act as an important element of the communication system of the brain and it has been widely accepted that depression is caused by an imbalance of neurotransmitters. The mechanism of action of citalopram is through influencing a neurotransmitter (i.e. serotonin) by preventing its uptake by nerve cells after it has been released. This can terminate the action of the released neurotransmitter on the adjacent nerves, leading to the stimulation of the nerve cells [1].

Some research has been focused on the determination of citalopram in biological and pharmaceutical [2-8] samples by various methods. However, potentiometric detection using ions selective electrodes (ISEs) presents advantages like easy procedures, short analysis and response times, good selectivity and wide linear dynamic ranges, and low costs, which have resulted in an increasing number of reports on ISEs, for different species including pharmaceutical analytes [9-12].

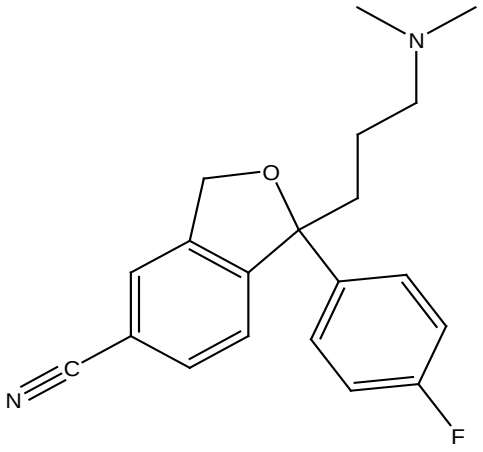

Fig. 1. Chemical structure of Citalopram

Ion-selective electrodes (ISEs) can be sub-classified into different categories, including PVC membrane electrodes (PME) [13-22], coated wire electrodes (CWE) [23-25], carbon paste electrodes (CPE) [26], all solid state electrodes (ASS) [27-32] and field effective transistors (FET). Structurally speaking these devices can be further categorized as being symmetric or asymmetric regarding the way the PVC membrane is placed on the transducer. Symmetric devices are those, in which the PVC is located between two internal and external solutions. In the latter category, however, the outer surface of the membrane is in contact

with a solution (sample solution), while it's inner surface is in contact with a solid state transducer. A symmetric membrane electrodes can easily remove during the long time treatments. A major difference of the two classes is that the symmetric devices do not usually reach very low detection limits and these values are usually in the order of 10^{-5} to 10^{-7} mol L^{-1} for symmetric devices, while asymmetric electrodes produce improved results in the order of 10^{-8} mol L^{-1} or lower.

All-solid-state polymeric membrane electrodes (ASS-PME) [27-32] in which a conductive solid state (e.g. a polymeric composite of graphite mixed with epoxy resin) internal contact consists the internal contact of the membrane are examples of asymmetric electrodes. The solid contact, is covered with a layer of the ion selective PVC membrane. Naturally ASS-PMEs provide improved results in terms of lower detection limits, and reasonable mechanical stability and structural simplicity.

In the light of the insight gained during previous work on symmetric devices, like a PME Citalopram [33], the focus of the present work was on the devise of a Citalopram ASS-PME based on ion-exchange mechanism, for the determination of the analyte in pharmaceutical samples, to gain advantages of wider linear ranges and lower detection limits. The selectivity of the membrane was due to the incorporation of an ion-pair compound in the PVC matrix.

2. EXPERIMENTAL SECTION

2.1. Measurements

To perform the measurements the devised ASS-PME was used as the indicator electrode in a cell assembly as below:

Cu wire | ASS layer | PVC membrane | sample solution || Ag-AgCl, KC1 (satd.)

Further using a reference electrode (Ag/AgCl; Azar-Elelectrode Co., Iran), which was connected to the indicator ASS-PME using an ion analyzer (with a 250 pH/mV meter with ±0.1 mV precision). The measurements were based on calibration using different standard solutions.

2.2. Materials and Reagents

The chemicals (i.e. sodium tetraphenyl borate (NaTPB), potassium tetrakis (p-chlorophenyl) borate (KTPClPB), dibutyl phthalate (DBP), nitrobenzene (NB), dibutyl sebacate (DBS), benzyl acetate (BA), *o*-nitrophenyloctylether (*o*-NPOE), 1-n-butyl-3-methylimidazolium tetrafluoroborate ([bmim]BF_4), 1-n-butyl-3-methylimidazolium hexafluorophosphate ([bmim]PF_6), tetrahydrofuran (THF), all the solvents and salts and graphite powder (1–2 μm)) were all of analytical reagent grade and were procured from

Merck Co High-molecular weight PVC was from obtained from Fluka Co. and the MWCNTs (10-40 nm diameters, 1-25 μm length, core diameter: 5-10 nm, SBET: 40-600 m^2/g, V_{total}: 0.9 cm^3/g, bulk density 0.1 g/cm^3, true density 2.1 g/cm^3 and with 95% purity) were obtained from the Research Institute of the Petroleum Industry, Tehran, Iran. The Epoxy (macroplast Su 2227) and the hardener (desmodur RFE) was obtained from Henkel and Bayer Ag (Germany) respectively. CP hydrobromide was obtained from Sigma-Aldrich. The pharmaceutical formulations were received from a local pharmacy (Tehran, Iran).

The CP.HBr solutions were prepared by preparing a 0.1 mol L^{-1} of this water soluble compound in distilled water as the stock solution. The rest of the solutions were prepared through the dilution of the stock solution to give solutions in the range of 1×10^{-9} to 1×10^{-2} mol L^{-1}. The solutions were stored in refrigerator (4 °C).

In the case of the real samples twenty 20 mg CP.HBr tablets were crushed and powdered. Next an amounts equivalent to the weight of 5 tablets were carefully weighed and transferred into a 100-mL volumetric flask, dissolved in distilled water and diluted with acetate buffer (0.1 mol L^{-1}; pH=4). THE solution was filtered through a Millipore filter (0.45 mm) and used as the stock solution.

2.3. Synthesis of the ion-pair

The ion-pair complex to be used in the PME was prepared by mixing solutions of CP-HBr (40 mg in 15 mL distilled water) and a solution of suitable an organic salt with hydrophobic large anions and small inorganic cations (e.g. sodium tetraphenyl borate (NaTPB) or potassium tetrakis (p-chlorophenyl) borate) at (40 mg in 5 mL distilled water). The resulting precipitate was filtered, and rinsed with distilled water, then dried in room temperature.

2.4. Constructions of the sensors

The initial step for the preparation of the ASS-PME includes the preparation of the PME by mixing known weights of the ion-pair compound, PVC, the plasticizer and ionic additive in tetrahydrofuran (THF). The mixture was next mildly heated to let the THF evaporate resulting in a viscous concentrated solution [10-15].

In the meantime the a conductive polymeric composite was also prepared through mixing graphite powder, MWCNTs, epoxy, and hardener in various weights and loading the resulting paste on the surface of the internal contact which was a copper wire. The investigations proved the best solid-state contact composition to be 0.30% w/w of the epoxy resin, 15% w/w of the hardener, 5% w/w of MWCNTs and 50% w/w of graphite powder. These ingredients were mixed in THF, and the resulting mixture was left to rest for about 20-30 min for the solvent to evaporate, and the resulting oily mixture was coated on the copper wire (0.5 mm diameter and 15 cm length), let dry for 10 h and then polished

well before it was immersed in the viscose mixture of PME prepared in the first step. Once the ASS was coated with the PME after 3 immersions, the whole device was allowed to dry in air for 24 h. The so prepared ASS-PME was finally conditioned in a 10^{-3} mol L^{-1} solution of CP.HBr.

3. RESULTS AND DISCUSSION

3.1. The PME Composition

The nature, amount and ratio of the components of the membrane should be optimized to optimize the performance. Table 1 presents an overview of the different membrane compositions tested and the subsequent results. It is known that the plasticizer/polymer ration should range from about 2 to 2.2 [34-40]. For the ease of calculations 33%wt. of PVC was used in all compositions.

Table 1. Compositions of the membranes used in preparation of CP sensor

No.	Composition of the membrane			Characterization of PME		
	Plasticizer	**Ion-pair**	**Ionic Additive**	**Slope mV/decade**	**LR (mol L^{-1})**	**Response time**
1	DBP, 65	2	-	19.2±0.3*	1.0×10^{-4}-1.0×10^{-3}	59 s
2	DBP, 63	4	-	39.1±0.4	1.0×10^{-4}-1.0×10^{-3}	57 s
3	DBP, 61	6	-	48.1±0.3	5.0×10^{-5}-1.0×10^{-3}	51 s
4	DBP, 60	7	-	50.2±0.2	5.0×10^{-5}-1.0×10^{-3}	50 s
5	DBP,59	8	-	50.2±0.3	5.0×10^{-5}-1.0×10^{-3}	47 s
6	DBP, 58	7	2 NaTPB	54.9±0.4	1.0×10^{-6}-1.0×10^{-3}	22 s
7	DBP, 57	7	3 NaTPB	55.7±0.2	1.0×10^{-6}-1.0×10^{-3}	21 s
8	DBP, 57	7	3 KpClTPB	55.8±0.3	1.0×10^{-6}-1.0×10^{-3}	20 s
9	DBP, 57	7	3 [bmim]PF_6	57.3±0.3	1.0×10^{-7}-1.0×10^{-3}	20 s
10	DBS,57	7	3 [bmim]PF_6	57.1±0.4	1.0×10^{-7}-5.0×10^{-3}	24 s
11	NB, 57	7	3 [bmim]PF_6	40.4±0.4	1.0×10^{-5}-1.0×10^{-3}	21 s
12	BA, 57	7	3 [bmim]PF_6	50.5±0.3	1.0×10^{-6}-1.0×10^{-3}	23 s
13	NPOE, 57	7	3 [bmim]PF_6	46.3±0.3	1.0×10^{-5}-1.0×10^{-3}	21 s

*standard deviation of five repeated measurements

The solvent mediator or plasticizer, which is a non-volatile water-immiscible solvent facilitates the mobility of the sensing material through the membrane phase based on its properties [40-45]. Different plasticizers with various dielectric constants have been used to this end. In this work dibutyl sebacate (DBS;DC: 4.5), dibutyl phthalate (DBP; DC: 6.4),

nitrophenyloctyl ether (*o*-NPOE; DC: 24), nitrobenzene (NB; DC: 35.7) and benzylacetate (BA; DC: 5.7) were used, and the results in Table 1 show that DBP, which was used in the composition with the optimal results (no. 9) leads to the best performance. This can be explained based on the fact that the hydrophobic CP cation is extracted better with plasticizers of lower DC.

Ionic additives that are commonly incorporated into the membrane composition for reducing the Ohmic resistance of the membrane were also used in this work. These compounds are used in relatively small amounts so that they do not interfere with the ion-exchange phenomena which are preferred to be solely performed by the ionophore. In this work room temperature ionic liquids (RTILs), that have recently been reported as promising ionic additives were also evaluated and among all additives tested, the RTIL [bmim]PF_6 was found to lead to the best response behavior (no. 9).

Based on the data in Table 1, membrane no. 9 containing 7%wt of the ion-pair, 57%wt of DBP, 33%wt of PVC, and 3%wt of [bmim]PF_6 revealed a Nernstian behavior with a slope of 57.3±0.3 mV per decade, and was hence used for further evaluations.

3.2. Calibration curves

During the measurements various concentrations of CP.HBr were measured using the ASS-PME and the calibration curve (E *vs.* –log [CP]), which followed the Nernst equation was depicted and illustrated in Figure 2. As it is sen the curve has a linear section 1.0×10^{-7} to 1.0×10^{-3} mol L^{-1} with a slope of 57.3±0.3 mV per decade.

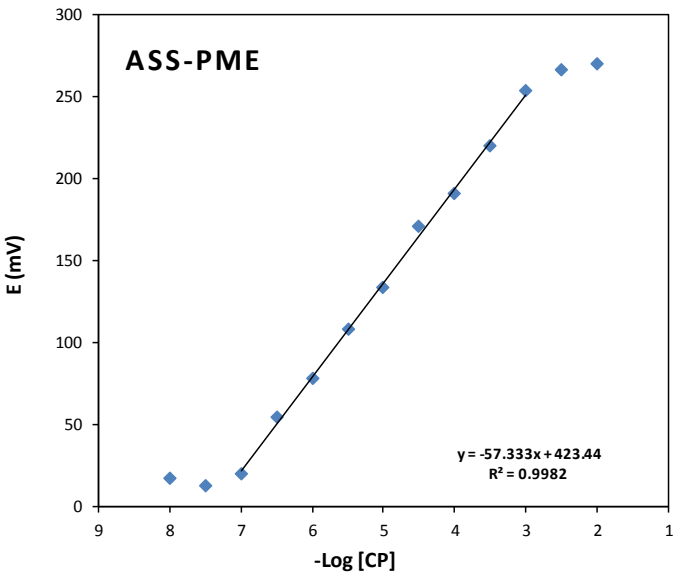

Fig. 2. The calibration curve of the CP ASS-PME (each point is the average of five replicate measurements)

With most of the PME sensors reported for pharmaceutical compounds [33-40] the linear range is in the order of 10^{-5} to 10^{-2} mol L^{-1}, which can be held proof as the improvement of the detection limit due to the properties of the asymmetric ASS-PME. Extrapolation of the two linear sections of the calibration curves at lower concentrations was used to evaluate the detection limit of the sensor which was found to be 7.0×10^{-8} mol L^{-1}.

3.3. Response Time

By definition, the response time of a potentiometric sensor is the time span between a 10 fold change in the concentration of the test solution and reaching an equilibrium potential within ±1 mV of the final potential. This value can be evaluated after successive immersions of the sensors in the analyte solutions [34-41]. In the case of the ASS-PME the test was performed using CP solutions of 1.0×10-7 to 1.0×10-3 mol L-1 and the response time was found to be 20 s.

3.4. pH-Potential behavior

The pH-potential behavior of the ASS-PME was evaluated in 1.0×10^{-5} mol L^{-1} CP solutions, by varying the pH was changed from 1.0 to 10.0 through the addition concentrated NaOH or HBr solutions, so as to avoid considerable volume and concentration changes. The results, presented in Figure 3, reveal that the potential is independent from pH in the range of 3.0 to 6.0. Pottential drifts above and below this range are due to the masking of the positive charge of the drug cations at higher pH values and reduced solubility of the drug in the solution and removal of the membrane components or the analyte in the solution at lower values.

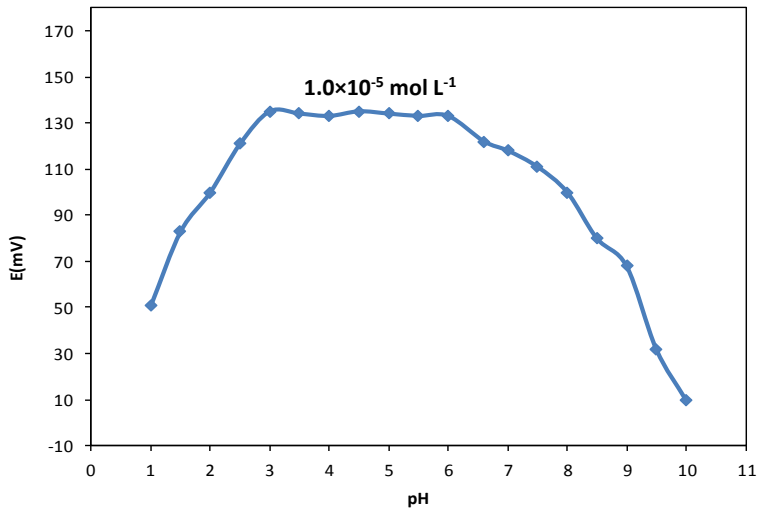

Fig. 3. pH range of the ASS-PME performance in the solutions of 1.0×10^{-5} mol L^{-1}

3.5. Life-time

The lifetime of potentiometric sensors, can be evaluated by studying the trend f changes in the Nernstian slope and detection limit over time. In this study three electrodes were used 1 hour per day, for 10 weeks and the results were recorded (Table 3). The life time of potentiometric sensors normally falls in the range of 4–10 weeks. In this case after 9 weeks of usage, the potential slope and detection limits gradually decreased and increased respectively. ASS-PME devices usually possess improved life-times, as compared to PMEs, due to their improved properties and properties and mechanical stability, however the main reason for the decay of the devices is the loss of the membrane ingredients due to chronic application.

Table 3. Lifetime of ASS-PME

Week	ASS-PME	
	Slope (mV per decade)	DL (mol L^{-1})
First	57.3±0.3	7.0×10^{-8}
Second	57.2±0.3	7.2×10^{-8}
Third	57.0±0.4	7.3×10^{-8}
Fourth	56.8±0.3	7.5×10^{-8}
Fifth	56.6±0.2	7.6×10^{-8}
Sixth	56.5±0.3	7.8×10^{-7}
Seventh	56.4±0.2	7.0×10^{-7}
Eighth	56.3±0.4	6.8×10^{-7}
Ninth	56.2±0.3	6.5×10^{-7}
Tenth	35.5±0.5	1.0×10^{-5}

3.6. Real-time evaluation of the ASS-PME

To assess the capability of the devices for use in real-time analysis, they were applied to the determination of CP in pure solutions and in pharmaceutical tablets. To validate the sensors the linear range, detection limit, selectivity, precision, accuracy, and ruggedness/robustness should be evaluated.

The ASS-PMEs were used in the analysis of CP in 20 mg tablets, through the calibration method (Table 4) and the results were compared with those of an HPLC standard method, which revealed no significant differences between the two sets of data.

The selectivity of an ion-selective electrode, defined as its tendency to respond to the analyte in the presence of interfering species, is expressed in terms of the selectivity

coefficients that are evaluated through various methods. In this case the matched potential method (MPM) was used [43-46] and the selectivity coefficients are given in Table 5. This data shows that the interferences from ionic and non-ionic species, on the results of the analysis of CP using the ASS-PME of this work is not significant.

Table 4. Measurement of CP.HBr in pharmaceutical formulations by the proposed sensors and standard methods

Sample	Labeled amount (mg/tab.)	Found by the ASS-PME* (mg/tab.) n=5	Standard method n=5	t-test (p-value: 0.05; $t_{theoritical}$: 2.31)
Sample 1	10	9.25±0.32	9.07±0.18	$t_{experimental}$= 1.10
Sample 2	10	11.13±0.35	10.94±0.26	$t_{experimental}$= 0.97
Sample 3	10	10.10±0.28	9.92±0.15	$t_{experimental}$= 1.27

* Averages of five repeated measurements

Table 5. Selectivity coefficients obtained for CP ASS polymeric membrane sensor

Interfering species	ASS-PME Log (K_{MPM})
Na^+	-3.4
K^+	-3.1
NH_4^+	-2.7
Ca^{2+}	-3.2
Mg^{2+}	-3.6
Cl^-	-3.7
NO_3^-	-4.0
Lactose	-4.5
Glucose	-4.1

The repeatability of the results was evaluated using 3 standard synthetic samples, which were subjected to measurements repeatedly. The RSD% obtained for the results of the ASS-PME was 3.15%. The ruggedness of the device was also studied by comparing its results of the two analysis intra- and inter-day studies in the same laboratory and comparing the

RSD% of the two analysis, which did not exceed 3.7%. Robustness was obtained while the important parameters (i.e. pH of the solution and the laboratory temperature) changed slightly. CP recoveries were also good under most conditions, and did not reveal any significant changes upon changing critical parameters.

4. CONCLUSIONS

A new citalopram selective ASS-PME was developed and successfully applied to the determination of the analyte. The best ion-pair based polymeric membrane used in the construction of the ASS-PME was composed of 7%wt of CP-tetraphenyl borate, 57%wt dibutyl phthalate, 33%wt of poly(vinyl chloride), and 3%wt of an ionic liquid. The ASS element was prepared based on a conductive composite of graphite, MWCNTs, and epoxy resin which was coated on a copper wire and was later coated with the optimized PME to yield the ASS-PME. The results included a Nernstian behavior (slope of 57.3±0.3 mV/decade) in a wide concentration range of 1.0×10^{-7} to 1.0×10^{-3} mol L^{-1}. The method was validated and it was found to be applicable for the quality control analyses of the analyte in pharmaceutical formulations.

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