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Highly Selective Coated–wire Potentiometric Sensor for Determination of Oxycodone in Plasma and Urine

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Abstract- The construction and electrochemical response characteristics of polyvinyl chloride (PVC) membrane sensor for the determination of oxycodone are described. An ion–pair of oxycodone–sodium tetraphenyl borate was used as an electroactive material and dibutyl phthalate (DBP) as an anion excluder in a PVC matrix in the percentage ratio of 2.70:61.30:36.00 (w/w). This potentiometric sensor exhibits a linear response to oxycodone in a concentration range of 1.0×10^{-6} to 1.0×10^{-2} M with a limit of detection of 9.4×10^{-7} M and with a slope of -56.10 mV/decade over the pH range 4.0–9.0. The selectivity coefficients of the sensor for oxycodone relative to a numbers of potential interfering compounds were investigated. The sensor is highly selective for oxycodone over a large number of similar compounds. The new sensor showing a fast response time of 15 s and was used over a period of 2 month with a good reproducibility. The sensor was successfully applied to determine oxycodone in urine and blood serum samples with satisfactory results.

Keywords- Potentiometric sensor, Oxycodone determination, Sodium tetraphenylborate

1. INTRODUCTION

Oxycodone, (5*R*,9*R*,13*S*,14*S*)-4,5α-epoxy-14-hydroxy-3-methoxy-17-methylmorphinan-6-one exhibits well known analgesic properties, which render the drug useful for the treatment of acute or chronic pain such as cancer pain [1,2]. Urine is the preferred biological fluid for the analysis of oxycodone. Oxycodone is extensively metabolized in human body with less than 10% of the oral dose excreted unchanged in the urine. The disadvantages of urine testing include concentration variations attributable to changing fluid intake and a greater possibility for adulteration or substitution [3]. The analysis of oxycodone in plasma is of interest due to human and animal pharmacokinetic studies, investigation of oxycodone abuse for epidemiological purpose, drug abuse control, the cause of intoxication and death in cases of clinical, pathological or forensic interest [4]. Since the clinical use of oxycodone has become increasingly common, it is important to find and use a selective and rapid method for the determination of oxycodone in human plasma or serum. Oxycodone has been determined in pharmaceutical preparations and biological fluids using several methods including, chromatography [5] high performance liquid chromatography [6-9], gas chromatography–mass spectroscopy [10-14], resonance light scattering technique [15], and voltammetry [16]. The reported chromatographic methods suffer from time–consuming, expensive instrumentation (with mass spectrometric detection) and costly sample pretreatment.

Potentiometric membrane electrodes show potential responses to certain substances. Many compounds have been detected by ion-selective electrodes (ISEs) [17-20]. The development of potentiometric sensors to determine pharmaceuticals in real samples based on the synthesis and characterization of different selective carriers [21]. Different advantages such as speed and ease of preparation and procedures, simple instrumentation, relatively fast response, wide dynamic range, reasonable selectivity, and low cost can be named for potentiometric detection based on ion–selective electrodes (ISEs) [22,23]. Those have wide applications in biomedical, clinical, and environmental research due to their capability for extreme miniaturization.

Based on our knowledge, there is not any report for determination of oxycodone using potentiometric method based ISE. In this paper, we introduced a new potentiometric sensor for selective and fast determination of oxycodone in real samples. The sensor works based on the use of the ion association complex between oxycodone with sodium tetraphenyl borate (NaTPB) as ion exchange site in a PVC matrix. The developed sensor was found to be simple, accurate and precise when compared with the reported chromatography methods.

2. MATERIALS AND METHODS

2.1. Chemicals

All chemicals used were of analytical reagents grade and were used without further purification. All solutions were prepared by dissolving the salts in distilled deionized water.

PVC of high relative molecular weight, dioctylsebacate (DOS), sodium tetraphenyl borate (NaTPB), dioctyl phthalate (DOP), dibutyl phthalate (DBP), tetrahydrofuran (THF), and all other chemicals were of highest purity available from Aldrich (Milwaukee, USA) and were used without further purifications except THF, which was distilled before using.

A stock solution of 0.010 M oxycodone was prepared by dissolving 34.0 mg of oxycodone sulfate solution (Prepared from Faran Shimi Company, Iran) in water in a 10-mLvolumetric flask, and the solution kept in a dark glass bottle and then stored at 4 °C. More dilute solutions of the bulk oxycodone were prepared by accurate dilution with water. The working standard solutions of oxycodone were stable for at least 2 h at room temperature.

2.2. Apparatus

Potentials were measured by direct potentiometry at 25±0.1 °C with the help of ceramic junction calomel electrodes and the cell set–up was as follow:

Hg/Hg_2Cl_2, KCl(sat'd) ‖ Sample solution ⎹ Membrane ⎹ Pt–wire

All potentiometric measurements were made with a pH/mV meter (WTW, Model 720, Nederland). All emf measurements were carried out in a 25–mL double walled glass cell with a constant magnetic stirring of the test solution. Response times were determined after the potential of the solution had become constant, and similar measurements were carried out in another solution of 100–fold lower concentration. The response time is defined as the time taken to reach a potential of 95% the potential difference in the two measurements.

2.3. Electrode preparation

For preparation of the membrane, 25 mL of 1.0×10^{-3} M oxycodone solution was added to 25 mL of 1.0×10^{-3} M NaTPB. The resulting precipitate (white color) was filtered, washed with deionized water, dried, and protected from light in a desiccators at room temperature. Then, about 2.70 mg of the precipitate (oxycodone–sodium tetraphenyl borate ion–pair) was mixed with 36.00 mg PVC and 61.30 mg DBP previously dissolved in 2.0 mL of THF. Dipping the Pt performed coating process of the platinum wire electrode–wire 20 times into the mixture. After each coating, the membrane was air dried for 10 min until a thin film was formed. The electrode was finally conditioned in a 1.0×10^{-3} M oxycodone solution for 10 min. The life time of the sensor was at least 2 months when conditioned by soaking in 1.0×10^{-3} M oxycodone solution for 30 min before measurements and stored in air when not in use. The oxycodone–coated–wire sensor was calibrated by immersion in conjunction with the reference electrode in a 25 mL cell containing 5 mL water of pH 7.0. Then a 0.50 mL aliquot of oxycodone solution was added with continuous stirring, to give final oxycodone concentration ranging from 1.0×10^{-8} to 1.0×10^{-2} M, and the potential was recorded after stabilization to ±0.1 mV. A calibration graph was then constructed by plotting the potentials

as a function of –log[Oxycodone]. The resulting graph was used for subsequent determination of unknown oxycodone concentration.

2.4. Sample preparation

The urine and/or human serum were taken from healthy volunteers. The sample of human serum was diluted two times with methanol until the protein of serum was participated. Then, the urine and blood samples were separately centrifuged at 3500 rpm to remove the blood cells and other dead cells. The serum was kept in a freezer at −20 ^{o}C until analysis. The samples of human urine and serum were diluted 2–times with water and were analyzed directly as described in the recommended procedure.

3. RESULTS AND DISCUSSION

In a preliminary experiment, different membranes with and without carrier were constructed. The results showed that the membrane with no carrier displayed insignificant selectivity toward oxycodone and their response was not reliable. However, in the presence of the ion–pair (oxycodone–tetraphenyl borate, oxycodone-TPB), the optimized membrane demonstrated Nernstian response and remarkable selectivity for oxycodone over several compounds. This means that oxycodone has a strong interaction with the ion–pair complex.

3.1. Influence of membrane composition

Besides the critical role of the nature of the ion–carrier in preparing membrane–selective sensors, some other important features of the PVC membrane such as amount of the ionophore, nature of the solvent mediator (plasticizer), amount of plasticizer to PVC ratio, and especially the nature of additives used are known to significantly influence the sensitivity and selectivity of the sensor [24,25]. In many potentiometric ion selective sensors, the ratio between PVC/plasticizer/ionophore is about 30–35/60–65/2–6 percent [21]. Thus, several membrane compositions were investigated by varying the ratio of PVC, plasticizer, and the ionophore (Table 1). The potential response of all the electrodes was studied in the concentration range 1.0×10^{-8}–1.0×10^{-2} M oxycodone. At least four criteria can be mentioned that determine the basic requirements for an adequate plasticizer with good performance: sufficient lipophilicity, no crystallization in the membrane phase, no exudation, and optimized selectivity for each application. Lengthening the alkyl residues enhances the lipophilicity of the plasticizer. As can be seen from Table 1, in our work, three plasticizers of different polarity including DOP (ε_T=5.0), DOS (ε_T=4.5) and DBP (ε_T=6.42) were used. The results showed that DBP gave the best sensitivity of the three plasticizers. It is also known that the presence of ionophore strongly influence the response of the oxycodone selective electrode.

Among the different compositions studied, the membrane incorporating 36.00% PVC in the presence of 2.70% carrier, and 61.30% DBP, exhibits the best response characteristics and shows the best sensitivity with a near Nernstian slope of 56.10 mV/decade of oxycodone concentrations in the range of 1.0×10^{-6}–1.0×10^{-2} M.

Table 1. The potentiometric response of the membrane at different conditions (number of replications=3)

No.	Composition (%)			Slope (mV/decade)	Dynamic range (M)	R^2
	PVC	Ion pair	Plasticizer			
1	30.10	4.40	65.50 DBP	52.89±0.51	1.0×10^{-6}–1.0×10^{-2}	0.983
2	34.7	2.80	62.50 DBP	53.32±0.12	1.0×10^{-6}–1.0×10^{-2}	0.981
3	36.00	2.70	61.30 DBP	56.10±0.10	1.0×10^{-6}–1.0×10^{-2}	0.991
4	35.15	2.65	62.20 DBP	55.40±0.20	1.0×10^{-6}–1.0×10^{-2}	0.989
5	35.00	2.60	62.40 DBP	54.20±0.21	1.0×10^{-6}–1.0×10^{-2}	0.980
6	29.60	2.70	67.70 DOS	62.16±0.14	1.0×10^{-6}–1.0×10^{-2}	0.971
7	33.00	4.10	62.90 DOS	61.87±0.15	1.0×10^{-6}–1.0×10^{-2}	0.963
8	29.70	2.70	67.60 DOP	40.87±0.12	1.0×10^{-4}–1.0×10^{-2}	0.870
9	30.68	4.82	64.50 DOP	38.60±0.58	1.0×10^{-4}–1.0×10^{-2}	0.836
10	36.00	----	64.00 DBP	No response		

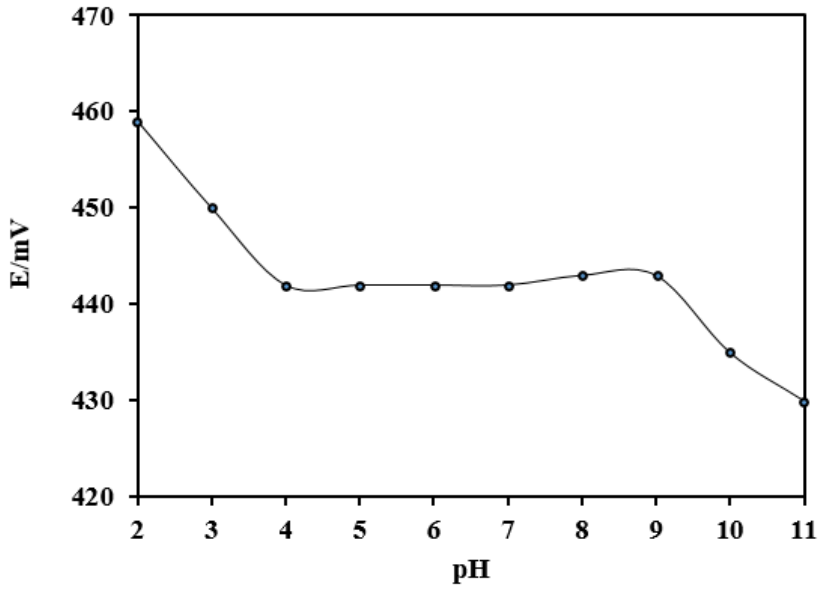

Fig. 1. Influence of pH on the response of the membrane (1.0×10^{-4} M oxycodone)

3.2. pH Effect

The effect of pH of the test solution (with 1.0×10^{-4} M oxycodone) on the sensor potential was investigated by following the potential variation of the sensor over the pH range of 2.0–11.0. The pH was adjusted by introducing very small drops of hydrochloric acid solution (0.050 M) and sodium hydroxide solution (0.050 M) (Fig. 1). The results showed that the sensor response does not depend on the solution pH in the pH range of 4.0 to 9.0. Under more acidic conditions, oxycodone may be protonated and thus increasing the sensor signal. In pH more than 9 the hydroxide ion may react with oxycodone to produce neutral species (precipitate).

3.3. Lifetime

The electrode was tested over a period of two months to investigate the stability. During this period, the electrode was in daily use and was stored in dry conditions when not in use. Before each measurement, the electrode was conditioned in 1.0×10^{-3} M oxycodone solution for 10 min. The lifetime of the sensor was worked out by performing calibrations periodically with standard solutions and calculating the slopes over the concentration ranges of 1.0×10^{-6}–1.0×10^{-2} M oxycodone. The experimental results showed that the lifetime of the present sensor was over 60 days (Table 2). No significantchange in the performance of the electrode was observed during this period.

Table 2. Response of the sensor during 75 days

Time (day)	Slope (mV/decade)	Dynamic range (M)
1	56.10	1.0×10^{-6}–1.0×10^{-2}
10	56.05	1.0×10^{-6}–1.0×10^{-2}
20	55.87	1.0×10^{-6}–1.0×10^{-2}
30	55.76	1.0×10^{-6}–1.0×10^{-2}
40	55.52	1.0×10^{-6}–1.0×10^{-2}
50	55.08	1.0×10^{-6}–1.0×10^{-2}
60	53.21	1.0×10^{-6}–1.0×10^{-2}

3.4. Response time

The average time for the electrode to reach a potential within ±1 mV of the final equilibrium value after successive immersion of the electrode in a series of oxycodone solutions, each having a 10–fold difference in their concentration, was measured. The static response time thus obtained was <15s over the entire concentration range of 1.0×10^{-6}–

2.0×10^{-2} M oxycodone (Fig. 2). The sensing behavior of the membrane electrode remained unchanged when the potentials recorded either from low to high concentration or vice versa.

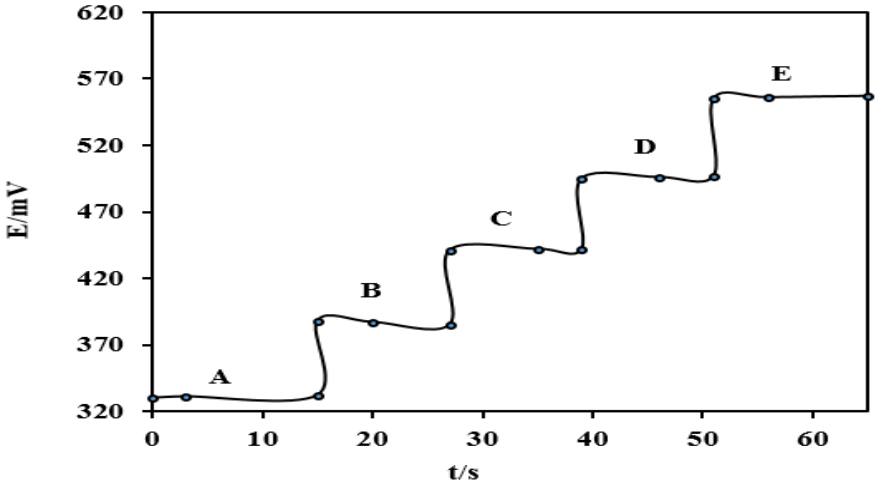

Fig. 2. Response time of the electrode; A) 1.0×10^{-2} M; B) 1.0×10^{-3} M; C) 1.0×10^{-4} M; D) 1.0×10^{-5} M; and E) 1.0×10^{-6} M oxycodone

3.5. Repeatability and reproducibility of the sensor

The electrode potentials were repeatable, and the standard deviation of potential for 10 replicate measurements for oxycodone concentrations of 1.0×10^{-3} and 1.0×10^{-4} M was ±1.2 and ±1.3 mV, respectively. The reproducibility of the calibration parameters was studied by making calibrations with the same membrane at different days (n=3) and with different membranes in one day (n=3). The standard deviation obtained for the slope was ±1.5 mV/decade with the same membrane and ±0.4 mV/decade with different membranes in one day.

3.6. Potentiometric selectivity

The selectivity behavior is obviously one of the important characteristics of any sensors in which reliable measurement of the target sample is determined to be possible or not. Considering that the developed methodology would be applied to the determination of oxycodone in pharmaceutical preparations and human fluids, the interference effect of several compounds commonly used as excipients was assessed. Potentiometric selectivity coefficients (K) describing the preference of the membrane for an interfering ion M^{n+} relative to oxycodone were determined by the match potential method (MPM) [26-28]. Table 3 lists the potentiometric selectivity coefficient data of the sensor for several substances relative to oxycodone.

Table 3. Values of selectivity coefficients of the selective electrode

Interfering substance	Log K	Interfering substance	Log K	Interfering substance	Log K
Ni^{2+}	–3.29	Ca^{2+}	–3.86	Glucose	–6.01
Na^{+}	–2.29	Cr^{3+}	–2.90	Sucrose	–6.25
Mg^{2+}	–3.73	Fe^{3+}	–3.93	Fructose	–6.35
Co^{2+}	–3.73	Cd^{2+}	–3.40	Uric acid	–6.25
Cu^{2+}	–4.51	Cl^{-}	–5.76	Methadone	–3.52
K^{+}	–3.21	IO_4^{-}	–6.89	Thebaine	–3.27

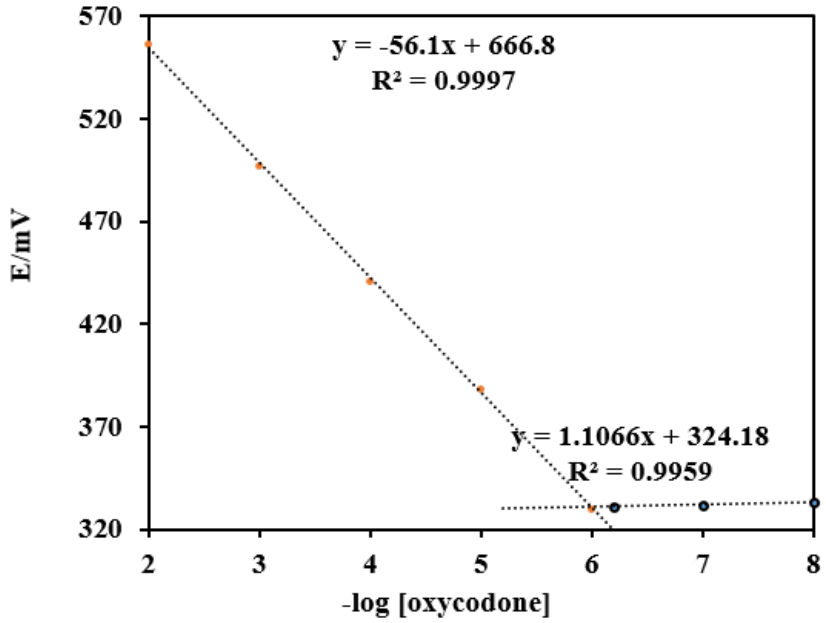

Fig. 3. Potentiometric response of the electrode

The selectivity coefficients clearly indicate that the sensor is highly selective to oxycodone over a numbers of other cations and natural compound, and therefore the sensor has been found to be chemically inert to other cations and natural compound. The inorganic cations do not interfere owing to the differences in ionic size, and consequently their mobilities and permeability, as compared with those of oxycodone. In addition, the pharmaceutical additives, diluents and ingredient commonly used in drug formulations did not show any interference. The response of the sensor for different cations and natural compound shows the best selectivity to oxycodone.

The limit of detection defined as the concentration of oxycodone obtained when extrapolating the linear region of the calibration curve to the base–line potential was 9.4×10^{-7} M oxycodone.

3.7. Analytical Applications

In order to evaluate the applicability of the proposed method for the determination of oxycodone in real samples, determining of oxycodone in plasma and urine samples tested its utility. Each sample was analyzed in triplicate, using the sensor by standard addition method. Urine and plasma samples were analyzed as described before by standard addition method. The results are given in Table 4, which shows that the amount of oxycodone covered with the help of the sensor are in good, thereby reflecting the utility of the proposed method.

Table 4. Recovery of oxycodonein urine and human plasma

Sample	Oxycodoneadded (M)	Oxycodone found (M)	Recovery (%)
Plasma	< LOD	—	—
Plasma	1.00×10^{-4}	$0.95\ (\pm0.3)\times10^{-4}$	95.0
Plasma	5.00×10^{-5}	$4.82\ (\pm0.2)\times10^{-5}$	96.4
Urine[a,b]	<LOD	—	—
Urine[a]	4.00×10^{-4}	$4.10\ (\pm0.2)\times10^{-4}$	102.5
Urine[a]	1.50×10^{-5}	$1.44\ (\pm0.3)\times10^{-5}$	96
Urine[b]	1.00×10^{-4}	$1.01\ (\pm0.1)\times10^{-4}$	101
Urine[b]	6.00×10^{-5}	$5.90\ (\pm0.3)\times10^{-5}$	98.3

[a]Urine of healthy woman
[b]Urine of healthy man
±Shows the standard deviation for 3 replicates analysis

4. CONCLUSION

This sensor has shown to have good operating characteristics including Nernstian response, reasonable detection limit, relatively high selectivity, wide dynamic range, and fast response for oxycodone determination. These characteristics and the typical applications presented in this paper make the sensor suitable for measuring oxycodone content in real samples without a significant interaction from concomitant cationic species and natural. The use of the proposed sensor offers the advantages of fast response, elimination of drug pretreatment or separation steps and direct determination of drugs in turbid and colored solutions. These characteristics and the typical applications presented in this paper make the sensor suitable for measuring oxycodone content in pharmaceutical samples without a

significant interaction from concomitant substances. It can therefore, be used for routine analysis of the drugs in quality control laboratories.

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