Potentiometric Determination of Raloxifene by PVC Membrane Sensor

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PVC membrane potentiometric sensor was made for determination of raloxifene, an oral selective estrogen receptor modulator, in pharmaceutical formulations. The electrode respond based on ion-exchange mechanism. The ion-pair used as a sensing element in PVC membrane composition was synthesized through the interaction of raloxifene hydrochloride and sodium tetraphenyl borate. The best PVC membrane sensor response was obtained by a membrane composition of 30% PVC, 63% DBP, and 7% ion-pair. The detection limit of the constructed sensor was calculated 8.0×10^{-6} M. The proposed sensor has a fast response time (less than 10 s). The proposed method was successfully applied in determination of raloxifene in some pharmaceutical formulations.

Keywords: Raloxifene, Potentiometric Sensor, PVC membrane Electrode, Ion-Pair complex

1. INTRODUCTION

Raloxifene (RLX), [6-hydroxy-2-(4-hydroxyphenyl) benzo[b]thien-3-yl]-[4-[2-(1-piperidinyl) ethoxy] phenyl] (Fig. 1), is a selective estrogen receptor modulator (SERM) which belongs to the benzothiophene class of compounds [1]. Raloxifene is an oral SERM that has estrogenic actions on bone. It is used in the prevention of osteoporosis in postmenopausal women [2]. In addition to its effects on bone, a number of beneficial non-skeletal effects have been reported on the breast, uterus and cardiovascular system. These findings were mainly derived from secondary end points and analyses of large osteoporosis studies on raloxifene [3].

There were very few methods that have been used for determination of RLX in pharmaceutical and biological systems, such as capillary electrophoresis [4], spectrophotometry [5], high-performance

liquid chromatography (HPLC) [6,7] and rayleigh scattering technique [8].
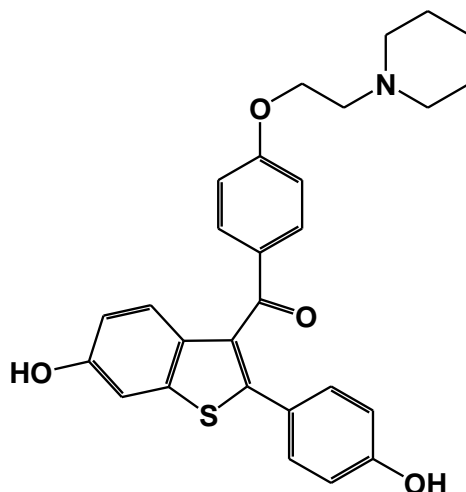
Figure 1. Chemical structure of Raloxifene

Different electrochemical measurement techniques were used for drug analysis during recent year but potentiometric methods using indicator electrodes have advantages of rapid and ease of preparation and procedures, fast response time, reasonable selectivity, wide linear dynamic range, and low cost. These characteristics have certainly led to the preparation of numerous sensors for several ionic species, and the list of available electrodes has grown largely over the past years [9-18]. PVC membrane electrodes are one of the subdivisions of potentiometric sensors which are widely used and have different application in analysis of various ionic species [19-29].

In this work, the sensing element used in the proposed electrode is an ion-pair complex which is made from the interaction between Raloxifene and sodium tetraphenyl borate and they respond according to the ion-exchange mechanism. The best PVC membrane composition was selected after series of experiments and optimizations.

2. EXPERIMENTAL SECTION

2.1. Apparatus

The glass cell where the RLX indicator PVC membrane electrode was located; consisted of two Ag/AgCl double junction reference electrodes (Azar-Elelectrode Co., Iran) as internal and external reference electrodes. 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

Chemicals of analytical reagent grade were high-molecular weight polyvinylchloride (PVC)

(Fluka Co., USA), sodium tetraphenyl borate (NaTPB), dibutyl phthalate (DBP), nitrobenzene (NB), benzyl acetate (BA) and tetrahydrofuran (THF) (Merck Co., Germany). All materials were of the highest available purity without further modification. Raloxifene hydrochloride (HCl) has the empirical formula of $C_{28}H_{27}NO_4S.HCl$, which corresponds to a molecular weight of 510.05 g/mol. Raloxifene HCl is off-white to pale-yellow solid. Raloxifene hydrochloride (RLX.HCl) and its pharmaceutical formulation were obtained from a local pharmaceutical manufacturer (Tehran, Iran) as gift samples.

2.3. Standard RLX solution preparation

RLX.HCl is sparingly insoluble in aqueous media. For maximum solubility of the RLX.HCl in an aqueous solution, it should be prepared by dissolving of RLX.HCl firstly in an organic solvent such as ethanol, dimethyl sulfoxide (DMSO) and dimethyl foramide (DMF) and dilution with water. Solubility of RLX.HCl in ethanol is approximately 0.1 g/ml and 10 mg/ml in DMSO and DMF. A stock solution of 0.01 M RLX.HCl solution was prepared by dissolving the solid of RLX.HCl in some milliliters of DMSO and then diluted with water. The working standard solutions (1×10^{-7} to 1×10^{-2} M) were prepared by appropriately dilution of the stock solution with distilled water.

2.4. Preparation of the ion-pair (RLX-TPB)

Sensing element used in the PVC membranes is an ion-pair compound made from the interaction of raloxifene and sodium tetraphenyl borate. It was prepared by mixing about 20 mL of 0.01 M acidic solution of RLX with 20 mL of tetraphenyl borate aqueous solution. The resulting precipitate was then filtered, washed with distilled water and dried in room temperature [12,14].

2.5. Preparation of PVC membrane Electrodes

General procedure to prepare PVC membrane was as follow: different amounts of ion-pair along with appropriate amounts of PVC, plasticizer and additive were dissolved in tetrahydrofuran (THF), and the solution was mixed well into a glass dish of 2 cm diameter. Then, THF was evaporated slowly until an oily concentrated mixture was obtained. A plastic tube (about 3 mm o.d.) was dipped into the mixture for about 10 s so a transparent membrane of about 0.3 mm in thickness was formed. The tube was then pulled out from the mixture and kept at room temperature for about 5 h. Afterwards, the tube was filled with an internal filling solution (1.0×10^{-3} M of RLX.HCl solution). The electrode was finally conditioned for about 20 h by soaking in the same solution [10-12].

2.6. The emf Measurements

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

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

These measurements were done using calibration of the PVC membrane electrodes with several standard solutions of RLX.HCl.

3. RESULTS AND DISCUSSION

3.1. PVC Membrane Composition Selection

The composition of the membrane affects on the sensor characterizations and responses [19-29]. Membrane composition effect on the potential responses of the electrode was tested. The operating characteristics of PVC membrane sensor can be significantly modified by changing the relative amount of the membrane components of the electrode. The main components of a membrane are PVC matrix, plasticizer and the ion-pair as a sensing material. Each membrane component plays a special role in the sensor response. Previous studies shows that the membrane prepared with a plasticizer/PVC ratio about 2.2 can show the best performance [30-35]. According to the previous studies, the optimum amount of PVC was selected 30 mg.

Table 1. Optimization of the PVC membrane ingredients

| No.	Composition (%)			Slope (mV per decade)	LR (M)	DL (M)	R^2
	PVC	Plasticizer	RLX-TPB				
1	30	DBP, 67	3	28.6±0.6	1.0×10^{-5}-5.0×10^{-3}	5.0×10^{-5}	0.917
2	30	DBP, 65	5	33.8±0.5	1.0×10^{-5}-5.0×10^{-2}	5.0×10^{-5}	0.959
3	30	DBP, 64	6	40.9±0.4	1.0×10^{-5}-1.0×10^{-2}	8.0×10^{-6}	0.983
4	30	DBP, 63	7	55.9±0.4	1.0×10^{-5}-1.0×10^{-2}	8.0×10^{-6}	0.995
5	30	DBP, 62	8	37.3±0.6	1.0×10^{-5}-1.5×10^{-2}	1.0×10^{-5}	0.976
6	30	DBP, 61	9*	34.6±0.5	1.0×10^{-5}-1.5×10^{-3}	1.0×10^{-5}	0.984
7	30	DBP, 60	10*	20.9±0.6	1.0×10^{-5}-1.5×10^{-3}	1.0×10^{-5}	0.981
8	30	NB, 63	7	7.2±0.5	1.0×10^{-4}-1.0×10^{-2}	1.0×10^{-4}	0.957
9	30	BA, 63	7	16.3±0.4	1.0×10^{-4}-5.0×10^{-2}	1.0×10^{-4}	0.966
10	30	DBP, 70	0	3.9±0.7	5.0×10^{-4}-5.0×10^{-3}	-	-

*After 48 h conditioning

The most important ingredient of a membrane which has the main and most effect on the selectivity of the sensor is sensing element. In this work, an ion-pair complex of RLX-TPB was synthesized and then applied for sensing element. 3-10 mg of RLX-TPB was tested. As shown in Table 1, 7 mg is a suitable amount. More amount of RLX-TPB causes the conditioning time of the membrane takes more, about 48 h.

Plasticizer which mainly acts as a membrane solvent mediator allowing homogeneous dissolution and diffusional mobility of the ion-pair compound inside the membrane [26-31]. The plasticizer should be water-immiscible liquid with low vapor-pressure, compatible with PVC, no

functional groups which can undergo protonation reactions. The selectivity of such electrode can be drastically influenced by the choice of the membrane solvent [24-34]. Nature of the plasticizer has also important effect on analytical responses of the membrane. Here, three plasticizers with different polarity (dielectric constant) were tested, dibutyl phthalate (DBP with DC of 6.4), nitrobenzene (NB with DC of 35.7) and benzylacetate (BA with DC of about 5.7), as listed in Table 1. The electrode responses showed that the membrane having DBP better respond. DBP among the used plasticizers provided an effective linear range and a lower detection limit due to the better extraction of RLX, which is a hydrophobic organic cation, from aqueous phase into the organic layer of the membrane. As it can be seen from Table 1, absence of ion-pair in the membrane causes a very poor response (membrane no. 10), which confirm significance of the ion-pair. The electrodes behavior show that the best Nernstian slope is 55.9±0.4 mV per decade. Finally, membrane no. 4 with the composition of 30% PVC, 7% ion-pair, and 63% DBP was the optimum one for the sensor design.

3.2. Calibration Graph and Statistical Data

The measuring range of a potentiometric sensor is the linear part of the calibration graph as shown in Figure 2.

$$y = 55.93x - 171.96$$
$$R^2 = 0.995$$
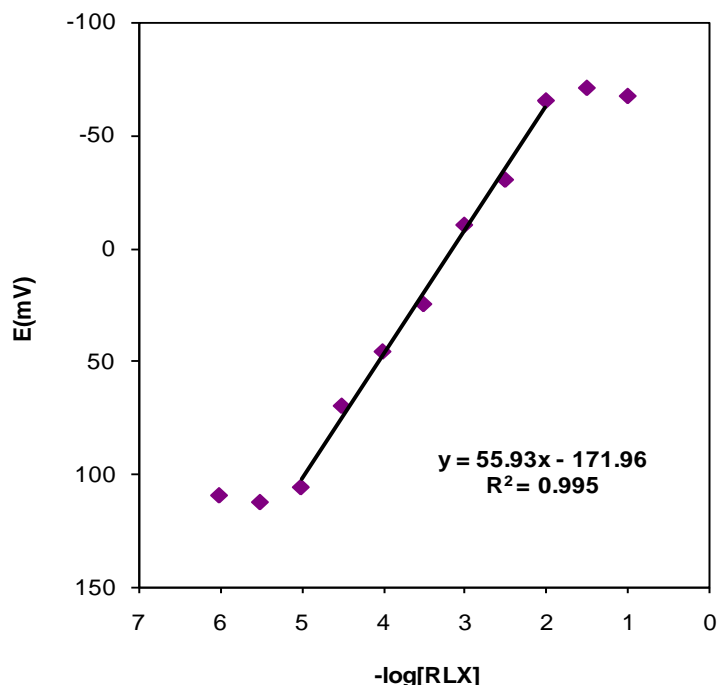
Figure 2. Calibration curve of PVC membrane electrode, the results are based on 5 replicate measurements.

One of the advantages of potentiometric sensors are their wide linear range. For many electrodes the measuring range can extend from 1 molar to 10^{-6} or even 10^{-7} molar concentrations [19-24]. However, it should be noted that more closely spaced calibration points are required for

more precise determinations. Calibration graph slope for PVC membrane electrode is 55.9 mV per decade of the Raloxifene concentration and a standard deviation of ±0.4 mV after five replicate measurements. A linear response towards the Raloxifene concentration was from 1.0×10^{-5}-1.0×10^{-2} M. In this work, detection limit of the PVC membrane sensor was 8.0×10^{-6} M which was calculated by extrapolating the two segments of the calibration curves.

3.3. 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 [25-30]. Its calculation involved the variation and the recording of the Raloxifene concentration in a series of solutions from 1.0×10^{-5} to 1.0×10^{-2} M. Sensor was able to quickly reach its equilibrium response in the whole concentration range. This time for PVC membrane electrode was less than 10 s in the concentrated solutions.

3.4. pH Effect on the Sensor

To examine the effect of pH on the electrode responses, the potential was measured at specific concentration of the raloxifene solution (1.0×10^{-4} M) from the pH value of 1.0 up to 10.0 (concentrated NaOH or HCl solutions were employed for the pH adjustment) by PVC membrane electrode. The results showed that the potential remained constant despite the pH change in the range of 4 to 6.5 which indicates the applicability of this electrode in the specified pH range.

Relatively noteworthy fluctuations in the potential *vs.* pH behavior took place below and above the formerly stated pH limits. In detail, the fluctuations above the pH value of 4 might be justified by removing the positive charge on the drug molecule. Fluctuations below the pH value of 4 were caused by removal of the membrane ingredients or analyte in the solution.

3.5. Life-time Study

Electrode lifetime was estimated by the calibration curve, periodical test of a standard solution and calculation of its response slope.

For this estimation, three electrodes were employed 1 hour per day for 10 weeks. After 5 weeks utilization of PVC membrane electrode, two changes were observed: a slight gradual decrease in the slope and an increase in the detection limit.

In PVC membrane electrodes after several time of usage, the membrane ingredients leak from the organic layer and affect the membrane response.

Table 3. Lifetime of the PVC membrane electrode

Week	Slope (mV per decade)	DL (M)
First	55.9	8.0×10^{-6}
Second	54.6	9.5×10^{-6}
Third	54.3	1.5×10^{-5}
Fourth	54.0	5.5×10^{-5}
Fifth	53.9	7.0×10^{-5}
Sixth	51.4	1.0×10^{-4}
Seventh	50.1	3.0×10^{-4}
Eighth	48.0	7.5×10^{-4}
Ninth	44.2	9.0×10^{-4}
Tenth	35.9	2.5×10^{-3}

3.6. Analytical Applications

Linearity, limit of detection, recovery test, selectivity, precision, accuracy, and ruggedness/robustness were the parameters used for the method validation.

3.6.1. Selectivity study

Table 4. Selectivity coefficients of various interfering compounds for Raloxifene sensor

Interfering ion	Log (K_{MPM})
Na^+	-3.1
K^+	-3.0
NH_4^+	-2.7
Ca^{2+}	-3.3
Mg^{2+}	-3.5
Cl^-	-3.4
NO_3^-	-3.7
Lactose	-4.0
Glucose	-3.9

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 Raloxifene sensor were evaluated by the matched potential method (MPM) [37-40]. The resulting values of the selectivity coefficients are shown in Table 4. The selectivity coefficients show that the interferences have negligible effect on the performance of the electrode in an assay.

3.6.2. Recovery Test from Tablet

The proposed sensor was evaluated by measuring the drug concentration in some pharmaceutical formulations (Raloxifene amount of some tablets) (Table 5). The drug concentration was determined using calibration method and the proposed sensor with membrane no. 4. The results are in satisfactory agreement with the labeled amounts.

Table 5. Potentiometric determination of Raloxifene in pharmaceutical formulations

Sample	Labeled amount (mg/per tab)	Found by electrode* (mg/per tab)
Sample 1	60	64.3±1.3
Sample 2	60	58.9±1.5
Sample 3	60	62.8±0.8
Sample 4	60	59.5±0.9
Sample 5	60	61.7±1.4

* The results are based on five replicate measurements.

3.6.3. Precision and accuracy

For repeatability monitoring, 3 standard samples were measured. The RSD values by PVC membrane were not exceeding 4.3%.

3.6.4. Ruggedness/Robustness

For ruggedness of the methods a comparison was performed between the intra- and inter-day assay results for raloxifene 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 4.6.%. On the other hand, the robustness was examined while the parameter values (pH of the solution and the laboratory temperature) changed slightly. Raloxifene recovery percentages were good under most conditions, and not showing any significant change when the critical parameters were modified.

4. CONCLUSIONS

In the present work, potentiometric electrode was constructed for determination of Raloxifene. The sensor demonstrated advanced performance with a fast response time, a lower detection limit of

8.0×10^{-6} M for PVC membrane electrode and potential responses across the range of 1.0×10^{-5}-1.0×10^{-2} M. The sensor enabled the raloxifene determination in some pharmaceutical formulations. Sensor respond based on ion-exchange mechanism. RLX-TPB was synthesized and used as sensing element in the PVC membrane. The best PVC membrane electrode performance was achieved by a membrane composition of 30% PVC, 63% DBP, and 7% RLX-TPB.

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