Anal. Bioanal. Electrochem., Vol. 6, No. 3, 2014, 355-366

Analytical & Bioanalytical Electrochemistry

Full Paper

Analysis of Levothyroxine in Pharmaceutical Formulation by a Novel Levothyroxine Potentiometric Membrane Sensor

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Received: 30 December 2014 / Received in Revised form: 10 May 2014 / Accepted: 13 May 2014 / Published online: 30 June 2014

Abstract- Observation of the patients used levothyroxine tablets but still have problem with their thyroid, leads to analysis of pharmaceutical formulation in the first step. Common instrumental methods for the active ingredient analysis of different pharmaceutics are time-consuming and pricy and require a skilled operator. In this study, for the first time, an electrochemical sensor which is capable of evaluating the active ingredient of Levothyroxine in formulations is introduced. The proposed sensor, which is a PVC membrane electrode, offers advantages of rapidity, inexpensively, and portability. The sensor responds based on ion-exchange mechanism. Levothyroxine-Hexadecyltrimethylammonium ion-pair (LEV-HTA) was employed as a sensing element in construction of the membrane electrode. PVC

membrane electrode was made after series of experiments. The best PVC membrane electrode performance was achieved by a membrane composition of 30% PVC, 60% DBP, and 7% LEV-HTA ion-pair. The proposed method was successfully applied in determination of levothyroxine in some pharmaceutical formulations. Five samples from the vary batches, coded differently, were sent for high performance liquid chromatography (HPLC) analysis. The results of two methods were compared using parametric tests. Pearson correlation test was used to assess the correlation between these findings. A strong correlation was found between the active ingredient content measured using electrochemical sensor and official method (HPLC) (ρ=0.98 P<0.05).

Keywords- Levothyroxine, Electrochemical Sensor, Potentiometry, Pharmaceutical analysis

1. INTRODUCTION

Levothyroxine (Fig. 1), L-thyroxine, synthetic T_4, or 3,5,3',5'-tetraiodo-L-thyronine, is a synthetic form of thyroid hormone (thyroxine), used as a hormone replacement for patients with thyroid problems. Levothyroxine is typically used to treat hypothyroidism. It may also be used to treat goiter via its ability to lower thyroid-stimulating hormone (TSH).

The instrumental techniques capable of identifying drugs include high performance liquid Chromatography (HPLC), ultraviolet spectroscopy (UV), electrochemical or fluorescent method, thin Layer chromatography (TLC), mass spectrophotometer (MS) and gas chromatography (GC). Considering the fact that many of these techniques are very sensitive and accurate, however, they are time consuming, expensive, and require sample preparations. There are some reports on levothyroxine analysis include HPLC and Mass Spectrophotometer Inductively Coupled Plasma (MS-ICP) [1-5].

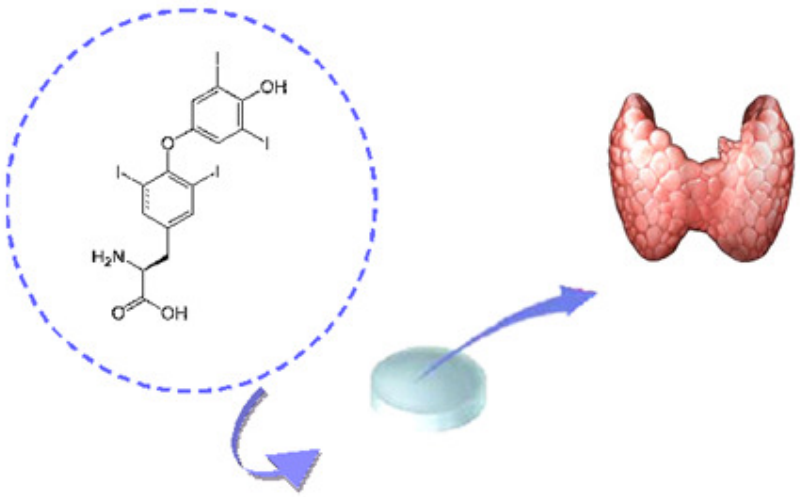

Fig. 1. Chemical structure of levothyroxine

Potentiometric sensors are playing an important role in pharmaceutical analysis and offer the advantage of speed and ease of preparation and procedures, relatively fast responses, reasonable selectivity thorough judicious choice of membrane active materials, wide linear dynamic range, and low cost. These characteristics have inevitably led to the preparation of numerous sensors for several ionic species, and the list of available electrodes has grown substantially over the past years [6-10]. More recently, however, they have been used in evaluating certain medications but they were never mass produced [8-16].

In this work, for the first time a potentiometric membrane sensor introduced for rapid analysis of levothyroxine in formulation. The present study, therefore, was designed to evaluate the validity of the electrochemical sensor in assessing the amount of the active ingredient available in levothyroxine tablets in pharmaceutical formulation.

2. EXPERIMANTAL SECTION

2.1. Apparatus

The glass cell, where the levothyroxine-selective electrode was placed, consisted of an R684 model Analion Ag/AgCl double junction reference electrode as the internal reference electrode and a double-junction saturated calomel electrode (SCE, Philips). 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 reagent grade) were: high-molecular weight polyvinylchloride (PVC) (Fluka Co.), hexadecyltrimethylammonium bromide (HTAB), benzyl acetate (BA), dibutyl phthalate (DBP), nitrobenzene (NB), nitrophenyloctyl ether (*o*-NPOE) and tetrahydrofuran (THF) (Merck Co.). All the materials were at the highest available purity and were used as received. Levothyroxine and its tablets were obtained from local pharmaceutical factory in Iran.

2.3. Preparation of Ion-Pair Compound

Ion-pair compound of Levothyroxine-Hexadecyltrimethylammonium ion-pair (LEV-HTA) was prepared as follow: About 10 mL of 0.01 mol L^{-1} fresh solution of alkali levothyroxine (pH=8.5) was mixed with 10 mL of 0.01 mol L^{-1} solution of hexadecyltrimethyl ammonium bromide under stirring. The resulting precipitate was filtered off, washed with distilled water and dried.

2.4. Preparation of the sensor

The general procedure to prepare the PVC membrane sensor was as follows: 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. Then, 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 plastic 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 pulled out from the mixture and kept at room temperature for about 5 h. The tube was then filled with an internal filling solution (1.0×10^{-3} mol L^{-1} alkali levothyroxine (pH=8.5)). The electrode was finally conditioned for 24 h by soaking in a 1.0×10^{-3} mol L^{-1} sodium levothyroxine solution [8-10].

2.5. Standard Levothyroxine Solutions

A stock standard solution of 0.02 mol L^{-1} levothyroxine sodium was prepared by dissolving 1.553 g of pure drug powder in 100 ml distilled water and adjusting pH to 8.5. The working solutions (1×10^{-6} to 1×10^{-1} mol L^{-1}) were prepared by appropriate dilution of the stock solution with water.

2.6. Emf Measurements

The following cell was assembled for conduction of emf (electromotive force) measurements; Ag–AgCl | internal solution, 1×10^{-3} mol L^{-1} alkali levothyroxine | PVC membrane | sample solution | Hg–Hg_2Cl_2, KC1 (satd.)

These measurements were preceded by the calibration of the electrode with several alkali levothyroxine solutions (working solutions).

2.7. Sample Preparation

All pills were extracted from their original packaging, coded, and given to an examiner, unaware of the characteristics of the original tablets, as 10 anonymous packages of 20 tablets. The batches sent to be evaluated using HPLC had a different code from those to be assessed with the electrochemical sensor. The tablets were kept in a dark and cool (<25 °C) environment before being tested. For electrochemical determination, 20 tablets of each package were thoroughly powdered. The certain amount of this powdered precisely weighed and transfer into a volumetric flask. The pH was adjusted to 8.5 and diluted to mark by distilled water. Then, the solution was filtered and used for analysis.

HPLC tests, on the other hand, were conducted in the laboratory of MoHME's Food and Drug Research Center. After assessing the normal distribution of HPLC and electrochemical sensor results with Kolmogrov Smirnov, parametric independent, one sample t-test, and ANOVA were performed. The correlation between the HPLC and sensor results was evaluated using Pearson correlation test.

3. RESULTS AND DISCUSSION

3.1. Membrane composition optimization

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 responses of the proposed sensor was studied. Actually, the operating characteristics of the PVC membrane electrode can be significantly modified by changing the relative proportions of the electrode membrane components. The main components of a membrane electrode of this type are PVC matrix, the plasticizer, the ion-pair and the nature of the additives used significantly influences the sensitivity and the selectivity of the ion-selective electrodes. Each membrane component plays a special role in the membrane function [5].

Using PVC in membrane electrodes has a long history in ion selective field since the name of this kind of potentiometric sensors called after that (PVC membrane Sensors). The idea to incorporate all membrane ingredients, into a PVC matrix to control site density, came from the work of Rene Bloch, Adam Shatkay, and H. A. Sharoff in 1967 [5]. Other polymers have been tested long ago and the recommendation was PVC as the best polymeric matrix. The unique properties of PVC (specially the fact that it can be plasticized with both polar and non polar water-immiscible solvents) made it the best candidate.

Table 1. Optimization of the membrane ingredients for PVC membrane electrode

No.	Composition, wt.%			Slope (mV decade^{-1})	Linear Range (mol L^{-1})
	PVC	Plasticizer	LEV-HTA		
1	30	DBP,65	5	50.23±0.3	5.0×10^{-5}-1.0×10^{-2}
2	30	DBP,63	7	58.6±0.3	1.0×10^{-5}-1.0×10^{-2}
3	30	DBP,61	9	55.3±0.4	3.0×10^{-5}-1.0×10^{-2}
4	30	BA,63	7	25.3±0.3	7.0×10^{-5}-5.0×10^{-2}
5	30	NB,63	7	13.7±0.4	5.0×10^{-5}-1.0×10^{-3}
6	30	NPOE,63	7	15.6±0.3	1.0×10^{-4}-1.0×10^{-3}
7	30	DBP,70	-	4.3±0.5	1.0×10^{-5}-1.0×10^{-2}

The plasticizer mainly acts as a fluidizer, allowing homogeneous dissolution and diffusion mobility of the ion-pair inside the membrane [12,13]. The nature of the plasticizer has a marked influence on the response slope, linear domain and also on the selectivity of the PVC membrane electrodes. Here, some common plasticizer types were tested, namely benzyl acetate (BA), dibutylphthalate (DBP), nitrobenzene (NB) and nitrophenyloctyl ether (*o*-

NPOE) as listed in Table 1. After their evaluation, DBP, having the lower dielectric constant than other plasticizers was chosen to be employed in the sensor construction, because it provided an effective linear range and a lower detection limit which is due to the better extraction of the levothyroxine in the organic layer.

As it can be seen from Table 1, the absence of the ion-pair in the membrane results 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, 7% ion-pair, 63% DBP was the optimum one for the sensor design.

3.2. Calibration Graph and Statistical Data

The measuring range of potentiometric electrodes includes the linear part of the calibration graph as shown in Fig. 2 [17-21]. Measurements can be performed in this lower range, but it must be noted that more closely spaced calibration points are required for more precise determinations. For many electrodes the measuring range can extend from 1 molar down to 10^{-5} or even 10^{-6} molar concentrations [5,17-21].

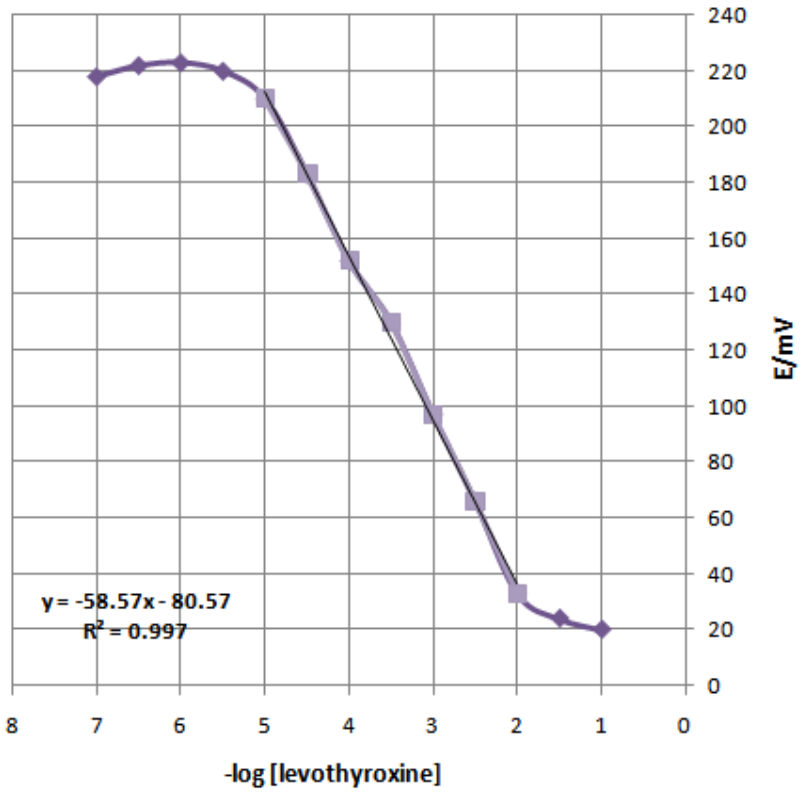

Fig. 2. Calibration curve of levothyroxine potentiometric sensors. The results are based on triplicate measurements

According to another definition, the measuring range of an ion-selective electrode is defined as the activity range between the upper and lower detection limits. According to the Fig. 2, a linear range of 1.0×10^{-5}-1.0×10^{-2} mol L^{-1} of levothyroxine concentration with a slope of 58.6±0.3 mV $decade^{-1}$ of the levothyroxine concentration and a standard deviation of ±0.3 mV after three replicate measurements were obtained. The detection limit was calculated from the intersection of the two extrapolated segments of the calibration graph. In this work, the detection limit of the proposed membrane sensor was 1.0×10^{-5} mol L^{-1} which was calculated by extrapolating the two segments of the calibration curve.

3.3. Dynamic Response Time of the Levothyroxine Sensor

Dynamic response time is the required time for the sensor to reach values within 90% of the final equilibrium potential, after successive immersions in the levothyroxine solutions [17-21]. Its calculation involved the variation and the recording of the levothyroxine concentration in a series of solutions from 1.0×10^{-5} to 1.0×10^{-2} mol L^{-1}. The sensor was able to reach quickly its equilibrium response (~30 s) in the whole concentration range.

3.4. Life-Time Study

The levothyroxine potentiometric electrode lifetime was estimated with the creation of its calibration curve, the periodical test of a standard solution (1.0×10^{-5}-1.0×10^{-2} mol L^{-1}, levothyroxine) and the calculation of its response slope.

For this estimation, four same electrodes were employed extensively (1 h per day) for 10 weeks. After their 6 week utilization, two changes were observed. Firstly, a slight gradual decrease in the slope (from 58.6±0.3 to 37.5±0.5 mV $decade^{-1}$) and, secondly, an increase in the detection limit (from 1.0×10^{-5} mol L^{-1} to 8.5×10^{-4} mol L^{-1}).

3.5. Analytical Performance

The linearity, limit of detection, selectivity, precision, accuracy, and ruggedness/robustness were the parameters which were used for the method validation.

As mentioned before, the measuring range of the levothyroxine sensor is between 1×10^{-5} and 1×10^{-2} mol L^{-1}. The detection limit of the sensor was calculated 1.0×10^{-5} mol L^{-1} (7 µg/ml).

3.5.1. 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 metochlopramide sensor were evaluated by the

matched potential method (MPM) [22]. The resulting values of the selectivity coefficients are given in Table 2. As can be seen from Table 2, in all cases the selectivity coefficients are about 10^{-3}, which seems to indicate negligible interferences in the performance of the electrode assembly.

Table 2. Selectivity coefficients of various interfering compound for levothyroxine sensor

Interference	Log K_{MPM}
Br^-	-3.20
Cl^-	-3.32
Na^+	-3.66
Mg^{2+}	-4.05
Ca^{2+}	-3.75
K^+	-3.86
CO_3^{2-}	-3.14
NO_3^-	-3.20
Glucose	-4.33
Lactose	-4.42
Cellulose	-4.41

3.5.2. Precision

The parameters of the repeatability and reproducibility were investigated in order to assess the precision of the technique. For the repeatability monitoring, 5 replicate standards samples 10, 100, 1000 µg/ml were measured using the proposed electrode by calibration method. Then, RSD values of 4.2, 3.9, and 2.8%, respectively. Regarding the inter-day precision, the same three concentrations were measured for 3 consecutive days; the associated RSD values of 4.5, 3.3, and 2.7% were obtained respectively.

3.5.3. Accuracy

The active ingredients of the collected batches were assessed using electrochemical potentiometric sensor. HPLC, however, was used to calculate the very variable only five batches of each series of the tablets. The characteristic of each drug along with the result of these tests are outlined in Table 3. The present study revealed a correlation between the active ingredients of levothyroxine tablets analyzed using official method and the electrochemical sensor. Comparison between the amount of the active ingredient in different tablets batch number using electrochemical sensor and HPLC demonstrated in Table 3. The difference

reported between the active ingredient found in Levoxine tablets from different manufacture series (batch number: 768-147-559) with different expiry dates were not statistically significant (p-value=0.544).

Table 3. Comparison between the amount of the active ingredient in different tablets batch number using electrochemical sensor and HPLC

Brand and Batch Number	**Electrochemical sensor Mean** *μg / tab*	**HPLC** *μg / tab*	**P. value**
Levoxine 559	104.4±8.5	103.7±4.11	**0.803**
Levoxine 147	102.9±6.4	105.6±4.91	**0.215**
Levoxine 768	106.5±6.7	100.2±2.13	**0.016**
L-Thyrox 801593	47.75±5.9	45.35±3.49	**0.834**
Eutirox c-27	105.1±7.7	111.8±2.32	**0.043**

3.5.4. Ruggedness/Robustness

For ruggedness of the method a comparison was performed between the intra- and inter-day assay results for levothyroxine obtained by two analysts. The RSD values for the intra- and inter-day assays of levothyroxine in the cited formulations performed in the same laboratory by the two analysts did not exceed 4.7%. On the other hand, the robustness was examined while the parameter values (pH of the solution and the laboratory temperature) were being slightly changed. Levothyroxine recovery percentages were good under most conditions, not showing any significant change when the critical parameters were modified.

3.5.5. Determination of Levothyroxine in formulations

The proposed sensor was evaluated by measuring the drug concentration in some pharmaceutical formulations. Comparison between the amounts of the active ingredient in similar Levoxine tablets collected from various pharmacies in Tehran demonstrated in Table 4. There was no significant difference between the active ingredient content of Euthyrox tablets provided from the importing company and from various pharmacies in Tehran (p-value =0.745). When comparing Synox with Levoxine 559 and Euthyrox 53997019, both of which had almost similar expiration date, there was no significant difference between the active ingredient content (p-value =0.157 and 0.593, respectively).

Table 4. Characteristic and the amounts of the active ingredient in levothyroxin tablets available in the Iranian Market

Brand	Batch Number	Expire Date	Measuring by Electrochemical sensor		
			Mean $\mu g.tab$	SD	95% CI
Levoxine	559	2010/6	104.4	8.4	98.3-110.5
	768	2011/3	106.5	6.7	101.7-111.3
	004	2011/9	100.6	3.3	98.2-102.9
	035	2011/11	108.2	4.7	104.8-111.6
	147	2012/1	102.9	6.4	98.3-107.5
	119	2012/1	99.9	2.1	98.3-101.4
Euthyrox	5399701	2010/7	96.8	3.9	94.0-99.6
	5337901	2011/6	104.3	7.4	99.0-109.6
	100730	2011/10	103	6.8	98.0-107.9
	101777	2012/2	110.7	7.5	105.3-116.0
L-Thyrox	801593	2009/2	45.75	5.9	41.5-50.0
Eutirox	c-27	2010/9	105.1	7.7	98.7-111.5
Synox	W10204	2010/11	98.5	9.2	92.0-105.1

According to US pharmacopeia (USP) the active ingredients of tablets should fall within the range of 90 to 110% of the label amount the tablet [23-25]. These characteristics can vary even in the batches produced by different pharmaceutical companies in different intervals of time. Different brands, regardless of having equal dosage of the medication, may similarly have dissimilar efficacies due to the differences found in their formulations and manufacturing environments [23,25].

The present study revealed non-significant differences in the active ingredient content of Levoxine tablets with diverse expiration dates, indicating constancy in the amount of the active ingredients found in these pills over time.

The present study had several limitations. Providing more batch numbers of a single product particularly those which were imported officially or unofficially was one of the main limitations of this study. It was also preferred to use HPLC to assess the active ingredient content of all the studied batches and brands in order to provide a better conclusion regarding

the reliability and precision of electrochemical sensor in comparison with HPLC in this regard.

4. CONCLUSION

The present study successfully revealed an electrochemical sensor in assessing the active ingredient content of levothyroxin using potentiometric electrodes. The proposed sensor is a PVC membrane electrode which offers advantages of rapidity, inexpensively, and portability. The sensor responds based on ion-exchange mechanism. Levothyroxine-Hexadecyl trimethylammonium ion-pair (LEV-HTA) was employed as a sensing element in construction of the membrane electrode. PVC membrane electrode was made after series of experiments. The best PVC membrane electrode performance was achieved by a membrane composition of 30% PVC, 60% DBP, and 7% LEV-HTA ion-pair. Standardizing the newly developed sensor can facilitate the process of evaluating the active ingredient content of levothyroxin tablets at the manufacturing time and during its shelf life while providing a data with a similar validity to that assessed with the use of the existing methods.

Acknowledgements

The authors express their appreciation to the Research Council of University of Tehran and Tehran University of Medical Sciences for financial support of this work.

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