ANALYSIS OF L-THYROXINE FROM PHARMACEUTICAL FORMULATIONS USING A POTENTIOMETRIC MICROSENSOR BASED ON IONIC LIQUID

Grigorina MITROFAN¹, Raluca Ioana STEFAN-VAN STADEN², Iuliana MOLDOVEANU³, Constantina P. KAPNISSI-CHRISTODOULOU⁴

A new enantioselective, potentiometric microsensor based on ionic liquid L-Ala-C₄-NO₃-L-lac was designed and used for the assay of L-thyroxine (L-T₄) in its pharmaceutical formulations. The matrix used for the design of the microsensor was carbon nanopowder. The limit of detection obtained for L-T₄ using this sensor was 5x10⁻¹₀ mol/L, the linear concentration range was between 10⁻⁹ and 10⁻⁷ mol/L L-T₄. The average recovery of L-T₄ in pharmaceutical formulations was 96.15%.

Keywords: L-thyroxine, potentiometric microsensors, pharmaceutical formulations, L-Ala-C₄-NO₃-L-lac ionic liquid, carbon nanopowder

1. Introduction

Thyroid hormones are among the most important hormones in the body due to their involvement in regulating the metabolic function [1]. Thyroid hormones play a significant role in prenatal and neonatal neurological development [2] and also in the regulation of the energy metabolism [3]. The major form of thyroid hormone in the blood is thyroxine/tetraiodothyronine (T₄), and its effects in vivo are mediated via triiodothyronine (T₃) (T₄ is converted to T₃ in target tissues) [4]. T₃ enters the cells and binds to nuclear receptors which triggers the production of proteins required for cellular respiration, thermogenesis, cellular growth and differentiation and metabolism of proteins, carbohydrates and lipids [4-6]. An abnormal secretion of T₄ from thyroid gland leads to a wide spectrum of thyroid diseases from hypothyroidism to hyperthyroidism. In order to diagnose this pathology, the free form of T₄ along with TSH (thyroid stimulating

¹ PhD student, Faculty of Applied Chemistry and Materials Science, University POLITEHNICA of Bucharest, e-mail: grigorina_mitrofan@yahoo.com
² Prof. habil. SR1, Faculty of Applied Chemistry and Materials Science, University POLITEHNICA of Bucharest, and Laboratory of Electrochemistry and PATLAB Bucharest, National Institute of Research for Electrochemistry and Condensed Matter, Bucharest, Romania, e-mail: ralucavanstaden@gmail.com
³ PhD chem., Laboratory of Electrochemistry and PATLAB, National Institute of Research for Electrochemistry and Condensed Matter, Bucharest, Romania, e-mail: iuli_0909@yahoo.com
⁴ Professor, University of Cyprus, Nicosia, Cyprus, e-mail: ckapn1@ucy.ac.cy
hormone) are the most used in clinics [7, 8]. Both T_{3} and T_{4} are used to treat thyroid hormone deficiency [7, 8]. For the treatment of underactive thyroid, levothyroxine sodium is used in order to replace or to provide more thyroid hormone. Levothyroxine is a synthetic levoisomer of T_{4} (L-T4), similar to the endogenous hormone produced by the thyroid gland [7]. Purity and enantiopurity are very important in the synthesis of pharmaceutical compounds.

Ionic liquids (IL) are defined as salt-like materials, made of organic cations and organic or inorganic anions, with the melting point close to the room temperature [9]. Their properties such as: high conductivity, low toxicity, non-volatility and wide potential window [10] make them suitable for sensory applications. An important subclass of IL is represented by chiral ionic liquids, having the ability of chiral discrimination [11], therefore they can be used as electroactive material in order to improve enantioanalytical capacity of the sensor [12]. Among other applications, IL have been used previously for chiral separation of different analytes such as α-cyclohexylmandelic acid enantiomers [13], ofloxacin enantiomers [14], D-phenylalanine [15], fucose and piperolic acid enantiomers [16].

When added a certain quantity of a ligand or a chiral selector, carbon nanopowder proved to be a very good matrix for electrodes construction, due to high selectivity for both organic and inorganic analytes [17]. It has been successfully used previously for the detection of different molecules such as S-captopril [18], S-perindopril [19] and L-proline [20].

Up to date the following techniques were proposed for the assay of L-T_{3} and L-T_{4} in pharmaceutical formulations: impedance spectroscopy [21], HPLC [22, 23], HPLC-UV-ICP-MS [24], differential pulse polarographic analysis [25], capillary electrophoresis [26], radioimmunoassay [27] and sequential-injection chemiluminescence [28]. Electrochemical sensors proved to be a very good alternative for the chromatographic methods, enabling their utilization for enantiopurity and purity tests of active compounds, as it was emphasized by Stefan van Staden and co-authors in several papers [29,30]. Due to the differences in pharmacokinetics and pharmacodynamics of the enantiomers of the same chiral pharmaceutical substance, there is a high need of reliable methods for enantiopurity tests in its pharmaceutical formulations.

The purpose of this work was to determine L-T_{4} using direct potentiometric technique from levothyroxine formulations using more sensitive and selective electrochemical sensors.
2. Experimental

2.1. Reagents and materials

3,3’5-Triiodo-L-thyronine (L-T₃), L-Thyroxine (L-T₄), D-Thyroxine (D-T₄), ionic liquid L-Ala-C₄-NO₃-L-lac, carbon nanopowder, monosodium phosphate and disodium phosphate (>99.0%) for the buffer solution pH 7.5 were purchased from Sigma Aldrich. Levothyroxine sodium (Euthyrox 25 µg) was acquired from Merck. Paraffin oil was supplied by Fluka. Deionized water obtained from a Millipore Direct-Q 3 System was used for the preparation of all solutions. Standard solutions (10⁻¹²–10⁻¹⁴ molL⁻¹) were obtained by serial dilution. All solutions were fresh prepared before measurements.

2.2. Apparatus

All potentiometric measurements were recorded using a PGSTAT 302N (Metrohm, Switzerland) potentiostat/galvanostat, linked to a computer via Eco Chemie s®oftware version 4.9. An Ag/AgCl (0.1 molL⁻¹ KCl) electrode served as the reference electrode in the cell.

2.3. Microsensors design

Carbon nanopowder was selected as the matrix for the electrochemical sensor. Ionic liquid L-Ala-C₄-L-lac was used as electroactive material in order to obtain a reliable design. 100 mg of carbon nanopowder were mixed with 30 µL of paraffin oil, followed by the addition of 100 µL from the electroactive material solution (10⁻³ molL⁻¹ in water). The modified paste was placed into a plastic tube with the inner diameter of 300 µm. The electric contact was obtained by inserting a silver wire. Before using the surface of the microsensor was wetted with deionised water and polished with aluminium foil. When not in use, the microelectrodes were stored in a dry state at 25°C.

2.4. Recommended procedure

Direct potentiometry was used for the measurements of the potential of each standard solution (10⁻¹²–10⁻⁴ molL⁻¹). The electrode was placed in stirred standard solution and also in pharmaceutical solutions of sodium levothyroxine. The potential was recorded and graphs of E (mV) versus pL-T₄ (pL-T₄ = -lg [C₄ L-T₄]) were plotted. From the calibration graphs the unknown concentrations were determined (Fig. 1).

2.5. Uniformity Content Test

Uniformity Content Test [31] was used in order to evaluate the consistency of the dosage units and the requirements for the weight variation were met. Ten tablets containing 25 µg sodium levothyroxine were weighed separately.
Each tablet was dissolved in a mixture 1:1 of buffer solution (pH=7.4) and deionised water. The unknown concentrations of levothyroxine were determined using the calibration graph of the sensor and the recommended procedure was described in paragraph 2.4.

3. Results and discussions

a) The response characteristics of the potentiometric microsensor

The response characteristics of the potentiometric microsensor based on L-Ala-C₄-L-lac were determined in standard conditions, at 25°C. Solutions of concentrations varying from 10⁻¹² to 10⁻⁴ molL⁻¹ were used. The linear concentration range was between 10⁻⁹ to 10⁻⁷ molL⁻¹ L-T₄, with a response/slope of 41.87 mV/decade of concentration. The limit of detection was determined experimentally, as the concentration value from which no change on the potential value was observed as of 5x10⁻¹⁰ molL⁻¹ L-T₄. The standard potential was -245.7 mV. The equation of calibration was:

\[ E = -245.7 + 41.87 \text{pL-T}_4 \]  

with the correlation coefficient 0.9992.

![Calibration graph obtained using the microsensor based on carbon nanopowder modified with L-Ala-C₄-L-lac for the detection of L-T₄](image)

Fig. 1. Calibration graph obtained using the microsensor based on carbon nanopowder modified with L-Ala-C₄-L-lac for the detection of L-T₄

a. Selectivity of the proposed microsensor

The selectivity of the proposed microsensor was determined using mixed solutions method. The ratio used between L-T₄ and interferent was 1:10. Enantioselectivity was determined versus D-T₄, and the selectivity versus L-T₃. The potentiometric selectivity coefficients found using the mixed solutions
method were: $5 \times 10^{-4}$ for L-T₃, and $<<1 \times 10^{-4}$ for D-T₄. The values demonstrated that the proposed microsensor is selective versus L-T₃ and enantioselective.

b. Analytical applications

The response characteristics as well as the selectivity test performed versus two possible interferences which are by-products in the synthesis of L-T₄ proved that the proposed microsensor based on the ionic liquid L-Ala-C₄-L-lac can be used for the purity and enantiopurity tests of the pharmaceutical formulations of L-T₄. The results of the Uniformity Content Test are presented in Table 1.

### Table 1

<table>
<thead>
<tr>
<th>Tablet nr.</th>
<th>L-T₄, µg</th>
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<tbody>
<tr>
<td>1</td>
<td>23.88</td>
</tr>
<tr>
<td>2</td>
<td>23.06</td>
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<tr>
<td>3</td>
<td>23.97</td>
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<td>4</td>
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<td>9</td>
<td>25.00</td>
</tr>
<tr>
<td>10</td>
<td>23.13</td>
</tr>
</tbody>
</table>

The average content of L-T₄ in the tablets was 96.15% which is situated within the ranges required by international pharmacopoeias: 92-98% from the content declared.

b) Conclusions

The slope, selectivity and enantioselectivity of the sensor were favorable for the reliable assay of L-T₄ on its pharmaceutical formulations. The average recovery of L-T₄ from its pharmaceutical formulations was 96.15. The features of the sensor is in the pharmaceutical analysis.

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REFERENCES


Analysis of L-thyroxine from pharmaceutical formulations using a potentiometric (…)


[28]. H. Silvaieh, R. Wintersteiger, G. M. Schmid, O. Hofstetter, V. Schurig, G. Gübitz, “Enantioselective sequential-injection chemiluminescence immunoassays for 3,3,5-

