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

Grigorina MITROFAN[1], Raluca Ioana STEFAN-VAN STADEN[2], Iuliana MOLDOVEANU[3], Constantina P. KAPNISSI-CHRISTODOULOU[4]

A new enantioselective, potentiometric microsensor based on ionic liquid L-Ala-C_4-NO_3-L-lac was designed and used for the assay of L-thyroxyne (L-T_4) in its pharmaceutical formulations. The matrix used for the design of the microsensor was carbon nanopowder. The limit of detection obtained for L-T_4 using this sensor was $5x10^{-10}$ mol/L, the linear concentration range was between 10^{-9} and 10^{-7} mol/L L-T_4. The average recovery of L-T_4 in pharmaceutical formulations was 96.15%.

Keywords: L-thyroxine, potentiometric microsensors, pharmaceutical formulations, L-Ala-C_4-NO_3-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/tetraiodo-thyronine (T_4), and its effects *in vivo* are mediated via triiodo-thyronine (T_3) (T_4 is converted to T_3 in target tissues) [4]. T_3 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 T_4 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 T_4 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: ckapni1@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 enatiomers [14], D-phenylalanine [15], fucose and pipecolic 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-T3 and L-T4 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-T_3), L-Thyroxine (L-T_4), D-Thyroxine (D-T_4), 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^{-4}-10^{-12} $molL^{-1}$) 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 software 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-C_4-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 25ºC.

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-T_4 (pL-T_4 = -lg [C L-T_4]) 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-C_4-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-T_4, 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-T_4. The standard potential was -245.7 mV. The equation of calibration was:

$$E=-245.7+41.87\ pL\text{-}T_4 \quad (1)$$

with the correlation coefficient 0.9992.

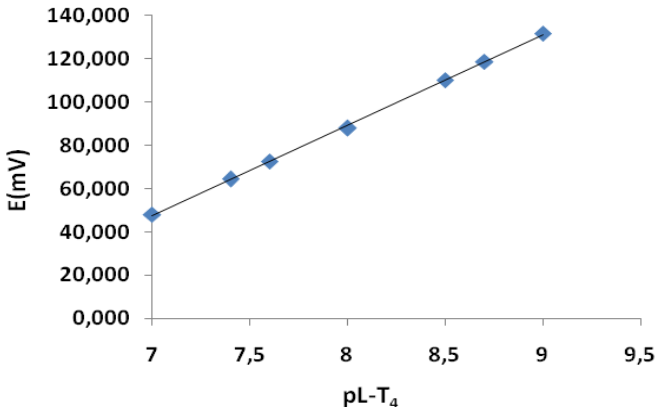

Fig. 1. Calibration graph obtained using the microsensor based on carbon nanopowder modified with L-Ala-C_4-L-lac for the detection of L-T_4

a. Selectivity of the proposed microsensor

The selectivity of the proposed microsensor was determined using mixed solutions method. The ratio used between L-T_4 and interferent was 1:10. Enantioselectivity was determined versus D-T_4, and the selectivity versus L-T_3. The potentiometric selectivity coefficients found using the mixed solutions

method were: $5x10^{-4}$ for L-T_3, and $<<1x10^{-4}$ for D-T_4. The values demonstrated that the proposed microsensor is selective versus L-T_3 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-T_4 proved that the proposed microsensor based on the ionic liquid L-Ala-C_4-L-lac can be used for the purity and enantiopurity tests of the pharmaceutical formulations of L-T_4. The results of the Uniformity Content Test are presented in Table 1.

Table 1

Determination of L-T_4 concentration in tablets

Tablet nr.	L-T_4, µg
1	23.88
2	23.06
3	23.97
4	24.91
5	25.00
6	25.00
7	23.38
8	23.06
9	25.00
10	23.13

The average content of L-T_4 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-T_4 from its pharmaceutical formulations was 96.15. The features of the sensor is in the pharmaceutical analysis.

Acknowledgments

This work was supported by UEFISCDI, PNII Program Ideas 2012-2014, Contract nr.100/27.10.2011, and Bilateral Project Romania-Cyprus 2014-2015.

R E F E R E N C E S

[1]. *J.H. Oppenheimer, H. Samuels,*" Molecular basis of thyroid hormone action", Academic Press, 1983, pp 36-63.

[2]. *S.P. Porterfield, C.E. Hendrich*, "The role of thyroid hormones in prenatal and neuronatal neurological development-current perspectives", Endocr. Rev., **vol. 94**, no. 2, 2014, pp. 355-382.

[3]. *R. Mullur, Y.Y. Liu, G.A. Brent,* "Thyroid hormone regulation of metabolism", Physiol. Rev., **vol. 94**, no. 2, 2014, pp. 355–382.

[4]. *A.C. Bianco, J.E. Silva,* "Intracellular convertion of thyroxine to triiodotyronine is required for the optimal thermogenic function of brown adipos tissue", J. Clin. Invest., **vol. 79**, no. 1, 1987, pp. 295-300.

[5]. *F. Goglia, M. Moreno, A. Lanni,* "Action of throid hormones at the cellular level: the mithocondrial target", FEBS Lett., **vol. 452**, no. 3, 1999, pp.115-120.

[6]. *Y. Halpelin, M.I. Surks, L.E. Shapiro,* "L-triiodothyronine (T3) regulates cellular growth rate, growth hormone production, and levels of nuclear T3 receptors via distinct dose-response ranges in cultured GC cells", Endocrinology, **vol. 126**, no. 5, 1990, pp. 2321-2326.

[7]. *J. Jonklaas, A.C. Bianco, A.J. Bauer, K.D. Burman, A.R. Cappola, F.S. Celi, S.D. Cooper, B.W. Kim, R.P. Peeters, M.S. Rosenthal , A.M. Sawka,* "Guidelines for the treatment of hypothyroidism: prepared by the American Thyroid Association Task Force on Thyroid Hormone Replacement", Thyroid, **vol. 24**, no. 12, 2014, pp. 1670-1751.

[8]. *R.S. Bahn, H.B. Burch, D.S. Cooper, J.R. Garber, M.C. Greenlee, I.K.P. Laurberg, I.R. McDougall, V.M. Montori, S.A. Rivkees, D.S. Ross, J.A. Sosa , M.N. Stan*, "Hyperthyroidism and other causes of thyrotoxicosis: management guidelines of the american thyroid association and american association of clinical endocrinologists", Endocr. Pract., **vol. 11**, no. 3, 2011, pp. 1-65.

[9]. *D. Wei, A. Ivaska,* "Applications of ionic liquids in electrochemical sensors", Anal. Chim. Acta., **vol. 607**, no. 2, 2008, pp.126-135.

[10]. *T. Welton,* "Room-temperature ionic liquids.Solvents for synthesis and catalysis". Chemical Reviews, **vol. 99**, no. 8, 1999, pp. 2071-2083.

[11]. *C. Baudequin, D. Brégeon, J. Levillain, F. Guillen , J.C. Plaquevent, A.C. Gaumont,* " Chiral ionic liquids, a renewal for the chemistry of chiral solvents? Design, synthesis and applications for chiral recognition and asymmetric synthesis", Tetrahedron: Asimmetry, **vol. 16**, no. 14, 2005, pp. 3921-3945.

[12]. *I.J. Stavrou, M. C Mavroudi, C. P. Kapnissi-Christodoulou,* "Chiral Selectors in CE: Recent Development and Applications", Electrophoresis, **vol. 36**, 2015**,** pp. 101-123.

[13]. *L.L. Chen, F.F. Li, Z.J. Tan,* "Chiral separation of α-cyclohexylmandelic acid enantiomers using ionic liquid/salt aqueous two-phase system", Chemical Papers, **vol. 69**, no 11, 2015, pp. 1465–1472.

[14]. *B. Wentao, T. Minglei, H.R. Kyung*, "Chiral separation and determination of ofloxacin enantiomers by ionic liquid-assisted ligand-exchange chromatography", Analyst**, vol. 136**, 2011, pp. 379-387.

[15]. *H.M. Marwani, Esraa M. B., A.T. Hamad, M.A. Abdullah, S.B. Khan*, "Enantioselective separation and detection of D-Phenylalanine based on newly developed chiral ionic liquid immobilized silica gel surface", Int. J. Electrochem. Sci., **vol . 9**, 2014, pp. 7948–7964.

[16]. *C. A. Hadjistasi, I. J. Stavrou, R.-I. Stefan-van Staden, H. Y.Aboul-Enein, C. P. Kapnissi-Christodoulou*, "Chiral separation of the clinically important compounds fucose and pipecolic acid using CE – Determination of the most effective chiral selector", Chirality, **vol. 25**, no. 9, 2013, pp 556-560.

[17]. *R.I. Stefan-van Staden, J.F. van Staden, H.Y. Aboul-Enein*, ''Electrochemical Sensors in Bioanalysis'', CRC Press, New York, 2001.

[18]. *R.I. Stefan, J.K. van Staden, H.Y. Aboul-Enein* , "A new construction for a potentiometric, enantioselective membrane electrode--its utilization to the S-captopril assay", Talanta, **vol. 48**, no. 5, 1999, pp. 1139-1143.

[19**].** *R.I. Stefan-van Staden, J.K. van Staden, H.Y. Aboul-Enein*, "*S*-perindopril assay using a potentiometric, enantioselective membrane electrode", Chirality, **vol. 11**, no. 8, 1999, pp. 631-634.

[20]. *R.I. Stefan-van Staden, J.K. van Staden, H.Y. Aboul-Enein*, "A new construction for potentiometric, enantioselective membrane electrodes, and use for L-proline assay", Analytical Letters, **vol. 31**, no. 11, 1998, pp. 1787-1794.

[21] *L. Bendo, M. Casanova, A.C. Figueira, I. PolikarpovI,V. Zucolotto*, " Nanostructured sensors containing immobilized nuclear receptors for thyroid hormone detection", J. Biomed. Nanotechnol., **vol. 10**, no. 5, 2014, pp. 744-50.

[22]. *H. Gika, M. Lammerhofer, I. Papadoyannis, W. Lindner*, " Direct separation and quatitative analysis of thyroxine and triiodothyronine enatiomers in pharmaceuticals by high-performance liquid chromatography", J. Chromatogr. B, **vol. 800**, no. 1-2, 2004, pp. 193-201.

[23]. *M. Takahashi, M. Nagashima, S. Shigeoka, H. Kamimura, K. Kamata*, " Determination of thyroid hormones in pharmaceutical preparations, after derivatization with 9-anthroylnitrile, by higj-performance liquid chromatography with fluorescence detection", J. Cromatogr. A, **vol. 958**, no. 1-2, 2002, pp. 299-303.

[24]. *S.S. Kannamkumarath, R.G. Wuilloud, A. Stalcup, J.A. Caruso, H. Patel, A. Sakr, "*Determination of levothyroxine and its degradation products in pharmaceutical tablets by HPLC-UV-ICP-MS", J. Anal. At. Spectrom, **vol. 19**, 2004, pp. 107-113.

[25]. *W. Holak, D. Shostak,* "Differential pulse polarographic analysis of thyroid hormone: Determination of iodine, thyroxine and liothyronine", J. Pharm. Sci., **vol. 68**, no. 3, 1979, pp. 338-342.

[26]. *D. Schmalzing, L. B. Kountny, T. A. Taylor, W. Nashabeh, and M. Fuchs*, "Immunoassay for thyroxine (T4) in serum using capillary electrophoresis and micromachined devices", J. Chromatogr. B., **vol. 697**, 1997, pp. 175–180

[27]. *A. Pacchiarotti, L. Bartalena, M. Falcone, L. Buratti,L. Grasso, E. Martino, A. Pinchera*, "Free thyroxine and free triiodothyronine measurement in dried blood spots on filter paper by column adsorption chromatography followed by radioimmunoassay", Journal Horm. Metab. Res., **vol. 20**, no. 5, 1988, pp. 293-297.

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

triiodothyronine (T3) and thyroxine (T4), Analytica Chimica Acta, **vol. 463**, 2002, pp. 5–14.

[29]. *R.I. Stefan, J.K. van Staden, H.Y. Aboul-Enein,* "Simultaneous determination of L-thyroxine (L-T4), d-thyroxine (D-T4), and L-triiodothyronine (L-T3) using a sensors/sequential injection analysis system", Talanta, **vol. 64**, 2004, pp. 151-155.

[30] *I. Moldoveanu, R.I. Stefan –van Staden, F.J. van Staden,G.L. Radu*, "Analysis of L-thyroxine and 3,3',5-triiodo-L-thyronine using potentiometric microsensors", U.P.B. Sci. Bull., Series B, **vol. 76**, no. 3, 2014, pp. 3-10.

[31]. U.S. Pharmacopeia 38 and National Formulary 33, USP official thyroxine monograph, – second supplement, U.S.Pharmacopeial Convention, Rockville, MD, USA, 2015.