ANALYSIS OF L-THYROXINE AND 3,3',5-TRIIODO-L-THYRONINE USING POTENTIOMETRIC MICROSENSORS

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*Potentiometric microsensors based on maltodextrin and α-cyclodextrin, were developed for the assay of 3,3',5-triiodo-L-thyronine (**L-T$_3$**) and L-thyroxine (**L-T$_4$**). The matrix used for the design of the potentiometric microsensors was carbon nanopowder. The limits of quantification obtained for 3,3',5-triiodo-L-thyronine using the microsensor based on maltodextrin was 10^{-10} mol/L, and 10^{-9} mol/L using the sensor based on α-cyclodextrin. For L-thyroxine, there were obtained the limits of quantification of 10^{-11} mol/L using the microsensor based on maltodextrin, and 10^{-10} mol/L using the microsensor based on α-cyclodextrin. The analytical application of the microsensors was for the analysis of thyroid hormones in whole blood.*

Keywords: potentiometric microsensors, 3,3',5-triiodo-L-thyronine, L-thyroxine, carbon nanopowder

1. Introduction

Triiodothyronine also known as **L-T$_3$**, and levothyroxine or thyroxine (**L-T$_4$**), are the naturally occurring thyroid hormones (Fig. 1). [1]

Thyroid hormones, triiodothyronine (**L-T$_3$**) and thyroxine (**L-T$_4$**), are critical for the development of the central nervous system [2]. The importance of thyroid hormones for normal central nervous system development is apparent from the serious mental retardation syndrome of cretinism [3, 4].

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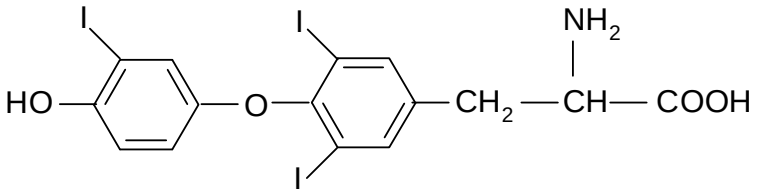
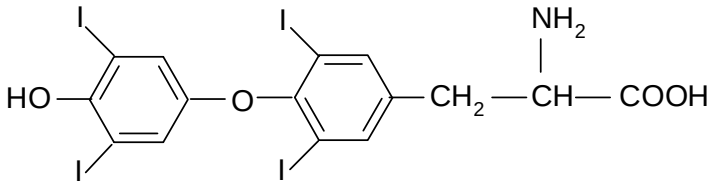


Triiodothyronine (L-T_3)



Thyroxine (L-T_4)

Fig. 1. Structures of **L-T_3** and **L-T_4**

The excess or lack of thyroid hormones in the body has a significant influence on the structural, biochemical, and functional development of the central nervous system. [5]

In 1952, 3,5,3'-triiodo-L-thyronine was found in human plasma by Gross and Pitt-Rivers [6]. **L-T_4** is converted in **L-T_3** in the peripheral tissues [6]. The interest of many researches to detect **L-T_3** has been stimulated by the following: the well known greater biological activity of **L-T_3** compared with **L-T_4**, the capability to measure **T_3** turnover rates in men [7], and the demonstration by Braverman and collaborators [8], that **T_4** is converted to **T_3** *in vivo*.

An amperometric biosensor based on L-aminoacid oxidase for the enantioselective analysis of **T_3** and **T_4** was proposed by H. Y. Aboul-Enein and coautors [9]. The selectivity is low when direct assay of the hormones in the blood was employed with this biosensor.

R.I. Stefan and H. Y. Aboul-Enein have described a reliable construction for an amperometric imunosensor based on the imobilization of the antibody on graphite paste, for the assay of **L-T_4** [10]. The sensitivity and selectivity of the proposed immunosensor are adequate for the purpose of analysis. Many chromatographic techniques - HPLC [11], UHPLC [12] capillary electrophoresis [13], radioimmunoassay [14], sequential-injection chemiluminescence immunoassays [1] - based techniques were proposed for the assay of **L-T_4** and **L-T_3**.

The purpose of this work was to determine **L-T_4** and **L-T_3** using direct potentiometric technique. For the construction of working microsensors we used carbon nanopowder as matrix and maltodextrin and α-cyclodextrin as modifiers.

The microsensors were used for the assay of **L-T_4** and **L-T_3** in whole blood samples. Their active surface can be regenerated by simple polishing on aluminium paper.

2. Experimental

2.1. Reagents and materials

3,3',5-Triiodo-L-thyronine (**L-T_3**) (>95,0%), L-Thyroxine (**L-T_4**) (>98,0%), L-Tryptophan (>99,5%), serotonin (>98,0%), carbon nanopowder (>99,0%), maltodextrin (**MD**), and α–cyclodextrin (**α-CD**) (>98,0%), were purchased from Sigma-Aldrich. Monosodium phosphate (>99,0%) and disodium phosphate (>99,0%) were purchased from Reactivul, Bucharest. Paraffin oil was supplied by Fluka. Deionized water obtained from a Millipore Direct-Q 3 System (Molsheim, France) was used for the preparation of all solutions. All solutions were buffered with phosphate buffer solution pH=7.04 prepared in our laboratory. Standard solutions of 1×10^{-3}mol/L of **L-T_3** and **L-T_4**, respectively were prepared by dissolving the necessary amount of **L-T_3** and **L-T_4** in buffer solution and deionized water 1:1 (v/v). The **L-T_3** and **L-T_4** solutions were prepared from respective standard solutions (10^{-3} mol/L), by serial dilution. All solutions were fresh prepared before measurements.

2.2. Apparatus

An Ivium potentiostat/galvanostat was used for all potentiometric measurements. Ag/AgCl (0.1 mol/L KCl) electrode served as reference electrode in the cell.

2.3. Microsensors design

Maltodextrin and α-cyclodextrin were used as electroactive materials in the design of the microsensors. The matrix selected was nanopowder of carbon in order to obtain a reliable design for the electrochemical sensors.

Paraffin oil and carbon nanopowder were mixed in a ratio of 1:4 (w/w), followed by the addition of the electroactive material solution (10^{-3}mol/L in water). 100μL from each electroactive material solution were added to 100 mg carbon paste. The modified paste was placed into a plastic tube. The diameter of the active surface of the microelectrode was 300μm. Electric contact was obtained by inserting a Ag/AgCl wire. The surface of the microsensor was wetted with deionised water and polished with alumina paper (polishing strips 30144-001, Orion) before using. When not in use, the microelectrodes were stored in a dry state at room temperature.

2.4. Recommended procedure

Direct potentiometry was used for the measurements of the potential of each standard solution (10^{-12} – 10^{-3} mol/L). The electrodes were placed in stirred standard solution while the potential was recorded, and graphs of E(mV) versus pL-T_3, and pL-T_4 respectively (pL-T_3 = -lg[$C_{L\text{-}T3}$]; pL-T_4 = -lg[$C_{L\text{-}T4}$]) were plotted. The unknown concentrations were determined from the calibration graphs (Fig. 2).

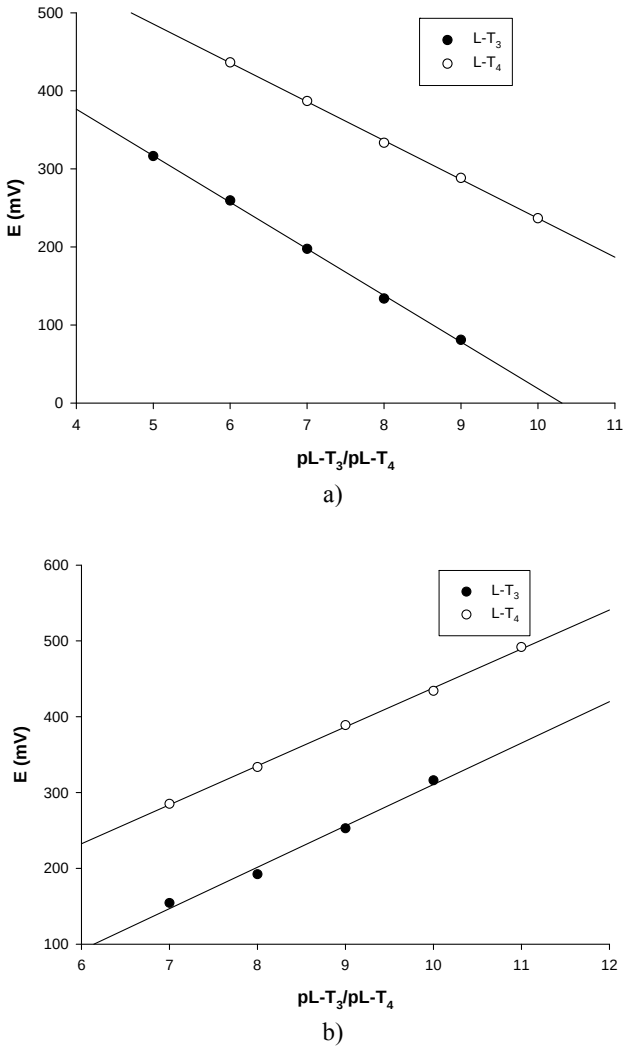


Fig. 2. Calibration graphs for (a) CD/C nanopowder; (b) MD/C nanopowder

2.5. Sample preparation for whole blood

Whole blood samples were taken from different patients. The apparatus cell was filled with the whole blood and the potential developed were measured. The unknown concentration was determined from the calibration graphs as described in the direct potentiometry method.

3. Results and discussion

3.1. Potentiometric microsensors response

Microsensors based on carbon nanopowder modified with **MD** or **α-CD** were tested using direct potentiometric method for the analysis of **L-T_3** and **L-T_4**. Both sensors showed linear and near-Nernstian response, so that they can be used for the analysis of **L-T_3** and **L-T_4**. Table 1 shows the response characteristics of the microsensors used in the assay of **L-T_3** and **L-T_4**. The microsensor based on carbon nanopowder modified with maltodextrin can be used for the assay of **L-T_3** in the range 10^{-10}–10^{-7} mol/L and for the assay of **L-T_4** in the range 10^{-11}–10^{-7} mol/L. The microsensor based on carbon nanopowder modified with α-cyclodextrin can be used for the assay of **L-T_3** on the range 10^{-9}–10^{-5} mol/L and for the assay of **L-T_4** on the range 10^{-10}–10^{-6} mol/L. The time of analysis was 5 minutes. The lowest limit of quantification was exhibited by the sensor based on carbon nanopowder modified with maltodextrin for assay of **L-T_4** (Table 1).

Table 1

Response characteristics of the potentiometric microsensors for the assay of L-T_3 and L-T_4

Potentiometric microsensors based on	E° (mV)	Slope (mV/decade of concentration)	Working concentration range. (mol/L)	Limit of quantification (mol/L)	Limit of detection (mol/L)
L-T_3					
CD/C nanopowder	615.1	59.63	10^{-9} – 10^{-5}	10^{-9}	4.8 x 10^{-11}
MD/C nanopowder	-235.1	54.58	10^{-10} – 10^{-7}	10^{-10}	4.9 x 10^{-11}
L-T_4					
CD/C nanopowder	734.8	49.81	10^{-10} – 10^{-6}	10^{-10}	1.76 x 10^{-15}
MD/C nanopowder	-75.89	51.40	10^{-11} – 10^{-7}	10^{-11}	3.3 x 10^{-13}

3.2. Selectivity of the proposed microsensors

Serotonin and L-tryptophan were considered for the tests of selectivity of the microsensors proposed for the assay of thyroid hormones. Mixed solution method was applied for potentiometric microsensors. The ratio between the analyte and interferent was 1:10 (mol:mol) in the mixed solution.

Potentiometric selectivity coefficients were calculated using the equation [15]:

$$K_{pot} = (10^{\frac{\Delta E}{S}} - 1) \times \frac{C_i}{C_j^{z_i/z_j}} \quad (1)$$

where ΔE is the difference between the potential recorded for mixed solution ($E_{i,j}$) and for the solution that contains only the main ion (E_i), $\Delta E = E_{i,j} - E_i$ (all recorded in mV); S is the slope of the electrode deducted from the equation of calibration (mV/ decade of concentration); C_i is the concentration of the main ion, i, C_j is the concentration of the interfering species, j; z_i is the charge of the main ion, i, z_j is the charge of interfering species, j.

Results for the potentiometric selectivity coefficients are shown in Table 2. The values show that the sensors cannot be easily used for the assay of the thyroid hormones in whole blood samples, because there are considerable interferences from tryptophan and serotonin.

Table 2

Potentiometric selectivity coefficients for the potentiometric microsensors

Potentiometric microsensors based on	K_{sel}^{pot}	
L-T_3		
	L-Tryptophan	Serotonin
CD/C nanopowder	1.75	0.18
MD/C nanopowder	0.99	0.96
L-T_4		
	L-Tryptophan	Serotonin
CD/C nanopowder	0.99	1.00
MD/C nanopowder	1.00	0.95

3.3. Analytical applications

To be able to use the proposed sensors for the analysis of thyroid hormones **L-T_3** and **L-T_4** in whole blood samples, further tests for the assay of one hormone in the presence of the other were performed. In this regard, synthetic mixtures containing the thyroid hormones in different ratios, were prepared. The results are shown in Tables 3 and 4.

Table 3

Recovery of L-T_3 in the presence of L-T_4

L-T_3: L-T_4 (mol/mol) Potentiometric microsensors based on	%, Recovery of **L-T_3**					
	2:1	1:1	1:2	1:4	1:9	1:99
CD/C nanopowder	128.47	49.20	71.90	77.41	100.74	65.91
MD/C nanopowder	24.96	34.30	57.67	62.16	82.04	44.70

*N=3

Table 4

Recovery of L-T_4 in the presence of L-T_3

L-T_4: L-T_3 (mol/mol) Potentiometric microsensors based on	%, Recovery of **L-T_4**					
	2:1	1:1	1:2	1:4	1:9	1:99
CD/C nanopowder	128.58	88.49	92.55	88.75	111.10	139.55
MD/C nanopowder	30.43	3.17	6.98	21.24	86.76	36.05

*N=3

The results presented in Tables 3 and 4, shown that for the assay of **L-T_3** in the presence of **L-T_4**, and for the assay of **L-T_4** in the presence of **L-T_3**, the best microsensor was the one based on CD and C nanopowder, although in both cases the accuracy of the results is influenced by the ratio (mol/mol) between the thyroid hormones.

The electrodes were used for the assay of thyroid hormones in real whole blood samples. The only microsensor that could have been used with good accuracy was the one based on CD, when the recovery of **L-T_3** was 95.35% from the value found using a standard method of analysis and for the recovery of **L-T_4** 93.57% from the value found using a standard method of analysis.

4. Conclusions

Although the proposed microsensors presented good and reliable response characteristics for the assay of thyroid hormones, their selectivity is low for other biological substances, e.g., serotonin, tryptophan. The assay of one hormone in the presence of the other, shown that the best microsensor is the one based on α-cyclodextrin, although even in this case, the accuracy of measurements is dependent on the ratio between the hormones. Only one microsensor was able to assay both thyroid hormones in whole blood samples, with acceptable accuracy. Given the low selectivity, one should consider the utilization of these sensors in simple matrices, like pharmaceuticals rather than for clinical analysis.

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R E F E R E N C E S

[1]. *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.

[2]. *L. Zhang, C. M. Cooper-Kuhn, U. Nannmark, K. Blomgren and H. G. Kuhn,* "Stimulatory effects of thyroid hormone on brain angiogenesis in vivo and in vitro", Journal of Cerebral Blood Flow & Metabolism, **vol.30**, 2010, pp. 323–335.

[3]. *J.W Smith A. T, Evans, B. Costall, J.W .Smythe,* "Thyroidhormones, brain function and cognition: a brief review".Neurosci Biobehav Rev., **vol. 26**, 2002, pp. 45–60.

[4]. *I.D. Papakostas and George A. Macheras*, "Thyroid Hormones and Peripheral Nerve Regeneration", J. of Thyroid Research, 2013, Article ID 648395, 5 pages.

[5]. *C.C. Thompson, G. B Potter,* "Thyroid hormone actionin neural development", Cereb. Cortex., **vol. 10**, 2000, pp. 939–945.

[6]. *J. Gross and R. Pitt-Rivers,* "The identification of 3:5 :3'-triiodothyronine in human plasma", Lancet., **vol. 1**, 1952, pp. 439.

[7]. *K.A, Woeber, R. J. Sobel, S. H. Ingbar, and K. Sterling,* "The peripheral metabolism of triiodothyronine in normal subjects and in patients with hyperthyroidism" J. Clin. Invest., **vol. 49**, 1970, pp. 643.

[8]. *L.E. Braverman, S. H. Ingbar, and K. Sterling*, "Conversion of thyroxine (T4) to triiodothyronine (T3) in athyreotic human subjects", J. Clin. Invest., **vol. 49**, 1970, pp. 855.

[9]. *H. Y. Aboul-Enein, R. I. Stefan, S. Litescu and G. L. Radu*, "Biosensor for the enantioselective analysis of the thyroid hormones (+)-3,3',5- triiodo-l-thyronine (T_3) and (+)-3,3',5,5'-tetraiodo-l-thyronine (T_4), J. Immunoassay and Immunochemistry, **vol. 23**, no. 2, 2002, pp. 181–190.

[10]. *R.I. Stefan and H. Y. Aboul-Enein*, "The construction and characterization of an amperometric immunosensor for the thyroid hormone (+)-3,3, 5,5'-tetraiodo-l-thyronine (L-T_4)", Journal of Immunoassay & Immunochemistry, **vol. 23**, no. 4, 2002, pp. 429–437.

[11]. *V.F, Samanidou, H. G. Gika, and I. N. Papadoyannis,* "Rapid HPLC analysis of thyroid gland hormones tri-iodothyronine (T3) and thyroxine (T4) in human biological fluids after SPE, J. Liq. Chromatogr. Relat. Technol., **vol. 23**, 2000, pp. 681–692.

[12]. *L.G. Luna, K. Coady, J. R. McFadden, D. A. Makham, M. J. Bartels*, "Quantification of total thyroxine in plasma from xenopus laevis", J. Anal. Toxicol, **vol. 37**, no.6, 2013, pp. 1-11.

[13]. *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.

[14]. *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.

[15]. *K. Ren*, "Selectivity problems of membrane ion-selective electrodes", Fresenius J. Anal. Chem., **vol. 365**, 1999, pp. 389-397.