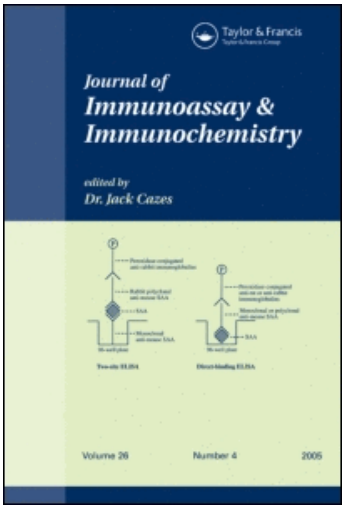

Journal of Immunoassay and Immunochemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597271

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)

Hassan Y. Aboul-Enein[a]; Stefan Raluca-Ioana[b]; Simona Litescu[c]; Gabriel Lucian Radu[c]
[a] Pharmaceutical Analysis Laboratory, Biological and Medical Research Department (MBC-03), King Faisal Specialist Hospital and Research Centre, Riyadh, Saudi Arabia [b] Department of Chemistry, University of Pretoria, Pretoria, South Africa [c] Department of Analytical Biochemistry, National Institute of Biological Sciences, Bucharest, Romania

Online publication date: 05 February 2002

To cite this Article Aboul-Enein, Hassan Y. , Raluca-Ioana, Stefan , Litescu, Simona and Radu, Gabriel Lucian(2002) '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)', Journal of Immunoassay and Immunochemistry, 23: 2, 181 — 190
To link to this Article: DOI: 10.1081/IAS-120003660
URL: http://dx.doi.org/10.1081/IAS-120003660

J. IMMUNOASSAY & IMMUNOCHEMISTRY, 23(2), 181–190 (2002)

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)

Hassan Y. Aboul-Enein,[1,*] Raluca-Ioana Stefan,[2] Simona Litescu,[3] and Gabriel Lucian Radu[3]

[1]Pharmaceutical Analysis Laboratory, Biological and Medical Research Department (MBC-03), King Faisal Specialist Hospital and Research Centre, P.O. Box 3354, Riyadh 11211, Saudi Arabia
[2]Department of Chemistry, University of Pretoria, Pretoria 0002, South Africa
[3]National Institute of Biological Sciences, Department of Analytical Biochemistry, Spl. Independentei 296, C.P. 17-16, Bucharest-6, 77748, Romania

ABSTRACT

An amperometric biosensor based on L-aminoacid oxidase is proposed for enantioselective assay of (+)-3,3′,5-triiodo-L-thyronine (L-T_3) and (+)-3,3′,5,5′-tetraiodo-L-thyronine (L-T_4), due to the fact that only the L enantiomer has the hormonal activity. The construction of the amperometric

*Corresponding author. E-mail: enein@kshrc.edu.sa

biosensor is simple and reproducible. The analytical information obtained from enantioselective analysis are reliable. The RSD <1% assured by using the amperometric biosensors for L enantiomers assay as raw materials, and from tablets, demonstrated their suitability for the analysis of T_3 and T_4 at ppb concentration levels.

INTRODUCTION

With normal thyroid function, about 28 to 50 μg of L-T_3((+)-3,3′,5-triiodo-L-thyronine) and 90 μg of L-T_4 (L-thyroxine or (+)-3,3′,5,5′-tetraiodo-L-thyronine) are produced daily. The unbound, or free, L-T_4 is the metabolically active thyroxine. Approximately 90% of the circulating T_3 is formed by deiodination of L-T_4 in peripheral tissues. This peripheral conversion of L-T_4 to L-T_3, and observed higher metabolic activity of L-T_3, indicate that L-T_3 is probably the major active thyroid hormone, with L-T_4 acting as a prohormone. The physiological effects of these hormones are profound. They affect the general oxygen consumption and caloric production in essentially all tissues. Therefore, thyroid dysfunction at any stage of development may have severe pathophysiological consequences.(1–4)

Thyroxine hormone (T_4) exists as enantiomers (Figure 1) that have different therapeutic effects. L-thyroxine (L-T_4) is mainly used as a

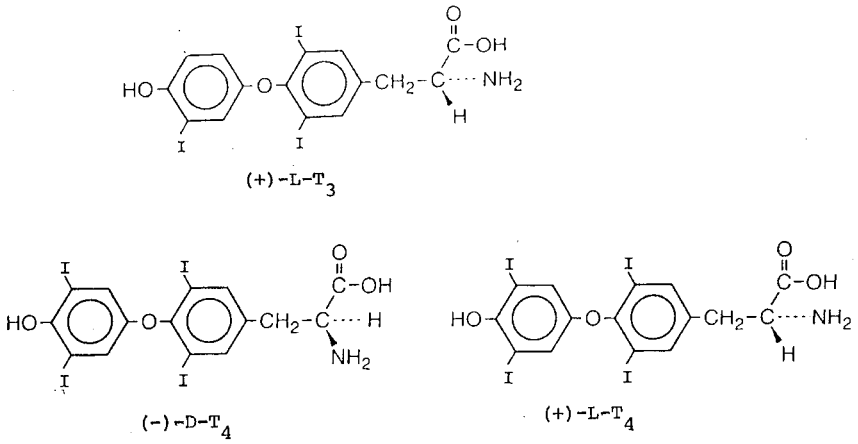

Figure 1. The absolute configuration of (+)-T_3, (−)-D-T_4, and (+)-T_4.

replacement therapy in hypothyroidism while D-thyroxine (D-T_4) is used as an anticholestraemic hypolidemic agent.(5,6) Therefore, the clinical use of L-T_4 requires a check on the optical purity of the enantiomer during storage of the bulk material and also in pharmaceutical formulation.

Several chromatographic techniques were published for the analysis of thyroxine without differentiation between their optical isomers. These assays are performed by means of gas chromatography following derivatization, (7) ion exchange chromatography,(8) and high-performance liquid chromatography (HPLC).(9–12) However, few studies have focused on the analysis of the individual enantiomers.(13–16) Aboul-Enein and Serignese (17) described an enantioselective assay of L-T_4 and D-T_4 in bulk material using exchange chiral thin layer chromatography.

The present study describes the development of a reliable analytical method for the enantioselective analysis of L-T_3 and L-T_4 as raw materials and in several pharmaceutical dosage formulations by using an amperometric biosensor based on L-aminoacid oxidase (L-AAOD). The sensor is based on the immobilization of L-AAOD in carbon paste. The reaction sequences between the analytes and the enzyme are described by Radu and Coulet (18) as follows:

$$\text{L-amino acid} + O_2 + H_2O \rightarrow \text{keto acid} + NH_3 + H_2O_2$$

$$H_2O_2 \xrightarrow[Pt/Ag-AgCl]{+650\,mV} O_2 + 2H^+ + 2e^-$$

EXPERIMENTAL

Reagents

L-AAOD (E.C. 1.4.3.2., from *Crotalus durissus terrficus*, Boehringer Mannheim, 7U/mg 25°C), L-T_3, L-T_4, and D-T_4 were purchased from Sigma Chemical Co. (St. Louis, MO, USA), Synthroid® injection (Levothyroxine Sodium, USP) (Boots Pharmaceuticals, Nottingham, UK) and Eltroxin® tablets (Glaxo Laboratories Ltd., Greenford, England).

Apparatus

Electrochemical experiments were performed using a PAR 273A potentiostat and Model 270 software (EG&G Princeton Applied

Research, Princeton, NJ). A platinum wire and Ag/AgCl (0.1 M KCl) served as the counter and reference electrodes in the cell.

Biosensor Design

Graphite powder (Fluka, cat. No. 50870) was heated at 700°C for 15 s in a Muffle furnace and cooled to ambient temperature in a desiccator. To 100 mg of activated graphite powder, were added 100 μL enzyme solution (1 mg enzyme/mL, made in 0.1 M phosphate buffer, pH 7.0). This mixture was allowed to react to 4°C for 2 h before drying in a vacuum for 4.5 h to remove water. An aliquot of 40 μL of nujol oil per 100 mg of graphite powder was added to the dry enzyme-modified graphite to prepare the paste. Plain graphite-nujol oil paste was filled into a plastic pipette, leaving about 3–4 mm empty in the top to be filled with the chemically modified carbon paste that contains L-AAOD. The diameter of the sensor was 1.5 mm. Electric contact was made by inserting a platinum wire in the carbon paste. The electrode tips were gently rubbed on fine paper to produce a flat surface. The biosensor was stored in a dry state at 4°C when not in use.

Calibration Plot for Amperometric Biosensors

The technique used for L-T_3 and L-T_4 assay was chronoamperometry, the potential applied being +0.65 V vs. Ag/AgCl. The working temperature was 25°C. The sensor was dipped into a thermostated cell (25°C) containing 10 mL of phosphate buffer, pH 7.0; a lot of aliquots from stock L-T_3 and L-T_4 solutions (of 34 and 30 ppm, respectively) were added to generate a series of concentration steps.

Content Uniformity Assay of L-Thyroxine-Eltroxin® Tablets and of Synthroid® Injection

Ten Eltroxin® tablets were each put into ten 100 mL calibrated flasks and dissolved in phosphate buffer. The apparatus cell was filled with the obtained solution (no filtration of the tablet excipients was necessary) and the current obtained was measured. The unknown concentration was determined from calibration plots.

Synthroid® injection was diluted with 0.19% NaCl solution to 5 mL. Ten aliquots of 0.2 μL synthroid solution were added to 10 mL phosphate buffer and the current obtained was measured. The unknown concentration was determined from calibration plots.

RESULTS AND DISCUSSION

Amperometric Biosensor Response

The equations of the calibration plots for L-T_3 and L-T_4 are:

L-T_3: $\log I = 1.61 + 0.28 \log c$; $r = 0.9978$
L-T_4: $\log I = 1.62 + 0.33 \log c$; $r = 0.9950$

where I is the intensity of current (μA) and c is the concentration (ppb). The correlation coefficients are better than 0.9900. The linear ranges are 0.34–17.00 ppb, with a limit of detection of 0.27 ppb for L-T_3, and 0.30–45.00 ppb, with a limit of detection of 0.12 ppb for L-T_4, respectively. Data presented represents the average of 10 determinations.

Optimum pH

The optimum pH range of the amperometric biosensor used was determined for the 0.68 and 0.60 ppb concentrations of L-T_3 and L-T_4, using various phosphate buffers (pH 5.8–7.8). The optimum pH ranges for L-T_3 and L-T_4 determinations were 6.8–7.4 and 6.2–7.2, respectively.

Selectivity

The selectivity of amperometric biosensors was chosen by both separate and mixed solution methods versus L-T_3, L-T_4, and D-T_4 and polyvinylpyrolidone (compression compound commonly used in tablets). The selectivity of the coefficient was calculated according to the method described by Wang.(19) The ratio between interfering species and the L-enantiomer was 1 : 4 L-T_3 and L-T_4. As can be seen in Table 1, the L-T_4 and L-T_3 cannot be determined simultaneously in presence of each other by using this amperometric biosensor, as they interfere with each other in the determination. However, a low amperometric selectivity coefficient was obtained for D-T_4, which confers to biosensors the enantioselective

Table 1. Amperometric Selectivity Coefficients

Interfering Species (J)	$K_{S,J}^{amp}$	
	L-T_3	L-T_4
L-T_3	–	3.1×10^{-2}
L-T_4	1.2×10^{-2}	–
D-T_4	4.8×10^{-3}	2.0×10^{-3}
Polyvinylpyrolidone	2.7×10^{-3}	7.7×10^{-3}

All values are the average of 10 determinations.

property. Also, the L-thyroxine can be determined from tablets in the presence of polyvinylpyrolidone which is used as compression compound.

Response Time

The response of 90% was achieved in 3 min after the biosensors were placed in the solution.

Amperometric Biosensor Stability in Time

The amperometric biosensor can be used daily for about three weeks. During this time, the response and linearity are maintained, as shown in Figure 2.

Analytical Applications

The response characteristics, as well as the enantioselectivity, make these amperometric biosensors useful for enantiomeric purity determination of L-T_3 and L-T_4 raw materials and for the content uniformity test of L-thyroxine tablets and injection formulations.

The results were obtained using the direct amperometric method through the calibration plot. The recovery tests demonstrate the suitability of the method for the validation, due to the RSD and recovery values obtained (Table 2).

The results of uniformity content tests for Eltroxin® tablets and Synthroid® injection are presented in Table 3. The relative standard deviation values attest to the reliability of the analytical information

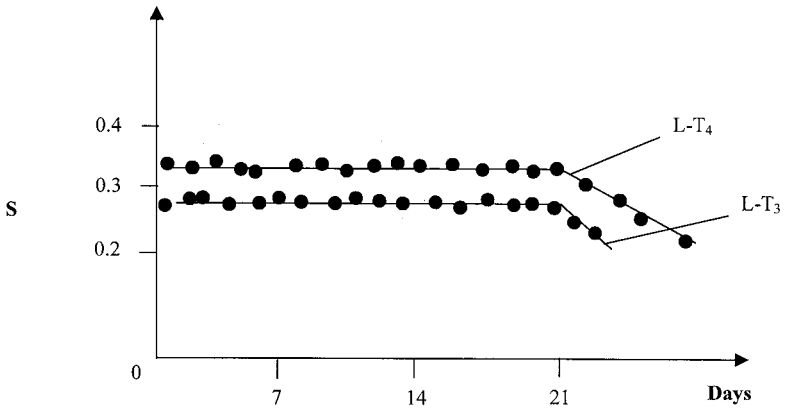

Figure 2. The stability of the biosensor.

Table 2. Results Obtained by Direct Amperometric Assay for the Recovery Test of T_3 and T_4

Sample	No.	Recovery (% of Nominal Value)*	RSD** (%)
L-T_3	1	99.72	
	2	100.00	0.12
	3	99.60	
L-T_4	1	99.72	
	2	100.00	0.12
	3	99.98	

*All values are average of three determinations.
**All values are average of 10 determinations.

obtained, as well as the ability to use the amperometric biosensor for enantioselective analysis of L-enantiomers.

CONCLUSIONS

The proposed amperometric biosensors, based on L-aminoacid oxidase, proved to be reliable for the enantiomeric purity assay of L-T_3 and L-T_4, as raw materials, and from pharmaceutical formulations

Table 3. Results Obtained by Direct Amperometric Assay of L-T_4 from Pharmaceutical Formulations Eltroxin® Tablets and Synthroid® Injection (Content Uniformity Assay)

Sample	No.	Recovery (% of Nominal Value)*	RSD** (%)
Eltroxin® tablets	1	99.77	
	2	99.60	0.19
	3	99.56	
Synthroid® injection	1	98.18	
	2	98.76	0.13
	3	98.76	

*All values are average of three determinations.
**All values are average of 10 determinations.

(the content uniformity test) at the ppb concentration level. The reproducible construction of these amperometric biosensors makes them suitable for validation for enantioselective analysis of these drugs.

REFERENCES

1. Cabello, G.; Wrutniak, C. Pituitary-Thyroid Axis Sensitivity and Neonatal Changes in Plasma Iodothyronine, Thyreostimulin, and Cortisol Levels in the Preterm Lamb: Comparison of Two Experimental Models. Reprod. Nutrit. Devel. **1989**, *29*, 387–402.
2. Wong, E.T.; Suffes, M.W. A Fundamental Approach to the Diagnosis of Diseases of the Thyroid Gland. Clinics Lab. Med. **1984**, *4*, 655–670.
3. Brennan, M.D. Series on Pharmacology in Practice. 5. Thyroid Hormones. Mayo Clin. Proc. **1980**, *55*, 33–44.
4. Bernal, J.; Refetoff, S. The Action of Thyroid Hormone. Clin. Endocrinol. **1977**, *6*, 227–249.
5. Gorman, C.A.; Jiang, N-S; Ellefson, R.D.; Elveback, L.R. Comparative Effectiveness of Dextrothyroxine and Levothyroxine in Correcting Hypothyroidism and Lowering Blood Lipid Levels in Hypothyroid Patients. J. Clin. Endocrinol. Metab. **1979**, *49*, 1–7.
6. Gless, K-H; Oster, P.; Hüfner, M. Influence of D-Thyroxine on Plasma Thyroid Hormone Levels and TSH Secretion. Hormon. Metab. Res. **1977**, *9*, 69–73.

7. Peterson, B.A.; Giese, R.W.; Lansen, P.R.; Karger, B.L. Measurement of Free Thyroid Hormones in Serum by Dialysis and Gas Chromatography. Clin. Chem. **1979**, *23*, 1389–1396.
8. Sorimachi, S.; Ui, N. Ion-Exchange Chromatographic Analysis of Iodothyronines. Anal. Biochem. **1975**, *67*, 157–165.
9. Rapaka, S.; Knight, P.W.; Prasad, V.K. Reversed-Phase High-Performance Liquid Chromatographic Analysis of Liothyronine Sodium and Levothyroxine Sodium in Tablet Formulations. J. Pharm. Sci. **1981**, *70*, 131–134.
10. Richheimer, S.L.; Amer, T.M. Stability-Indicating Assay, Dissolution, and Content Uniformity of Sodium Levothyroxine in Tablets. J. Pharm. Sci. **1983**, *72*, 1349–1351.
11. Brower, J.F.; Toler, D.Y.; Reepmeyer, J.C. Determination of Sodium Levothyroxine in Bulk, Tablet, and Injection Formulations by High-Performance Liquid Chromatography. J. Pharm. Sci. **1984**, *73*, 1315–1317.
12. Garnick, R.L.; Burt, G.F.; Long, D.A.; Bastian, J.W.; Aldred, J.P. High-Performance Liquid Chromatographic Assay for Sodium Levothyroxine in Tablet Formulations: Content Uniformity Applications. J. Pharm. Sci. **1984**, *73*, 75–77.
13. Hay, I.D.; Annesley, T.M.; Jiang, N.S.; Gorman, C.A. Simultaneous Determination of D- and L-Thyroxine in Human Serum by Liquid Chromatography with Electrochemical Detection. J. Chromatogr. **1981**, *226*, 383–390.
14. Gübitz, G.; Juffmann, F. Resolution of the Enantiomers of Thyroid Hormones by High-Performance Liquid-Exchange Chromatography Using a Chemically Bonded Chiral Stationary Phase. J. Chromatogr. **1987**, *404*, 391–393.
15. Dalgaard, L.; Hansen, J.J.; Pederson, J.L. Resolution of the Enantiomers of Thyroid Hormones by High-Performance Liquid Chromatography Using Immobilized Albumin as Chiral Stationary Phase. Determination of the Optical Purity of Thyroxine in Tablets. J. Pharm. Biomed. Anal. **1989**, *7*, 361–368.
16. Abou-Basha, L.I.; Aboul-Enein, H.Y. Enantiomeric Separation and Optical Purity Determination of Thyroxine Enantiomers in Bulk and Pharmaceutical Formulations. Pharma. Acta. Helv. **1995**, *70*, 237–240.
17. Aboul-Enein, H.Y.; Serignese, V. Optical Purity Determination of Thyroxine Enantiomers in Bulk Materials by Chiral Thin Layer Chromatography. Biomed. Chromatogr. **1994**, *8*, 317–318.
18. Radu, G.L.; Coulet, P.R. Amperometric Peptide Sensor for Protein Determination. Anal. Lett. **1993**, *26*, 1321–1332.

19. Wang, J. Selectivity Coefficients for Amperometric Sensors. Talanta **1994**, *41*, 857–863.

Received June 19, 2001
Accepted July 22, 2001
Manuscript 3040