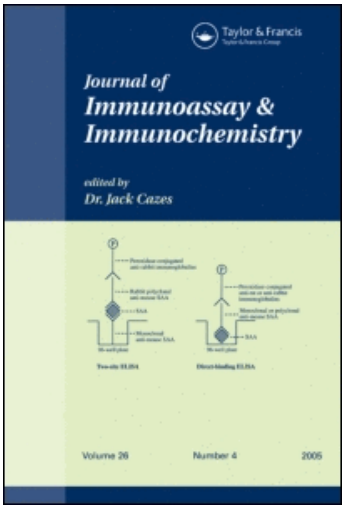

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THE CONSTRUCTION AND CHARACTERIZATION OF AN AMPEROMETRIC IMMUNOSENSOR FOR THE THYROID HORMONE (+)-3,3′,5,5′-TETRAIODO-L-THYRONINE (L-T_4)

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ABSTRACT

A novel amperometric immunosensor, based on graphite paste (graphite powder and paraffin oil), has been constructed for the assay of L-T_4. The graphite paste is impregnated with mouse monoclonal anti-(+)-3,3′,5,5′-tetraiodo-L-thyronine (anti-L-T_4). The immunosensor can be reliably used for the assay of L-T_4 in thyroid and in drugs, using chronoamperometry technique, at ppt up to ppb concentration levels. The potential used for L-T_4 assay was +450 mV vs. Ag/AgCl electrode. The surface

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of the immunosensor can be regenerated by simply polishing, obtaining fresh immunocomposite ready to be used in a new assay.

Key Words: (+)-3,3′,5,5′-Tetraiodo-L-thyronine; Amperometric immunosensor; Enantioanalysis

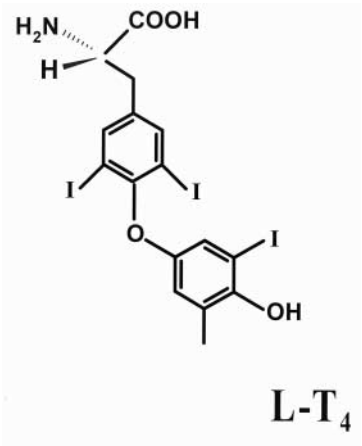
INTRODUCTION

Immunosensors are characterized by high selectivity, sensitivity, and versatility. The selection of the transducer will highly influence the sensitivity and selectivity of the immunosensor.[1,2] Using potentiometric immunosensors only the ppm concentration level can be detected, due to the low sensitivity of potentiometric electrodes.[3–5] The most sensitive transducers are the piezoelectric ones;[6,7] however, their problem is that the selectivity decreases, due to their high sensitivity. In the last few years, several amperometric immunosensors were constructed. The amperometric transducers assure the best sensitivity and selectivity balance of the analytical signal obtained from the antigen–antibody reaction.[8–17]

The reliability of immunosensor construction influences the reliability of the analytical information and it will also contribute to the validation of the immunosensors for clinical and pharmaceutical analysis. Santandreu et al.[18] proposed an amperometric immunosensor based on rigid biocomposite materials (graphite powder, rabbit IgG, and methacrylate or epoxy resins). The proposed construction method is a reproducible one, but it used an enzyme-labeled system followed by a loosely binding affinity of antigen over the antibody. In this case, the antigen determination is an indirect one.

This paper describes a reliable construction for an amperometric immunosensor, based on the immobilization of the antibody on a graphite paste. The immunosensor was constructed for the assay of a thyroid hormone, (+)-3,3′,5,5′-tetraiodo-L-thyronine (L-T_4), known also as L-thyroxine. The main advantages of the proposed immunosensor vs. the amperometric immunosensor for L-thyroxine proposed by Yao et al.[19] are higher sensitivity and lower limit of detection.

With normal thyroid function, about 90 μg of L-T_4 are produced daily in thyroid. Circulating L-T_4 (99.9%) is bound to three serum proteins (thyroid-binding globulin [TBG], thyroid-binding prealbumin [TBPA], and serum albumin). The unbound, or free L-T_4, is the metabolically active thyroxine. Approximately 90% of the circulating L-T_3 is formed by



deiodination of L-T_4 in peripheral tissues. The peripheral conversion of L-T_4 to L-T_3, and the observed higher metabolic activity of L-T_3, indicate that L-T_3 is the major active thyroid hormone with L-T_4 functioning as prohormone.

EXPERIMENTAL

Amperometric Immunosensor Design

The antiserum was diluted to a working dilution of 1 : 30 in 0.01 mol/L phosphate buffered saline, pH = 7.4, containing 0.1% sodium azide. The graphite powder was heated at 700°C for 15 s in a muffle furnace and cooled to ambient temperature in a dessicator. The parafin oil and graphite powder were mixed in a ratio of 1 : 4 (w/w) and then it was added to the diluted anti-L-T_4 to obtain a final composition of 0.9% (w/w) in anti-L-T_4. The carbon paste (graphite powder and paraffin oil) was filled into a plastic pipette tip, leaving about 3 to 4 mm empty in the top to be filled with the chemically modified carbon paste, that contains anti-L-T_4. The diameter of the immunosensor was 3 mm. Electrical contact was made by inserting a silver wire into the carbon paste.

Before each use, the surface of the electrode was wetted with double distilled water and then polished with an alumina paper (polishing strips 30144-001, Orion). When not in use, the amperometric immunosensor was stored in a dry state at 5°C.

Apparatus

A 663 VA stand (Metrohm, Herisau, Switzerland), connected to a PGSTAT 20 and a software version 4.4 were used for all amperometric measurements. A platinum electrode and a Ag/AgCl (0.1 mol/L KCl) electrode served as counter and reference electrodes in the cell.

Reagents and Materials

The immunological system, composed from L-T_4 and monoclonal anti-L-T_4 was supplied by Sigma (St. Louis, MO, USA). Synthroid® (Levothyroxine Sodium, USP injection) was supplied by Bots Pharmaceuticals (Nottingham, UK) and Eltroxin® (tablets) was supplied by Glaxo Laboratories, Ltd. (Greenford, UK). Graphite powder with a particle size of 50 μm was supplied by Merck (Darmstadt, Germany). Paraffin oil was supplied by Fluka (Buchs, Switzerland). All other reagents were of the highest analytical grade. All the solutions were prepared using doubly distilled water.

Recommended Procedures. Cyclic Voltammetry

Cyclic voltammetry scans were performed using the new amperometric immunosensor as working electrode, jointly with a reference electrode (Ag/AgCl), and an auxiliary platinum electrode.

Direct Amperometric Assay

The technique used for the direct amperometric assay was chronoamperometry; the potential applied was +450 mV vs. Ag/AgCl. The working temperature was 25°C. The sensor was dipped into a thermostated cell (25°C) containing 10 mL of phosphate buffered saline, pH = 7.4, and containing 0.1% sodium azide. Different aliquots of stock L-T_4 solution ($c = 30$ μg/mL) were added to generate a series of concentration steps.

Content Uniformity Assay of Eltroxin® Tablets and Synthroid® Injections

Ten Eltroxin® tablets are individually placed in ten 100 mL calibrated flasks, and solved in phosphate buffer. The apparatus cell was filled with the

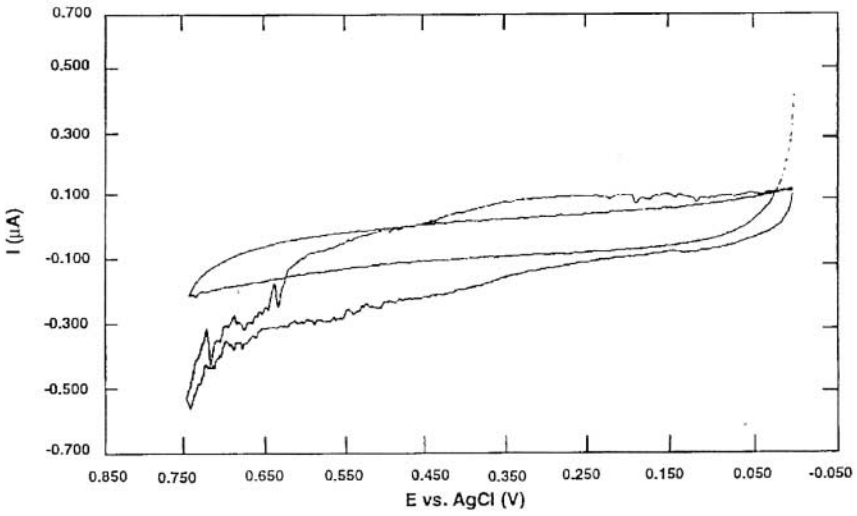


Figure 1. Cyclic voltammogram for phosphate buffer saline, pH = 7.4, containing 0.1% sodium azide, (a) before, and (b) after, the addition of L-T_4 solution ([L-T_4] = 600 ppt). The sweep rate was 0.05 V/s.

prepared solution and the current developed was measured. The unknown concentration was determined from the calibration graph.

Synthroid® injections are diluted with 0.19% NaCl solution to 5 mL. Ten aliquots of 0.2 μL solution were diluted to 10 mL using the phosphate buffer, and the current developed was measured. The unknown concentration was determined from the calibration graph.

RESULTS AND DISCUSSION

In order to optimize the working conditions for the chronoamperometric technique, cyclic voltammograms of the phosphate buffered saline, pH = 7.4 (containing 0.1% sodium azide), before and after adding of L-T_4, were recorded (Fig. 1). The optimum working potential was established to be +450 mV vs. Ag/AgCl electrode.

Amperometric Immunosensor Response

The equation of calibration for L-T_4 is:

$$\log I = 1.46 + 0.28 \log c$$

where I (nA) is the intensity of the current recorded and c is the concentration (ppb) of L-T_4. The correlation coefficient, r, is 0.9988. The linear concentration range for the immunosensor is between 60 ppt and 15 ppb, with a limit of detection of 18 ppt.

Response Time of the Amperometric Immunosensor

The response time was determined for a 600 ppt concentration of L-T_4. The plateau was reached after 90 s from the immersion of the electrodes in the solution. Before 90 s, the intensity of the current has a linear variation with the time, and it is following the equation:

$$I = 49.60 + 0.16t$$

where I is the intensity of the current (nA) and t is the time passed from the immersion of the electrodes in the solution (s). The correlation coefficient, r is 0.9367.

Selectivity of the Amperometric Immunosensor

The selectivity of the amperometric immunosensor was checked using both separate and mixed solution methods, vs. L-T_3 ((+)-3,3′,5-triiodo-L-thyronine), D-T_4 (the pair enantiomer of L-T_4) and polyvinylpyrolidine (PVP). The selection of L-T_3 for the selectivity test is to prove the suitability of the method to be used for the assay of L-T_4 in the presence of L-T_3 in blood samples. It is also essential to determine L-T_4 in the presence of D-T_4 for the enantiopurity tests of the raw materials of drugs containing L-T_4 as active substance. Polyvinylpyrolidine is one of the most used compression compound for tablets; therefore, it may influence the current intensity during the uniformity content test of tablets containing L-T_4 as active substance.

Amperometric selectivity coefficients were determined following the method proposed by Wang.[20] The amperometric selectivity coefficients values obtained for L-T_3, D-T_4, and PVP demonstrate the specificity of the immunosensor for the assay of L-T_4 (Table 1). The inorganic cations such as Na^+, K^+, Ca^{2+} do not interfere in the assay of L-T_4.

Table 1. Amperometric Selectivity Coefficients

Interfering Species (J)	
L-T_3	1.2×10^{-4}
D-T_4	1.7×10^{-4}
Polyvinylpyrolidone	1.3×10^{-3}

All measurements were made at 250°C; all values are the average of ten determinations collected during one week.

Table 2. The Results of the Recovery Test of L-T_4

Sample No.	Recovery (%)	RSD (%)
1	100.00	–
2	100.00	0.03
3	99.95	–

All measurements were made at 250°C; all values are the average of ten determinations.

Stability of Time of the Amperometric Immunosensor

The amperometric immunosensor can be used daily for one week. During this period of time, the RSD value for all the response characteristics do not exceed ±1.00%.

Analytical Applications

The response characteristics, as well as the specificity, of the immunosensor made it suitable for both clinical and drug analysis. The recovery test demonstrated the suitability of the direct amperometric method for the assay of L-T_4, due to the low RSD values (less than 0.5%), with a recovery of 99.98% of the L-T_4-raw material (Table 2).

The results obtained for the uniformity content test are presented in Table 3. L-T_4 can be reliably assayed from the tablets and injection with a high average recovery and low RSD% values. The results are in good agreement with those obtained using the U.S. Pharmacopoeia method: $99.38 \pm 1.00\%$ and $98.89 \pm 1.50\%$ for the pharmaceutical formulations: Eltroxin® and Synthroid®, respectively.[21] The advantage of the proposed

Table 3. The Results Obtained by Direct Amperometric Assay of L-T_4 from the Pharmaceutical Products (Content Uniformity Assay)

Sample	No.	Recovery (%)	RSD (%)
	1	99.49	–
Eltroxin®	2	99.26	0.43
	3	99.30	–
	1	98.79	–
Synthroid®	2	98.19	0.34
	3	99.00	–

All measurements were made at 250°C; all values are the average of ten determinations.

method vs. the one recommended by the U.S. Pharmacopoeia is the simplicity and higher precision due to the lower values of the RSD (%).

CONCLUSIONS

The amperometric immunosensor provides excellent features for the immunoassay of L-T_4 in pharmaceutical products as well as in thyroid or blood samples. The design of the immunosensor is simple, fast and reproducible. The reliability of the analytical information is assured by the low RSD values obtained in the recovery tests, by the fast response of the amperometric immunosensor, and by its broad working concentration range. The proposed amperometric immunosensor is very sensitive.

REFERENCES

1. Stefan, R.I.; van Staden, J.F.; Aboul-Enein, H.Y. *Electrochemical Sensors in Bioanalysis*; Marcel Dekker, Inc.: New York, USA, 2001.
2. Aboul-Enein, H.Y.; Stefan, R.I.; Baiulescu, G.E. *Quality and Reliability in Analytical Chemistry*; CRC Press: Boca Raton, Florida, USA, 2000.
3. Ghindilis, A.L.; Atanasov, P.; Wilkins, E. Sens. Actuat. B **1996**, *34*, 528–532.
4. Morgan, C.L.; Newman, D.J.; Price, C.P. Clin. Chem. **1996**, *42*, 193–209.
5. Pfeifer, U.; Baumann, W. Fresenius' J. Anal. Chem. **1996**, *343*, 541–549.

6. Carter, R.M.; Mekalanos, J.J.; Jacobs, M.B.; Lubrano, G.J.; Guilbault, G.G. J. Immunol. Meth. **1995**, *187*, 121–125.
7. Cohen, Y.; Levi, S.; Rubin, S.; Willner, I. J. Electroanal. Chem. **1996**, *417*, 65–75.
8. Ghindilis, A.L.; Krishnan, R.; Atanasov, P.; Wilkins, E. Biosens. Bioelectron. **1997**, *12*, 415–423.
9. Ivnitski, D.; Rishpon, J. Biosens.Bioelectron. **1996**, *11*, 409–417.
10. Kalab, T.; Skladal, P. Anal. Chim. Acta **1995**, *304*, 361–368.
11. Katz, E.; Willner, I. J. Electroanal. Chem. **1996**, *418*, 67–72.
12. Krishman, R.; Ghindilis, A.L.; Atanasov, P.; Wilkins, E. Anal. Lett. **1995**, *28*, 2459–2474.
13. Krishman, R.; Ghindilis, A.L.; Atanasov, P.; Wilkins, E. Anal. Lett. **1996**, *29*, 2615–2631.
14. Medyntseva, E.P.; Babkina, S.S.; Budnikov, G.K.; Fedorova, I.L.; Ibragimova, N.N. Zh. Anal. Khim. **1992**, *47*, 1101–1106.
15. Skladal, P. Electroanalysis **1997**, *9*, 737–745.
16. Tiefenauer, L.X.; Kossek, S.; Padeste, C.; Thiebaud, P. Biosens. Bioelectron. **1997**, *12*, 213–223.
17. Aizawa, M. Immunosensors. In *Biosensors Principles and Applications*; Blum, L.J., Coulet, R.P., Eds.; Marcel Dekker, Inc.: New York, USA, 1991.
18. Santandreu, M.; Cespedes, F.; Alegret, S.; Martinez-Fabregas, E. Anal. Chem. **1997**, *69*, 2080–2085.
19. Yao, T.; Rechnitz, G.A. Biosens. **1987–1988**, *3*, 307–312.
20. Wang, J. Talanta **1994**, *41*, 857–863.
21. *The United States Pharmacopoeia XXII*; US Pharmacopoeia Convention Inc.: Rockville, MD, 1990.

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