RSC Advances

PAPER

View Article Online
View Journal | View Issue

Cite this: *RSC Adv.*, 2017, **7**, 43567

Fast screening of whole blood samples for early detection and monitoring of thyroid diseases

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Received 7th August 2017
Accepted 5th September 2017

DOI: 10.1039/c7ra08724a

rsc.li/rsc-advances

This paper described a fast and reliable method for the screening of whole blood for TSH, L-T_4, L-T_3 and D-T_4 using stochastic sensors. The main advantage of this method is the possibility of tracing the precise diagnosis of thyroid dysfunction from a single whole blood sample by multianalyte screening in one run, with low cost. Six stochastic microsensors based on mixtures between inulins: Frutafruit TEX (TEX) and inulin Inutec (IN), and ionic liquids: L-phenylalanine-*tert*-butyl-ester-lactate (L-PheC$_4$-Lac), L-alanine-*tert*-butyl-ester-L-lactate (L-AlaC$_4$-Lac) and L-alanine-*tert*-butyl-ester-nitrate (L-AlaC$_4$-NO$_3$), physically immobilized in a diamond paste matrix were designed and used for the screening tests. The tests were reliable for the assay of TSH, L-T_4, L-T_3 and D-T_4 in whole blood samples, with recoveries higher than 98.00% with RSD values lower than 1.00% being recorded.

Introduction

The analysis of different biomolecules is of prime importance for life science research and medical diagnostics. Therefore, molecular medicine could represent an important step to personalized medicine, developing also the possibility of early diagnosis.[1]

Stochastic sensors represent a new class of single-molecule detectors of increasing interest due to their capacity for assessing both qualitatively and quantitatively the analyte of interest, being able to detect simultaneously more than one biomarker.

The stochastic technique consists of measuring the electrical fluctuations of ions that pass through the nanopore at a fixed applied potential. The modulation of the ion current is induced by reversibly binding analytes of interest to the wall of the channel. From the diagrams associated with the individual binding events the signature of the analyte can be identified (the value of t_{off}) and its concentration revealed by t_{on}.

This type of analysis allows the usage of chemical modifiers in order to improve their potential to detect a wide range of molecules.[2] Thus in this paper we demonstrated the ability of a mixture made by different types of ionic liquids and inulins to assess four thyroid hormones: thyroid stimulating hormone (TSH), levothyroxine (L-T_4), dextrothyroxine (D-T_4) and triiodothyronine (L-T_3). The enantiorecognition ability of ionic liquids or inulins for the detection of thyroid hormones has been demonstrated elsewhere.[3,4]

One of the most frequent endocrine diseases are those of thyroid.[5] The key assays that are used to detect thyroid dysfunction are serum thyroid stimulating hormone (TSH) and the main circulating thyroid hormones thyroxine (f-L-T_4) and triiodothyronine (f-L-T_3).[1] The level of TSH is used by physicians as a screening test for thyroid diseases. Elevated concentrations of TSH usually represents a sign of a decreased L-T_4 or L-T_3 production, while suppressed levels can point an excessive activity of thyroid gland.[1] The determination of TSH serves not only as a preliminary test for thyroid status, but the high level of TSH is associated with increased thyroid cancer incidence and advanced-stage disease.[6]

For L-T_4 and L-T_3 equilibrium dialysis and ultrafiltration represented the gold-standard methods[7,8] but they are no longer used, being expensive and time consuming. Other methods such as MS[9] LC-MS,[10] HPLC,[11] RIA[12] and CLEIA[13] have been developed. Also for the assessment of TSH the most used methods were developed based on immunoassay techniques such as ECLIA,[14,15] ELISA,[16] IRMA,[17] EIA[18] and RIA.[19] Improved or newer technologies are proving to be viable alternatives, sometimes offering better sensitivity, increased throughput and lower matrix interference. Thus, L-T_3, L-T_4 and D-T_4 have been analyzed with different electrochemical sensors,[20–22] biosensors[23] and immunosensors.[24] For the assay of TSH, immunosensors,[25] biosensors[26] and optical sensors[27] have been reported. Recently, reliable methods of analysis were proposed for assay of biomarkers specific to different illnesses.[28–30]

This paper proposed a fast and reliable method for the screening of whole blood for TSH, L-T_4, L-T_3 and D-T_4 using stochastic sensors. The main advantage of this method is the possibility of tracing the precise diagnosis of thyroid

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dysfunction from a single whole blood sample by multianalyte assay in one run, with low cost. Six stochastic microsensors based on mixtures between inulins: Frutafruit TEX (TEX) and inulin Inutec (IN), and ionic liquids: L-phenylalanine-*tert*-butyl-ester-lactate (L-PheC$_4$-Lac), L-alanine-*tert*-butyl-ester-L-lactate (L-AlaC$_4$-Lac) and L-alanine-*tert*-butyl-ester-nitrate (L-AlaC$_4$-NO$_3$) physically immobilized in a diamond paste matrix were designed and used for the screening tests.

Experimental

Materials and methods

Reagents and materials. The standard solutions of free L-T$_3$ (f-L-T$_3$), L-T$_4$ (f-L-T$_4$), D-T$_4$ (f-D-T$_4$) and TSH were prepared in phosphate buffer solution (pH = 7.50). The pH was adjusted using small amounts of 0.1 mol L^{-1} NaOH or HCl solution. Deionised water obtained from a Millipore Direct-Q 3 System

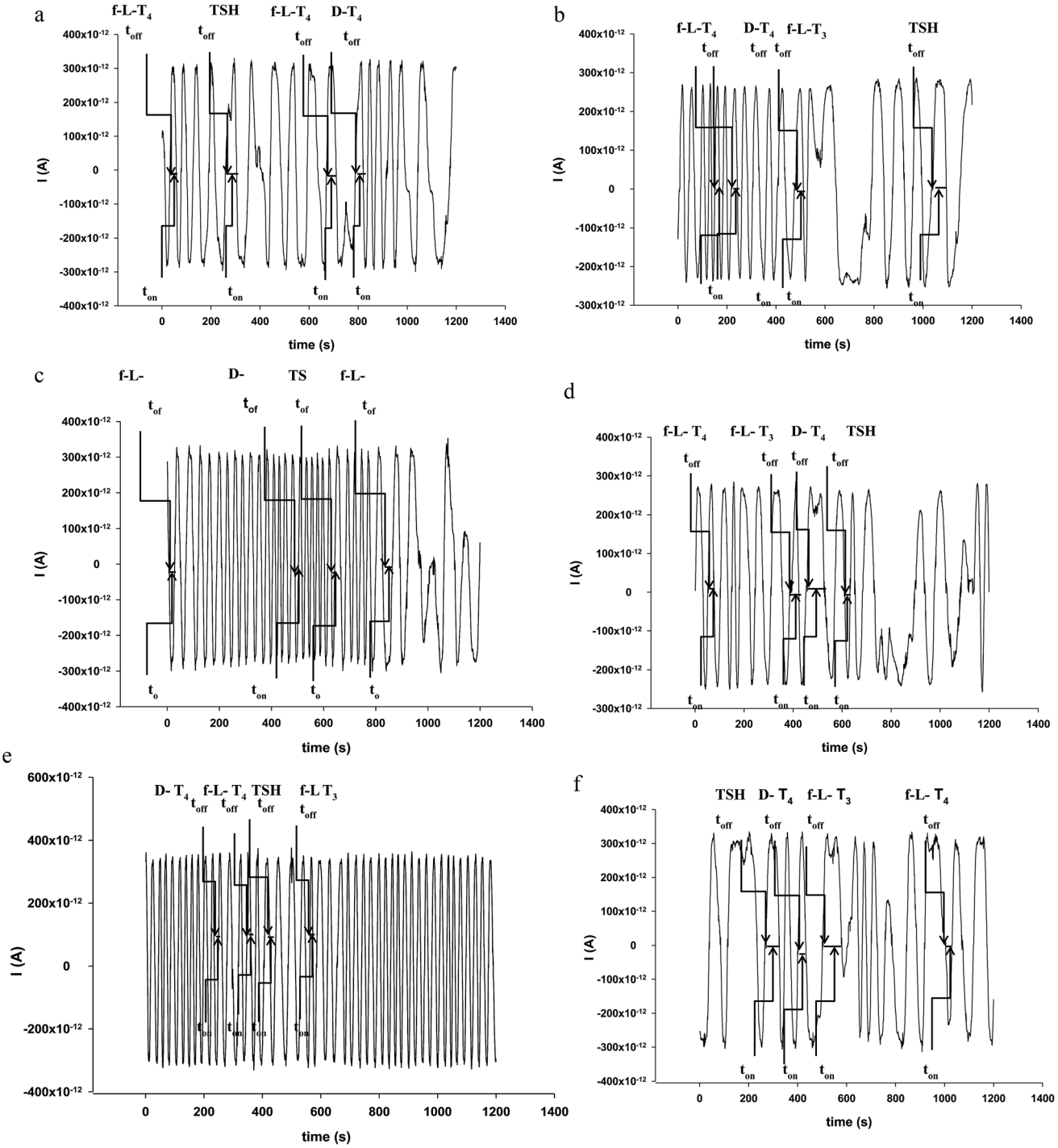

Fig. 1 Screening the whole blood for f-L-T$_3$, f-L-T$_4$, f-D-T$_4$ and TSH using the microsensor based on: (a) IN-L-Ala-C$_4$-NO$_3$; (b) IN-L-Ala-L-Lac; (c) IN-L-Phe-C$_4$-L-Lac; (d) TEX-L-Ala-C$_4$-NO$_3$; (e) TEX-L-Ala-L-Lac; (f) TEX-L-Phe-C$_4$-L-LAC.

(Molsheim, France) was used for all solutions preparation. Natural diamond powder with particle size of 1 μm (99.9%), free L-T_3 (f-L-T_3), L-T_4 (f-L-T_4), D-T_4 (f-D-T_4), TSH, monosodium, disodium phosphate and ionic liquids L-phenylalanine-*tert*-butyl-ester-lactate (L-PheC_4-Lac), L-alanine-*tert*-butyl-ester-L-lactate (L-AlaC_4-Lac) and L-alanine-*tert*-butyl-ester-nitrate (L-AlaC_4-NO_3) were purchased from Sigma Aldrich (Milwaukee, USA). Inulin Frutafruit TEX (TEX) was provided by Sensus (Roosendaal, Netherlands) and inulin Inutec (IN) was provided by Orafti Non Food (Oreye, Belgium). Paraffin oil was purchased from Fluka (Buchs, Switzerland).

Standard solutions of different concentration were obtained by serial dilution. All solutions were fresh prepared before measurements. When not in use, all the solutions were stored in the freezer at 2–8 °C.

Apparatus. PGSTAT 302 was used for the recording of all the chronoamperometric measurements using a software Ecochemie version 4.9. The conventional electrochemical cell was constituted by the working electrode, Ag/AgCl (0.1 mol L^{-1} KCl) as reference electrode and Pt as counter electrode.

Microsensors design. The matrix selected was modified diamond paste (DP). Ionic liquids such as L-phenylalanine *tert*-butyl ester lactate (L-PheC_4-Lac), L-alanine *tert*-butyl ester L-lactate (L-AlaC_4-Lac), L-alanine *tert*-butyl ester nitrate (L-AlaC_4-NO_3) and inulins TEX and IN were selected as modifiers. For the preparation of diamond paste we used natural monocrystalline diamond powder mixed with paraffin oil; 12 μL of each ionic liquid solution (10^{-3} mol L^{-1}) and 12 μL of each inulin solution (10^{-3} mol L^{-1}) were added to 100 mg diamond paste to give six modified pastes.

Each modified paste was places in a plastic tube with an internal diameter of the active surface of 300 μm. The electric contact was obtained using an Ag wire inserted into the modified paste. The surface of the microsensor was renewed by polishing with aluminum paper and wetted with deionised water before using. When not in use, the microelectrodes were stored in a dry state at room temperature.

Recommended procedures: stochastic method. All the stochastic measurements were performed using chronoamperometry at a constant potential of 125 mV. The electrochemical cell was filled with blood samples without any pretreatment. The t_{off} values (signatures of thyroid hormones) were identified for TSH, f-L-T_4, f-L-T_3 and f-D-T_4 (Fig. 1). The values of t_{on} were determined and the unknown concentrations of TSH, f-L-T_3, f-L-T_4 and f-D-T_4 were obtained by inserting the value $1/t_{on}$ in the related equation of calibration ($1/t_{on}$ = f (conc)).

Sample preparation

Whole blood samples. Whole blood samples were obtained from 9 patients, who have been previously evaluated in the Elias Emergency University Hospital Bucharest. All experiments were performed in compliance with the EU guidelines, and approved by the medical ethics committee at University of Medicine and Pharmacy "Carol Davila", Bucharest, Romania (Ethics committee approval no. 11/2013). Informed consents were obtained from human participants of this study. The apparatus cell was filled with the whole blood samples and the stochastic measurements were performed. The unknown concentrations were determined from the calibration graphs as described above in the Recommended procedure section.

Results and discussions

Response characteristics of the stochastic sensors

The response of stochastic sensors is based on channel conductivity, when a fixed potential is applied. Accordingly, for the assay of the thyroid hormones a potential of 125 mV *vs.* Ag/AgCl was applied. The measurement process of a single molecule is taking place in two stages: the first stage on which the molecule is entering the channel, blocking it – when the current is dropping to zero value (the time designed as t_{off}, that the molecule needs to go into the channel is called signature of the analyte and depends on the size and geometry of the molecule, its capacity of unfolding and of velocity needed to enter the channel); the second stage is the one on which binding with the wall of the channel are taking place (the time spend for these processes is called t_{on} – which is measured between two t_{off} events):[3,4]

$$Ch_{(i)} + \text{f-L-}T_{3(i)} \Leftrightarrow Ch\cdot\text{f-L-}T_{3(i)}$$

$$Ch_{(i)} + \text{f-L-}T_{4(i)} \Leftrightarrow Ch\cdot\text{f-L-}T_{4(i)}$$

$$Ch_{(i)} + \text{f-D-}T_{4(i)} \Leftrightarrow Ch\cdot\text{f-D-}T_{4(i)}$$

$$Ch_{(i)} + TSH_{(i)} \Leftrightarrow Ch\cdot TSH_{(i)}$$

where Ch is the channel, and i is the interface, followed by redox processes. In the case of simultaneous investigation of multi-analytes, each of them entering the channel in an order given by their size, geometry, stereochemistry, capacity of unfolding and velocity of passing through the opening of the channel; therefor for each of them their signature (t_{off} value, Fig. 1) followed a peak are seen in the diagram obtained after screening of whole blood.[31–33]

Response characteristics are shown in Table 1. Different signatures of TSH, f-L-T_4, f-L-T_3 and f-D-T_4 were obtained for the proposed sensors; there are only two sensors for which the differences between the signatures of TSH, f-L-T_4, f-L-T_3 and f-D-T_4 are very small: the sensors based on IN-L-Ala-NO_3, and TEX-L-Phe-C_4-L-Lac and therefore difficult to assess in real samples. All sensors exhibited high sensitivity for the simultaneous assay of TSH, f-L-T_4, f-L-T_3 and f-D-T_4. The lowest limit of determination for f-L-T_3 was achieved using the sensor based on IN-L-Ala-C_4-L-Lac, while for f-L-T_4 was achieved using the sensor based on IN-L-Phe-C_4-L-Lac, and for f-D-T_4 the lowest limit of determination was given by the sensor based on TEX-L-Ala-C_4-L-Lac. For TSH assay, no differences in limit of determination were recorded when the six sensors' response characteristics were determined. Regarding all response characteristics as well as the signatures of the hormones, the sensor of choice for simultaneous assay of thyroid hormones is the one based on TEX-L-Ala-C_4-NO_3-L-Lac.

The sensors were used for more than 3 months when their sensitivities' RSD (%) values did not exceed 1.00%, proving

Table 1 Response characteristics of the stochastic microsensors based on inulins and ionic liquids stochastic for the assay of f-L-T_4, f-D-T_4, f-L-T_3 and TSH

Microsensors based on:	Signature of the enantiomer (t_{off})	Sensibility	Linear concentration range	Quantification limit:	Equation of calibration; correlation coefficient
f-L-T_3 (mol L^{-1} s^{-1})					
IN-L-Ala-C_4-L-Lac	1	3.08×10^{10}	4×10^{-13} to 10^{-12}	4×10^{-13}	$1/t_{on} = 0.04 + 3.08 \times 10^{10} \times C$; $r = 0.9967$
IN-L-Phe-C_4-L-Lac	1	4.02×10^{9}	8×10^{-12} to 4×10^{-12}	8×10^{-12}	$1/t_{on} = 0.02 + 4.02 \times 10^{9} \times C$; $r = 0.9977$
IN-L-Ala-C_4-NO_3-L-Lac	1	3.00×10^{10}	2×10^{-12} to 8×10^{-12}	2×10^{-12}	$1/t_{on} = 0.05+3 \times 10^{10} \times C$; $r = 0.9295$
TEX-L-Ala-C_4-L-Lac	1.7	2.63×10^{9}	6×10^{-12} to 10^{-11}	6×10^{-12}	$1/t_{on} = 0.03 + 2.63 \times 10^{9} \times C$; $r = 0.9959$
TEX-L-Phe-C_4-L-Lac	0.7	7.21×10^{10}	10^{-12} to 4×10^{-13}	1×10^{-12}	$1/t_{on} = 0.01 + 7.21 \times 10^{10} \times C$; $r = 0.9973$
TEX-L-Ala-C_4-NO_3-L-Lac	0.7	2.28×10^{7}	10^{-9} to 10^{-11}	1×10^{-9}	$1/t_{on} = 0.05 + 2.28 \times 10^{7} \times C$; $r = 0.0986$
f-L-T_4 (mol L^{-1} s^{-1})					
IN-L-Ala-C_4-L-Lac	0.7	5.44×10^{8}	8×10^{-12} to 10^{-10}	8×10^{-12}	$1/t_{on} = 0.03 + 5.44 \times 10^{8} \times C$; $r = 0.9833$
IN-L-Phe-C_4-L-Lac	0.7	5.93×10^{9}	10^{-12} to 4×10^{-12}	1×10^{-12}	$1/t_{on} = 0.03 + 5.93 \times 10^{9} \times C$; $r = 0.9833$
IN-L-Ala-C_4-NO_3-L-Lac	0.7	5.41×10^{9}	8×10^{-13} to 2×10^{-12}	8×10^{-13}	$1/t_{on} = 0.01 + 5.41 \times 10^{9} \times C$; $r = 0.9561$
TEX-L-Ala-C_4-L-Lac	1.3	5.62×10^{6}	10^{-10} to 10^{-8}	1×10^{-10}	$1/t_{on} = 0.01 + 5.62 \times 10^{6} \times C$; $r = 0.9985$
TEX-L-Phe-C_4-L-Lac	0.5	1.21×10^{8}	8×10^{-12} to 10^{-10}	8×10^{-12}	$1/t_{on} = 0.03 + 1.21 \times 10^{8} \times C$; $r = 0.9361$
TEX-L-Ala-C_4-NO_3-L-Lac	1.1	5.97×10^{8}	8×10^{-12} to 10^{-10}	8×10^{-12}	$1/t_{on} = 0.01 + 5.97 \times 10^{8} \times C$; $r = 0.9937$
D-T_4 (mol L^{-1} s^{-1})					
IN-L-Ala-C_4-L-Lac	2.1	2.63×10^{4}	10^{-6} to 10^{-8}	1×10^{-6}	$1/t_{on} = 0.03 + 2.63 \times 10^{4} \times C$; $r = 0.9758$
IN-L-Phe-C_4-L-Lac	1.1	2.2×10^{9}	6×10^{-12} to 10^{-11}	6×10^{-12}	$1/t_{on} = 0.02 + 2.2 \times 10^{9} \times C$; $r = 0.9991$
IN-L-Ala-C_4-NO_3-L-Lac	0.4	3.56×10^{9}	4×10^{-12} to 8×10^{-12}	4×10^{-12}	$1/t_{on} = 0.02 + 3.56 \times 10^{9} \times C$; $r = 0.9543$
TEX-L-Ala-C_4-L-Lac	2.4	4.8×10^{10}	4×10^{-13} to 10^{-12}	4×10^{-13}	$1/t_{on} = -0.007 + 4.8 \times 10^{10} \times C$; $r = 0.995$
TEX-L-Phe-C_4-L-Lac	1.0	3.14×10^{2}	10^{-6} to 10^{-4}	1×10^{-6}	$1/t_{on} = 0.04 + 3.14 \times 10^{2} \times C$; $r = 0.9411$
TEX-L-Ala-C_4-NO_3-L-Lac	1.8	9.48×10^{9}	8×10^{-13} to 2×10^{-12}	8×10^{-13}	$1/t_{on} = 0.01 + 9.48 \times 10^{9} \times C$; $r = 0.9981$
TSH (UI L^{-1})					
IN-L-Ala-C_4-L-Lac	0.4	2.96×10^{-1}	5.6×10^{-4} to 5.6×10^{-2}	5.6×10^{-4}	$1/t_{on} = 0.02 + 2.96 \times 10^{-1} \times C$; $r = 0.9994$
IN-L-Phe-C_4-L-Lac	1.2	5.89×10^{-4}	5.6×10^{-1} to 5.6×10^{1}	5.6×10^{-1}	$1/t_{on} = 0.03 + 5.89 \times 10^{-4} \times C$; $r = 0.9998$
IN-L-Ala-C_4-NO_3-L-Lac	0.6	3.52×10^{3}	5.6×10^{-8} to 5.6×10^{-6}	5.6×10^{-8}	$1/t_{on} = 0.03 + 3.52 \times 10^{3} \times C$; $r = 0.9994$
TEX-L-Ala-C_4-L-Lac	0.3	4.34×10^{3}	5.6×10^{-8} to 5.6×10^{-6}	5.6×10^{-8}	$1/t_{on} = 0.02 + 4.34 \times 10^{3} \times C$; $r = 0.9996$
TEX-L-Phe-C_4-L-Lac	1.2	2.24×10^{-1}	5.6×10^{-6} to 5.6×10^{-4}	5.6×10^{-6}	$1/t_{on} = 0.03 + 2.24 \times 10^{-1} \times C$; $r = 0.9993$
TEX-L-Ala-C_4-NO_3-L-Lac	0.4	7.06×10^{3}	5.6×10^{-8} to 5.6×10^{-6}	5.6×10^{-8}	$1/t_{on} = 0.02 + 7.06 \times 10^{3} \times C$; $r = 0.9788$

that the sensors are stable for this period, when accurate and precise determinations were performed for whole blood samples.

The selectivity of sensors is given for this type of sensors by the signatures recorded for each compound analysed with the same sensor. The sensors were tested for f-L-T_3, f-L-T_4, f-D-T_4, TSH and thyroid hormone receptor; different values were obtained for each of the compounds when the same sensor was used. The different values obtained for the signatures made possible the correct identification of the signal of the hormones in the diagrams (Fig. 1), and accordingly reliable qualitative analysis followed by quantitative analysis.

Table 2 Determination of f-L-T_4, f-D-T_4, f-L-T_3 and TSH in whole blood samples using the stochastic microsensors

Sample no.	Microsensor based on	f-L-T_3 (ng dL^{-1})	f-L-T_4 (ng dL^{-1})	f-D-T_4 (ng dL^{-1})	TSH (μUI mL^{-1})
1	IN-L-Ala-C_4-L-Lac	53.99 ± 0.21	6.99 ± 0.01	0.16 ± 0.01	0.056 ± 0.003
	IN-L-Phe-C_4-L-Lac	54.05 ± 0.20	6.00 ± 0.05	0.14 ± 0.01	0.061 ± 0.002
	IN-L-Ala-C_4-NO_3-L-Lac	54.21 ± 0.19	6.81 ± 0.05	0.14 ± 0.02	0.060 ± 0.003
	TEX-L-Ala-C_4-L-Lac	53.93 ± 0.20	7.08 ± 0.03	0.17 ± 0.02	0.067 ± 0.003
	TEX-L-Phe-C_4-L-Lac	54.28 ± 0.18	6.06 ± 0.02	0.13 ± 0.03	0.049 ± 0.002
	TEX-L-Ala-C_4-NO_3-L-Lac	54.10 ± 0.10	6.78 ± 0.01	0.19 ± 0.02	0.046 ± 0.002
2	IN-L-Ala-C_4-L-Lac	108.06 ± 0.18	1.38 ± 0.05	0.09 ± 0.02	1.440 ± 0.009
	IN-L-Phe-C_4-L-Lac	107.95 ± 0.18	1.20 ± 0.02	0.06 ± 0.01	1.400 ± 0.004
	IN-L-Ala-C_4-NO_3-L-Lac	107.21 ± 0.21	1.80 ± 0.07	0.10 ± 0.01	1.600 ± 0.005
	TEX-L-Ala-C_4-L-Lac	107.33 ± 0.19	1.28 ± 0.04	0.12 ± 0.02	1.610 ± 0.004
	TEX-L-Phe-C_4-L-Lac	108.21 ± 0.20	1.30 ± 0.02	0.13 ± 0.02	1.609 ± 0.004
	TEX-L-Ala-C_4-NO_3-L-Lac	108.08 ± 0.11	1.27 ± 0.02	0.09 ± 0.01	1.590 ± 0.003
3	IN-L-Ala-C_4-L-Lac	143.00 ± 0.18	1.000 ± 0.005	0.011 ± 0.003	2.020 ± 0.005
	IN-L-Phe-C_4-L-Lac	143.02 ± 0.15	1.020 ± 0.007	0.010 ± 0.002	1.920 ± 0.005
	IN-L-Ala-C_4-NO_3-L-Lac	145.60 ± 0.13	0.991 ± 0.003	0.015 ± 0.002	2.190 ± 0.003
	TEX-L-Ala-C_4-L-Lac	145.69 ± 0.13	0.978 ± 0.005	0.015 ± 0.005	2.000 ± 0.004
	TEX-L-Phe-C_4-L-Lac	143.78 ± 0.12	0.956 ± 0.006	0.017 ± 0.002	1.940 ± 0.003
	TEX-L-Ala-C_4-NO_3-L-Lac	142.56 ± 0.10	0.966 ± 0.003	0.016 ± 0.001	1.720 ± 0.002
4	IN-L-Ala-C_4-L-Lac	11.72 ± 0.17	3.90 ± 0.03	0.12 ± 0.02	0.028 ± 0.005
	IN-L-Phe-C_4-L-Lac	11.22 ± 0.15	4.10 ± 0.03	0.11 ± 0.02	0.023 ± 0.005
	IN-L-Ala-C_4-NO_3-L-Lac	11.36 ± 0.17	4.66 ± 0.07	0.15 ± 0.01	0.030 ± 0.002
	TEX-L-Ala-C_4-L-Lac	11.98 ± 0.12	4.25 ± 0.02	0.12 ± 0.01	0.024 ± 0.002
	TEX-L-Phe-C_4-L-Lac	11.24 ± 0.12	4.52 ± 0.05	0.17 ± 0.03	0.029 ± 0.007
	TEX-L-Ala-C_4-NO_3-L-Lac	12.08 ± 0.11	4.88 ± 0.03	0.11 ± 0.01	0.023 ± 0.001
5	IN-L-Ala-C_4-L-Lac	106.21 ± 0.20	1.38 ± 0.02	0.05 ± 0.01	0.330 ± 0.012
	IN-L-Phe-C_4-L-Lac	104.96 ± 0.18	1.37 ± 0.02	0.05 ± 0.02	0.390 ± 0.012
	IN-L-Ala-C_4-NO_3-L-Lac	106.00 ± 0.18	1.37 ± 0.03	0.06 ± 0.01	0.330 ± 0.015
	TEX-L-Ala-C_4-L-Lac	105.92 ± 0.22	1.27 ± 0.05	0.06 ± 0.01	0.360 ± 0.013
	TEX-L-Phe-C_4-L-Lac	105.33 ± 0.15	1.39 ± 0.01	0.07 ± 0.02	0.330 ± 0.017
	TEX-L-Ala-C_4-NO_3-L-Lac	106.02 ± 0.11	1.39 ± 0.03	0.05 ± 0.01	0.308 ± 0.011
6	IN-L-Ala-C_4-L-Lac	170.02 ± 0.22	1.08 ± 0.02	0.08 ± 0.01	0.451 ± 0.011
	IN-L-Phe-C_4-L-Lac	179.21 ± 0.15	1.31 ± 0.04	0.07 ± 0.02	0.414 ± 0.012
	IN-L-Ala-C_4-NO_3-L-Lac	178.24 ± 0.15	1.13 ± 0.03	0.08 ± 0.01	0.482 ± 0.012
	TEX-L-Ala-C_4-L-Lac	176.66 ± 0.18	1.20 ± 0.02	0.08 ± 0.01	0.445 ± 0.021
	TEX-L-Phe-C_4-L-Lac	177.22 ± 0.10	1.24 ± 0.03	0.09 ± 0.02	0.443 ± 0.010
	TEX-L-Ala-C_4-NO_3-L-Lac	173.23 ± 0.12	1.27 ± 0.02	0.08 ± 0.01	0.453 ± 0.010
7	IN-L-Ala-C_4-L-Lac	163.34 ± 0.18	1.25 ± 0.05	0.09 ± 0.02	1.410 ± 0.008
	IN-L-Phe-C_4-L-Lac	164.00 ± 0.18	1.22 ± 0.03	0.10 ± 0.01	1.370 ± 0.008
	IN-L-Ala-C_4-NO_3-L-Lac	161.40 ± 0.15	1.34 ± 0.03	0.08 ± 0.01	1.390 ± 0.009
	TEX-L-Ala-C_4-L-Lac	162.50 ± 0.17	1.21 ± 0.05	0.08 ± 0.01	1.430 ± 0.007
	TEX-L-Phe-C_4-L-Lac	164.06 ± 0.11	1.30 ± 0.02	0.09 ± 0.02	1.360 ± 0.007
	TEX-L-Ala-C_4-NO_3-L-Lac	164.03 ± 0.12	1.30 ± 0.02	0.10 ± 0.01	1.359 ± 0.005
8	IN-L-Ala-C_4-L-Lac	55.48 ± 0.11	0.776 ± 0.009	0.017 ± 0.002	7.200 ± 0.009
	IN-L-Phe-C_4-L-Lac	55.59 ± 0.12	0.771 ± 0.012	0.015 ± 0.002	7.150 ± 0.009
	IN-L-Ala-C_4-NO_3-L-Lac	55.62 ± 0.12	0.771 ± 0.011	0.014 ± 0.003	7.610 ± 0.011
	TEX-L-Ala-C_4-L-Lac	55.27 ± 0.13	0.729 ± 0.009	0.017 ± 0.003	7.470 ± 0.012
	TEX-L-Phe-C_4-L-Lac	55.53 ± 0.11	0.731 ± 0.011	0.015 ± 0.002	7.000 ± 0.009
	TEX-L-Ala-C_4-NO_3-L-Lac	54.09 ± 0.11	0.729 ± 0.013	0.010 ± 0.002	7.240 ± 0.009
9	IN-L-Ala-C_4-L-Lac	59.93 ± 0.11	1.12 ± 0.03	0.011 ± 0.003	2.149 ± 0.011
	IN-L-Phe-C_4-L-Lac	59.98 ± 0.11	1.15 ± 0.02	0.012 ± 0.003	2.180 ± 0.012
	IN-L-Ala-C_4-NO_3-L-Lac	60.60 ± 0.12	1.11 ± 0.01	0.011 ± 0.023	2.200 ± 0.012
	TEX-L-Ala-C_4-L-Lac	60.67 ± 0.13	1.13 ± 0.01	0.010 ± 0.003	2.198 ± 0.015
	TEX-L-Phe-C_4-L-Lac	60.22 ± 0.10	1.16 ± 0.02	0.010 ± 0.001	2.230 ± 0.013
	TEX-L-Ala-C_4-NO_3-L-Lac	60.63 ± 0.08	1.17 ± 0.01	0.009 ± 0.001	2.159 ± 0.013

Table 3 Recovery tests of f-L-T_4, f-D-T_4, f-L-T_3 and TSH in whole blood samples

Microsensor based on	f-L-T_3%, recovery	f-L-T_4%, recovery	f-D-T_4%, recovery	TSH%, recovery
IN-L-Ala-C_4-L-Lac	98.92 ± 0.09	98.70 ± 0.05	99.22 ± 0.04	99.21 ± 0.02
IN-L-Phe-C_4-L-Lac	98.72 ± 0.09	98.99 ± 0.05	99.05 ± 0.04	99.20 ± 0.02
IN-L-Ala-C_4-NO_3-L-Lac	98.90 ± 0.08	98.01 ± 0.06	98.98 ± 0.05	99.43 ± 0.03
TEX-L-Ala-C_4-L-Lac	98.50 ± 0.07	98.20 ± 0.07	98.32 ± 0.05	99.32 ± 0.02
TEX-L-Phe-C_4-L-Lac	99.01 ± 0.07	99.03 ± 0.07	99.99 ± 0.02	99.99 ± 0.02
TEX-L-Ala-C_4-NO_3-L-Lac	99.24 ± 0.08	99.56 ± 0.04	99.93 ± 0.04	99.98 ± 0.02

Analytical applications

The response characteristics, selectivity, and reliability of the microsensors made possible the simultaneous assay of f-L-T_3, f-L-T_4, f-D-T_4, and TSH in whole blood samples. The whole blood samples were analysed as collected from the nine patients, immediately after they were collected from patients. Diagrams were recorded (Fig. 1), and signatures (t_{off} values) identified for f-L-T_3, f-L-T_4, f-D-T_4 and TSH in each diagram. After identifying the signature for each biomarker (f-L-T_3, f-L-T_4, f-D-T_4 and TSH), the corresponding t_{on} value was read (see Fig. 1) and introduced in the equation of calibration of each sensors, to obtain the value of the concentration.

Results presented in Table 2 shown a good correlations between the results obtained for the assay of f-L-T_3, f-L-T_4, f-D-T_4, and TSH in whole blood samples. Recovery tests were performed by comparing the amounts of f-L-T_3, f-L-T_4, f-D-T_4, and TSH determined in whole blood samples using ELISA (the standard method), and the proposed microsensors. The values of recoveries (Table 3) shown for each sensor, and each compound a very good correlation, given by the amount of compound found using the proposed microsensors reported to the amount of the same compound found in the sample blood sample using ELISA, the recovery being expressed as % recovery. All relative standard deviations recorded were less than 1.00%, proving a high precission and reliability of the measurements.

Conclusion

Six stochastic microsensors based on diamond paste modified with inulins and ionic liquids were proposed for the assay of f-L-T_4, f-D-T_4, f-L-T_3 and TSH in whole blood samples. The microsensors were selective, and enantioselective and presented high sensitivity and reliability for the assay of thyroid hormones in whole blood samples. Regarding all response characteristics as well as the signatures of the hormones, the sensor of choice for simultaneous assay of thyroid hormones is the one based on TEX-L-Ala-C_4-NO_3-L-Lac.

Compared with ELISA and chemiluminescence methods used in clinical laboratories for their determination, the main advantages of the proposed method are: there is no need for sample pretreatment before assay, samples being used as taken from the patient, all four hormones can be determined in one run, low cost, decreased time of determination, high sensitivities and lower limits of determination.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

This work was supported by UEFISCDI PNIII Program PCE Contract nr. 46/2017.

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