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New method for the molecular recognition of thyroid hormones

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SUMMARY. The development of new methods that can detect a broad range of biomarkers became essential in modern medicine. The most frequent endocrine disorders include thyroid pathology. Stochastic sensors represent a unique class of single-molecule detectors and a promising candidate in biomedical analysis due to their ability to determine in one run more than one analyte. In clinical practice the main analytes used for the diagnosis and evolution of thyroid disease are free L-T_3, L-T_4 and TSH. A fast screening method based on stochastic sensors was proposed for the enantiorecognition of free L-T_3, L-T_4, D-T_4 and TSH. Stochastic microsensors based on a mixture between two inulins (IN, TEX) and two ionic liquids (IN-L-Ala-C_4-L-lac, IN-L-Phe-L-lac) immobilized on diamond paste (DP) were used for the assessment of thyroid hormones in whole blood samples. IN-L-Phe-C_4-L-lac based microsensors showed the highest sensitivity for the assay of D-T_4, L-T_4 and TSH, while the highest sensitivity for L-T_3 was obtained by using the stochastic microsensors based on IN-L-Ala-L-lac. The quantification limits obtained for thyroid hormones were: 10^{-12} mol/L for L-T_4, $4x10^{-13}$ mol/L for L-T_3, $6x10^{-12}$ for D-T_4 mol/L and $5x10^{-15}$ g/mL for TSH.

The microsensors determined the thyroid hormones in whole blood samples with high reliability: recoveries higher than 95.00%, and RSD (%) lower than 1.00%. The microsensors had great features in biomedical analysis for pattern recognition of thyroid hormones. This will help early detection of related diseases.

Keywords: Enantiorecognition, inulins, stochastic microsensors, thyroid hormones

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Introduction

Stochastic sensors represent a promising candidate in early diagnosis and prevention due to their capacity to perform qualitative and quantitative analysis (Movileanu, 2014). The principle of the stochastic sensors is based on the channel conductivity and consists of modulation of the ionic current induced by reversibly binding analytes of interest to the wall of the channel (Stefan-van-Staden *et al.*, 2013). From the obtained diagram (Fig. 1) it could be recognized the signature of the analyte expressed by t_{off} and its concentration revealed by t_{on} values. Using diamond paste as a matrix in the construction of stochastic sensors represents a relatively new method, having the advantage of a better fixation of the analyte passing the channel.

ELISA and CLEIA/ECLEIA are the most frequently used methods in clinical practice for the determination of F-T4, F-T3 and TSH. In order to detect the three hormones, three different detection kits are needed, while using our method one can determine in one run all the analytes with a lower cost. Several methods that have been used for the assay of free L-T3, free L-T4 and TSH include high performance liquid chromatography (Gondova *et al.*, 2011; Jin *et al.*, 2007), radioimmunoassay (Giorgiou *et al.*, 1994), liquid chromatography (Wang *et al.*, 2003), equilibrium dialysis (Sapin *et al.*, 2003) and electrochemical methods (Wang *et al.*, 2014).

In this paper we demonstrate the ability of the proposed microsensors to fast detect free triiodothyronine (L-T_3), levothyroxine (L-T_4), dextrothyroxine (D-T_4) and thyroid stimulating hormone (TSH) in whole blood samples.

Materials and methods

Two diamond paste sensors based on a mixture of inulins and ionic liquids (IN-L-Phe-C_4-L-lac and IN-L-Ala-L-lac) were designed by modifying diamond paste. The concentration ranges of standard solutions were obtained by serial dilution. All the chronoamperometric measurements were recorded using a PGSTAT 302, software Ecochemie version 4.9. The unknown concentrations of L-T_3, L-T_4, D-T_4 and TSH were determined by inserting the value $1/t_{on}$ in the related equation of calibration.

Results and discussions

The response characteristics of the enantioselective electrochemical sensors based on inulins and ionic liquids are shown in Table 1. The linear concentration range of both microsensors based on IN-L-Phe-C4-L-lac and IN-L-Ala-L-lac covers the normal range of f-L-T_3 in serum given by ECLIA method. Also these two microsensors can detect the presence of L-T_4 with a very low limit of determination, but with a

linear concentration range under the normal used range by ECLIA method. Compared with the most frequently used techniques for the assessment of TSH, such as ELISA, the limit of determination obtained using the microsensors based on IN-L-Phe-C4-L-lac and IN-L-Ala-L-lac ($4x10^{-15}$ g/mL) is lower than the one reported using ELISA kit ($2,74x10^{-12}$ g/ml).

Conclusions

IN-L-Phe-C4-L-lac and IN-L-Ala-L-lac microsensors had the best sensitivity and limit of quantification, having the linear concentration range suitable for direct enantiorecognition of L-T3 in blood samples; therefore they can be used reliably as tools in the diagnostic of thyroid diseases. Compared with ELISA and chemiluminescence methods used in clinical laboratories for their determination, the main advantages are: (1) there is no need for sample pretreatment before assay, samples being used as taken from the patient; (2) low cost; (3) decreased time of determination.

Table 1.

Response characteristics of the microsensors

Microsensor based on	Signature of the enantiomer t_{off} (s)	Sensitivity (mol/L s^{-1})	Linear concentration range (mol/L)	Limit of quantification (mol/L)	Limit of detection (mol/L)	Equation of calibration; correlation coefficient*
L-T4						
IN-L-Phe-C4-L-lac/DP	0.7	$5.93x10^{9}$	10^{-12}-$4x10^{-12}$	10^{-12}	$7.81x10^{-14}$	1/ton=0.05+$5.93x10^{9}$; R=0.9831
IN-L-Ala-L-lac/DP	0.7	$5.44x10^{8}$	$8x10^{-12}$-10^{-10}	$8x10^{-12}$	$1.3x10^{-12}$	1/ton=0.03+$5.44x10^{8}$; R=0.9833
D-T4						
IN-L-Phe-C4-L-lac/DP	1.1	$2.2x10^{9}$	$6x10^{-12}$-10^{-11}	$6x10^{-12}$	$1.35x10^{-14}$	1/ton= $2.2x10^{9}$-0.01; R=0.999
IN-L-Ala-L-lac/DP	2.1	$2.45x10^{4}$	10^{-8}-10^{-6}	10^{-8}	$3.41x10^{-8}$	1/ton=0.03+$2.45x10^{4}$; R=0.941891
L-T3						
IN-L-Phe-C4-L-lac/DP	1	$4.02x10^{9}$	$4x10^{-12}$-$x10^{-12}$	$4x10^{-12}$	$8.16x10^{-13}$	1/ton=0.05+$4.02x10^{9}$; R=0.92953
IN-L-Ala-L-lac/DP	1	$3.08x10^{10}$	$4x10^{-13}$-10^{-12}	$4x10^{-13}$	$4.85x10^{-15}$	1/ton=0.03+$3.08x10^{10}$ R=0.9967
TSH						
		g/mL	**g/mL**	**g/mL**	**g/mL**	
IN-L-Phe-C4-L-lac/DP	0.5	$3.58x10^{10}$	$5x10^{-15}$-$5x10^{-13}$	$5x10^{-15}$	$1.01x10^{-16}$	1/ton=0.02+$3.58x10^{10}$; R=0.9972
IN-L-Ala-L-lac/DP	0.3	$3.36x10^{10}$	$5x10^{-15}$-$5x10^{-13}$	$5x10^{-15}$	$2.6x10^{-14}$	1/ton=0.03+$3.36x10^{10}$; R=0.9393

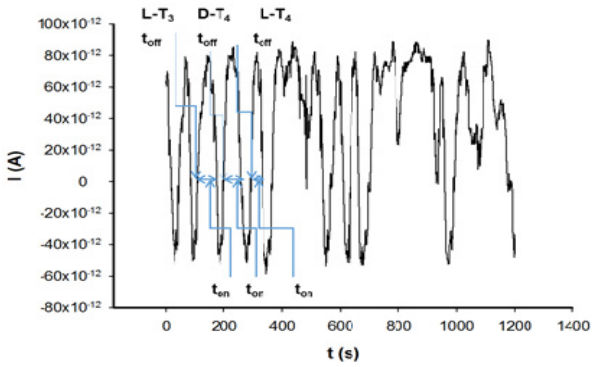

Figure 1. Example of a diagram recorded for the assay of f-L-T_3, f-L-T_4, and f-D-T_4

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