Biosensors and Bioelectronics 82 (2016) 217–223

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

A novel optical biosensor for direct and selective determination of serotonin in serum by Solid Surface-Room Temperature Phosphorescence

Teresa Ramon-Marquez [a], Antonio L. Medina-Castillo [b,*], Alberto Fernandez-Gutierrez [a], Jorge F. Fernandez-Sanchez [a,*]

[a] Department of Analytical Chemistry, University of Granada, Avd. Fuentenueva s/n, 18071 Granada, Spain
[b] NanoMyP®, Nanomateriales y Polimeros S.L., Spin-Off Company of the UGR, BIC Building, Avd. Innovacion 1, E-18016 Granada, Spain

ARTICLE INFO

Article history:
Received 12 February 2016
Received in revised form
17 March 2016
Accepted 4 April 2016
Available online 8 April 2016

Keywords:
Nanotechnology
Tiss®-Link
SS-RTP
Nanofibre mat
Serum
Phosphorescence
Serotonin
Protein clean-up

ABSTRACT

This paper describes a novel biosensor which combines the use of nanotechnology (non-woven nanofibre mat) with Solid Surface-Room Temperature Phosphorescence (SS-RTP) measurement for the determination of serotonin in human serum. The developed biosensor is simple and can be directly applied in serum; only requires a simple clean-up protocol. Therefore it is the first time that serotonin is analysed directly in serum with a non-enzymatic technique. This new approach is based on the covalent immobilization of serotonin directly from serum on a functional nanofibre material (Tiss®-Link) with a preactivated surface for direct covalent immobilization of primary and secondary amines, and the subsequent measurement of serotonin phosphorescent emission from the solid surface. The phosphorescent detection allows avoiding the interference from any fluorescence emission or scattering light from any molecule present in the serum sample which can be also immobilised on the nanofibre material. The determination of serotonin with this SS-RTP sensor overcomes some limitations, such as large interference from the matrix and high cost and complexity of many of the methods widely used for serotonin analysis.

The potential applicability of the sensor in the clinical diagnosis was demonstrated by analysing serum samples from seven healthy volunteers. The method was validated with an external reference laboratory, obtaining a correlation coefficient of 0.997 which indicates excellent correlation between the two methods.

1. Introduction

Serotonin or 5-hydroxytryptamine (5-HT) is a biogenic amine that acts as a neurotransmitter in the central and peripheral nervous systems (Berger et al., 2009; Jacobs and Azmitia, 1992). It controls a wide variety of physiological and behavioural processes, including depression, mood, sleep, anxiety, addiction, sexual activity, aggression and cognition as well as memory or perception (Berger et al., 2009; Deemyad et al., 2013; Hoyer et al., 2002; Julius, 1998; Makris et al., 2016; Meneses and Liy-Salmeron, 2012; Piszczek et al., 2015; Ramboz et al., 1998; Udupa and Chen, 2015).

Atypical concentrations of 5-HT in physiological fluids (serum and plasma) and body tissues have been related as a critical factor to several mental disorders such as Alzheimer's disease, schizophrenia, depression, infantile autism and obsessive compulsive disorder (Berger et al., 2009; Hoyer et al., 2002; Tecott et al., 1995). Other studies have also shown that high levels of 5-HT can affect the cardiovascular and gastrointestinal systems, and can be related with the carcinoid syndrome (Berger et al., 2009; Brand and Anderson, 2011; Kema et al., 2001; Meijer et al., 2000; Pussard et al., 1996).

Due to the multiple roles played by the serotonergic systems, it is important for pathological investigations and clinical diagnostics to establish sensitive, highly selective, rapid and simple methods for determining 5-HT levels in biological samples, mainly in serum.

Many methodologies have been developed for the determination of 5-HT in biological samples, including enzyme immunoassays (Chauveau et al., 1991; Lee et al., 2014; Nichkova et al., 2012), chromatographic (Danaceau et al., 2003; de Jong et al., 2010; Kema et al., 2001; Pussard et al., 1996; Yi et al., 1994; Yoshitake et al., 2004; Yubero-Lahoz et al., 2014), capillary electrophoresis (Peterson et al., 2004; Román et al., 2004), electrochemical (Goyal and Agrawal, 2012; Gupta and Goyal, 2014; Hasanzadeh et al., 2013; Mazloum-Ardakani

* Corresponding authors.
E-mail addresses: amedina@nanomyp.com (A.L. Medina-Castillo), jffernan@ugr.es (J.F. Fernandez-Sanchez).

http://dx.doi.org/10.1016/j.bios.2016.04.008
0956-5663/

and Khoshroo, 2014; Wang et al., 2013; Xue et al., 2014) and spectrofluorometric (Bracamonte and Veglia, 2011; Peng and Jiang, 2007) methods.

Both enzyme immunoassay (Darwish, 2006; Wu, 2006) (EIA) and enzyme-linked immunosorbent assay (Comley, 2012; Gan and Patel, 2013; Grange et al., 2014) (ELISA) methods are widely used as diagnostic tools in medicine and biomedical researches to analyse 5-HT, among other analytes, in serum samples (Chauveau et al., 1991). These assays require the immobilization of antigens, haptens, or antibodies on a solid surface, involving some disadvantages such as: 1) the presence of natural inhibitors in the samples which can provide wrong results, 2) nonspecific binding of the antibody or antigen to the solid support which leads to false-positive results, and 3) the enzyme reactions are very sensitive to temperature (Berg and Stryer, 2002; Coolbear et al., 1992; Hedstrom, 2001; Iyer and Ananthanarayan, 2008). Moreover, these assays need of many steps of reaction (Chauveau et al., 1991; Darwish, 2006), incubation processes (Li and Cassone, 2015; Nichkova et al., 2012) and washing steps (Huisman et al., 2010; Kim et al., 2009) which increase the analysis time and cost (Kim et al., 2008; Nichkova et al., 2013) as well as require experimented technicians.

Separative methods, in special HPLC with fluorescence detection, are also commonly used for routine clinical analysis of 5-HT in serum. However, chromatographic determination of 5-HT shows significant drawbacks: they usually require previous solid phase, liquid-liquid extractions or protein precipitations (de Jong et al., 2010; Golubchik et al., 2009; Zhen et al., 2011), pre or post-column derivatization reactions (Hirowatari et al., 2004; Mao et al., 2009; Ohkawa et al., 2005), use of relatively large amounts of solvents (Mao et al., 2009; Sa et al., 2012), long preparation times (Hirowatari et al., 2004; Mao et al., 2009; Teradaira et al., 2007) and expensive equipments.

Electrochemical methods are sensitive enough to estimate the concentration of 5-HT in biological samples, but they are not available in clinical laboratories due to problems of maintenance, limited life time of the electrodes and variability on sensitivity (Babaei et al., 2013; Hasanzadeh et al., 2013; Li and Lin, 2007; Myers and Lee, 2008).

Spectrofluorometric methods have also been developed to determine 5-HT in serum, but optical measurements are not selective enough to develop a good routine method in this field. One of the main problem of the analysis of 5-HT in serum samples by spectrofluorometric methods is the complexity of the matrix, which contains potential interfering compounds such as proteins, antibodies, antigens, hormones, etc. Many of these compounds show weak natural fluorescence and, therefore can affect the reliability of the results. In fact, many proteins show intrinsic fluorescence emission due to the excitation of tryptophan residues (Gorinstein et al., 2000; Kowalska-Baron et al., 2015).

On the other hand, there are only few components of the serum that show intrinsic phosphorescence emission, where 5-HT, tryptamine (TRYP) and tryptophan (TRYPH) are included (Bruxvoort et al., 1993; de Ribamar et al., 1995). They are structurally and metabolically related (D'Andrea et al., 2015). Therefore, phosphorescence could be a good alternative to much more complex methods to determine 5-HT in human serum. Phosphorescence sensors have been successfully used for the direct, quick and easy (in one step) determination of different molecules in complex matrices, solving the main drawbacks of the enzymatic and separative techniques previously discussed (Bi et al., 2014; Ramon-Marquez et al., 2016; Wu et al., 2010).

Since 5-HT has native phosphorescence, in this paper, we have developed a novel biosensor for the direct determination of 5-HT in human serum samples with satisfactory results, by taking advantage of the benefit of the functional material Tiss®-Link (Medina-Castillo et al., 2011a, 2011b; Ramon-Marquez et al., 2016), and the high sensitivity and selectivity of Solid Surface-Room Temperature Phosphorescence (SS-RTP). Tiss®-Link is a non-woven nanofibre mat made by electrospinning which has been demonstrated to be useful for covalent immobilization of amines from complex matrices (Medina-Castillo et al., 2011a, 2011b; Ramon-Marquez et al., 2016). The use of Tiss®-Link combined with SS-RTP simplifies the procedure to analyse 5-HT directly in serum samples (it only requires minimal clean-up of the samples), reducing considerably the time and cost of the analysis. In addition, the developed biosensor is highly selective and sensitive in the determination and quantification of 5-HT and therefore, it is a good candidate to be used as routine tool in diagnosis and clinical labs.

2. Experimental

2.1. Reactives and materials

Tiss®-Link, and PolymP®-Pyridine were kindly supplied by NanoMyP® (http://www.nanomyp.com), Tween 20, potassium iodide (KI), sodium carbonate (Na_2CO_3), potassium phosphate (K_2HPO_4), tryptamine (TRYP), serotonin (5-HT), tryptophan (TRYPH) and human serum albumin (HSA) were all purchased from Sigma Aldrich and used without further purification. Human serum samples were obtained from seven healthy volunteers and stored frozen until assay.

Tiss®-Link is a non-woven nanofibre mat manufactured by electrospinning using PolymBlend® (http://nanomyp.com/es/page.cfm?id=212&title=polymblend%C2%AE) as raw material. PolymBlend® is a polymeric blend formulated with an optimum mixture of two high molecular weight statistic copolymers, containing a high percentage of hydroxyl groups in their structure (40%). Therefore, the nanofibre mats produced with PolymBlend® have high hydrophilicity. During the fabrication process of Tiss®-Link, part of its hydroxyl groups have been functionalized with active vinyl groups, thus the final material has two functionalities: the non-functionalized hydroxyl groups which provide a high hydrophilicity, and the active vinyl groups (330 μmol/g) which can be used for covalent immobilization of primary and secondary amines.

The hydrophilicity of Tiss®-Link was determined by calculating the ω parameter (amount of absorbed water per mass of material) (Stathopoulos et al., 2010). The obtained ω parameter was 2.0, indicating that Tiss®-Link can absorb 2 g of water per gram of material. On the other hand, the pK_a of the primary hydroxyl groups of the material (which are the only one which can provide ionic charge) is 15, thus, at pH=10 the material is uncharged. Therefore, the properties are kept at pH=10.

Tiss®-Link has excellent mechanical properties (high mechanical strength, high consistency and high flexibility). It is non-luminescent, uncharged, insoluble in aqueous media as well as in apolar solvents (oil, toluene, etc.), showing a high robustness and stability in a wide range of pHs (at least between pH 5 and 10 up to 24 h), and temperature resistance (up to 100 ° C).

PolymP®-Pyridine is a cationic ion exchange material with format of monodisperse and spherical polymeric particles of 2.5 μm of diameter. They have a high surface density of accessible pyridine groups (250 μmol g^{-1}) with a pK_a of 6. These particles are suitable for protein immobilization in a pH range between 3 and 6.

Supporting material (SM) (see Fig. SM-1) shows images of scanning electron microscopy (SEM) in which the microstructure and morphology of Tiss®-Link and PolymP®-Pyridine are shown.

2.2. Apparatus and measurements

All SS-RTP measurements were performed on a Varian Cary-Eclipse luminescence spectrophotometer equipped with a homemade flow cell. A general description of the equipment for SS-RTP measurements has previously been specified (Ramon-Marquez et al.,