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Application of antibody–nanogold–ionic liquid–carbon paste electrode for sensitive electrochemical immunoassay of thyroid-stimulating hormone

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ABSTRACT

A novel electrochemical immunosensor based on carbon paste electrode (CPE) composed of ionic liquid (IL) and graphite was constructed. It demonstrated good efficiency for quick (each test in 30 s) determination of thyroid stimulating hormone (TSH). Electrode surface was modified by gold nanoparticles in order to immobilize of the thyroid stimulating hormone antibody (anti-TSH) on the CPE. The immunoassay structure was established by sandwiching the antigen (TSH) between the thyroid stimulating hormone antibody on the CPE surface modified with gold nanoparticles and the secondary antibody, polyclonal anti-human-TSH labeled with horseradish peroxidase (HRP-labeled anti-TSH). The signal of differential pulse voltammetry (DPV) was used as a basis for the determination of TSH concentration. This signal is generated by the reaction between O-aminophenol (OAP) and H_2O_2 catalyzed by HRP. The proposed immunosensor is able to measure the concentration of TSH in a linear range between 0.2 and 90.0 ng/mL with a detection limit 0.1 ± 0.02 ng/mL. In addition, high sensitivity and acceptable stability were achieved by this immunosensor which is promising in the clinical assay of TSH.

1. Introduction

Thyroid stimulating hormone (TSH), as an essential hormone in the human body, is a glycoprotein which is secreted by thyrotroph cells in the pituitary gland. It stimulates the synthesis and secretion of thyroid hormones including triiodothyronine (T3) and thyroxine (T4) into the blood. The value and balance of T3 and T4 hormones are particularly effective on the metabolism of human body (Smaniotto et al., 2017; Amandeep Kaur et al., 2014; Rudge et al., 2015). A low level of TSH with the normal serum concentration of T4 and T3 can be an indicator of subclinical hyperthyroidism. Although it is an asymptomatic disease, un-treatment of subclinical hyperthyroidism causes serious consequences including heart failure, atrial fibrillation, cardiac dysfunction, symptoms of neuropsychiatric, osteoporosis, and overt hyperthyroidism. TSH also acts as a biomarker for the early diagnosis of thyroid cancer. The normal TSH level in adults is 0.4–4.2 $\mu IU\ mL^{-1}$ (milli-international units per liter) (Choi et al., 2017). Due to this low range values of TSH, its determination in human serum is difficult as well as the development of a biosensor with the capability of accuracy, precision, reproducibility, and quick monitoring of TSH. In addition, in the cases of pituitary gland tumor, the TSH level is considerably higher than normal level. Therefore, the development of rapid, reliable and sensitive techniques for sensing TSH is imperative in the prevention of thyroid disease. A high sensitive analysis of TSH provides the early diagnosis of hyperthyroidism as well as assessment of its severity (Liu et al., 2015; Jung et al., 2013; Wang et al., 2015). An immunosensor as a type of biosensor is a compact analytical device which its function is based on the formation of antigen-antibody complexes that is detectable and convertible to an electrical signal via a transducer. In fact, the reaction between an antibody and antigen is the basis for the determination of proteins through a sensitive and selective technique provided by immunosensors. These sensors provide molecular recognition in a direct monitoring simply on the surface of a chip (Park and Kricka, 2014; Dong et al., 2016). Various immunosensors have been fabricated with different transducers which operate based on changes in heat, mass, optical and electrochemical characteristics. The most frequently used reagents for fabrication of immunosensors are antibodies, enzymes, and fluorescence labels which are in some cases expensive. In addition, some samples such as infant blood or spinal fluid are valuable and scarce. Therefore, the development of diagnostic devices in miniature size with no reduction in their sensitivity or detection limit is highly favorable (Shim et al., 2017; Zhang et al., 2015; García-González et al., 2016).

With the advent of new concepts in electrochemistry such as micro-

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and nano-fabrication and lab-on-chip, novel high performance immunosensors have being developed with advantages of quick analysis, portability, automation and integration (Wang et al., 2017). In this regard, electrochemical immunosensors have attracted more attention among the various developed immunosensors since they are able to provide high sensitivity, simplicity, cost effectiveness, quick analysis, and facility of miniaturization. In these series, sandwich-type electrochemical immunosensors are highly desirable due to their high sensitivity and specificity (Serafín et al., 2014; Tao et al., 2013). In the cases where immobilization of immunological reagents is desired, the carbon paste, glassy carbon and screen-printed electrodes are commonly used to play the role of electrode transducer (Liu et al., 2001; Padeste et al., 1998; Huet and Bourdillon, 1993; Kaláb and Skládal, 1997; Fahnrich et al., 2003). Since the first report of electrode modification through the chemical approaches in 1979, it is still an open area of research in electrochemistry (Eissa et al., 2018). During the progress in the modification process, various modifiers including polymers, nanoparticles and ionic liquids (ILs) have been employed to promote the efficiency of common metal, carbon, and semiconductor-based electrodes (Movlaee et al., 2017; Molaakbari et al., 2017a, 2017b; Beitollahi et al., 2016a, 2016b, 2017a, 2017b; Al Aqad et al., 2018)

In the past two decades, ionic liquids (ILs) with their peculiar characteristics became applicably attractive in the various fields of electrochemistry, electrodeposition, catalysis, energy management, bioscience, biomechanics, and material chemistry. ILs have diverse properties featuring wide electrochemical window, electrically conductive, very low vapor pressure, high thermal stability as well as tunable characteristics via the changes of cations and anions. In the electrochemical applications, ILs have found a key role as a conductive binder in the fabrication of carbon paste electrodes (CPEs) which are known as carbon ionic liquid electrodes (CILEs) (Ren et al., 2017). Formerly, carbon particles and organic oil were mixed to make a CPE. However, the organic oil has two main disadvantages as a binder. The first one arises from its origin as a product of refined petroleum or processed crude oil with some unwanted ingredients which may be effective in the analysis performance. The second flaw is related to its non-conductivity which has a negative effect on the electrochemical response of CPE and detection limit, as well (Huang et al., 2010; Maleki et al., 2006).

Accordingly, replacing the organic oil with a sufficient viscose, chemically inert and electrochemically conductive liquid to overcome the mentioned limitations of former binder has been a matter of concern in the improvement of carbon paste electrode. In this regard, ILs are a promising alternative for the traditional paraffin binders since they provide high sensitivity, good anti-fouling ability, stable electrochemical responses, and inherent electrocatalytic activity (Sun et al., 2014; Atta et al., 2015). The progress in biochemistry, chemistry, nanotechnology and electronics is indispensable for improving potentialities, reliability, mobility and functionality of biosensors based on the ionic liquids. The development of techniques for the production of suitable materials and immobilization will make it ready to implement and commercialize biosensors based on different biological materials in many practical applications (Wasilewski et al., 2017).

Nowadays, the direct immobilization of the antibody fragments in a well-oriented way is accessible on the gold nanoparticles. Comparing with micro beads, nanomaterials have the advantage of higher capture efficiency because of their large surface-to-volume ratio, faster kinetic reactions, and minimum sample requirement (Luo et al., 2014; Cheng et al., 2015).

In this study, a novel electrochemical immunosensor is constructed based on ionic liquids, gold nanoparticles and graphite for the determination of TSH.

2. Experimental

2.1. Apparatus

The electrochemical measurements were carried out using an Autolab potentiostat/galvanostat (PGSTAT 302N, Eco Chemie, the Netherlands). Controlling the experimental conditions was performed by General Purpose Electrochemical System (GPES) software. A conventional three electrodes cell was used at 25 ± 1 °C. An Ag/AgCl/KCl (3.0 M) electrode, a platinum wire, and the HPR-Ab/TSH/Ab/TGA/nano-Au/CILE were used as the reference, counter and working electrodes, respectively. All the electrochemical experiments were performed at ambient temperature of 25 ± 2 °C. A Metrohm 710 pH meter was employed for pH measurements.

2.2. Materials

Bovine serum albumin (BSA)-fraction was purchased from AiBi Chemistry Preparation (China). *O*-Aminophenol (OAP), H_2O_2, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide solution (EDC) and *N*-hydroxysuccinimide solution (NHS) were purchased from Sigma. The ionic liquid, *N*-butylpyridinium hexafluorophosphate ($BPPF_6$, 97%, melting point 65 °C) was purchased from Sigma. Graphite powder (particle size < 30 μm) was obtained from Merck and used without further treatment. Phosphate buffered saline (PBS, 0.1 M, pH 7.0) was used as supporting electrolyte solution. All other reagents were of an analytical grade. Doubly distilled water was used for the preparation of all solutions.

2.3. Procedure

2.3.1. Construction of CPE and CILE

The fabrication of ionic liquid carbon paste electrode (CILE) was performed through the following procedure: in a mortar, 0.9 g graphite powder, 0.2 mL ionic liquid $BPPF_6$ and 0.7 mL paraffin oil was mixed thoroughly until a homogeneous carbon paste was formed. Then, the end of a glass tube (inner diameter 0.35 cm, and 15 cm long) was filled with 0.08 gr of the paste. In order to establish an electrical contact, a copper wire was inserted through the opposite end of the corresponding glass tube. The surface of electrode became mirror-like, prior to use by polishing the electrode on a weighing paper.

2.3.2. Electrodeposition of gold nanoparticles onto CILE

In order to electrodeposit gold nanoparticles onto CILE, the electrode was immersed into a solution of $HAuCl_4$ (6 mM) containing 0.1 M KNO_3, while deoxygenated by bubbling nitrogen. Then, a constant potential of − 0.4 V versus Ag/AgCl was applied for 400 s. Finally, the modified electrode (nano-Au–CILE) was thoroughly washed with doubly distilled water and dried completely.

2.3.3. Fabrication of the immunosensor

Prior the fabrication procedure, the nano-Au/CILE was continuously scanned to be cleaned by cyclic voltammetry within a potential range of 0.1–1.5 V in 0.1 M H_2SO_4 at a scan rate of 0.1 V s^{-1}. Then, nano-Au/CILE was immersed in 5 mM thioglycolic acid (TGA) aqueous solution for 24 h to form a self-assembled monolayer. Afterwards, to remove the residue of TGA, it was rinsed several times with doubly distilled water. In the next step, the electrode was inverted and activated through the rinsing by 20 μL of a solution containing EDC and NHS in 50 mM PBS (pH 7.0), and then dried after the evaporation. After that, the thyroid stimulating hormone immunosensor was developed through the conjugation of TSH/Ab by a covalent bond to TGA on the nano-Au modified electrode. Later, the unreacted groups on the TGA-activated surface were passivated through the reaction with BSA (1% w/w) at room temperature for 2 h.

The TSH immunosensor was established on the basis of sandwich