Journal of Electroanalytical Chemistry 799 (2017) 278–284

Contents lists available at ScienceDirect

Journal of Electroanalytical Chemistry

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

Carbon black as a glassy carbon electrode modifier for high sensitive melatonin determination

Joanna Smajdor, Robert Piech*, Magdalena Pięk, Beata Paczosa-Bator

Faculty of Materials Science and Ceramics, AGH UST University of Science and Technology, 30-059 Kraków, al. Mickiewicza 30, Poland

ARTICLE INFO

Keywords:
Melatonin
Voltammetry
Carbon black
Hormones determination

ABSTRACT

Carbon nanomaterials are becoming a significant part of science development nowadays. In this work, carbon black nanopowder was used as an electrode modifier in order to improve sensitivity of glassy carbon electrode. A rapid and high sensitive method of melatonin determination was successfully developed on glassy carbon electrode modified with carbon black using differential pulse voltammetry. The electrochemical behaviour of melatonin electro oxidation on carbon black layer was investigated using cyclic voltammetry. A linear voltammetric response was obtained for analyte in the concentration range from $0.05{\cdot}10^{-6}$ mol L^{-1} ($1.16{\cdot}10^{-5}$ g L^{-1}) to $12.0{\cdot}10^{-6}$ mol L^{-1} ($2.78{\cdot}10^{-3}$ g L^{-1}), with a detection limit of $1.9{\cdot}10^{-8}$ mol L^{-1} for preconcentration time of 45 s. Repeatability of method was determined as RSD % for melatonin concentration of $2{\cdot}10^{-6}$ mol L^{-1} as 1.3% (n = 7). The proposed method was successfully applied and validated by studying the recovery of melatonin in different pharmaceutical products. Amperometric measurements of melatonin were performed as well to indicate the possibility of its fast and accurate determination.

1. Introduction

Melatonin (ML) (*N*-acetyl-5-methoxy tryptamine), a serotonin derivative, is an endogenous hormone, primarily responsible for maintaining circadian rhythm of organisms [1]. It is mainly known as synthetized by mammals, but also by microorganisms, plants and fungi, and by other vertebrates and invertebrates [2]. It is proven that mainly pineal gland is responsible for melatonin secretion, but some other tissues in guts or skin along with leukocytes also has ability of producing melatonin [3]. Melatonin is the first – line drug in cases of sleep disorder, like insomnia, or to reduce the effects of jet lag [4–6]. Not only it is responsible for regulating circadian and seasonal rhythms, but also express major effects on immune system [7–10] and as an anti-inflammatory [11,12] and an antioxidant agent [13,14]. It is known that melatonin influences course of mental disorders [15,16] and supports treatment of breast cancer [17,18].

Melatonin determination in pharmaceutical formulation and body fluids is the main research topic for many scientists. So far numerous methods of ML determination have been developed. Among them, the numerous chromatography techniques coupled with various detectors, such as mass spectrometry [19–21], UV – VIS [22,23] or diode array detector [24] is performed. Other popular methods of melatonin determination includes capillary electrophoresis with electrochemical detection [25], enzyme-linked immunosorbent assay (ELISA) [26] and spectrofluorometric method [27,28]. However, these methods are usually complex and time consuming, not to mention that used equipment is often very expensive. In order to eliminate these inconveniences, voltammetry techniques have been proposed.

Among the wide variety of working electrodes used in voltammetry, melatonin has been determined mainly on the solid electrodes and on hanging mercury drop electrode [29]. Solid electrodes have become more popular in modern electrochemistry, due to its low impact on environment and possibility of applying different modifications. According to the literature, melatonin determination on unmodified solid electrode has been performed on boron doped diamond electrode [30,31], carbon paste electrode [32,33] and glassy carbon electrode [23,33,34] so far. In order to obtain high sensitivity of performed measurements, different types of electrode modifications are developed nowadays. The most popular includes multi walled carbon nanotubes [35,36], palladium nanoparticles [37], nanorods of ZnO_2 [38] or graphene [39–41]. Especially carbon nanomaterials, such as carbon nanotubes, graphene and carbon black are particularly interesting in the electrochemistry field. One of them, that has been used for the first time in 2012, is carbon black (CB) [42,43]. It is a new carbon nanomaterial, attracting much attention in electrochemistry field, due to its unique properties, such as high surface development, high reversible capacity, low density and disordered structure [44–46]. As a consequence of high surface development, using carbon black as a modification layer for

* Corresponding author.
E-mail address: rpiech@agh.edu.pl (R. Piech).

http://dx.doi.org/10.1016/j.jelechem.2017.06.013
Received 26 January 2017; Received in revised form 11 May 2017; Accepted 7 June 2017
Available online 08 June 2017
1572-6657/

electrodes may cause in achieving lower detection limits [47–53]. Carbon black also provides stable signals with high precision and repeatability of measurements. Since the first use of CB in the electrochemistry, the number of reports with its use is consequently increasing, proving the attractiveness of its properties and wide range of possible applications, both for organic and inorganic compounds.

In this paper melatonin determination on carbon black modified glassy carbon electrode is performed using voltammetric and amperometric method. To the best of our knowledge, such modification has never been used before for melatonin determination. Proposed method was successfully applied for sensitive and accurate melatonin determination in pharmaceutical samples. Carbon black glassy carbon electrode was also applied for melatonin determination in flow injection system with great repeatability of obtained signal.

2. Experimental

2.1. Measuring apparatus

A multipurpose Electrochemical Analyzer M161 and the electrode stand M164 (both MTM-ANKO, Poland) with the EAGRAPH software were used for all voltammetric measurements. The typical three-electrode quartz cell, volume 20 mL, consisted of a glassy carbon electrode modified with carbon black layer (CBGC) as the working electrode, a silver reference electrode Ag/AgCl/KCl (3 mol L^{-1}) with replaceable outer cover (3 mol L^{-1} KCl) and a platinum rod as an auxiliary electrode. During the preconcentration time the stirring was performed using a magnetic Teflon coated bar, rotating at approximately 500 rpm. Flow injection analysis along with the amperometric detection were carried out using Autoburette Abu12 (Radiometer, Copenhagen) as a pump and Electrochemical Analyzer M161. Measurements were performed in three-electrode thin-layer flow cell using the same electrode as for voltammetry. The flow cell body was fabricated with teflon. pH measurements were performed with laboratory pH-meter (N-512 elpo, Polymetron, Poland). All experiments were carried out at room temperature.

2.2. Chemicals

All reagents were of analytical grade and used without further purification. Standard stock solution (10 mL) of $2{\cdot}10^{-3}$ mol L^{-1} melatonin (Sigma Aldrich) was obtained by dissolution of an appropriate amount of this reagent in equal proportion of water and ethanol and then stored in fridge. KH_2PO_4, K_2HPO_4 were obtained from Merck. In measurements 0.1 mol L^{-1} phosphate buffer of pH 6.2 was used as supporting electrolyte. Carbon black nanoparticles was obtained from 3D-nano (surface area: 100 m^2 g^{-1}, average particle size: 30 nm, Poland) and dimethylformamide (DMF) was obtained from Sigma Aldrich. Triton X-100 was purchased from Windsor Laboratories Ltd, UK.

All solutions were prepared with double-distilled water.

2.3. Working electrode preparation

A 1 mg mL^{-1} carbon black dispersion was prepared in following steps:

1. weighting 5 mg of carbon black nanopowder (3D-nano, Poland);
2. quantitative transfer of carbon black into the 5 mL volumetric flask;
3. addition of 5 mL of DMF to the volumetric flask;
4. dissolving of carbon black using ultrasonic washer for 1 h.

DMF was chosen as the solvent in order to improve the dispersibility of carbon black and ensure good traction of the layer to the electrode surface.

After preparing carbon black dispersion, electrodes could be prepared. The glassy carbon film electrode was prepared following the procedure:

1. cleaning glassy carbon electrode surfaced in order to remove scratches by polishing it on the polishing cloth with alumina powder (particle size of 0.3 μm) (Buehler Micropolish II, USA);
2. homogenisation of carbon black suspension by using ultrasonic washer for 7 min;
3. applying 10 μL droplet of carbon black mixture onto the glassy carbon electrode surface;
4. drying electrodes for about 12 h in room temperature. After solvent evaporation, electrode was ready to work.

2.4. Sample preparation

Pharmaceutical formulation: Melatonina (LEK-AM, Poland), and diet supplements: Zdrowosen Melatonina (Natur Produkt Pharma, Poland) and Olimp Forsen Forte with melatonin (Olimp Laboratories, Poland) containing 5, 5 and 1 mg of melatonin respectively was obtained from local pharmacy. For the analysis of melatonin contents in tablet formulation, three of them were crushed in a mortar, then quantitatively transferred and dissolved in ethanol and water (proportion 1:1) in 10 mL volumetric flask and sonicated for 5 min. After complete dissolving, solution was filtered in order to remove tablet powder remaining and ready to analyse.

The content of melatonin in tablet samples was determined by the standard addition method and its suitability was validated using recovery parameter.

2.5. Standard procedure of measurements

Quantitative measurements of melatonin concentration were performed using differential pulse voltammetry (DPV). Carbon black glassy carbon electrode (CBGC) was coated day before planned measurements and stability of such layer was very good. A CBGC electrode obtained in this way was used for sensitive melatonin determination in medical formulation, urine and serum samples in the supporting electrolyte consisted of 0.1 mol L^{-1} phosphate buffer with pH of 6.2 (total volume 10 mL). The measurement procedure was performed in an uninterrupted sequence of the following steps: (a) cleaning CBGC electrode surface by applying potential of 1100 mV for 3 s (b) the melatonin preconcentration step: E_{acc} = 300 mV; t_{acc} = 5 s; (c) after the rest period of 3 s; voltammograms were recorded in the potential range from 300 mV to 1100 mV.

Other conditions for the DPV mode were as follows: step potential 6 mV (0.3 V s^{-1}); pulse amplitude 50 mV; time step potential 20 ms (10 ms waiting and 10 ms sampling time).

3. Results and discussion

3.1. Voltammetric behaviour of melatonin on CBGC electrode

To the best of our knowledge, carbon black suspended in dimethylformamide on glassy carbon electrode has never been used for melatonin determination. Morphology of this modification layer used in following researches versus bare GC electrode is presented in Fig. 1.

In order to investigate the melatonin oxidation process on the carbon black glassy carbon electrode linear sweep voltammetry was used. The effect of the scan rate in the range from 5 to 100 mV s^{-1} on the melatonin oxidation peak is presented in Fig. 2. Considering absence of reduction peak it is possible to say that the melatonin electrode reaction is irreversible. In order to determine mechanism of melatonin oxidation, dependences of CV peak current versus scan rate and square root of the scan rate value were analysed. Linear response was obtained between melatonin peak current on the square root of scan rate and described as in Eq. (1):