Sensors and Actuators B 252 (2017) 803–812

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

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

Dopamine sensing with fluorescence strategy based on low temperature co-fired ceramic technology modified with conducting polymers

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ARTICLE INFO

Article history:
Received 15 March 2017
Received in revised form 8 June 2017
Accepted 11 June 2017
Available online 12 June 2017

Keywords:
Fluorescence biosensor
Dopamine
LTCC
GQDs
Conducting polymer
Laccase
Microfluidic devices
FRET

ABSTRACT

A convenient fluorescence sensing strategy for dopamine (DA) detection was developed based on polydopamine (poly(DA)) formed on the surface of graphene quantum dots (GQDs). The prepared GQDs were highly luminescent due to the planar structure in aromatic molecules. The ceramic-based miniature biosensor was designed and constructed through the immobilization of laccase in an electrochemically synthesized polymer – poly[dithienotetraphenylsilane] based on low temperature co-fired ceramics technology (LTCC). This sensing system utilized the catalytic oxidation of DA to dopamine-*o*-quinone (DOQ), and then to poly(DA) (in alkaline conditions), which can selectively quench the strong luminescence of GQDs owing to Förster resonance energy transfer (FRET). The detection process was based on substarte oxidation in the presence of the enzyme – laccase. In optimized conditions, the analytical performance illustrated high sensitivity, selectivity in a broad linear range with detection limit of 80 nM. Moreover, the method was successfully applied to test labeled pharmacological samples for DA.

1. Introduction

Dopamine (3,4-dihydroxyphenyl ethylamine, DA), a catecholamine neurotransmitter, regulates many physiological processes in the central nervous, hormonal, renal and cardiovascular systems [1,2]. Changeable DA level in biological fluids (i.e. urine, blood plasma, and extracellular fluid of the central nervous system) can be a marker of several disorders such as schizophrenia and Huntington's and Parkinson's diseases [3]. Because of its significance in analytical techniques and diagnostics, sensitive and selective detection of DA is increasingly attracting a lot of attention in various areas of bio-analysis or biomedical research. DA concentration is currently monitored with such techniques as chromatography [4], spectroscopy [5], microdialysis techniques [6], electrochemical sensing [7], or colorimetric probes [1]. These methods, however, have some limitations. For instance chromatographic methods are time-consuming, labor intensive, and expensive. Similarly, the synthesis of fluorescent or colorimetric probes for dopamine sensing involves complicated procedures [8].

Recently, fluorescent techniques for detection of DA have attracted interests due to their reasonable cost, convenient control, and maneuverability in biological environments [9]. Although here seems to be a strong need for a sensitive and selective DA sensor, the development of a suitable method (for DA) is still limited [9].

A simple and quick alternative for dopamine detection is monitoring the oxidation product of dopamine [10] – a quinone derivative (of dopamine). The quinone (dopamine-*o*-quinone, DOQ) is (often) a product of DA oxidation in the presence of oxidoreductase (i.e. laccase) – Fig. 1. This derivative, however, is unstable [11] and swiftly polymerized in alkaline conditions to polydopamine (poly(DA)), which is structurally similar to the biological eumelanin polymers [12].

A crucial factor in the construction of a biosensor is the need to achieve adequate and effective enzyme immobilization. The use of conducting polymers (CPs) for the construction of different biosensors has recently been studied extensively because of their redox, optical, mechanical and electrical properties. Conducting polymers are durable and stable, and are considered among the most suitable materials for biosensors [13]. Biosensor efficiency depends mainly on the surface architecture, interaction between the enzyme and electrode surface, and the protection of 3D struc-

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http://dx.doi.org/10.1016/j.snb.2017.06.073
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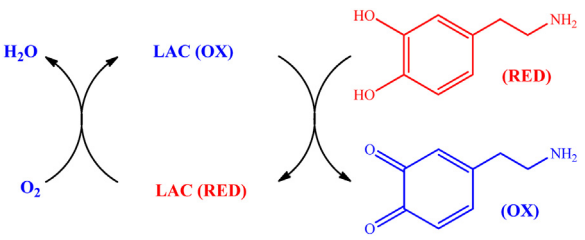

Fig. 1. Biocatalytic oxidation of dopamine (DA).

ture of a biocatalyst. Therefore, CPs have turned out to be one of the most useful transducers because of their simple fabrication. CPs operate as a three-dimensional substrate for biomolecule deposition. Their charge transfer capacity acts as an excellent matrix for biomolecules providing enzyme mimetic environment [14].

In particular, the biosensor should be coated extensively with poly(3,4-ethylenedioxythiophene) (PEDOT) to improve its function and biocompatibility [15]. Luo group used PEDOT doped with carbon nanotubes (CNT) [16] and PEDOT doped graphene [17] coated GC electrodes for the detection of dopamine. Xue et al. modified the electrode with polyaniline composite to detect the serotonin in human serum [18].

Due to the presence of aromatic units in the polymer backbone, the immobilization was performed with the help of π–π stacking interactions of the polymer and enzymatic protein. These strong interactions stabilized the tertiary structure of proteins effectively [19]. Furthermore, sulfur-containing heteroatom materials were compared for their capacity to act as an immobilization matrix and for their redox mediator properties [20].

Graphene quantum dots (GQDs) have demonstrated several attractive properties of standard carbon-based nanosized materials, such as low toxicity, biocompatibility, and photostability. In comparison with other carbon nanomaterials, GQDs exhibit a relatively high quantum yield [21]. Several studies on GQDs have recently focused on their properties, but research on the analytical adoption of this tool is still limited [9,22].

GQDs obtained in the process of pyrolysis of citric acid [23] have a passivated surface, and therefore, exhibit good fluorescence properties. The change in the surface of GQDs can cause FRET (Förster resonance energy transfer) and quench fluorescence intensity (of the designed biosensor). Despite the fact that some sensors proposed for DA detection are based on fluorescence strategy, the mechanism of DA detection still needsto be improved.

In this study, we report the fluorescence route for selective dopamine detection.

The detection system involved laccase immobilized in CP matrix (poly[dithienotetraphenylsilane]) and GQDs. The obtained quantum dots were used as fluorescent markers for quantitative detection of dopamine.

The designed sensor was made with LTCC (low temperature co-fired ceramics) technology [24]. The LTCC material is temperature and pressure resistant. A large majority of solvents, bases and moderately concentrated acids are inert to the fired LTCC material [25]. Moreover, various 3D structures (e.g. channels) can be made inside the LTCC-based substrate using laser, hot embossing or mechanical milling [26–28]. As a result, the LTCC-based substrate seems to be a convenient substrate for the construction of the lab-on-chip (LOC).

The presented LTCC-based biosensor for monitoring of dopamine consisted of a microfluidic chip and a chip holder with integrated optoelectronic components. The biosensor operated on the principle of measuring an efficient fluorescence quenching of GQDs. Thus the concentration of dopamine was monitored easily and inexpensively.

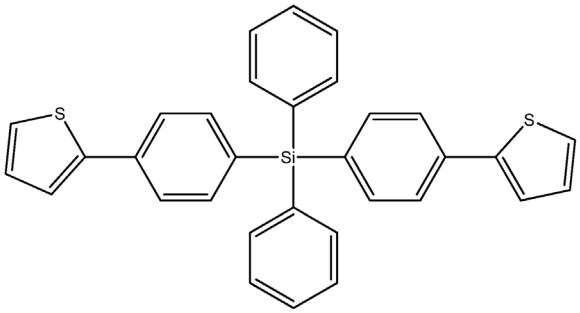

Fig. 2. Structure of dithienotetraphenylsilane.

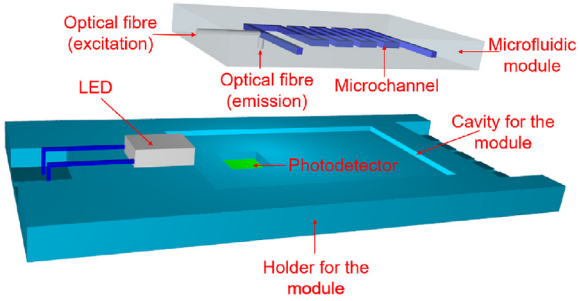

Fig. 3. Model of the fluorescence-based biosensor.

2. Experimental part

2.1. Reagents and materials

Laccase (from *Cerrena unicolor*, EC 1.10.3.2, ≥10 U/mg) and all other reagents (dopamine hydrochloride, citric acid, tetrabutylammonium tetrafluoroborate – TBA-TFB) were purchased from Sigma-Aldrich Co. All chemical reagents were of analytical grade and were not purified further before use. The phosphate-citrate buffer solution (McIlvaine, pH 5.2) was prepared by mixing the citric acid and sodium phosphate solutions. The water used throughout the experiments was purified by Milipore system.

The silane derivative, dithienotetraphenylsilane (Fig. 2) was synthesized as in the previous experiment [29].

2.2. Synthesis and characterization of GQDs

GQDs stock solution was prepared using 4 g of pyrolyzed citric acid as described in the reported literature [23]. The pH (value) of quantum dots was adjusted to 10. The obtained GQDs were used as stock solution and stored at 4 °C when not used. The size and homogeneity of the obtained GQDs were determined with AFM (Atomic Force Microscope) method in tapping mode (AFM NanoScope V Veeco). The measurements were performed at ambient air (25 °C and relative humidity of 35%). The AFM used was the cutting edge TESP type, 10 nm size and scanning rate of 3 μm/s. The Fourier Transform Infrared Spectroscopy (FTIR) spectrum was executed on Nicolet iS10 spectrometer. Emission spectrum of GQDs was measured using SILVER-Nova high performance spectrometer (StellarNet).

2.3. Technology

The fluorescence-based biosensor was constructed using LTCC technology (similar to the one presented in [24]). It was composed of 2 parts – a microfluidic module and a holder for the module – as presented in Fig. 3.