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Neurotransmitters detection using a fluorescence-based sensor with graphene quantum dots

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In the paper, the development and performance of an optical sensor for detection of neurotransmitters (dopamine) is presented. The concentration of dopamine is measured basing on fluorescence quenching of graphene quantum dots. In the sensor, the dopamine molecules coat the graphene quantum dots surface – in result, the quenching of fluorescence occurs due to the Förster resonance energy transfer. The changes in fluorescence correspond to specific concentrations of the neurotransmitter in tested samples, so it is possible to accurately determine the concentration of dopamine in the sample.

Keywords: sensor, dopamine, fluorescence, Förster resonance energy transfer, quantum dots.

1. Introduction

Nanomaterials are an attractive group of interest because of their unique properties. They can be divided into several types: *e.g.*, nanotubes, nanowires or quantum dots (QDs). Quantum dots are small-size semiconductor nanocrystals (size 2–10 nm), with a high fluorescence feature. They consist of a limited number of atoms and have a few nanometers of diameter. In a consequence, the quantum dots are characterized by unusual properties of absorption and emission of radiation. Quantum dots exhibit luminescence characteristics and electronic properties such as wide and continuous absorption spectra, narrow emission spectra, and high light stability [1]. Graphene quantum dots (GQDs) possess, in comparison with casual quantum dots or organic dyes, much better photoluminescent properties, better bioavailability and chemical inertness. Because of that, quantum dots inspired extensive studies in photovoltaic devices [2], anti-cancer drug carriers [3], or biosensor studies [4].

On the other hand, a sensor is a device which consists of two main parts: receptor layer, where is proceeded the recognition of an analyte and converter, which receives a signal from the receptor layer and converts it to a measurable signal. A biosensor is a chemical sensor, in which the sensitive layer is a biologically active material. Through these two elements, the biosensors can be divided into two categories: due to the recognition element (*e.g.*, immunosensor) and due to the transducer (*e.g.*, optical sensor). The optical sensor transforms changes of optical phenomena, which are the result of an interaction between the analyte and the receptor part. There can be optical sensors based on, *e.g.*, fluorescence, where the positive emission effect caused by irradiation is measured.

Catecholamines play an important role in the human body as neurotransmitters and hormones. The main mediators of the autonomic nervous system, which stimulates the sympathetic nervous system are catecholamines: dopamine, epinephrine, adrenaline and noradrenaline. These compounds are synthesized in the neurons (nerve endings of the sympathetic neurons) and the adrenal medulla.

Dopamine (DA) is a very important neurotransmitter, which mainly occurs in the brain and central nervous system of mammals. DA is responsible for the transmission of information through the nervous system and plays an important role in processes of learning or memory. Detection of dopamine is significant for diseases associated with the central nervous system such as Parkinson or schizophrenia [4]. This is why rapid and sensitive detection of dopamine is extremely important in modern medicine. The detection system for DA can be based on the optical sensor, however, dopamine has no fluorescence properties. It is well known that dopamine tends to self-polymerize to polydopamine (pDA) in alkaline environment, which is highly controllable by adjusting the pH value. This pDA complex possesses fluorescence capability and can be used for the optical measurements. This kind of mechanism assumes the ability of DA to be attached onto surfaces of solids, including nanomaterials, to form thin pDA film. Thus, in such detection process, the addition of DA induces its self-polymerization forming pDA, which is then spontaneously adsorbed on the surface of GQDs – which induce Förster resonance energy transfer (FRET) and fluorescence quenching [5].

FRET describes an energy transfer mechanism between two fluorescent molecules. A fluorescent donor is excited at its specific fluorescence excitation wavelength. By a long-range dipol–dipol coupling mechanism, this excitation is nonradiatively transferred into another molecule (the acceptor), while the donor returns to the electronic ground state. The FRET occurs when: fluorescence emission spectrum of the donor overlaps with the absorption spectrum of the acceptor and when the distance between the donor and the acceptor is smaller than 10 nm. In this case, the polydopamine molecule is the energy acceptor, and quantum dots – the energy donor [6].

In the presented optical sensor for detection of dopamine, the graphene quantum dots (GQDs) are used. In the sensor, the dopamine molecules coat the GQD surface – as a result, the quenching of fluorescence due to FRET occurs. The aim of this research was to examine the possibility of application of GQDs to determine pDA concentration in the nanoliter volume range. The presented optical sensor was fabricated with LTCC

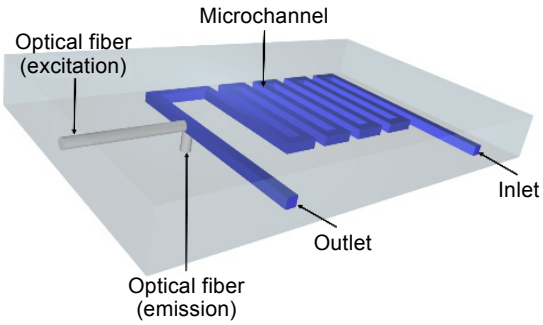

Fig. 1. Model of the optical sensor.

(low temperature co-fired ceramics) technology [7]. This technology has already been successfully applied to the fabrication of similar sensors [8, 9]. The presented optical sensor consisted of a microfluidic channel and two optical fibers connected with a light emitting diode (LED) and photodetector. 3D model of the sensor is presented in Fig. 1.

2. Technology and methods

2.1. Technology

The fluorescence-based sensor was fabricated using LTCC technology. It was composed of eight ceramic layers. Each of them was made of 254 μm-thick DP951 PX LTCC tape. Microchannels for the analyte and openings for optical fibers were cut in unfired LTCC material using UV laser (LPKF Protolaser U). Structured ceramic tapes were laminated using an isostatic press (5 MPa, 70°C) and co-fired (air atmosphere, T_{max} = 850°C). In the final step, two PMMA optical fibers were glued to the fired LTCC structure. The LED (Optosource 260019) with peak wavelength at λ_{max} = 370 nm and spectral halfwidth equal to $\Delta\lambda_{1/2}$ = 12 nm was used as a source of the excitation light. The emission was measured by a light-to-digital converter (TCS 3414) attached to the optical fiber. The TCS 3414 photodetector has a maximum spectral responsivity at λ_{max} = 460 nm with $\Delta\lambda_{1/2}$ = 35 nm.

2.2. Methods

2.2.1. Preparation of graphene quantum dots (GQDs)

To obtain graphene quantum dots, crystal citric acid was used. In a beaker 2 g of citric acid was melted (200°C) in 30 min. The next step was the dispersion of melted citric acid in NaOH solution (100 ml). To the beaker with sodium hydroxide, which was stirred on a magnetic stirrer, small portions taken with a pipette were dispersed in solution of NaOH.

2.2.2. Fluorescence intensity measurement

Firstly, dopamine solutions were prepared. In Tris buffer pH = 9.5 dopamine was dissolved to obtain several various concentrations (1–40 μM). To each solution was added a solution of GQDs in proportion 9:1. Each sample was closed with parafilm, wrapped

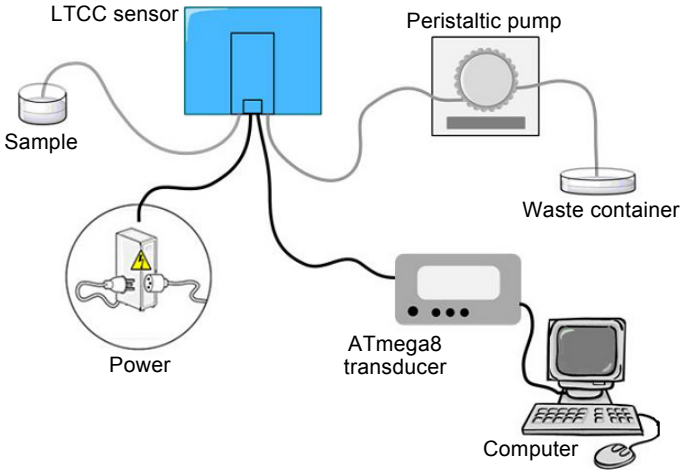

Fig. 2. Measurement setup.

in aluminum foil (to avoid dopamine oxidation) and incubated for 1 hour in 60°C on a magnetic stirrer. The measurement setup, presented in Fig. 2, consisted of a peristaltic pump, DC power supply and computer for data acquisition. UV light emitting diode, and light-to-digital converter were supplied by DC signal (max. LED current 15 mA). Measurement of the output digital signal was performed using the computer. The detection of the substrate, in this case pDA with quantum dots, was performed in the flow system. Test solution was pumped to the LTCC module, wherein a light emitting diode was illuminating the sample flowing through the flow channel, causing the fluorescence of the compounds present in the solution. The florescent light was collected by the light-to-digital converter connected to ATmega8-based transducer. It transmitted a digital signal using two wire interfaces (TWI) to the computer. More details about the transducer can be found in [10].

3. Results

In order to examine the absorption range of polydopamine and its fluorescence ability, UV–vis spectra of pDA were recorded. As it is shown in Fig. 3, the maximum of absorbance is around 380 nm, in UV range, and decreases with increasing concentration of polydopamine.

Atomic force microscope (AFM) was used to investigate the prepared graphene quantum dots. The exemplary image of GQDs is presented in Fig. 4. As it can be seen, the dots are characterized by a rounded shape and the height of a single dot is around 3.5–4.0 nm, therefore its dimension is in the nanocrystal range.

In order to investigate the influence of polydopamine concentration on quantum dots quenching, fluorescence intensity changes were measured. The preliminary measurement showed that the applied UV LED was sufficient to excite the GQDs and to

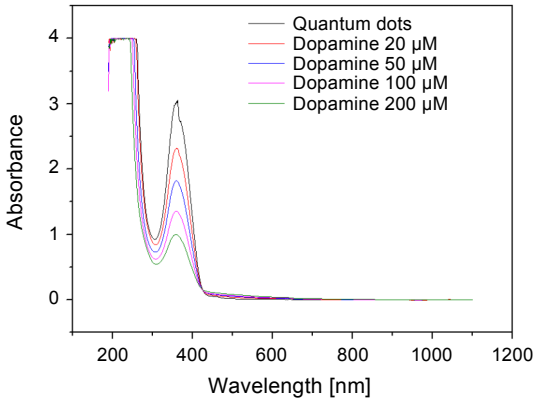

Fig. 3. UV–vis spectra of quantum dots and dopamine concentrations equal to 20, 50, 100 and 200 µM.

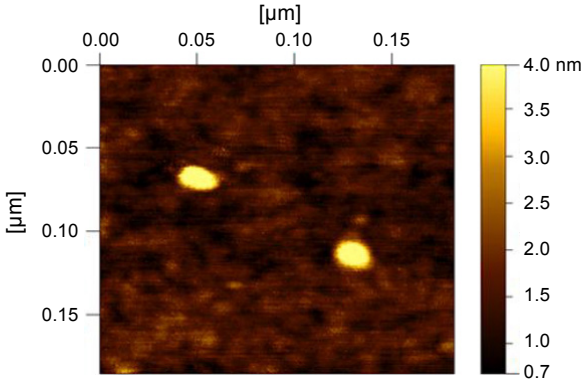

Fig. 4. The AFM image of the exemplary GQDs.

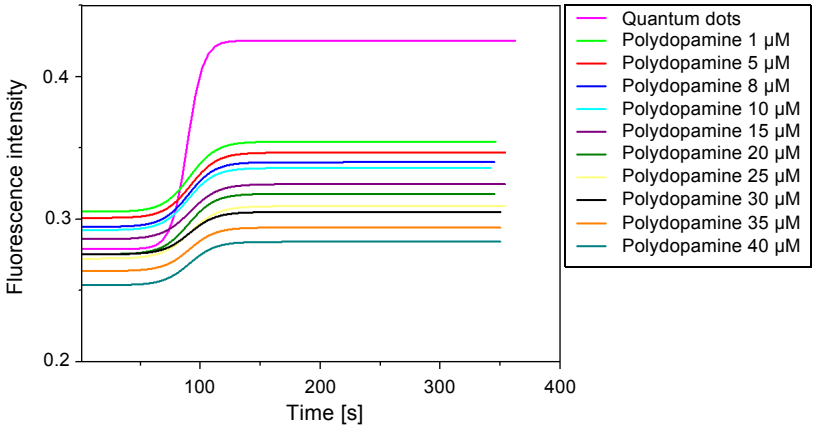

Fig. 5. QDs quenching with various concentrations of pDA.

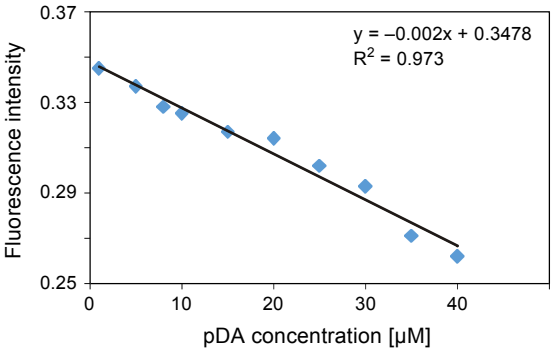

Fig. 6. Calibration curve of the LTCC-based optical sensor.

measure their fluorescence using an applied light-to-digital converter. The aim of the measurements was to investigate the effect of pDA presence on fluorescence quenching of graphene quantum dots. As it is shown in Fig. 5, fluorescence quenching is quite proportional to the initial concentration of pDA in the test sample – the higher concentration, the lower fluorescence intensity.

The calibration curve calculated according to the dynamic response of the sensor is presented in Fig. 6.

The obtained characteristics was almost linear ($R^2 = 0.973$). Its slope (sensitivity) was equal to 0.002 μM^{-1}. The standard deviation of the population (fluorescence intensity) was 1.47×10^{-5}. For the results of quantum dots quenching with various concentrations of polydopamine, the limit of detection (LOD) was calculated as:

$$\mathrm{LOD} = 3\sigma/a$$

where σ is a standard deviation of population, and a is a slope of the regression line (the absolute value). The LOD was estimated to be 0.022 μM.

The obtained result allows for quite precise detection of polydopamine presence in the sample using the proposed method, which is based on fluorescence quenching of graphene quantum dots.

4. Conclusions

The paper reported the design, fabrication and characterization of an optical sensor for determination of polydopamine concentration in a liquid sample. The device was fabricated using a well-known LTCC microelectronic technology.

For detection of the pDA, the method basing on fluorescence quenching of the graphene quantum dots was applied showing that changes in fluorescence correspond to specific concentrations of the neurotransmitter in the test sample. Due to that, it was possible to accurately determine the dopamine concentration in the sample, what can be helpful for recognition of, *e.g.*, Parkinson's disease.

The presented optical LTCC-based sensor utilizes low-cost, miniature and commercially available optoelectronic components (*e.g.*, UV LED, light-to-digital converters, polymeric optical fibers) for operation. Therefore, it can be used to construct a universal and inexpensive microfluidic system for neurotransmitters detection using other biochemical compounds.

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