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# Fluorescent ZnCdS nanoparticles for glucose sensing

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**Abstract.** The effect of glucose on fluorescence of synthesized ZnCdS nanoparticles in the presence of glucose oxidase or in a mixture of glucose oxidase and peroxidase has been investigated. Behavior of fluorescence characteristics of ZnCdS nanoparticles with nonstabilized surface and coated with polymer shell is compared. It has been shown that, for uncoated ZnCdS nanoparticles, hydrogen peroxide formed by glucose oxidation with glucose oxidase causes static quenching of the nanoparticle fluorescence. A quenching mechanism is proposed in which surface centers of fluorescence, which include cationic vacancies, trap oxygen ions supplied by hydrogen peroxide. It has been shown that the linear Stern–Volmer plot has no threshold within the investigated concentrations of glucose. The sensitivity of ZnCdS nanoparticles to glucose, determined from the slope of linear Stern–Volmer plot, is maximum for polymer-coated nanoparticles and is 12.2 ml/mg. With peroxidase, there is a threshold concentration of glucose (160  $\mu$ M) below which the nanoparticles become insensitive to glucose. © 2014 Society of Photo-Optical Instrumentation Engineers (SPIE) [DOI: 10.1117/1.JBO.19.1.011020]

Keywords: glucose; glucose oxidase; peroxidase; hydrogen peroxide; fluorescent semiconductor ZnCdS nanoparticle; spectroscopy; quenching.

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## 1 Introduction

At present, nanomaterials are widely used in biosensors. Properties of various nanostructures (nanotubes, nanofibers, nanorods, and nanoparticles) are studied for their application in biosensors.

At the moment, nanoparticles and nanofilms are the most studied. Optical biosensors are most common among nanoparticle biosensors. Optical biosensors have several advantages over other types of sensors: a high speed and sensitivity, chemical and thermal stabilities, and insensitivity to electromagnetic and radiated interference. They are pretty accurate and have a small size and relatively low cost.

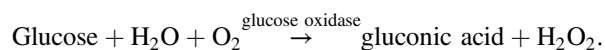
Among optical biosensors, fluorescent ones are the most sensitive because of the absence of background and significant signal-to-noise ratio. The basis of such sensors is an analysis based on photoluminescence excited by UV radiation, the sources of which are mercury-quartz and xenon lamps and lasers. Fluorescence is detected visually and by photoelectric method with selection of the spectral range of fluorescence. Fluorescence characteristics change under the effect of the environment. This suggests that certain substances have an effect on the fluorescent nanoparticles. Quantitative analysis is based on the dependence of the fluorescence intensity of nanoparticles on the amount of these substances.

Recent efforts of several researchers have focused on the development of fluorescence sensors based on nanoparticles, in particular semiconductor nanoparticles, for glucose detection.<sup>1–9</sup> Besides fluorescent nanoparticles, components which are directly responsive to the presence of the analyte are necessary. One way to recognize glucose is to use glucose oxidase.

The response time can be decreased, and shelf life of the biosensor can be increased, by using immobilization of enzyme onto the nanoparticles.<sup>10</sup> It should be noted that such immobilization affects enzyme activity.

The working principle of the optical biosensor based on fluorescent semiconductor nanoparticles is quite simple. Prepared suspension of nanoparticles with glucose oxidase (in this case), a drop of blood, and light-emitting diode-photodiode system with a recording device are sufficient to determine the glucose concentration in blood.

Glucose oxidase catalyzes the oxidation of glucose with the formation of gluconic acid and hydrogen peroxide:



In the presence of hydrogen peroxide, nanoparticle fluorescence quenching occurs, the efficiency of which depends on the amount of glucose in blood. To describe the changes in fluorescence intensity, various mechanisms are used. For stabilized nanoparticles, it is assumed that oxidation of shell molecules with removal of oxidation products leads to degradation of shielding of the surface, thus resulting in the loss of fluorescence efficiency. This mechanism is considered, in particular, for thioglycolic acid-capped CdTe nanoparticles<sup>3</sup> or for mercaptopropionic acid-capped CdTe/CdS nanoparticles.<sup>4</sup> The authors<sup>3</sup> also note the possibility of simultaneous multiple mechanisms of fluorescence quenching of the nanoparticles using glucose oxidase. Also proposed is a mechanism based on reversible capture of an electron of hydrogen peroxide by the nanoparticle.<sup>5,6</sup> This creates O<sup>-</sup> anion localized in an anion vacancy and nonfluorescing in the study spectral region. For CdSe/ZnS nanoparticles used as sensors, fluorescence has a recombination character, and fluorescence quenching is due to decreased concentration

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of the charge carriers generated by exciting radiation. At the same time, using enzyme mixture (glucose oxidase and peroxidase), it is proposed to determine glucose concentration from increasing the intensity of glucose oxidase fluorescence (in the region of 525 nm), due to resonance energy transfer from the nanoparticle to the enzyme.<sup>7</sup> Quenching of the enzyme fluorescence is also possible.

Because all the above quenching processes occur on the nanoparticle surface, we should expect greater sensitivity for particles whose fluorescence is caused by surface defects and not by recombination processes. In this case, quenching is due to the changes in the structure of these defects or reducing their amount. To achieve high sensitivity, it requires a significant amount of surface defects nonstabilized by coating with a layer of a stabilizer. At the same time, the use of nonstabilized nanoparticles is limited because of the low intensity of their fluorescence. In this case, we should expect different sensitivity for nonstabilized nanoparticles and for nanoparticles partially stabilized by coating with a porous polymer shell. The porous coating improves fluorescence intensity of surface defects and does not inhibit diffusion of oxygen anions.

The purpose of this study is to investigate the sensitivity of ZnCdS nanoparticles with fluorescent surface defects to glucose using glucose oxidase. Concentration and temperature dependences for nanoparticles with nonstabilized and stabilized surface are investigated.

## 2 Experimental Procedures

Nonstabilized ZnCdS nanoparticles were synthesized from a mixture of 0.2 ml aqueous solution of cadmium chloride ( $\text{CdCl}_2$ , 91 mg/ml) and 1 ml aqueous solution of zinc chloride ( $\text{ZnCl}_2$ , 157.85 mg/ml) and by the addition of 2 ml sodium sulphide solution ( $\text{Na}_2\text{S}$ , 194 mg/ml) at room temperature. In this case, the stoichiometric ratio between the concentration of sulfur and total concentrations of zinc and cadmium was maintained. Zn/Cd ratio specifying the type of the lattice and accordingly of fluorescence centers was 6.79. The nanoparticle surface was not stabilized. We also used ZnCdS nanoparticles coated with polyacrylic acid shell. Polymer shell was formed simultaneously with the nanoparticle synthesis by UV (365 nm) irradiation of the reactor. Synthesis was carried out in a mixture of initial reagents and acrylic acid aqueous solution containing 200 ppm *p*-methoxyphenol inhibitor. The polymerization was induced by radiation of 250-W mercury lamp for 15 min with continuous mixing of the solution. It should be noted that under irradiation of acrylic acid solution without nanoparticles in the same regime no polymerization occurred. Activation of polymerization with the nanoparticles is explained by the capability of semiconductor nanoparticles for photoinitiating polymerization via a free-radical pathway. The coating thickness is automatically stabilized when access for the reagent through a polymer layer is shut off.

The coating stabilizes the fluorescence properties of the nanoparticles and increases the fluorescence intensity. Therefore, aggregation of the polymer-coated nanoparticles does not change their fluorescence properties. The sensitivity of the aggregated uncoated nanoparticles to glucose is decreased. Heating the nanoparticle suspension to 70°C destroys these aggregates. For all types of nanoparticles, this is confirmed by decreasing the nanoparticle size measured. Heating the ZnCdS nanoparticle suspension as well as measuring the nanoparticle size were carried out using a meter of nanoparticle size

based on dynamic light scattering (ZetasizerNano-Z, Malvern Instruments Ltd., Malvern, United Kingdom) with a 532-nm laser.

Synthesized nanoparticles were repeatedly centrifuged at 2000 rpm and were washed with distilled water after each centrifugation. We used analytical grade reagents as such.

For the investigations, the following reagents and materials were used: (a) suspension of ZnCdS nanoparticles in water; (b) aqueous solution of a mixture of glucose oxidase (12.5 U/ml) and peroxidase (2.5 U/ml); (c) aqueous solution of glucose oxidase (Sigma G7016) (2 U/ml); and (d) aqueous solution of 1.3 mg/ml glucose.

To determine the influence of peroxidase, all experiments were performed with two aqueous solutions: (1) solution of a mixture of glucose oxidase and peroxidase; and (2) solution of pure glucose oxidase. Both solutions were mixed with a suspension of nanoparticles.

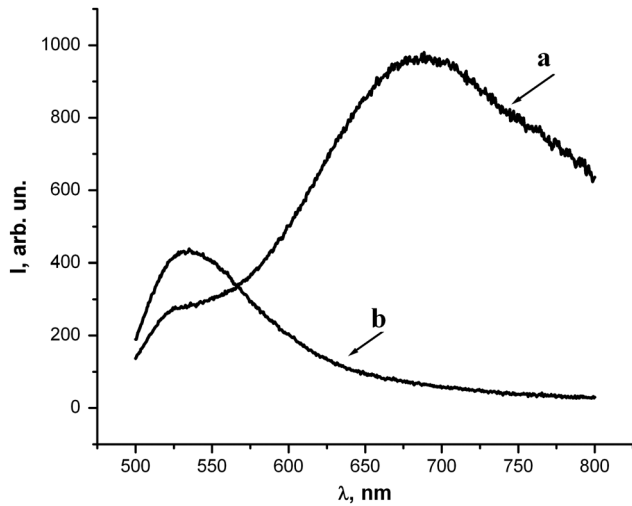
The absorption spectra of the synthesized nanoparticles were recorded by means of a double-beam Lambda 950 spectrophotometer (PerkinElmer, Waltham) using the integrating sphere. The spectra of fluorescence and excitation were measured using a LS-55 luminescence spectrometer (PerkinElmer, Waltham). The spectra were corrected taking into account the power of exciting radiation and the spectral sensitivity of the device. These spectra were recorded with removal of the scattered component by the use of polarizing elements. The last reduces the background, improves the accuracy of measurement, and reduces the measurement limit. Sample fluorescence was excited at a wavelength of 400 nm. This value corresponds to the position of the absorption edge of nanoparticles obtained from the absorption and excitation spectra of the samples. All measurements were carried out at room temperature.

The quenching process was determined from reducing the intensity of fluorescence spectrum of the sample at the maximum with increasing the concentration of glucose in steps of  $1.5 \times 10^{-2}$  mg/ml (83  $\mu\text{M}$ ).

## 3 Experimental Results and Discussion

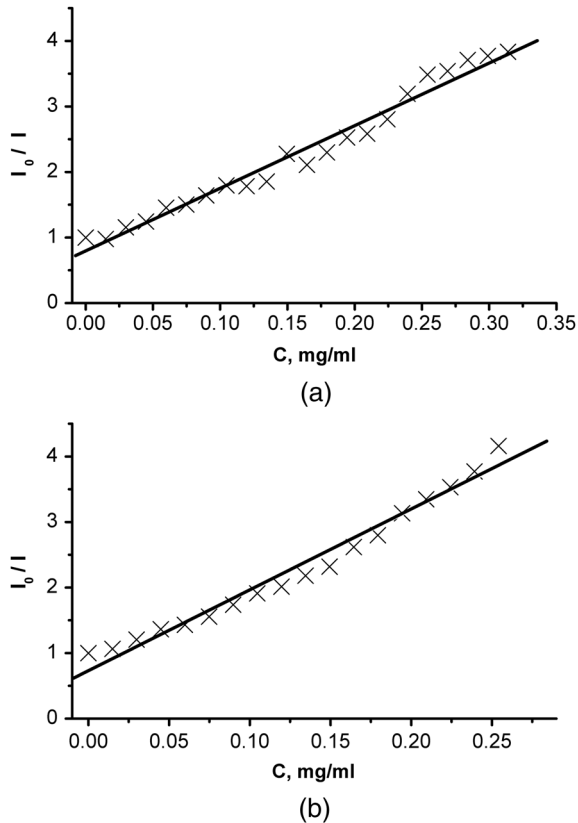
During the experiments, it was found that the glucose without the enzyme does not affect the fluorescence intensity of the ZnCdS nanoparticles.

Figure 1 shows the changes in a fluorescence spectrum of the sample containing uncoated nanoparticles and glucose oxidase, caused by the addition of glucose. Without glucose, a band with a maximum at 690 nm and a shoulder at a wavelength of 530 nm are observed (curve *a*). The fluorescence in the region of 530 to 560 nm is explained by the presence of oxygen-cation vacancy complexes, as synthesis was carried out under air without evacuating oxygen from the solution. The band in the region of 690 to 700 nm can be attributed to isolated cation vacancies. When we add glucose into the sample, the fluorescence band with the maximum at 690 nm is gradually suppressed, and the band with the maximum at 530 nm increases (curve *b*). This suggests the capture of oxygen ions from hydrogen peroxide by cation vacancies. As a result, the concentration of isolated cation vacancies decreases with increasing concentration of oxygen-vacancy complexes. Similar changes occur, with significantly higher sensitivity, in the spectra of the polymer-coated nanoparticles. This suggests that the fluorescence-quenching processes are similar for the uncoated and coated ZnCdS nanoparticles.



**Fig. 1** Fluorescence spectra of the mixture of uncoated ZnCdS nanoparticles and glucose oxidase (a) in the absence and (b) in the presence of 3 mM of glucose.

Glucose concentration can be determined by the changes in the fluorescence intensity of the solution of glucose oxidase with ZnCdS nanoparticles by adding glucose. With the increase in the concentration of glucose, proportional decrease of the fluorescence yield occurs. When Stern–Volmer variables are used (quenching efficiency  $I_0/I$  of the fluorophore as a function of quencher concentration  $C$ , where  $I_0$  and  $I$  are the fluorescence intensities of fluorophore in the absence and presence of



**Fig. 2** Stern–Volmer plots for glucose quenching of suspended ZnCdS nanoparticles (a) uncoated and (b) polymer coated, with the addition of glucose oxidase.

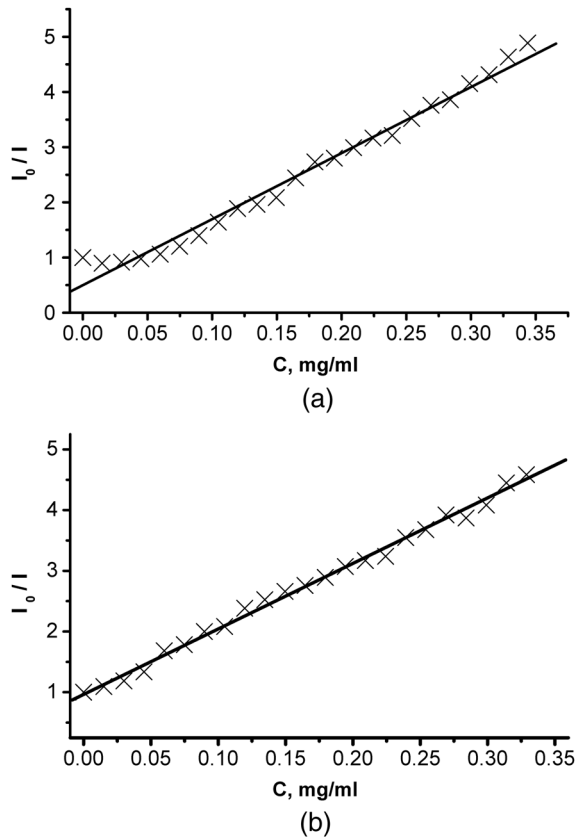
quencher, respectively), the experimental data for uncoated nanoparticles are approximated by a linear dependence [Fig. 2(a)]. Linearity is observed in the glucose concentrations 0.015 to 0.36 mg/ml. This linear dependence indicates the existence of one type of fluorophores in the solution,<sup>11</sup> which are quenched by hydrogen peroxide. In this case, the fluorescence quenching can be the result of both dynamic processes, namely the diffusional collisions between the fluorophore and quencher and the formation of nonfluorescent complexes of the fluorophore and quencher in the electronic ground state (static quenching). However, the above data on changes in the fluorescence spectra of the solutions in the quenching process favor the static quenching. For the polymer-coated nanoparticles [Fig. 2(b)], the quenching does not follow a linear Stern–Volmer plot. This can be explained by a variation of the degree of polymer shell swelling and, therefore, the accessibility of hydrogen peroxide to the nanoparticle surface.<sup>12</sup> The presence of the two processes leads to a distortion of linearity.

Differences in the plot behavior are observed first of all for the type of the enzymes used. When pure glucose oxidase is used, linear dependence is observed starting with the minimum glucose concentration used in the experiment ( $83 \mu\text{M}$ ). The presence of peroxidase leads to the appearance of a threshold concentration of glucose ( $160 \mu\text{M}$ ) below which the nanoparticles become insensitive to glucose. It is our assumption that the threshold is due to the oxidation of hydrogen peroxide by peroxidase in the sample, which reduces the interaction between hydrogen peroxide and the nanoparticle surface. The sensitivity of ZnCdS nanoparticles to glucose was defined as a proportionality coefficient between the relative fluorescence intensity ( $I_0/I$ ) of the nanoparticles and glucose concentration, i.e., was determined from the slope of linear Stern–Volmer plot. It was nine times high as using only glucose oxidase (1.05 ml/mg for the enzyme mixture and 9.68 ml/mg for glucose oxidase). Nonstabilized nanoparticles easily aggregate, increasing the distance between aggregates. Heating the nanoparticles at  $70^\circ\text{C}$  led to a change in their size from 776 to 60 nm. For heated nanoparticles, sensitivity increased slightly and leveled for both types of nanoparticles (12.1 and 10.7 ml/mg, respectively) (Fig. 3). However, the threshold concentration remains, when the mixture of enzymes is used.

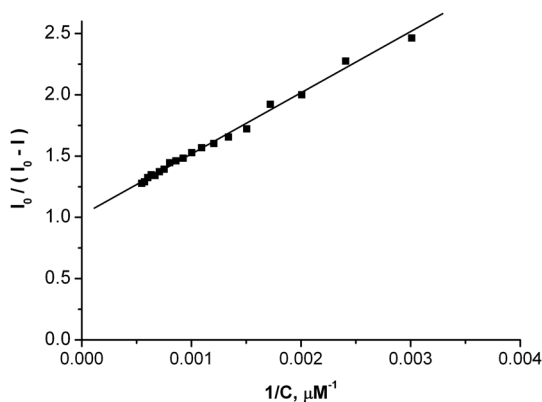
When polymer-coated nanoparticles and glucose oxidase are used, sensitivity increases slightly (Fig. 2) and becomes comparable with the values obtained for the heated nanoparticles. This is due to their stabilization, small size, and extended surface. Sensitivity relation remains almost the same as for the unheated particles (1.43 ml/mg for the mixture of enzymes and 12.2 ml/mg for glucose oxidase). It should be noted also that the fluorescence intensity is much higher for coated nanoparticles, which increases the measurement stability.

To test the assumption that in the solution there is one type of fluorescence centers quenched by  $\text{H}_2\text{O}_2$ , we have presented the experimental data for uncoated ZnCdS nanoparticles [Fig. 3(b)] as  $I_0/(I_0 - I)$  versus  $1/C$ , i.e., using the modified Stern–Volmer coordinates<sup>9</sup> (Fig. 4).

In the case of two types of fluorescence centers, the total fluorescence in the absence of quencher is given by sum of one or more partial fluorescence intensities. Under the action of the quencher, the intensity of the quenched fraction is decreased according to the Stern–Volmer equation. The second type of centers is not quenched. Therefore, the recorded intensity is given by<sup>11</sup>



**Fig. 3** Stern–Volmer plots for glucose quenching of suspended ZnCdS nanoparticles uncoated heated up to 70°C, with the addition of (a) the mixture of enzymes and (b) glucose oxidase.



**Fig. 4** Modified Stern–Volmer plot for glucose quenching of suspended ZnCdS nanoparticles uncoated heated up to 70°C, with the addition of glucose oxidase.

$$\frac{I_0}{I_0 - I} = \frac{1}{f_a K_a C} + \frac{1}{I_a},$$

where  $K_a$  is the Stern–Volmer quenching constant, and  $f_a$  is the fraction of the initial fluorescence centers that are accessible to quencher.

$$f_a = \frac{I_{0a}}{I_{0b} + I_{0a}}.$$

This modified Stern–Volmer equation allows to determine  $f_a$  and  $K_a$ . A plot of  $I_0/(I_0 - I)$  versus  $1/C$  yields  $f_a$  as an

intercept and  $(f_a K_a)^{-1}$  as a slope. The intercept represents the extrapolation to infinite quencher concentration ( $1/C = 0$ ). The value of  $I_0/(I_0 - I)$  at this quencher concentration represents the reciprocal of the fluorescence that was quenched. At high-quencher concentration, only the inaccessible fraction will fluoresce. The plot yields a straight line. Y-intercept is equal to 1, and therefore 100% of the fluorescence in the region of 690 nm is accessible for quenching by  $H_2O_2$ . It is arguable that there is only one mechanism of fluorescence quenching of the investigated nanoparticles, and there are no quenched centers fluorescing in the region of 690 nm.<sup>11</sup> Slope angle gives the same value of the quenching constant as for normal Stern–Volmer coordinates ( $500 \mu M^{-1}$ ).

## 4 Conclusions

It is shown that, for uncoated and polymer-coated ZnCdS nanoparticles, hydrogen peroxide formed by glucose oxidation with glucose oxidase causes quenching of surface centers of fluorescence through trapping oxygen ions by cationic vacancies. The linear Stern–Volmer plot has no threshold within the investigated concentrations of glucose. The sensitivity of ZnCdS nanoparticles to glucose, determined from the slope of linear Stern–Volmer plot, is maximum for polymer-coated nanoparticles and is 12.2 ml/mg. With peroxidase, there is a detection limit of 160  $\mu M$  for analyzing glucose.

## Acknowledgments

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