USE OF Gd-DOPED ZnO SURFACES TO INCREASE FLUORESCENCE OF GLOBULAR PROTEINS

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Abstract

Novel ZnO - Gd^{3+} materials were prepared by sol-gel method and photoluminescence properties of their films were studied for biomedical application. Biosensor's properties ZnO - Gd^{3+} films were investigated under the action of rabbit immunoglobulin G (IgG) in concentrations 10^{-9}, 10^{-11} and 10^{-13} M in solution depositing on the surface of film. The doping of ZnO film by Gd^{3+} in an amount from 1-12 mass.% with increments of 1 mass. % results in significant variations of fluorescence spectra ZnO - Gd^{3+} films. The ZnO substrate without of Gd^{3+} compounds allows detecting IgG in a concentration of 10^{-11} M by fluorescence spectroscopy method by registration in UV region (320-450 nm). By using ZnO - Gd^{3+} system it was obtained the increase the concentration limit of immunoglobulin (10^{-13}M) detection. In the only case (the content of Gd^{3+} in ZnO film - 8 mass. %) was observed batochromic shift of fluorescence band maximum by 20 nm (from 364 to 385nm) when interacting with immunoglobulin regardless of the concentration of the last.

Keywords: ZnO, gadolinium, sol-gel method, photoluminescence, fluorescence, immunoglobulin G, IgG, biosensor

1. INTRODUCTION

Nowadays attention of scientists mostly devoted to physical and optical properties of thin films of transition elements. Zinc oxide is one of the commonly used compounds for the creation of thin films where surfaces are structured on the nanosized level [1]. The presence of the narrow intensive line of ultraviolet fluorescence (UF) in the nanosized films of oxide zinc [2] serves as basis for the development of sensing devices [3,4]. The properties of ZnO films depend on conditions of its preparation [5], presence of dopants [6-9], and size of particles on its surface [1]. In general, the doping by the certain ions is the effective mean of enhance of properties of semiconductor nanoparticles. Doped nanoparticles of the oxide zinc stimulate great interest due to their unique optical priorities and high quantum yield of radiation [8, 9].

In the present work for the first time, we obtained zinc oxide films doped with various amounts of gadolinium (ZnO (Gd)) by sol-gel method and analyzed the optical characteristics by fluorescence spectroscopy. The application of immunoglobulin G (IgG) on their surface was studied, the dependence of the intensity of UV films of ZnO (Gd) on the concentration of IgG was determined.

2. EXPERIMENT

ZnO thin films doped gadolinium (Gd) were synthesized by using sol-gel method [10]. For the preparation of 0.5 M precursor sol zinc nitrate $Zn(NO_3)_2 \cdot 6H_2O$ and gadolinium nitrate $Gd(NO_3)_2 \cdot 6H_2O$ were dissolved in ethanol (CH_3CH_2OH) at room temperature for a period of one hour. Monoethanolamine ($HOCH_2CH_2NH_2$) was added as a stabilizer at a molar ratio of 1.0.

For the deposition of zinc oxide films, doped gadolinium, a 20x20 mm quartz substrates were used. All quartz substrates were cleaned to create a hydrophilic surface.

The prepared sol was deposed by method of spin coating with the rotation speed of 3000 rpm in the centrifuges. Then, films were preheated at 130°C for 15 min to eliminate the organic residuals. Finally, the obtained films were annealed in air at 450°C for one hour in a muffle furnace.

Rabbit immunoglobulin (Sigma-Aldrich) was used in concentrations of 10^{-9}M, 10^{-11}M and 10^{-13}M obtained from a base solution of 10^{-6} M by a sequential dilution method. 20 µl of IgG solution was applied on the surface of ZnO (Gd) films on a quartz substrate by spin coating method at a 2000 rpm spin speed in the centrifuges "Elekon" CLMN-P10-02 (Russia). Fluorescence spectra of the samples were measured with a spectrofluorometer RF-5300pc (Shimadzu).

Fluorescence intensity was registered on 0.2 nm intervals, with slits of excitation and registration of 3 and 5 nm, respectively. Origin 8.1 software was used for data processing. Spectra processed by an “adjacent averaging” curve smoothing method (number of pixels for averaging is 20) were used for the calculation of maxima of fluorescent bands. The integral fluorescence was evaluated as an area under a curve of a fluorescence intensity vs wavelength relationship at a 320-450 nm wavelength range. The use of integral intensity is convenient for unifying the results, including situations in which various devices with different optical characteristics were used.

3. RESULTS AND DISCUSSION

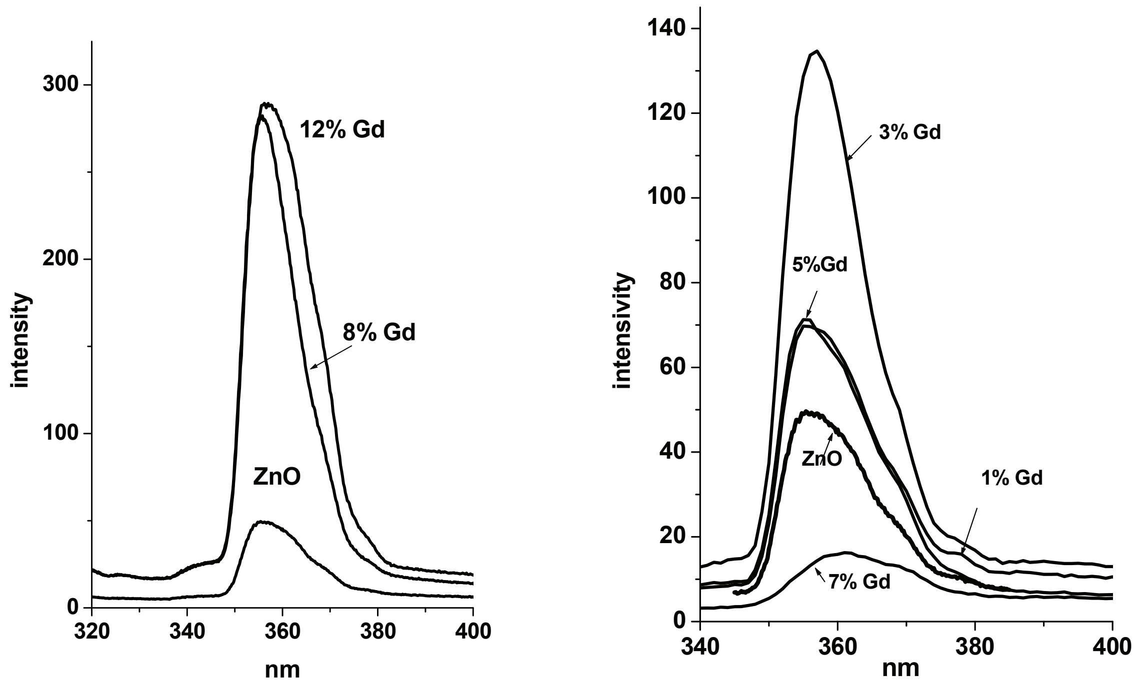

Figure 1 Spectra of fluorescence thin films ZnO and ZnO doped with different concentration of gadolinium

In **Figure 1** spectra of fluorescence thin films of ZnO and ZnO, doped with different concentration of gadolinium with an excitation wavelength of 280 nm are presented (maximum of rabbit immunoglobulin absorption band). Comparing fluorescence’s spectra of Gd-doped samples with the spectra of undoped ZnO sample there can be seen the fact of the increase of intensification of UV band photoluminescence but in the case with of the ZnO - 7% Gd the quenching can be observed. There is inconsiderable shift fluorescence’s spectra in ZnO samples doped Gd^{3+} on average 2 nm in comparison with ZnO film. Small quantities of Gd^{3+} dopant at 1, 3, 5 % result to increase of intensification of fluorescence at 1.5-3 times, Gd^{3+} dopant of 7 % quench the fluorescence at 3 times in comparison with fluorescence of undoped ZnO sample, but in the Gd^{3+} samples of 8 % and 12 % the intensity of fluorescence is increased by six times.

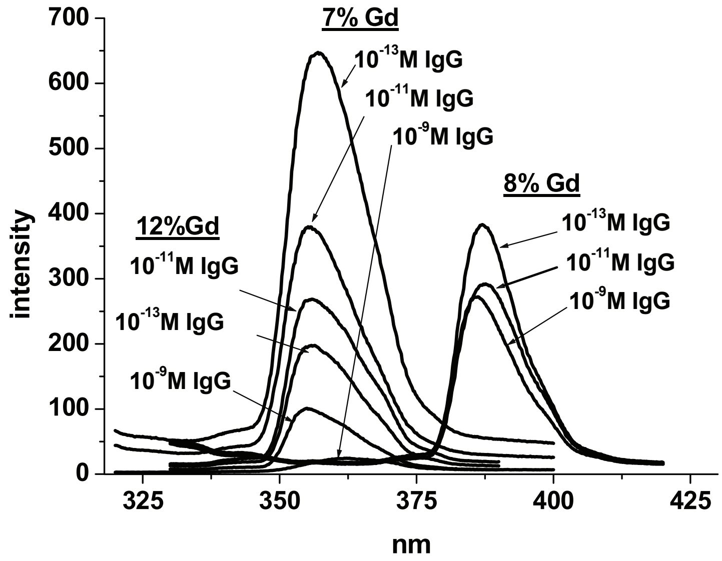

Figure 2 Dependence of fluorescence intensity with different concentration of immunoglobulin on the ZnO films, which contain different concentration of gadolinium

In **Figure 2** the dependence of the fluorescence intensity with different concentrations of immunoglobulin on the ZnO films, which contain different concentration of gadolinium, is presented.

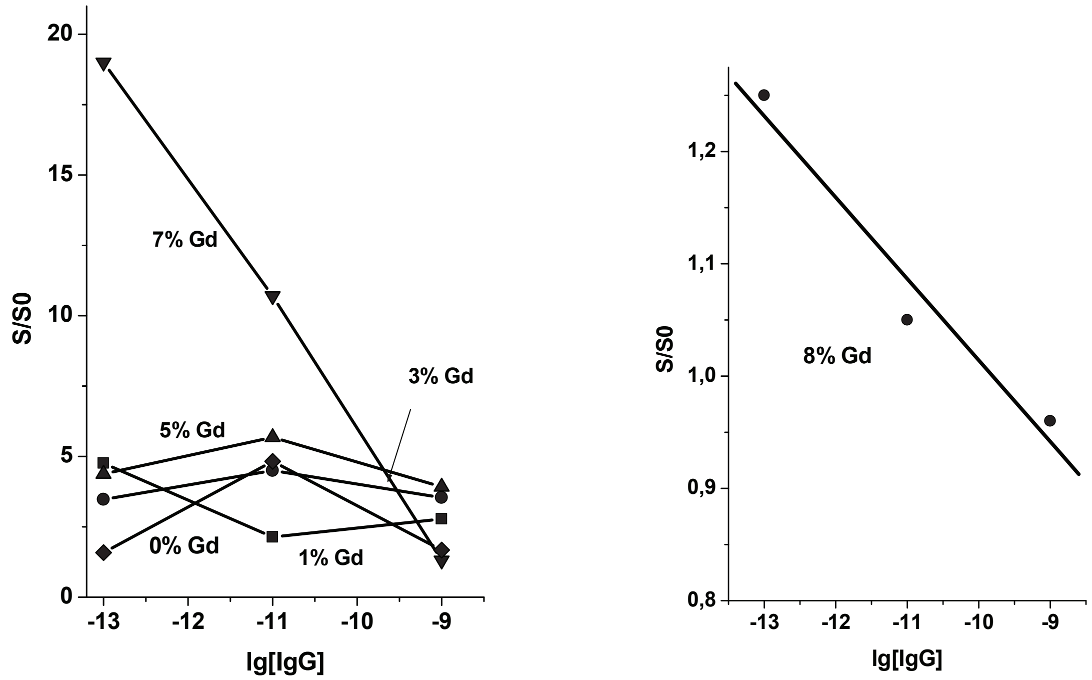

Figure 3 Dependence of the relative integrated fluorescence intensity with the immunoglobulin concentrations on the ZnO and ZnO films' surface, doped with different concentration of Gd

In samples ZnO containing 8 % Gd and 7 % Gd, the increase of UF intensity are observed in the order of decrease of concentration of immunoglobulin 10^{-9}, 10^{-11} and 10^{-13} M. The interaction between ZnO - 8 % Gd and IgG results to the shifts of UF spectra for all 3 concentrations of immunoglobulin at 32 nm (355 nm for 8%

Gd^{3+} without IgG and 387 nm after the depositing of analyzed IgG concentrations). However, in ZnO - 12 % Gd and ZnO - 7 % Gd samples such effect is absent.

Figure 3 shows the change in the relative integrated fluorescence intensity (S/S_0) in accordance with the concentration of immunoglobulin on the ZnO and ZnO film's surface, doped with different concentration of Gd with the excitation of 280 nm.

The dependences for films of zinc oxide alone and doped Gd -12 %, 8 %, 5 %, 3 % are monotonous itself the, but not linear - at the point 10^{-11} M there is a bend. In samples containing the 7 % and 8 % Gd there is linear dependence. It should be noted that for samples of 7 % and 8 % Gd the fluorescence intensity increases with the decrease of IgG concentration from 10^{-9} M to 10^{-13} M in 1.5 and 12 times correspondingly. The near-linear dependences of fluorescence intensity by the protein concentration and the enhance of fluorescence with decreasing IgG concentration in the analytical samples for 7 % and 8 % Gd films facilitate quantitative analysis for small concentrations. The shift of the fluorescence maximum on 32 nm at interacting with different quantities of immunoglobulin, what is also observed in system ZnO - 8 % Gd, is perspective for the quantitative and qualitative determination of small quantities of immunoglobulin in the sample, what is very important for the development of biosensors.

4. CONCLUSION

The significant changes of UF intensity and shifts of its maximums in nanosized ZnO(Gd) films in case of changes of IgG concentration up to 10^{-13} M permits to speak about a potential opportunity of the quantitative determination of immunoglobulins in case of their interaction with the films' surface.

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