Chapter 22
Metal Oxide Based Biosensors for the Detection of Dangerous Biological Compounds

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Abstract In this report an application of some metal oxide nanostructures as a biosensor platform for the detection of dangerous biological compounds (Bovine leucosis, Salmonella) have been discussed. The attention is paid to the TiO_2 nanoparticles and ZnO nanorods deposited on the flat surface. The changes in photoluminescense signal from nanostructured surface were applied as biosensor response to detect the analytes. The detection range of TiO_2 based biosensor for Bovine leucosis antibodies was in the range of 2–10 μg/ml. The detection range of ZnO based biosensor for Salmonella antigens was 10^2–10^6 cells/ml. The obtained results provide a good basis for the use of optical properties of metal oxide based semiconductor nanostructures in biosensor technology.

22.1 Introduction

During last decades metal oxide nanostructures based on TiO_2 and ZnO are the materials that attract a lot of attention due to their optical, catalytic and sensing applications. Physico-chemical properties of these nanomaterials that can be controlled and changed by growth methods or by modification of nanostructures can play crucial role in sensing application. For these metal oxides, quantum confinement effect caused by nanoscale size, have resulted not only in band gap increase and improved photocatalytic activity but also in photoluminescence peaks appearance at room temperature. Being wide band gap semiconductors that have a good affinity to biological compounds, TiO_2 and ZnO nanostructures are promising materials to be used as optical biosensor transducers [1–5].

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Among various types of biosensors, an immune biosensor is a type, based on specific interaction between antibody (Ab) – antigen (Ag) couple [6]. The reaction between this couples has high specificity and sensitivity to detecting analytes what makes immune biosensors suitable for accurate and precise tests with electrochemical, optical, magnetic and piezoelectric transducers [6–8]. As it is known from the range of works, optical methods of detection based on absorbance, reflectance and photoluminescence demonstrate simple, fast and accurate detection of target analyte [9–14]. In particular, photoluminescence from nanostructured metal oxides is a promising property that can be used for the detection of chemical and biologic compounds [4, 9, 10, 13, 14]. Therefore, the photoluminescence from TiO_2 nanoparticles was used for the detection of Bovine leucosis antibodies.

22.1.1 Application of Photoluminescence TiO_2 Nanoparticles for the Detection of Bovine Leucosis

Bovine leucosis virus (BLV) – is the highly foetal neoplasia of the cattle characterized by the abnormality maturation process of the blood cells [15]. Diagnosis of the BLV infection based on the clinical signs alone is difficult because of the wide range of symptoms. The traditional immune methods have high specificity and sensitivity, but they take a lot of time and require additional parameters such as labelled molecules [16]. To overcome such drawbacks we need to use the modern instrumental analytical devices based on the biosensor technology.

TiO_2 is a material, which is widely applied for different applications [17–22] including sensors and biosensors [20, 23–25]. TiO_2 shows good stability in aggressive environment what makes it attractive for chemical sensors applications [18, 20]. TiO_2 is a wide band gap semiconductor with indirect optical transitions [19]. TiO_2 has low isoelectric point pH = 5.5 what makes some advantages to protein immobilization on its surface [25].

22.2 Experimental

Anatase nanoparticles with mean size 32 nm were used as biosensor template. TiO_2 nanoparticles were solved in water to prepare sols (with concentration 0.05 mg/ml). TiO_2 layers were formed on glass substrates by dropping TiO_2 sols and drying at room temperature with post annealing treatment at 300 C for 1 h was provided to remove water from the samples. SEM measurements showed that nanoparticles formed high surface area porous structure (Fig. 22.1a) that is suitable platform for immobilization of biological species.

Raman spectrometer with Ar/Kr laser (Jobin Yvon-Labram 1B, $\lambda = 647.1$ nm) and spectral resolution 1 cm^{-1} were used for the study of Raman spectra. Raman spectrum of TiO_2 nanostructures, deposited on glass substrates is shown in Fig. 22.1b. The peaks were found at 392, 512 and 634 cm^{-1}, which correspond to B_{1g}, A_{1g} and E_g modes of anatase phase of TiO_2. Surface morphology of

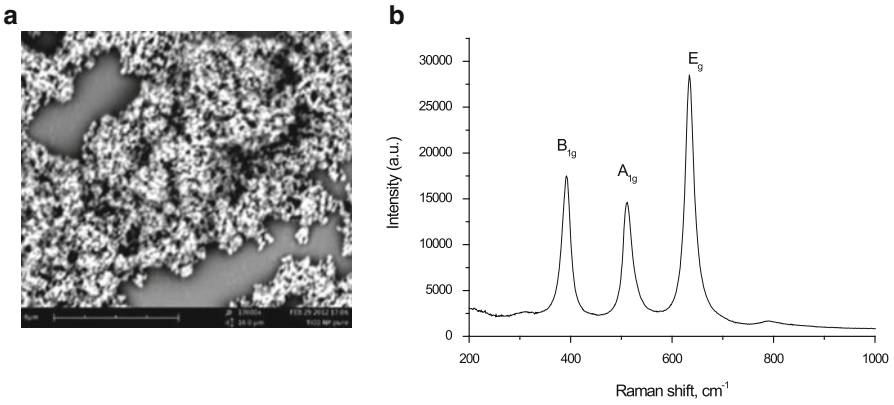

Fig. 22.1 (**a**) – SEM image of TiO_2 nanostructures deposited on glass; (**b**) – Raman spectrum of TiO_2 nanostructures

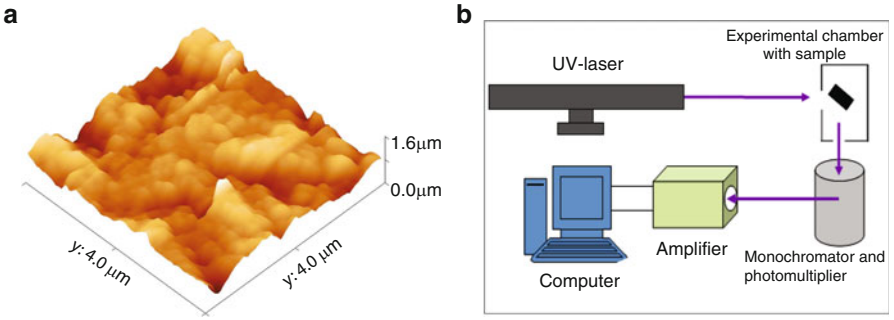

Fig. 22.2 (**a**) – AFM image of surface of TiO_2 nanostructures (Asylum Research MFP-3D); (**b**) – photoluminescence setup

deposited samples has been investigated with AFM (Fig. 22.2a). The obtained TiO_2 nanostructures had high active surface area. Mean square surface roughness (Rsq), measured with free software Gwiddion, was 140 nm for prepared TiO_2 nanostructures. Photoluminescence spectra were measured by setup shown on Fig. 22.2b. The photoluminescence was stimulated by UV laser LCS-DTL-374QT with excitation wavelenth $\lambda = 355$ nm. The emission spectra were amplified and recorded in the range of 370–800 nm.

22.3 Results and Discussion

To fabricate biosensitive layer, the antigens of BLV were immobilized on TiO_2 surface. TiO_2nanostructures were exposed to water solution of BLV antigens (Ag) for 10 min and then were washed two times in distilled water and dried in air at room temperature. The backside of TiO_2 sample was sealed to prevent immobilization of Ag on it.

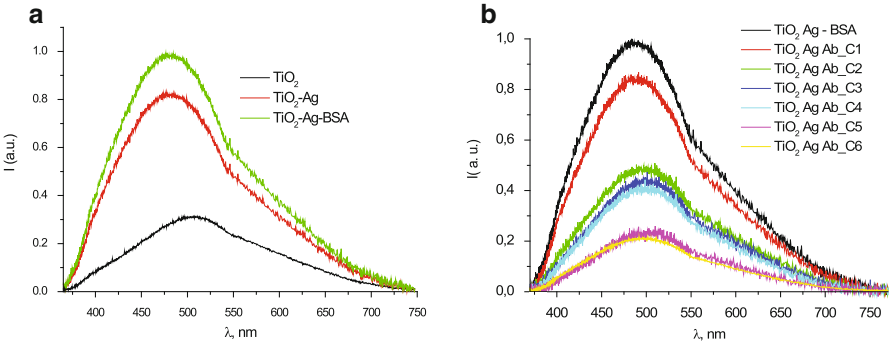

Fig. 22.3 (**a**) – PL spectra of TiO_2 nanoparticles before and after immobilization of BLV antigens, and after BSA adsorption; (**b**) – PL spectra of TiO_2-Ag-BSA layer under different concentrations of BLV Ab

The photoluminescence spectrum of pure TiO_2 samples is characterized by broad peak centered at 510 nm. A number of papers reported on room temperature PL in TiO_2 nanostructures [22, 26–28]. Usually TiO_2 nanostructures demonstrate emission in the range 430–560 nm. Two mechanisms of luminescence are proposed: self-trapped excitons (STE) (430–510 nm) and oxygen vacancies (530–560 nm).

Photoluminescence (PL) spectra of TiO_2 nanoparticles before and after immobilization of antigens are shown on Fig. 22.3a. As one can see, the immobilization of BLV antigens lead to the significant changes of PL spectra in intensity and peak position. It was found that after Ag immobilization PL spectra was shifted to shorter wavelengths, what can be a proof of formation links between TiO_2 and Ag. Increase of PL intensity could result from charge transfer between Ag molecules and conductance band of TiO_2. And the UV shift of PL maximum can be explained by additional dipole-dipole interaction, what can change energetic position of recombination centers into TiO_2. After immobilization of antigens, the bovine serum albumin (BSA) was deposited on the biosensitive layer as a blocking agent to prevent nonspecific protein adsorption. The intensity of PL after BSA adsorption has been increased.

After forming of biosensitive layer by immobilization BLV antigens, the BLV antibodies (which play role of analyte) were deposited on the functionalized surface from water solutions with different concentrations. PL spectra of TiO_2-Ag-BSA biosensor, measured under different Ab concentrations are shown in Fig. 22.3b. It was found that PL intensity decreased with the increase of analyte concentration. At the same time, peak position moved to higher wavelengths.

Thus, the biosensor response to leucosis Ab can be a function of two parameters: PL intensity and position of PL peak. To analyze the sensor response we calculated the changes biosensor signal S according the following equation

$$S = \frac{S_{\text{Ag-BSA}} - S_{\text{Ab}}}{S_{\text{Ag-BSA}}}, \tag{22.1}$$

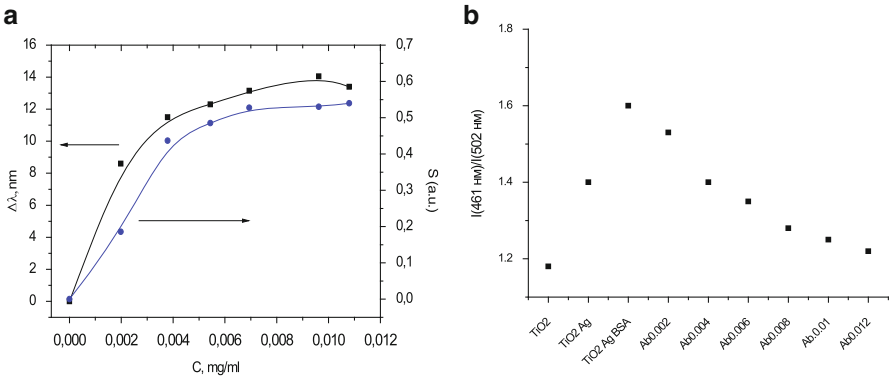

Fig. 22.4 (**a**) – Response of the biosensor signal S and peak shift to different concentrations of BLV antibodies; (**b**) Ratio of $I_{STE}/I_{V[O]}$ for TiO_2 NP before and after interaction with biomolecules

where $S_{Ag\text{-}BSA}$ and S_{Ab} are PL peak's intensities of TiO_2 nanostructures with immobilized BLV antigens before and after interaction with BLV antibodies, correspondently.

The changes of peak position after adsorption of Ab were calculated according following equation:

$$\Delta\lambda = \lambda_{Ag\text{-}BSA} - \lambda_{Ab}, \tag{22.2}$$

where $\lambda_{Ag\text{-}BSA}$ and λ_{Ab} are PL peak positions of TiO_2 nanostructures with immobilized BLV antigens before and after interaction with BLV antibodies, correspondently.

The results, obtained with the use of equations (22.1) and (22.2) are plotted in Fig. 22.4a. The analysis of the results showed that the changes of biosensor parameters had similar behavior. The obtained experimental curves increased at the range of Ab concentrations from 2–10 mkg/ml. The further increase of Ab concentration led to the saturation of signal changes.

It is known that photoluminescence spectrum of TiO_2 nanostructures can be split into two peaks, related to self-trapped excitons (STE) and oxygen vacancies $V_{[O]}$. We performed the fitting of the obtained PL spectra before and after interaction with biological molecules (the fitting is not shown here) and plotted the ratio of PL intensities related to STE and oxygen vacancies (Fig. 22.4b). It was found that the positions of both peaks have not been changed for all steps of the experiment (1–1.5 nm). At the same time the redistribution of the integrated intensity between peaks caused by STE at 461 nm and oxygen vacancies $V_{[O]}$ at 502 nm was observed.

The formation of biosensitive layer and BSA molecules adsorption was accompanied by an intensity increase of the peak at 461 nm and a decrease of the peak at 502 nm, leading to a shift in the overall spectrum to shorter wavelengths. This indicates that the biomolecules adsorption by the surface of TiO_2 reduces

the rate of radiative recombination caused by oxygen vacancies and increases the photoluminescence intensity caused by STE. After immune reaction between antigens and antibodies, the ratio of PL intensities related to STE and oxygen vacancies decreased with increasing concentration of antibodies.

The formation of antibody-antigen complex can lead to the changes in molecular structure of antigens previously immobilized on TiO_2 surface which is accompanied by link changes between antigens and TiO_2 surface.

Successful results were obtained for Salmonella antigens detection using similar procedure and biosensor, based on photoluminescence from ZnO nanorods [29–31]. The studied ZnO nanorods can be used as transducers in optical biosensors for Salmonella detection. The optimal response of the fabricated biosensor is observed at concentrations 10^2–10^6 cells/ml.

22.4 Mechanism of Interaction Between TiO_2 Surface and Bio-molecules

Reaction of TiO_2 with proteins (Ab, Ag and BSA) is due to non-covalent binding. Van der Waals and hydrophobic bonds are suggested as the mechanism of interaction between TiO_2 surface and BLV molecules. An increase of PL emission after biomolecules immobilization is observed as a result of charge transfer from bio-molecules to TiO_2 surface. The proteins are bound to the surface by several functional groups affecting the surface band bending. The changes in UV PL emission after target molecules adsorption could occur due to the immune reaction between Ag-Ab couple. The decrease in intensity of the PL peak after target molecules adsorption is due to elimination and/or weakening of link between TiO_2 and bio-molecules, caused by structural modification of previously adsorbed Ab molecules as a result of antigen-antibody reaction. However, the mechanism of interaction cannot be determined from only measurements of PL. Additional methods, such as XPS confocal microscopy are needed to investigate it.

22.5 Conclusions

Specific selective interaction between immobilized antibodies and antigens couples (‘key’-‘lock’ principle) can be monitored by PL of TiO_2 and ZnO. Photoluminescence from these nanomaterials is a suitable method for characterizing sample surface, surface defects and the changes of the surface properties as a result of biological impact. TiO_2 and ZnO nanostructures can be successfully used as a platform for the immobilization of biologically active substances on their surface which is confirmed by the changes in PL intensity and PL peak shift. Photoluminescence method of analyte detection is not the highest one comparably to SERS, SPR or

fluorescence however it is easier to be applied, does not need label system and can become the next generation of sensing devices. Obtained results provide a basis for the prospective application of metal oxide based nanostrucutures in immune biosensors for rapid diagnosis of such viruses as Bovine leucosis, Salmonella and other dangerous biological species.

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