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Application of Room Temperature Photoluminescence from ZnO Nanorods for Salmonella Detection

Roman Viter, Volodymyr Khranovskyy, Nikolay Starodub, Yulia Ogorodniichuk, Sergey Gevelyuk, Zanda Gertnere, Nicolay Poletaev, Rositza Yakimova, Donats Erts, Valentyn Smyntyna, and Arnolds Ubelis

***Abstract*— ZnO nanorods grown by gaseous-disperse synthesis are confirmed by XRD analysis to have the wurtzite crystal structure. The obtained crystallites, as found from SEM studies, are 57 ± 9 nm in diameter and 470 ± 30 nm long on the average. Two emission bands of photoluminescence from ZnO nanorods observed at room temperature are centered at 376 and 520 nm. A biosensitive layer is prepared by immobilization of *anti-Salmonella* antibodies from liquid solutions on the ZnO surface. Immobilization of the biosensitive layer onto ZnO nanorods is found to increase the intensity of PL. After further reaction with *Salmonella* antigens (Ags), the PL intensity is found to decrease proportional to Ag concentrations in the range of 10^2– 10^5 cell/ml. The possible mechanism of biosensor response is suggested and discussed.**

***Index Terms*— ZnO nanorods, biosensors, photoluminescence, sensor phenomena, characterization.**

I. Introduction

ZINC Oxide (ZnO) has attracted interest of semiconductor material studies during last decades [1]. Due to a wide and direct band gap (3.3 eV), high exciton binding energy (∼60 meV) and band gap engineering possibility from 2.1 to 3.5 eV it is regarded as one of the most prospective materials for photonics and optoelectronics [2]. Furthermore, ZnO nanostructures are expected to be non-toxic and biocompatible, which makes them promising transducers for biosensors [3]–[9].

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Particularly, ZnO nanostructures show good affinity to biological compounds [6]–[8]. Immobilization of biologic compounds has been demonstrated to change electrical properties of the ZnO nanostructures [6], [7]. The forces, involved in interactions between biomolecules and metal oxide nanoparticles have been reported as Van der Waals forces, London dispersion forces (hydrophobic interaction), hydrogen bonds, polarization and lone pair electrons [9]. Attention in recent reports is mostly paid to application of ZnO nanostructures in electrochemical biosensors [4], [5] without regard to good electrical and optical properties.

Salmonella sp. infections is a global problem for humans. These infections may cause mortality of humans as well as of productive animals. *Salmonella sp*. is typically transmitted among humans and animals through the consumption of contaminated food or water. A prompt and accurate detection of these bacteria can prevent the harmful effects.

Essential elements of *Salmonella* monitoring and control are sensitive and highly-specific laboratory and express methods of isolation, identification, and serotyping of the bacteria. These methods have to be rapid, inexpensive, easily reproducible, sensitive, and specific [10], [11]. None of the current laboratory methods satisfies all these criteria and the optimal methods vary depending on the source of specimen and the target serotype.

Our previous research was focused on development of rapid, sensitive and highly-specific biosensor devices for *Salmonella* detection based on different approaches such as surface plasmon resonance (SPR) [12], total internal reflection ellipsometry (TIRE) [13], [14], and ion sensitive field-effect transistors (ISFET) [15]. Nevertheless, all the mentioned immune biosensors have some disadvantages, e.g. high cost of the chips, complicated measurement procedure, etc. Furthermore, the signal recorders are very expensive and do not allow performing the desired number of repeated analysis. The problems motivate looking for design of the other types of biosensors for express immune detection of *Salmonella*.

Photoluminescence (PL) of ZnO is a promising property, that can be used for detection of chemical and biologic compounds [16]. Usually emission spectra of ZnO consist of two luminescence bands: a narrow UV near band edge excitonic emission (NBE) and a broad deep-level emission (DLE) in

the visible range, so called "green" luminescence related to interstitial Zn atoms (Zni) and/or oxygen vacancies (VO) [16].

Recently, photo-luminescent ZnO nanostructures have been successfully used for detection of organic compounds [17], [18]. Adsorption of organic compounds changes the defect concentration and surface band bending. UV and visible PL of ZnO nanostructures quenched as a result of chemical bonding between ZnO surface and the adsorbed molecules [17], [18]. Thus, quenching of photoluminescence by adsorption, bio-functionalization and band bending can be applied for novel immune biosensor applications for detection of toxins.

The present paper reports recent results of photoluminescence of ZnO nano-rods at room temperature used to detect *Salmonella*. As grown ZnO nano-rods were bio-functionalized with biosensitive layer (*Anti-Salmonella* antibodies). The biosensor response to *Salmonella* antigens was obtained by the change of PL intensity of ZnO nano-rods. The mechanisms of interaction between biomolecules and ZnO surface are suggested and discussed.

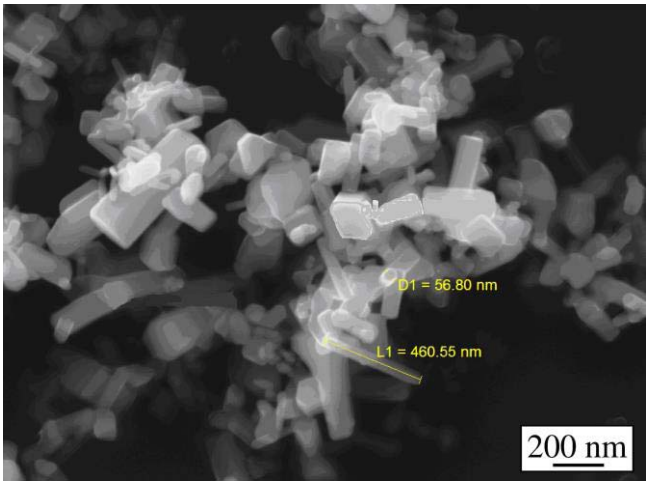

Fig. 1. SEM image of ZnO nano-rods. Diameter and length (D1, L1) are given on the image.

II. Experiment

A. Preparation of ZnO Nano-Rods

ZnO nano-rods were obtained by the gaseous-disperse synthesis (GDS) [19]. The method comprises specially organized two-phase flames of dust clouds of the corresponding metals (pure metals, mechanical mixtures or alloys of different metals). The final product is obtained as a result of condensation of gaseous phase products of burning of metals in the oxidizing ambient.

As grown nano-rods of a powder-like structure were further deposited onto quartz substrate (1×1 cm^2), preliminary processed by ultrasound in in acetone and dried in a nitrogen flow. ZnO nanorods (1 mg) were ultrasonically treated and dispersed in 1-butanol (5 ml) to prepare alcohol solution of zinc oxide nano-rods. The as-prepared solution was dropped on quartz substrate and dried at room temperature for 2 hours. The ZnO nano-rods formed a layer on the substrate, which was further annealed at 300 °C in air for 1 hour.

B. Characterization of Structural and Optical Properties of ZnO Nano-Rods

Structural properties of ZnO nano-rod powder have been investigated by X-ray diffraction spectroscopy (XRD) on a Rigaku Ultima XRD-setup (CuKα, $\lambda = 0.154$ nm). Conventional powder diffraction analysis was performed by acquiring the θ-2θ spectra in the θ range from 10 to 80°.

The microstructure of the obtained nanostructures was studied by scanning electron microscopy a TESCAN microscope being used to obtain microstructure images and to calculate the dimensions of the prepared nano-rods.

Photoluminescence spectra of ZnO nano-rods excited by a LCS-DTL-374QT (Russia) 355 nm solid state laser source at 15 mW/cm^2 were measured by steps of 1 nm in the 370–800 nm range at room temperature.

C. Biosensor Testing

Salmonella typhimurium from State Scientific-Research Control Institute of Veterinary Preparations and Feed Additives of Ukraine was obtained as autoclaved substance dissolved in the 0,85% of NaCl solution in 10^6 cells per 1 ml. *Anti-Salmonella* serum was purchased from St. Petersburg, Russia, Research Institute of Vaccines and Serums. Before deposition of bio-sensitive layer the surface of ZnO nano-rod layer was cleaned by washing with ethanol, then with PBS solution (pH 7.4) and dried in the air flow. Solution of 20 μg/mL of *Anti-Salmonella* serum (*Anti-Salmonella* antibodies (Ab)) was applied for 20 min on the surface of ZnO then washed away with PBS solution the surface being dried as described above. By the procedure the *Anti-Salmonella* antibodies (Ab) on the ZnO surface were immobilized by physical sorption. To prevent non-specific adsorption, Bovine serum albumin (BSA) solution was applied on ZnO surface after immobilization of the *Anti-Salmonella* Ab. After washing and drying the ZnO nano-rod layers were immersed for 20 min into the 0.85% of NaCl solution containing controlled concentration of *Salmonella* antigen (Ag). The appropriate specific immune reaction between *Salmonella* antigens and surface bound antibodies taking place at this stage further ZnO nano-rod layer was washed with PBS solution and dried described above. The PL spectra were recorded before and after each of the preparatory stage.

III. Results and Discussion

A SEM image of the obtained ZnO nano-rods deposited on a quartz substrate is shown in Fig. 1. The nano-rods are of hexagonally faceted elongated shape. The average dimensions of the nano-rods, assessed from SEM study are 470 ± 30 nm long and 57 ± 9 nm in diameter (Fig. 1).

The XRD spectrum of the studied nano-rods consists of reflection peaks at 2θ 31.6°, 34.3°, 36.1°, 47.4°, 56.4°, 62.7°, 67.8°, 72.3° and 76.8° (Fig. 2). The obtained XRD pattern reveals the wurtzite crystal structure of zinc oxide the observed peaks corresponding to reflections from crystal planes: (100), (002), (101), (102), (110), (103), (200), (112), (004), (104) and (202) (Fig. 2). From the Braggs equations for hexagonal crystalline structure we have calculated the lattice parameter

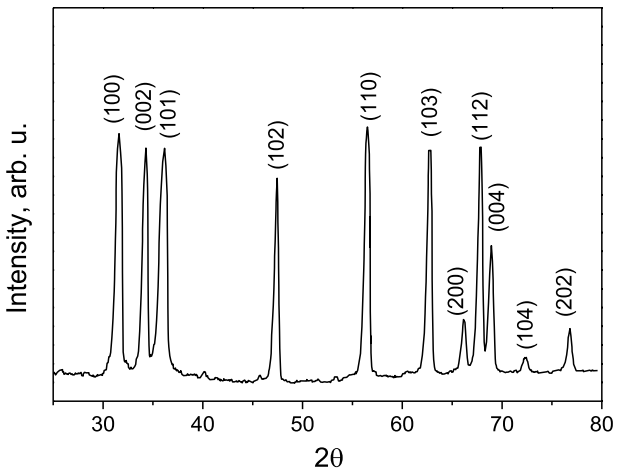

Fig. 2. XRD spectrum of as-prepared ZnO nano-rods: the observed peaks are indicated as reflections from respective crystallographic planes.

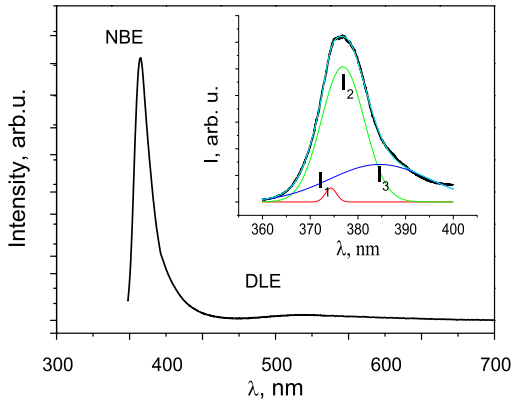

Fig. 3. Room-temperature PL spectrum of as-prepared ZnO nano-rods. The NBE and DLE bands are located at ∼376 and ∼520 nm, respectively. The three components (I1, I2, I3) of the NBE peak separated by Gauss fitting are presented in the inset.

$c = 5.215$ Å. The obtained value agrees well with the reported lattice parameter of ZnO nanostructures [20].

The room temperature PL spectrum of as prepared ZnO nanorods is shown in Fig. 3. The obtained PL spectrum demonstrates an intense maximum at ∼376 nm and a wide non-symmetric peak, centered around ∼520 nm.

Weak intensity of visible luminescence and a high NBE to DLE ratio suggest a good stoichiometry and the concentration of defects in the material being low. A detailed analysis of the NBE peak has shown its multicomponent nature. Three components of different intensities (I_1, I_2, I_3) located at 374, 376 and 384 nm (Inset of Fig. 3) of the resulting peak have been separated by Gaussian fitting. According to reported data, RT PL emission of ZnO is attributed to free and neutral donor bound excitonic transitions (FX and D^0X, respectively), followed by their multiple longitudinal phonon (LO) replicas (1^{st}, 2^{nd} and etc.). The contribution of the single peaks to integral NBE peak strongly depends on the quality and purity of the material (FX vs D^0X) or morphology and surface area of the material (LO replicas intensity). Peak I_1 at 374 nm (∼3.31 eV) can be attributed to FX emission [16] while next intense peak on the lower energy side I_2 at 376 nm (3.298 eV) can be attributed due to the neutral donor bound excitonic emission (D^0X). The third peak I_3 at 384 nm (3.228 eV) can be attributed to the 1^{st} LO phonon replica of the D^0X peak. The distance between D^0X peak and 1^{st} LO phonon replica is around 70 meV, which is close to the respective value for ZnO (∼72 meV).

Detailed analysis of the DLE peak has shown that it is consisted on two components (fitting is not shown here), located at ∼520 nm and 548 nm suggesting different contributions of the different transitions responsible for it. However, since the ratio between NBE and DLE intensities is around 100, we did not focus on this issue in the present study. Thus, NBE peak was chosen as main criterion for the study of PL bio-sensing.

The structural, optical and electrical properties of ZnO nanostructures are strongly interrelated [21]–[23]. Deviation from stoichiometry results in defects associated with zinc or oxygen electric conductivity and DLE intensity increasing in oxygen deficient ZnO nanostructures decreasing NBE to DLE ratio [21]–[23]. The NBE/DLE ratio of ZnO nanostructures increases with improvement of the stoichiometry [23]. However, the light emission properties of ZnO nanostructures are affected by surface layer depletion in case of a high surface to volume ratio [24]. The NBE and DLE intensities are reduced as photo-generated electrons are captured on surface increasing the band bending and excitons dissociate in the electrical field, induced by the surface charge [6], [7], [23]. Therefore, since PL properties of ZnO strongly depend on the electronic structure and the shape of surface band bending, the PL spectra of as grown ZnO nano-rods were recorded to compare with the spectra of ZnO layers immobilized by *Anti-Salmonella* antibodies and spectra obtained after adding *Salmonella* antigens of different concentrations.

To test biosensor response the PL spectra were recorded from pure ZnO, from ZnO with a layer of immobilized *Anti-Salmonella* antibodies, from ZnO with BSA blocking agent, and finally after exposure of ZnO-*Anti-Salmonella* Ab layer to the target *Salmonella* antigens.

Concentration of *Anti-Salmonella* antibodies was chosen to provide significant coverage of the ZnO surface and formation of bio-sensitive layer, while the optimal concentration of *Salmonella* antigens for response was taken as the average value of $\sim 10^5$ cells/ml.

The NBE part of the PL spectrum of ZnO nano-rods after immobilization of *Anti-Salmonella* Ab, BSA blocking and interaction of the biosensor with *Salmonella* antigens is shown in [Fig. 4(a)].

The PL intensity was found to increase after immobilization of antibodies and a weak blue shift being observed mostly of the NBE I_2 component revealing adsorption-assisted increase of exciton-phonon interaction.

The BSA coating of ZnO further increases the PL intensity. Reaction of ZnO:Ab system with *Salmonella* antigens stimulates changes reversing the PL intensity of ZnO. After interaction with antigens the PL intensity decreases being the actual response of the biosensor to *Salmonella*.

Taking into account the complex structure of PL peak of ZnO as revealed by Gauss fitting (Fig. 3) the *Anti-Salmonella*

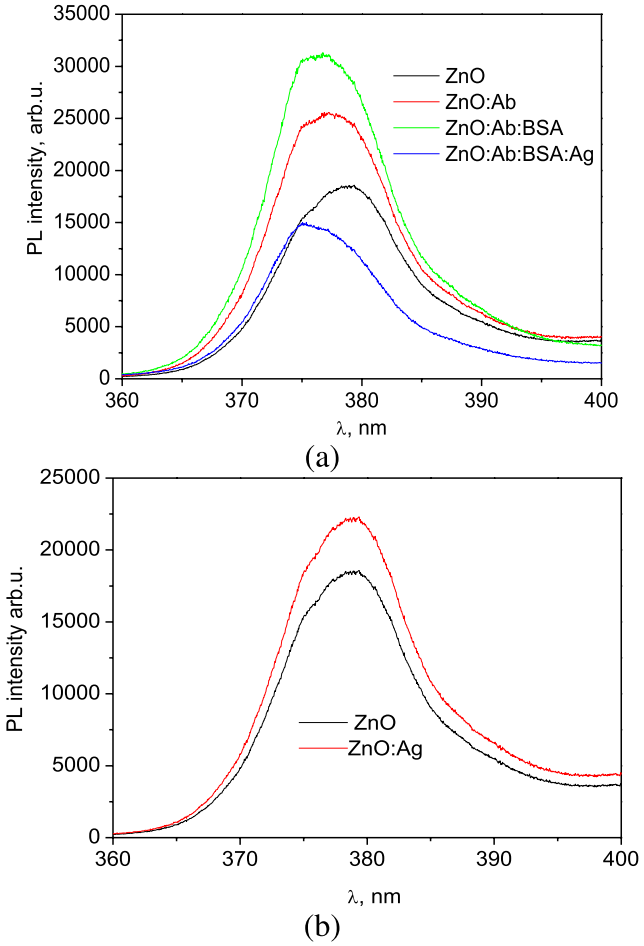

Fig. 4. (a) PL spectra of as-prepared ZnO nano-rods, after immobilization of Anti-Salmonella Ab (ZnO:Ab), after BSA (ZnO:Ab:BSA), and after immune reaction with Salmonella Ag (ZnO:Ab:BSA:Ag). (b) PL spectra of as-prepared ZnO nanorods and after immobilization of *Salmonella* Ag (ZnO:Ag).

Ab immobilization instigates an increase of all the components (I_1-I_3) of NBE PL signal. The detailed results of the testing experiment are summarized in Table I.

To analyze the influence of the immune reaction on PL of ZnO nano-rods, an additional test was performed–the PL spectrum was recorded after the *Salmonella* antigens were immobilized on ZnO nano-rods [Fig. 4(b)].

Immobilization of *Salmonella* Ag increases the NBE emission of ZnO nano-rods [Fig. 4(b)]. We suppose the observed reaction of ZnO with proteins (Ab, Ag and BSA) is due to non covalent binding. The proteins are bound to the surface by several functional groups affecting the surface band bending. Thus, the changes of ZnO PL emission after *Salmonella* Ag adsorption could occur due to the immune reaction between *Salmonella* Ag and *Anti-Salmonella* Ab [Fig. 4(a)].

After the probe testing experiments the sensitivity of the prepared biosensor was studied in a wide range of *Salmonella* Ag concentrations from 10^1 to 10^6 cell/ml. As BSA was used as a blocking agent to prevent non-specific interactions, the PL intensity after BSA immobilization was used as an initial point for calculation of the sensor response (Fig. 5).

Response R estimated as the difference of integrated spectral intensities of the NBE PL of ZnO with immobilized

TABLE I

COMPARISON OF THE GAUSS FITTED NBE PL PEAK POSITIONS VERSUS INTENSITIES FOR PURE ZnO NANORODS, IMMOBILIZED BY ANTIBODIES AND AFTER REACTION WITH ANTIGENS (~10^5 CELLS/ml)

ZnO		ZnO:Ab		ZnO:Ab:Ag	
Peak position , nm	Intensity, a.u.	Peak position, nm	Intensity ,a.u.	Peak position , nm	Intensity, a.u.
374	7710	374	7940	374	4310
376	286059	377	326754	377	17023
384	185811	384	167832	384	8190

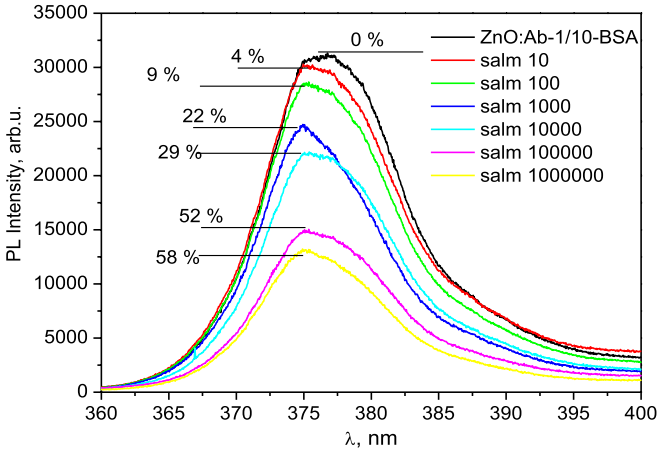

Fig. 5. Sensitivity of the NBE part of PL spectra to *Salmonella* Ag at different concentrations.

Anti-Salmonella Ab and BSA and after Ag adsorption is presented in Fig. 5. The sensitivity is calculated as:

$$S = \frac{I_{Int}^{Ab} - I_{Int}^{Ab:Ag}}{I_{Int}^{Ab}}.100\%,$$

where I_{Int}^{Ab} is the spectral integral intensity of NBE PL after Ab immobilization and BSA coating and $I_{Int}^{Ab:Ag}$ is the spectral integral intensity of NBE PL after reaction of biosensor with Ag.

Intensity of NBE PL decreases with the increase of Ag concentration. The detection threshold of the NBE signal is 10^2 cells/ml of Ag concentration. The sensitivity is almost linear only at the highest values of *Salmonella* antigen concentration (10^2 – 10^5 cell/ml) (Fig. 6).

The minor changes of the biosensor signal at 10 cell/ml could be in the range of the measurement error. Thus, we postulate that the prepared ZnO nano-rod biosensor shows good sensitivity to *Salmonella* in the 10^2 – 10^5 cell/ml range of the concentrations. The biosensors signal saturates at concentrations higher than 10^5 cell/ml.

Mechanisms of interaction between ZnO and biomolecules could be based on resonance energy transfer, charge transfer, hydrophobic and electrostatic interaction [9]. Layers of *Salmonella* Ab, *Salmonella* Ag and BSA deposited on glass substrates, showed no light emission within the studied

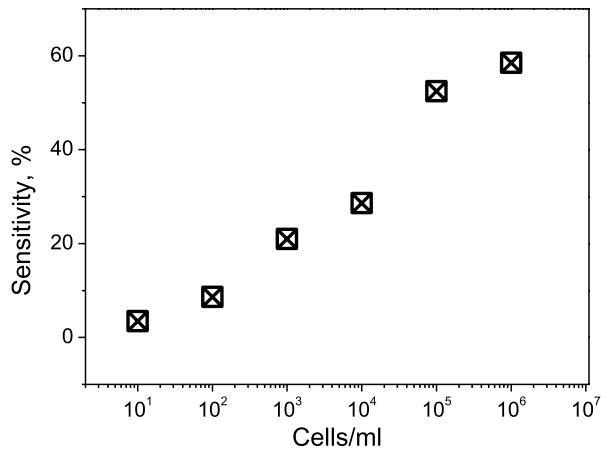

Fig. 6. Sensitivity of the ZnO Nanorod biosensor UV PL to Salmonella Ag at different concentrations (10^1 - 10^6 cell/ml).

spectral region. Therefore, the resonance energy transfer between ZnO and biomolecules can be excluded.

The oxygen vacancies on ZnO surface are found to act as adsorption sites forming bridging bonds with organic molecules [5]–[7]. Organic molecules can be anchored to the ZnO surface via phosphonic acid and carboxylate groups [25].

Calculations of interaction of the nucleotide bases of deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) with ZnO surface, carried out within the framework of density-functional theory have been reported by *V. Shewale et al.* [26]. Theoretical models show that in all cases ZnO surface prefers to bind with a ring nitrogen atom having a lone electron pair relative to the other possible binding sites of the bases. It is shown that the interaction between the ZnO-cluster and nucleobases is dominated by covalent and weak van der Waals forces.

From the above mentioned we assume that lone electron pair density moves from the ring nitrogen atom of *Anti-Salmonella* Ab molecules to uncoordinated surface Zn atoms (oxygen vacancies). As result, the concentration of free carrier density increases. The increase of free electrons concentration diminishes the depletion layer and stimulates the increase of the NBE band luminescence [21], [23].

We suppose that adsorption adsorption of proteins (Ab, Ag, BSA) could also stimulate changes in exciton-phonon coupling. The exciton peaks are shown to increase with the increase of the density of surface states and a consequent decrease of electric resistivity [27]. Antibodies and antigens are complex proteins with specific functional groups. Biological reaction between antigens and antibodies occurs as 'key'-'lock' interaction, demonstrating a high selectivity. The antigen-antibody reaction is supported by structural modification of previously adsorbed Ab molecules and elimination and/or weakening of ZnO-Ab link decreasing intensity of the NBE PL.

The obtained results confirm the key ideas of binding protein molecules on metal oxide nano-rods by Van der Waals and hydrophobic bonds, proposed by *V. Shewale et al.* [26] and *Y. Zhao et al* [27]. The increase of phonon assisted emission suggests a stable binding of protein molecules to ZnO surface. The studied ZnO nano-rods are a prospective material as optical biosensor transducers for *Salmonella* detection. However, several issues still have to be investigated in the future, such as optimization of the bio-sensitive layer by covalent binding, etc. eVentually, before considering practical application of the biosensor, lifetime of the bio-sensitive layer has to be clarified.

IV. Conclusion

UV and visible emission bands of ZnO nano-rods have been studied at room temperature luminescence of ZnO nano-rods demonstrates are related to near band edge excitonic emission and defect related deep level emission, respectively. The oxygen vacancies are suggested as active sites for protein adsorption. Adsorption of biologically active molecules on ZnO surface causes changes in its photoluminescence spectrum. Immobilization of *Anti-Salmonella* antibodies is assisted by charge transfer from protein to ZnO surface. As a result, an increase of UV emission is observed. Adsorbed *Anti-Salmonella* antibodies stimulate exciton-phonon interaction in ZnO nano-rods, resulted in increase of first exciton replica emission. Specific selective interaction between immobilized antibodies and antigens of *Salmonella* ('key'-'lock' principle) can be monitored by PL of ZnO. The studied ZnO nano-rods 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. Van der Waals and hydrophobic bonds are suggested as the mechanism of interaction between ZnO surface and *Salmonella* molecules.

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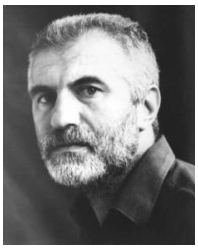

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