IOP Conf. Series: Materials Science and Engineering **145** (2016) 042030 doi:10.1088/1757-899X/145/4/042030

ZnO thin film as MSG for sensitive biosensor

N Iftimie[1], A Savin[1], R Steigmann[1,2], D Faktorova[3] and I Salaoru[4]
[1]Nondestructive Testing Department, National Institute of R&D for Technical Physics, Iasi, Romania
[2]Faculty of Physics, Alexandru Ioan Cuza University, Iasi, Romania
[3]Department of Measurement and Applied Electrical Engineering, University of Žilina, Žilina, Slovak Republic
[4]WMG, University of Warwick, Coventry, UK

E-mail: niftimie@phys-iasi.ro

Abstract. In this paper, we investigate the cholesterol sensors consisting of a mixture of cholesterol oxidase (ChOx) and zinc oxide (ZnO) nanoparticles were grown on ITO/glass substrates by vacuum thermal evaporation method and their sensing characteristics are examined in air. Also, the interest in surface waves appeared due to evanescent waves in the metallic strip grating in sub-wavelength regime. Before testing the transducer with metamaterials lens in the sub-wavelength regime, a simulation of the evanescent wave's formation has been performed at the edge of Ag strips, with thicknesses in the range of micrometers.

1. Introduction

The stability of ZnO nanostructures under physiological conditions, being important for sensing and biosensing applications, depends on the crystal quality of the nanostructures but can enable structures grown through thermal evaporation methods [1-3]. Nanostructures made of ZnO are easy and reliable to produce in a wide manner of different forms and structures [4]. Depending on the form and the shape of the deposited thin films, ZnO can used in many applications, like Surface Acoustic Wave (SAW) devices [5], bulk acoustic wave devices, gas sensors [6, 7], infrared detectors, tactile sensor arrays and enzyme biosensors [8]. ZnO nanomaterials demonstrate specific features that are encouraging for biological applications. In particular, via combining their fundamental material properties and a developed surface, ZnO nanostructures may possess particular characteristics, such as controllable hygroscopic. The modification and control of surface hygroscopic of different materials has become attractive in scientific and technological areas. Especially for biological systems and microfluidic systems, the hydrophobic and hydrophilic surface have important role in the mediation of a solute (e.g. protein) adsorption and cell adhesion [9, 10].

We investigate the possibility to excite plasmons at lower or equivalent frequencies in complex structures containing metallic strip gratings (MSGs). Their ability to store and propagate the EM energy at subwavelength scales is essential for many applications such as biosensing, photonic circuits and optical data storage. Actually, some metal oxide nanoparticles such as ZnO, Fe_2O_3, as well as carbon nanotube and graphene having a large surface-to-volume ratio, high catalytic efficiency, high surface reaction activity and strong adsorption ability were proved to be useful for improving sensor stability and sensitivity. In this paper, in order to confirm the availability of ZnO nanostructures for convenient high-efficiency biosensors, we investigated the cholesterol sensing performance via the electrical properties to be measured in air. Also, the interest in surface waves appeared due to evanescent waves in the metallic strip grating in subwavelength regime. Before testing the transducer with metamaterials lens in the subwavelength regime, a simulation of the evanescent wave's formation has been performed at the edge of Ag strips, with thicknesses in the range of micrometers.

2. Experimental section

Figure 1 shows the process flow of the procedure required in conducting the experiment for the structures obtained [8]. To sensing mechanism for cholesterol sensor designed in this study purpose the voltage applied across the two electrodes causes a current to flow via electron tunneling through the potential barriers between nanoparticles. The high surface to volume ratio of ZnO nanostructures provides large specific surface area for the adsorption of ChOx, and thus comparatively more active sites for catalysis. The depletion region at the surface of the film, produced from a mixture of cholesterol oxidase (ChOx) and ZnO nanostructures, is extended by the electrical field of electrons generated by reaction between ChOx and cholesterol (figure 2). The structure of cholesterol oxidase reveals deeply buried sites occupied by water molecules in the out of its substrate steroids. The current for the ChOx-ZnO film decreases proportionally with the number of cholesterol molecules.

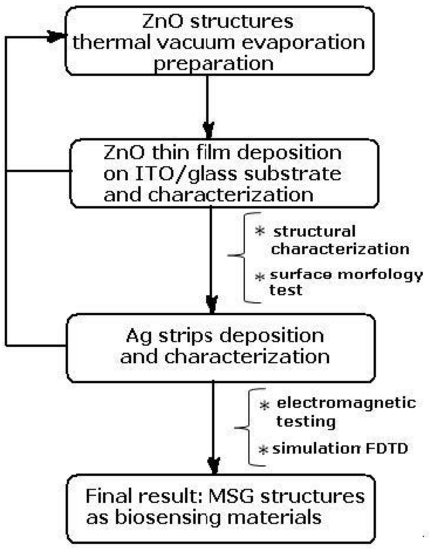


Figure 1. Process flow of the experiment.

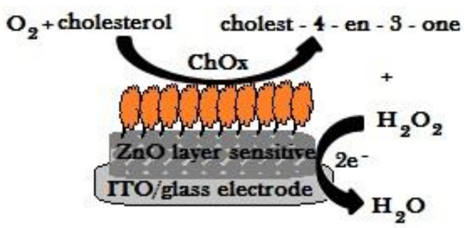


Figure 2. Electrocatalytic reaction of cholesterol by ChOx/ZnO/ITO/glass electrode.

3. Results and discussions

The X-ray diffraction analysis was making out to study the crystal quality and orientation of the synthesized ZnO thin films. All diffraction peaks in XRD spectra were matched with the reference spectra (JCPDS 36-1451) of standard ZnO, demonstrating the formation ZnO crystals.

The XRD patterns of the obtained structures were carried out on a Shimadzu XRD 6000 diffractometer using CuKα radiation (λ=1.5406 Å) (figure 3). The samples were analyzed in the range of $2\theta=5^{o}\div80^{o}$ with a scanning angle rate of 0.02 and a 2 s/step count time. The experimental XRD patterns were identified using Crystallographica Search-Match programme. The XRD peaks for Ag, ZnO and ITO crystalline phases are visible. The reflection peaks, at (100), (002), (101), (110), (103) and (112) were indicative of the hexagonal wurtzite ZnO nanostructure. The ZnO film is polycrystalline in nature having a preferred grain growth orientation along (002) and (101) planes which correspond with the peaks at 34.4 and 36.2^{o}. The weakness of the peaks is related to the thickness of the thin films. This is due the nature of the source material and it is assumed that only nanoparticles migration from the source to the substrate takes place.

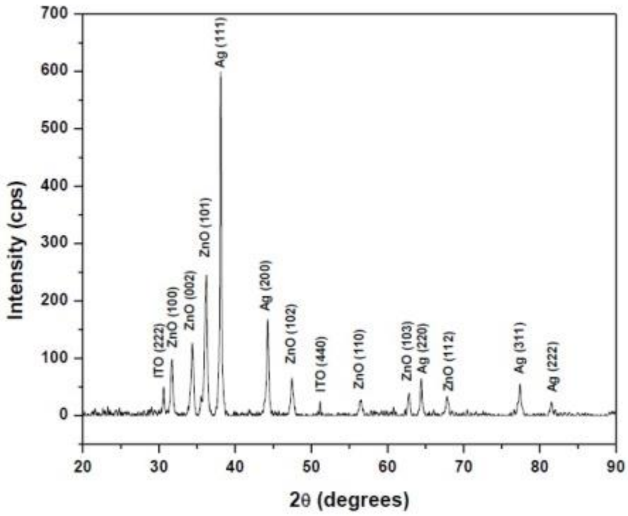
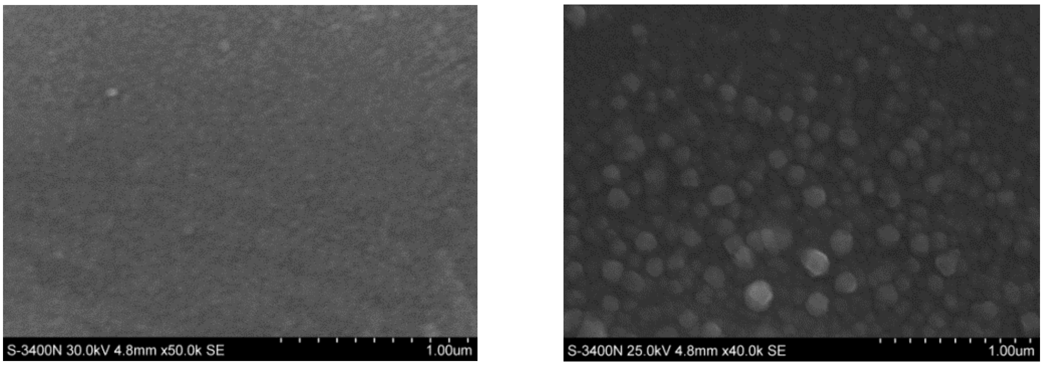


Figure 3. X-ray diffraction pattern to Ag/ZnO/ITO/glass structure.

For structural characterization of Ag/ZnO/substrate nanostructures powder, scanning electron microscopy (SEM) investigations were performed. The SEM showed that the structure of the films of ZnO is columnar (figure 4). We can show also that of ZnO nanostructured without bound enzyme had a uniform film, while ZnO nanostructured with bound enzyme had many globular structures. These observations confirmed immobilization of enzyme. The surfaces are without inclusions and defects, fact that make them appropriate for radio frequencies applications.



a b

Figure 4. The SEM images of cholesterol oxidase bound ZnO nanostructures: (a) without enzyme; (b) with immobilized cholesterol oxidase.

3.1. Evanescent waves. FDTD simulation.

Due to experimental difficulties in obtaining a perfect lens, the manipulation of evanescent modes can be made with an electromagnetic sensor with MMs lenses that have, at the operation frequency, either μ_{eff} = -1, and the lens can focus magnetic evanescent modes [11-17]. The spatial resolution of the system was verified according to [13] and the analysis of data obtained show that the realization of

MM lenses in the radiofrequency range is possible using CSR, whose distortions are minimal and whose calculation are based on Fourier optic principles [18].

The transducer and experimental set-up is presented [8, 19]. Generation and detection of evanescent waves in slits are made using the electromagnetic transducer lens. The rectangular frame used for the generation of TMz polarized waves is identical with rectangular frame used for the simulation model and the lens is made from a CSR [20, 8]. The working frequency was 474 MHz. The simulation we make using XFDTD 6.3 software produced by REMCOM [21]. In figure 5 we show the result of simulation with XFDTD. The E_y component, which counts in our case, is displayed. In [22] it has been presented the behavior of the field with air in the slits, it can be shown that, for cholesterol between the strips, the amplitude of the electric field has the same behavior as in [16] but the amplitude decreasing due to electrical permittivity above 20 of the cholesterol. Thus, the symmetrical maxima appear in the middle of the slits, decreasing to the minimum value, on the strips edges. Inside the strips, other pair of maxima appears, followed by the decreasing to middle of the strip.

The figure 6 shows the dependency of e.m.f. amplitude induced in the reception coil of sensor at the scanning of the MSG taken into study, the image showing that the type of sensor allows the correct emphasizing of extremely thick conductive strips and eventual interruptions.

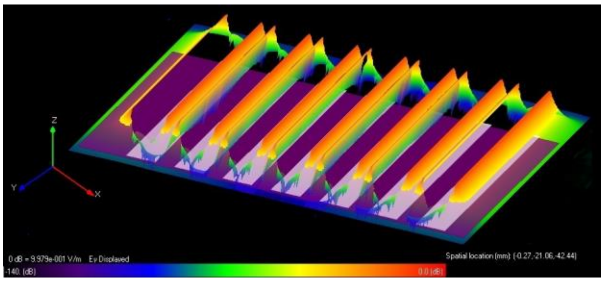


Figure 5. Numerical results for electric-field amplitude distribution near the seven strips; the field values are normalized to the amplitude of the incident field.

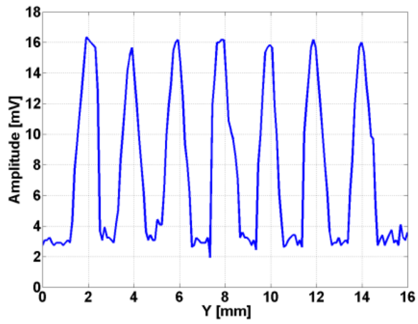


Figure 6. Amplitude of e.m.f. induced in the reception coil at the scanning of silver strip grating.

The existence of a single evanescent mode, theoretically foreseen, is experimentally confirmed by the existence of a local maximum in the middle zone of the slits, followed by an accentuated decreasing on the edge of the strips. The results are in good concordance with theoretical estimations, that confirms good adhesion of silver on ZnO/ITO/glass, also good alignments of strips.

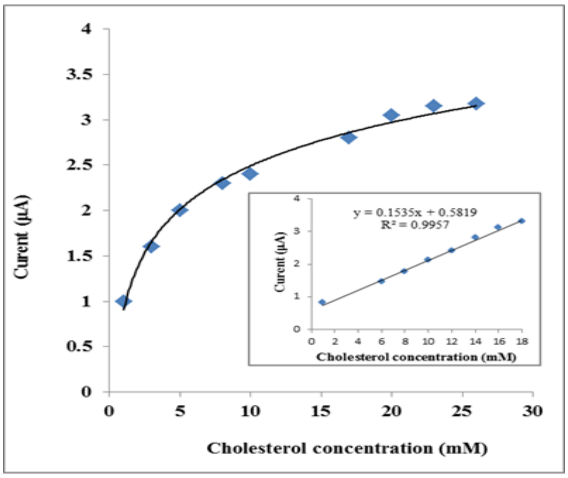
3.2. Experimental results.

ZnO nanostructured have excellent prospects for interfacing biological recognition events with electronic signal transduction as a new generation of biosensors in which active enzymes sites are coupled directly with a nanostructured ZnO electrode resulting in direct electron transfer between the enzyme and nanostructured ZnO with improved biosensing properties.

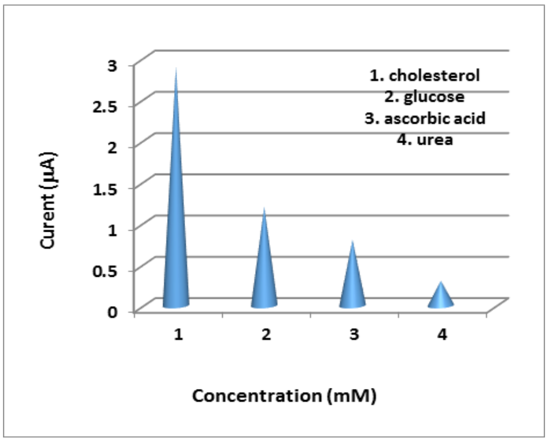
The initial experimental dates were carried out using a multimeter device because of its versatility in measuring resistance, voltage and ampere. The multimeter probes were immersed into 5 ml sodium phosphate buffer (0.02 M, pH 7.0) in a beaker. The reaction was started by adding 0.5 ml of enzyme solution. Based on the current produced during the chemical reaction the number of molecules of cholesterol and consequently the amount of cholesterol present in the sample could be determined.

In order to obtain a large working range, the current has been measured by variation of cholesterol concentration. To study effect of cholesterol concentration, the concentration was varied from 50 to 1000 mg/dl and amperometric measurements were made after 0.5 ml of cholesterol oxidase was added. The probe was sunk into the solution and the current has been recorded function of solution concentration.

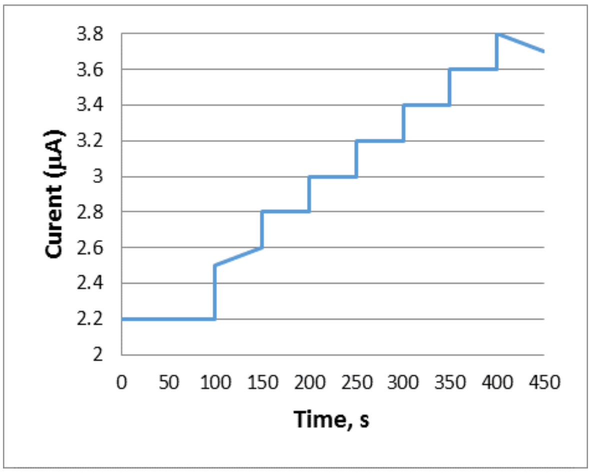
Figure 7a shows the relation between the response current and cholesterol concentration for the element biosensing. It is clearly seen from the graph that the response current increases as the concentration of cholesterol increases and saturated at high concentration of cholesterol which suggests the saturation of active sites of the enzymes at those cholesterol levels. The selectivity study suggests that the presence of interferants, like glucose, ascorbic acid and urea, have a negligible effect on the performance of the Ag/ZnO/ITO/glass architecture toward sensing of cholesterol (figure 7b). The response time is fast, which exhibits a high electron communication feature of the used ZnO (figure 7c). The calibration curves were obtained in the range of 50-1000 mg/dl (1.3-26.0 mM), with coefficient of determination (R^2) was estimated to be $R^2 = 0.9957$. Under optimized conditions, the steady-state current showed a linear dynamic range of 50-700 mg/dl (1.3-18.2 mM) (figure 7a).



a



b



c

Figure 7. (a) Variation in the current at a fixed potential of 0.04 V with changing cholesterol concentration at; the inset is calibration curve at λ = 0.6 m; (b) Study of Ag/ZnO/ITO/glass architecture biosensing; (c) Response time curve for the sensor structure.

4. Conclusions

A study of sensitive cholesterol biosensing material by ZnO nanostructures deposited on ITO/glass substrate by vacuum thermal evaporation was performed. The simulation of evanescent waves using the FDTD software and the presumption that the substrate of Ag/ZnO structure doesn't influence the formation of evanescent waves was confirmed by the experimental tests. The Ag strip grating thickness of 14 μm is comparable with the depth penetration of electromagnetic wave of 474 MHz, so that evanescent waves appear at the edge of strips and their manipulation can improve the resolution power of the sensor. The experimental measurement was making with a transducer with metamaterials lens improvement. We proved that transducers with metamaterials lens can be used in amplitude evaluation of the evanescent waves formed at the edge of strips of Ag/ZnO/substrate structure. The calibration curves were obtained in the range of 1.3 to 26.0 mM, with coefficient of determination was estimated to be $R^2 = 0.9957$ and the limit of detection was 12.6 μA/cm^2•mM. By results obtained we show that the element biosensing structure investigated is rapid and sensitive for the detection of cholesterol. One can concluded that due to the simple synthesis and electrode fabrication, good sensitivity, low detection limit and fast response, the ZnO nanostructured opens a way for the fabrication of highly efficient cholesterol biosensors.

References

[1] Zhou J, Xhu N S and Wang Z L 2006 *Adv. Mater.* **18** 2432
[2] Cheng C, Xin R, Leng Y, Yu D and Wang N 2008 *Inorg. Chem.* **47** 7868
[3] Israr M Q, Sadaf J R, Nur O, Willander M, Salman S and Danielsson B 2011 *Appl. Phys. Lett*. **98** 253705
[4] Wang Z L 2004 *J. Phys. Condens. Matter*. **16** R829-58
[5] Fu Q, Deng J, Zhou D, Gong S, Zhong S and Luo W 2012 Influence of Zinc oxide films structure on biological protein adsorption for SAW biosensors *IMCS 2012 – The 14th International Meeting on Chemical Sensors*, 905 - 908 DOI 10.5162/IMCS2012/P1.2.7
[6] Iftimie N, Iacomi F and Rezlescu N 2008 *JOAM* **10** 1810
[7] Rambu A P, Sirbu D, Iftimie N and Rusu G I 2011 *Thin Solid Films*, **520(4)** 1303
[8] Steigmann R, Iftimie N and Savin A 2015 *Appl. Mech. Mater.* **62**
[9] Kekkonen V, Hakola A, Kajava T, Sahramo E, Malm J, Karppinen M and Ras R H A 2010 *Appl Phys Lett* **97** 044102
[10] Papadopoulou E L, Zorba V, Pagkozidis A, Barberoglou M, Stratakis E and Fotakis C 2009 *Thin Solid Films* **518** 1267
[11] Pendry J B, Holden A J, Stewart W J and Youngs I 1996 *Phys. Rev. Lett*. **76** 4773
[12] Pendry J, Holden A J, Robbins D J and Stewart W J 1999 *IEEE Trans. Microw. Theory Tech,* **47** pp 47-58
[13] Gribic A and Eleftheriades G V 2003 *Applied Physics Letters* **82(12)** 1815
[14] Savin A, Steigmann R, Bruma A and Sturm R 2015 *Sensors* **15** 15903
[15] Grimberg R and Tian G Y 2012 *Proc. R. Soc.* A **468** 3080–99
[16] Savin A, Steigmann R and Bruma A 2014 *Sensors* **14(7)** 11786
[17] Savin A and Steigmann R 2014 patent RO129801-A
[18] Born M and Wolf E1975 Principle of Optics *5th ed. Pergamon Press Oxford UK*
[19] Grimberg R, 2013 *Mater. Sci. Eng.* B **178** 1285
[20] Grimberg R, Savin A, Steigmann R, Serghiac B and Bruma A 2011 *INSIGHT* **53(3)** 132-37
[21] Lagendijk A, Van Tiggelen B and Wiersma D S 2009 *Physics Today* **62(8)** pp 24-29
[22] Iftimie N, Tascu S, Salaoru I, Steigmann R, Savin A, Irimia M and Iacomi F 2015 *Materials Today: Proceedings* **2:10** 3486- 52