Detection of Salmonella Typhimurium in water and meat using nanosensor

Qsay kamil Hadi Al-Atify1), Prof. Dr. Jihad Deiyb Mahal Al-Janabi2) and Prof. Dr. Basim Abd Al-Hassan Al-Mayahi3)

University of Tikrit / College of Education for Pure Sciences

University of Kufa / Faculty of Science / Department of Ecology

1) qsayhadi@gmail.com
3) basim.almayahi@uokufa.edu.iq

Abstract:

The sensor dimensions are chosen (10 * 10 mm) and manufactured by the laser engraving system as a dimension of the fine copper die and installed on the glass substrate coated with the nano material and the mould is coated with silver by thermal vacuum evaporation and the fixation of the connecting electrodes. The surface properties and the structural, optical and electrical properties of the thin films were studied. Samples prepared with an X-ray diffraction device (XRD) were examined to ensure the presence of elements or compounds entering the coating layer, and an examination with an atomic force microscope (AFM) to identify the crystal size of the coating compound and a scanning electron microscope. Field (SEM) is to prove the existence and proportions of active ingredients. The UV nano composite spectra were studied to investigate the optical behavior of ZnO-rGO membranes (transmittance and absorption). The sensing properties were determined by measuring changes in the conductivity of the prepared bacteria present in water and flesh using current and voltage measurement I-V by two mass device methods and the results were accurate. The results proved that ZnO-rGO has high sensitivity towards bacteria and is made of thin films of nano composites and has a high performance to detect bacteria in water and meat. The goal of this study is to design and manufacture a nano sensor with high efficiency to detect a type of bacteria that the thin films deposited in the vacuum have properties excellent for accurate detection. The microbial detection devices based on thin materials are inexpensive and do not require trainers to use them, and they operate at room temperature. Thus they have the advantage of remote positioning and monitoring in dangerous places.

1. Introduction

Diverse applications in recognizing sicknesses, improvement of cells and microscopic organisms, harm and checking of sickness development may conceivably advantage from biosensor gadgets [1]. Electrochemical biosensors talk to an imperative subclass of chemical sensors, where an anode is utilized as transduction component [2]. They are cheap, sensible for arranging facilitates micro systems and quick response [3]. The resistive characteristics of materials and examination of capacitive (or inductive) in response to the small adequacy of sinusoidal excitation hare are combined by electrochemical impedance [4]. The instrument of impedance discovery is based on the estimation of impedance change as a result of definitive of target particles to receptors (proteins, antibodies, DNA...
and other bio-recognition components) taken after onto electrodes’ surfaces [5–7]. Biosensors are charming and successfully utilized in a couple of applications like restorative assurance [8], clinical chemistry [9], normal ranges [10], defilement assurance [11], food industry [12] and DNA hurt [13]. Different regions of development and science had applied inter-digitated anode (IDE) to function as actuators and/or transducers with particular accentuations inside the ranges of chemical sensors [14], organic detecting [15], biosensors [16] and gas identifying [17]. ZnO has been tried point by point to store in various ways counting warm vanishing, beat laser articulation, sprinkle pyrolysis19 and metal normal chemical vapour articulation (MOCVD) [18]. Subsequently, the spin-coating method has fascination due to its straightforwardness and moo fetched [20]. Within the current ponder an arrangement of viable, basic and effective stages permit the creation of biosensor gadgets onto ZnO-rGO nanostructure was made. The ZnO- rGO nanostructure is ready and after that it is stored onto glass substrate at toughening temperature of 350 °C spin-coating method. Besides, the ZnO -rGO nanostructure’s basic, morphological and optical characteristics are examined by utilizing UV–vis spectrometer, Nuclear Drive Microscopy (AFM), Filtering Electron Microscopy (SEM) and X-ray diffractometer (XRD), separately. Elaboration in plan and manufacture, and nitty gritty portrayal of test steps were made. In addition, I–Vmeasurements have characterized their electrical properties.

2. Reagents and Materials

The materials used for the preparation of ZnO-rGO nano composite; zinc acetate dehydrates were purchased from (India  PHD ) and polyvinyl alcohol from (TEDIA USA). Graphene oxide was supplied by (India- Oxoid). Ethanol 95% was bought from HmbG Chemicals and de ionized water as a solvent. All the chemicals were used in reagent grade and used without further purification

**Synthesis of ZnO-rGO nanocomposite**

Apparent composition of ZnO-GO nano composite incline film was orchestrated by a sol - gel technique utilizing the turn coating onto glass substrates and tests were carried out at room temperature. The predecessors 0.1M zinc acetic acid derivation dehydrates [Zn (CH3CO2)2.2H2O)] were broken up in ethanol and included to the polyvinyl liquor arrangement in refined water, mixed until a gel, straightforward and homogeneous arrangement was got. The composite blends with of GO (1.00 vol%) were arranged by slowly including the GO arrangement (brownish dim colour) to the ZnO solution and sonicator for 1 hour at room temperature to ensure that GO was dispersed well. The glass substrates were first cut into 20 mm x20 mm x 1 mm piece and cleaned with methanol and acetone. Then the substrates were rinsed with de ionized water. The solution was spin-coated on glass substrate using Laurell Technologies Corporation photo resist spinner, with the speed of 2000 rpm for 15 s. Then, the coated substrates were heated at 100°C for 10 minutes after each coating to evaporate the solvent and remove organic residuals. By repeating the above procedure five times, 5 layers of coatings were obtained. Having completed the 5 layers of coatings, the thin film samples were annealed at 350°C for 1 h in a furnace to achieve the crystal phase annealing process which reduces the GO into the graphene [21] and also gives the ZnO-rGO nano composite material a uniform coating and removes the residual precursors from the ZnO-rGO nano composite
Figure (1): The shape of the base material illustrates the base used in the manufacture of the nano sensor.

Manufacture of the sensor component

The copper wires were connected to the silver electrode as a connection between the thin film and the measuring instrument of the thin film sensor equipped with comb-type electrodes. The wires were fixed with silver paste material on the electrodes deposited on the glass pieces

The process of preparing bacteria

The *salmonella* were selected as a bacterial sample. 4 gm of nutrient broth powder was dissolved in 500 ml of distilled water, and mixed until a homogeneous solution was obtained. The broth was sterilized using autoclave at 121 °C for 15 minutes. From isolated primary agar dishes, new colonies were inoculated in nutrient broth (10 ml) and incubated for 24 hours at 37 °C. After that, 5 ml of isolate culture (10 ml) were diluted to 250 ml using nutrient broth and incubated with shaking it at 200 rpm for 24 hours at 37 °C. CFU was calculated by a viable counting method. The concentration of *salmonella* used in this experiment was constant (10⁸ cfu / ml) for each sensor 25 grams of ground meat bought from the local market. 10 grams of meat was taken and mixed in 90 ml of distilled water and sterilized at a temperature of 120 -180 degrees Celsius for one hour.

The isolates of *salmonella* were activated on the agar nutrient medium and the isolates were incubated for (24) hours in the incubator. The meat was filtered by filter paper.

Detection for salmonella

The sensor was examined with a Keithley electrical conductivity device by measuring the electrical conductivity of the thin film at room temperature. The change in the electrical conductivity properties with Ohm contacts was used to prepare the sensor for *salmonella*. Since the thickness of the vacuum-deposited films is 1000 °C, measurements at low voltages (<4 V) were required.

3. Result and Discussion

Structural properties of zinc oxide films are with graphene oxide and polyvinyl alcohol thin film nano particles. The results of X-ray diffraction (XRD) of zinc oxide nano particles prepared on glass substrates as in Fig. (2) shows that zinc oxide is multi crystalline in nature and its crystal structure is
hexagonal and in conformity with (JCPDS card No. 36-1451). The main peaks of the substance appeared at $2\theta = 31.45^\circ$, $34.1^\circ$, $36.05^\circ$, $45.95^\circ$, $56.8^\circ$, $60.05^\circ$, $66.2^\circ$, $67.45^\circ$, $70.1^\circ$ and $71.1^\circ$, represented by the levels (100), (002), (101), (102), (110), (103), (002), (112), (201) and (004). This is consistent with previous studies [22].

Figure (2): shows the X-ray diffraction pattern of thin zinc oxide nano films prepared on glass substrates.

It is evident from Fig. (2) that the natural location of the characteristic peaks (100) and other peaks have been driven as reported by [23]. The high temperature annealed film will show all sharp peaks of the printing oxide and no other phase, making it a marker to the last formation. The oxide structure is higher in crystallization.

The crystal sizes of the ZnO membrane models were calculated using the Debi-Sharr equation,[ 24] $D = \frac{k\lambda}{(\beta \cos \theta)}$

**Surface structure analysis**
The results of the microscopic study shows the surface strength of zinc films with graphene oxide and polyvinyl alcohol nano particles with a homogeneous and smooth surface structure. Figure (3) shows force microscopy images of thin films of zinc oxide with graphene oxide and polyvinyl chloride nano particles. On glass substrates, the average grain size was obtained from the results of bureau force microscopy (45 nm).
Figure (3): shows atomic force microscopy images of zinc oxide thin film with graphene oxide and polyvinyl alcohol nano particles prepared on glass substrates, (A), (B), (C) a modulus of granular sizes.

**Surface Morphology ZnO-rGO Doped PVA Nanocomposite Thin Film Analysis**

The SEM images ZnO-rGO doped with PVA thin film showed that the surface morphology consists of homogeneous and densely packed distribution of well-defined grains. Figures (4) show SEM images ZnO-rGO doped with PVA nano composite thin film prepared at reaction temperatures (350)°C respectively, which include small and large particles that have regular shape large and small with fine particle size as indicated in Figures are round in shape.

Figure (4) SEM images ZnO-rGO doped PVA Nanocomposite thin film

**Optical properties of zinc oxide film with graphene oxide and PVC nanostructures prepared on glass substrates**

The transmittance, absorbance and reflectivity of zinc oxide films were studied with graphene oxide and polyvinyl alcohol prepared on glass substrates. The transmittance and absorbance were recorded within the wavelength from (200-1000 nm) at room temperature.

**Permeability of Zinc Oxide Thin Films to Glass Substrates**
Optical transmittance is defined as the ratio of the intensity of the transmitted light to the intensity of the incident light. The permeability spectrum depends on the chemical and crystalline composition, the thickness and the surface morphology of the films, also depends on the important parameter effect on the permeability of the films.

![Absorbance vs Wavelength](image.png)

Figure (5) shows the transmittance diagram of the prepared thin zinc oxide film on glass substrates.

**Reflectivity of Zinc Oxide Thin Films on Glass Substrates**

The light reflection was defined as the ratio of the reflection image to the incident intensity, and the ZnO reflection can be calculated using the equation.
Figure (6) shows the reflectivity scheme of a thin zinc oxide film prepared on glass substrates.

**Electrical properties of ZnO -r GO, PVA / glass thin films Current voltage measurements**

Figure (8 - 9) shows the current-voltage characteristics (I-V) of ZnO - rGO, PVA / glass thin film preparation.

The interaction of electrical transmissions on the cell wall of the polymeric suspended microbe, especially in thin films, is the basis for the detection of salmonella by the polymeric sensor. The micro electric cell wall has a charge [25] as shown in Figures (8,9) current (IV) voltage characteristics of ZnO-rGO and PVA / glass thin films when exposed to Salmonella at room temperatures. The results can be interpreted from shifts in charge carrier concentrations around ZnO -rGO, PVA / thin films. Vitreous after exposure to microorganisms exhibited intravenous properties. The sensors are watertight and the metal ions on the surface of the film react to the bacteria. The positive charge of Zn2 + and G2 + can be linked to positive salmonella [26-27]. Zn + 2 nano particles are released from classes of negatively charged bacteria. Cell wall cause its explosion according to [28]. By the release of Zn2 + upon surface oxidation, or by means of an electrostatic molecule between the ions emitted from and the negatively charged bacterial cell wall, the ZnO nano composite can interact directly with the bacterial cell membrane.
Figure (7) shows the current voltage characteristics (I-V) for preparing ZnO-rGO, PVA / Glass Thin Film for detection of Salmonella in meat.

Figure (8) illustrates the current-voltage characteristics (I-V) of preparing ZnO-rGO, PVA / Thin glass for detection of Salmonella in water.

**Conclusion**

The ZnO-GO sensors were developed using the Sol - gel method at 350 ° for the synthesis of GO-ZnO nano particles on the substrate. The films have outstanding composition, morphology, and electrical properties. The appearance of a peak in the concentration suggests high crystallinity according to X-ray diffraction analysis. The prevalence of Salmonella in the water and meat was measured by calculating variations in thin film conductivity using I-V tests, which were observed. Sensor performance in the presence of bacteria in water and meat has been successfully detected. Various, the concentration of 10^8 was detected by the sensor at room temperature. Salmonella was present in water and meat respectively. High sensitivity and dynamic repeatability were seen in these sensors.
The sensors reveal that ZnO-GO nano composites promise sensitive and reliable sensors to detect the presence or absence of water and meat. They are cheap and easy to use.

Acknowledgments
First of all praise be to Allah, the almighty, the most gracious and the most merciful, Who strengthened me with the capability to complete this research.

I would like to extend my thanks to my supervisor Prof. Dr. Jihad Deiyb Mahal Al-Janabi and Prof. Dr. Basim Abd Alhassan Almayahi for suggesting the idea of this work and his moral support during the completion of this research work.

I would like to express my deep gratitude and appreciation to the staff in the Collage of Education for Pure Sciences, University of Tikrit and Department of Ecology, Faculty of Science, University of Kufa.

Reference
[1] MacKay S, Hermansen P, Wishart D, Chen J. Simulations of interdigitated electrode interactions with gold nanoparticles for impedance-based biosensing applications. Sensors2015;15(9):22192–208.
[2] Hanrahan G, Patil DG, Wang J. Electrochemical sensors for environment monitoring: design, development and applications. J Environ Monit 2004;6(8):657–64.
[3] Radke SM, Alocilja EC. A high density microelectrode array biosensor for detection of E. Coli O157: H7. Biosens Bioelec 2005;20(8):1662–7.
[4] Guan JG, Miao YQ, Zhang QJ. Impedimetric biosensors. J Biosci Bioeng 2004;97(4):219–26.
[5] Yang L, Li Y, Erf GF. Interdigitated array microelectrode-based electrochemical impedance sensor for detection of Escherichia coli O157: H7. Anal Chem 2004;76(4):1107–13.
[6] Radke SM, Alocilja EC. Design and fabrication of a microimpedance biosensor for bacterial detection. IEEE Sens J 2004;4:434–40.
[7] Yang L, Li Y. AFM and impedance spectroscopy characterization of the immobilization of antibodies on indium–tin oxide electrode through self-assembled monolayer of epoxysilane and their capture of Escherichia coli O157: H7. Biosens Bioelec 2005;20(7):1407–16
[8] Baraket A, Lee M, Zine N, Sigaud M, Bausells J, Errachid A. A fully integrated electrochemical biosensor platform fabrication process for cytokines detection. Biosens Bioelec 2017;93:170–5.
[9] Zhao W, Xu J, Chen H. Y. Electrochemical biosensors based on layer-by-layer assemblies. Electroanalysis 2006;18(18):1737–48.
[10] Larsen LH, KjærT, Revsbech NP. A microscale NO3-biosensor for environmental applications. Anal Chem 1997;69(17):3527–31.
[11] Pan Y, Sonn GA, Sin ML, Mach KE, Shih MC, Gau V, et al. Electrochemical immunosensor detection of urinary lactoferrin in clinical samples for urinary tract infection diagnosis. Biosens Bioelec 2010;26(2):649–54.
[12] Terry LA, White SF, Tigwell L.J. The application of biosensors to fresh produce and the wider food industry. J Agric Food Chem 2005;53(5):1309–16.
[13] Brett AM, Diculescu VC, Chiorcea-Paquim AM, Serrano SH. DNA-electrochemical biosensors for investigating DNA damage. Compr Anal Chem 2007;49:413–37.
[14] Dias CJ, Igreja R. A method of recursive images to obtain the potential, the electric field and capacitance in multi-layer interdigitated electrode (IDE) sensors. Sens Actuators A Phys 2017;256:95–106.
[15] Rana S, Page RH, McNeil CJ. Impedance spectra analysis to characterize interdigitated electrodes as electrochemical sensors. Electrochim Acta 2011;56(24):8559–63.
[16] Brandenburg A, Kita J, Groß A, Moos R. Novel tube-type LTCC transducers with buried heaters and inner interdigitated electrodes as a platform for gas sensing at various high temperatures. Sens Actuators B Chem 2013;189:80–8.
[17] Varshney M, Li Y, Srinivasan B, Tung S. A label-free, microfluidics and interdigitated array microelectrode-based impedance biosensor in combination with nanoparticles immunoseparation for detection of Escherichia coli O157: H7 in food samples. Sens Actuators B Chem 2007;128(1):99–107.
[18] Srinatha N, Nair KG, Angadi B. Effect of Fe doping on the structural, optical and magnetic properties of combustion synthesized nanocrystalline ZnO particles. Adv Powder Technol 2017;28(3):1086–91.
[19] Ewaid, S.H.; Abed, S.A.; Al-Ansari, N. Water Footprint of Wheat in Iraq. Water 2019, 11, 535.
[20] Rana N, Chand S, Gathania AK. Tailoring the structural and optical properties of ZnO by doping with Cd. Ceram Int 2015;41(9):12032–7.

[21] Raghu P, Srinatha N, Naveen CS, Mahesh HM, Angadi B. Investigation on the effect of Al concentration on the structural, optical and electrical properties of spin coated Al: ZnO thin films. J Alloys Compd 2017;694:68–75.

[22] Haichao Su, Qiang Ma, Kun Shang, Tao Liu, Huanshun Yin, Shiyun Ai. Gold nanoparticles as colorimetric sensor: a case study on E.coliO157: H7 as a model for Gram-negative bacteria, Sens. Actuators B 161 (2012) 298–303.

[23] Dixit, V., Tewari, J.C., Sharma, B.S., 2006. Detection of E. Coli in water using semiconducting polymer thin film sensor. Sens. Actuators, B120, 96-103.

[24] H.G. El-Shobaky, A.S. Ahmeda, N.R.E. Radwan. Effect of irradiation and ZnO-doping of CuO/TiO2 system on its catalytic activity in ethanol and isopropanol conversion, Colloids Surf. A: Physicochem. Eng. Aspects 274 (2006) 138–144.

[25] Salam Hussein Ewaid et al 2020 J. Phys.: Conf. Ser. 1664 012143.

[26] F. S. Ghoreishi, V. Ahmadi, M. Samadpour, Synthesis and characterization of GrapheneZnO nanocomposite and its application in photovoltaic cells, JNS. 3 (2013) 453-459.

[27] Tariq Jan, Javed Iqbal, Muhammad Ismail, Noor Badshah, Qaisar Mansoor, Aqsa Arshad, Qazi M. Akham, Synthesis, physical properties and antibacterial activity of metal oxides nanostructures, Materials Science in Semiconductor Processing 21 (2014) 154 - 160.

[28] F. S. Ghoreishi, V. Ahmadi, M. Samadpour, Synthesis and characterization of GrapheneZnO nanocomposite and its application in photovoltaic cells, JNS. 3 (2013) 453-459.

[29] Ahmed Alaa Kandoh et al 2021 IOP Conf. Ser.: Earth Environ. Sci. 790 012073

[30] Tariq Jan, Javed Iqbal, Muhammad Ismail, Noor Badshah, Qaisar Mansoor, Aqsa Arshad, Qazi M. Akham, Synthesis, physical properties and antibacterial activity of metal oxides nanostructures, Materials Science in Semiconductor Processing 21 (2014) 154.

[31] Salam Hussein Ewaid et al 2021 IOP Conf. Ser.: Earth Environ. Sci. 722 012008

[32] Pandiyarajan, T., Udayabhaskar, R., Vignesh, S., Arthur James, R., Karthikeyan, B., 2013. Synthesis and concentration dependent antibacterial activities of CuO Nanoflakes. Mater. Sci. Eng., C33,2020-2024.

[33] Ewaid, S.H.; Abed, S.A.; Al-Ansari, N.; Salih, R.M. Development and Evaluation of a Water Quality Index for the Iraqi Rivers. Hydrology 2020, 7, 67.

[34] Salam Hussein Ewaid et al 2021 IOP Conf. Ser.: Earth Environ. Sci. 790 012075

[35] Matai, Ishita, Sachdev, Abhay, Dubey, Poornima, Uday Kumar, S., Bhushan, Bharat, Gopinath, P., 2014. Antibacterial activity and mechanism of Ag-ZnO nanocomposite on S.aureus and GFP-expressing antibiotic resistant E. coli. Colloids Surf., B 115,359–367.

[36] Haichao Su, Qiang Ma, Kun Shang, Tao Liu, Huanshun Yin, Shiyun Ai. Gold nanoparticles as colorimetric sensor: a case study on E.coliO157: H7 as a model for Gram-negative bacteria, Sens. Actuators B 161 (2012) 298-303.

[37] Kareem Abass Al-Hassani, M. (2019). SEROLOGICAL DETECTION OF COXIELLA BURNETII CHRONIC INFECTION- PHASE 1 IN SERUM OF HUMAN AND SHEEP AT AL-QADISIYAH PROVINCE, IRAQ. Al-Qadisiyah Journal Of Pure Science, 24(1), 13 - 20.

[38] Jalil Abed, M. (2019). Synthesis and Characterization and evaluation of biological activities of some new pyrrole compounds. Al-Qadisiyah Journal Of Pure Science, 24(1).

[39] Abdul-Hamza, H. kadhum, & Mohammed, G. J. (2019). The inhibitory effect of some nanoparticles on biofilm formation of Streptococcus agalactiae. Al-Qadisiyah Journal Of Pure Science, 24(2).

[40] Hussein Al-abedi, K. J., & Abd Al-Mayahi, F. (2019). Molecular detection of metallo-β-lactamase genes in carbapenem-resistant isolates of Pseudomonas aeruginosa recovered from patients in Al-Diwaniyah province, Iraq. Al-Qadisiyah Journal Of Pure Science, 24 (2).