Impedimetric Sensor of Leptospira Bacteria based on Mixed Metal Alloys-Polyaniline Films

J. Jurait, H. Abdullah, I. Yahya, S.K Bejo and N.J Azman

***Abstract*—** The detection of Leptospira bacteria in water using polyaniline (PANI) nanocomposites thin films doped with metal alloys using sol-gel method is studied in this research. The relationship between bacteria concentration in regards to current and impedance were studied and measured based on current-voltage (I-V) and electrochemical impedance spectroscopy (EIS shows the sensor's performance). The morphologies and elementary characteristics of the thin film were characterized using FESEM, and AFM. Based on the result, the developed nanocomposite thin films are compatible in detecting the presence of pathogenic Leptospira bacteria. According to the FESEM results, irregularity of nanoparticles in terms of substrate size which is in the range of 80 nm to 120 nm can been observed and this confirms the presence of the metal particles. In addition, the PANI metal alloys nanocomposite thin film has shown higher sensitivity towards pathogenic Leptospira detection. Furthermore, the AFM images have provided a specific morphology structure for each bacteria detection. Therefore, these results affirmed the potentials of polyaniline metal alloys nanocomposite thin films to be used for Leptospira bacteria detection in water.

***Keywords*—** Leptospira; Polyaniline; I-V measurement; Thin Film; Sensor

I. Introduction

Leptospirosis disease became a global health issue in tropical rainforest and subtropical countries like Indonesia, Malaysia, and Brunei [1][2]. Leptospirosis is a zoonotic disease that can be spread through animals which act as carriers and intermediate hosts where this disease is currently considered as an emerging global public health issue. Leptospirosis disease leads to kidney and internal organs failure [3][4][5]. This infectious disease is caused by the pathogenic strain form the genus Leptospira interrogans [6]. The genus could be spread directly or indirectly from animals to humans. Most people get infected through contacts with polluted water, food, urine and soil from the host animals [7]. Leptospira bacteria consist of two types. The first type is known as the pathogenic Leptospira which causes the disease and the second type is named saprophytic where it is a dormant type without adverse effects [8].

Generally, the polymerase chain reaction (PCR) test could be taken to diagnosis the Leptospirosis in a biological laboratory by sampling the bacteria from blood, urine, tissues and serum [9][10]. This test also uses the enzyme-linked immunosorbent assay (ELISA) to complete the diagnostic of Leptospira specimen [11]. The polymerase chain reaction (PCR) method requires a specific instrument, spacious laboratory and also high expertise personnel. On the hand, microscopic agglutination test (MAT) is defined as a test which marks out antibodies in the serum of a patient [12]. Samples of polluted water and soil could be isolated and experimented for growth of Leptospira in which they take few weeks or months to complete the test. Thus, this induces more researchers to study on new innovations to overcome the particular time constraint. It will be an important contribution if improvement is achieved for surveillance, prevention, and control of this disease to protect humans.

In recent years, researchers have investigated several detection methods which include optical, electrochemical, thermal and mass methods, achieving a high accuracy by using biosensor which is required to detect and monitor the microbial population in polluted waters. Currently, polymers are widely used in biosensor electrochemistry applications because of their high conductivity in regards to molecular biology characteristics, stability of environment and basic monomer synthesis [13][14]. Conducting polymers are used by various applications in electrochemical biosensors for monitoring the environment in order to detect bacteria in the water. PVA is an important water-soluble polymer and are extensively used in industries because of its inspired chemical and physical properties, non-toxicity, attractive chemical resistance, excellent film establishment potential and also has high crystal structure [15][16]. Polyaniline (PANI) has become a significant conducting polymer due to its morphological and electrical properties. Furthermore, PANI has a greater advantage because it is rather inexpensive [17][18]. In addition to this, it has a doping process and is able to perform as an appropriate sensor that can interact with the microbes even on a thin film surface. The interactions of the bacteria with the surface of PANI metal alloys could produce a signal in the form of current-voltage (I-V) and can be characterized using electrochemical impedance spectroscopy (EIS) [19][20]. The conductivity of the biosensor flows through from the dopant to the polymer. The subsequent interaction between polyaniline and metal has been an added advantage to be used in a wide range of applications. By adding metal alloys doped with the polymers, particular area of materials is increased in terms of its sensitivity and accuracy [21][22][23]. PANI incorporated with metal alloys was expected to provide high sensitivity and stable biosensor with, quick response time which is suitable for a certain targeted bacteria [24].

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Thus, in this paper, the PANI metal alloys nanocomposite thin film was synthesized using sol-gel method using the spin-coating technique. The thin film was fabricated with a low cost and simple procedure based on a microbial sensor where the performance of the thin film will be evaluated based on current-voltage (*I-V*) and electrochemical impedance spectroscopy (EIS) to detect pathogenic Leptospira bacteria in the water.

II. Materials and Method

A. Reagents and Chemicals

The precursors of iron nitrate ($Fe(NO_3)_3$), nickel nitrate ($Ni(NO_3)_2$), aluminum nitrate $(NO_3)_3$, manganese nitrate ($Mn(NO_3)_2$), polyvinyl alcohol (PVA) and aniline (C_6H_7N) were used as the starting materials. Deionized (DI) water was used for all the preparation provided by ELGA Equipment.

B. Synthesis of Samples

The polyvinyl alcohol (PVA) was dissolved in 30 ml of deionized water (DI) and heated at 80 ^{0}C for 24 hours. 0.5 gram of mixed iron nitrate and metal nitrate (such as manganese, aluminum and, nickel) were added to each of the PVA solution and was stirred continuously. 1.25 mL of aniline and acid nitric 1.0 M were added to each of the PVA metal alloys and stirred continuously at 90 ^{0}C until the color changes to dark brown indicating that the solution has become PANI metal alloys nanocomposite.

C. Fabrication of PANI metal alloys Sensor

The nanocomposites solution was spin-coated on glass substrates (20 mm × 25 mm) at a speed of 2000 rpm for 18-20 s using a spin coater (model WS-400BX) from Laurell Technologies Corporation (LTC). The thin films were annealed in a furnace at 250 ^{o}C for 24 h. The thin films were then sputtered by silver following a comb-structure (silver layer at 2.2 - 2.4 mm and 1.2 - 1.4 mm) using J.Lesker Model PVD 75 silver magnetron sputtering equipment. The terminal electrodes of the thin films were soldered with a copper wire to connect it to the the measuring device fom the brand GAMRY-Physical Electrochemistry as shown in Fig. 1.

D. Bacteria Samples

The samples of pathogenic Leptospira are categorized at 10^8 CFU mL^{-1} (colony forming units per milliliter). These samples were obtained from the Department of Veterinary Pathology & Microbiology, Faculty of Veterinary, University Putra Malaysia.

E. Current-Voltage (I-V) and Impedance Measurement for Sensor Performance

The current-voltage (*I-V*), and electrochemical impedance spectroscopy (EIS) characteristic were measured using the GAMRY-Physical Electrochemistry equipment. The measurement was obtained whenever the thin films were immersed in water which contained a certain concentration of Leptospira bacteria where in this case was 10^8colony forming units (CFU mL^{-1}).

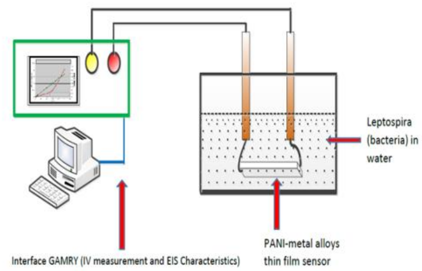

Fig. 1: Experiment Setup

III. Results and Discussion

A. FESEM and AFM analysis

FESEM images in Fig. 2(a, b and c) shows the morphology of sheet-like structure consisting of large spheres [25][26]. The obtained size of the spheres ranges from 80nm to 120nm. This is due to the combination of metal alloys embedded into PANI where it becomes a coated square [20]. The structure coated into the PANI looked inner dark in color. Meanwhile, the films show several cavities like structure which is highly beneficial for electrochemical sensing applications [27]. The reaction between Leptospira bacteria and thin film occurs easily by binding themselves into porous structures of the thin films. At this moment, aniline monomer of PANI particles adsorbed together with metal alloys particles is caused by polymerization attractions [28].

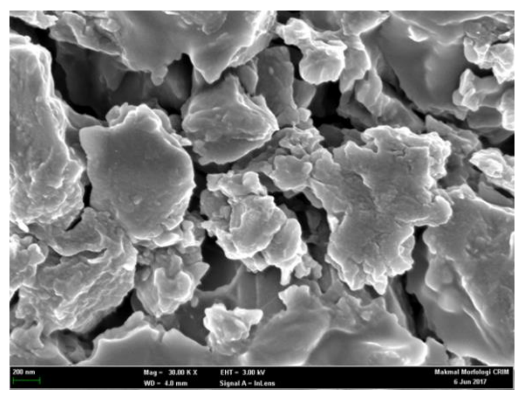

Fig. 2a : FESEM image of PANI-Fe-Ni nanocomposite thin film

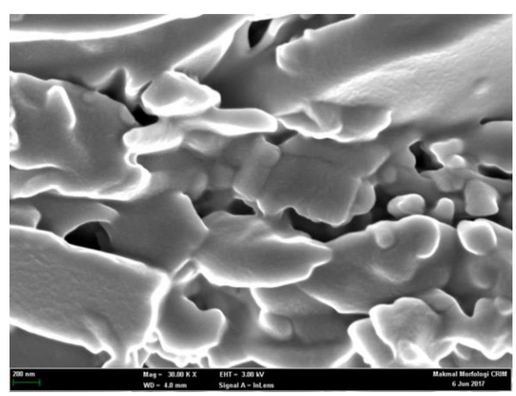

Fig. 2b : FESEM image of PANI-Fe-Al nanocomposite thin film

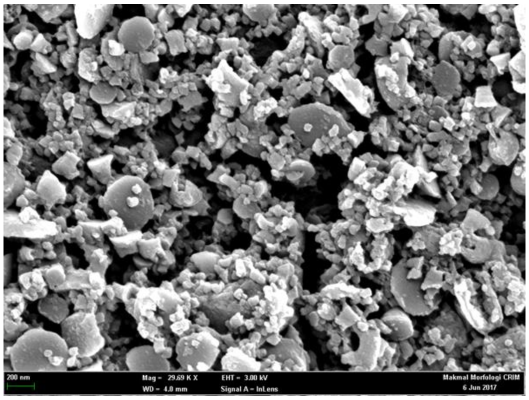

Fig. 2c : FESEM image of PANI-Fe-Mn nanocomposite thin film

AFM images in Figure 3(a, b and c) shows the morphology of the films with a successful dispersion of metal alloys nanoparticles [29]. The distribution metal alloys on the PANI substrate are comparatively uniform on the glass slides prepared by spin coating [30]. This could improve the stability of a device fabrication to be used as a sensor. The average roughness of the surfaces was recorded to be in between 40 nm to 60 nm [31]. The atom radius of Ni is greater than Al and Mn; thus the combination with Fe material will increase the film roughness to produce less smooth surface. This morphology assured in facilitating the adsorption of Leptospira bacteria into the film surface [32]. It could as well increase the responses of the sensor.

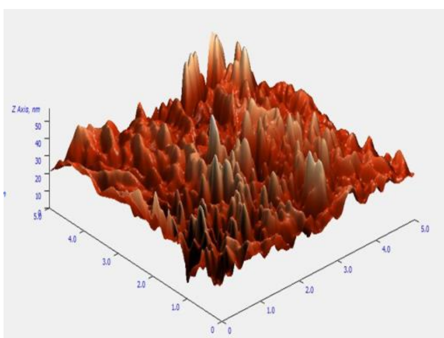

Fig. 3a : AFM image of PANI-Fe-Ni nanocomposite thin film

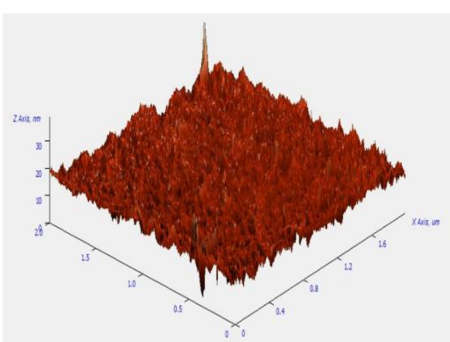

Fig. 3b : AFM image of PANI-Fe-Al nanocomposite thin film

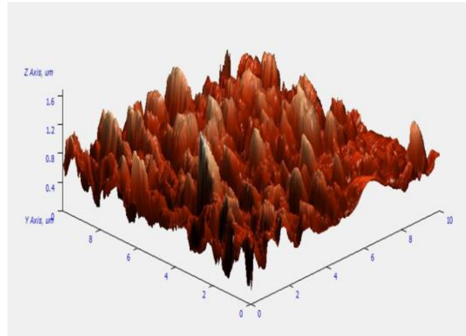

Fig. 3c : AFM image of PANI-Fe-Mn nanocomposite thin film

B. I-V measurements

The current voltage curve was obtained from the output terminal of the electrode sensor when thin films were immersed into Leptospira bacteria: 10^8 CFU mL^{-1}. Fig. 4 shows the current-voltage (*I-V*) characteristics of PANI-Fe-Ni nanocomposite thin film, PANI-Fe-Mn nanocomposite thin film and PANI-Fe-Al nanocomposite thin film. When 5V of voltage was applied on the PANI-Fe-Ni thin films during the experiment, the current drawn increases to 2.0 mA at the output terminal. For PANI-Fe-Mn nanocomposite thin film, the current drawn was 1.0 mA and for PANI-Fe-Al nanocomposite thin film, the current drawn was 1.2 mA, respectively [33]. Interactions between thin films with Leptospira was confirmed by the presence of an electrical conductivity that was incorporated due to active polyaniline and metal alloys. The result indicated that the interaction between the cell wall of Leptospira which are gram-negative bacteria [16]. The corresponding calibration curve presented a linear relationship between the peak current and the value of Leptospira concentration 1 x 10^8 CFU mL^{-1}. The PANI-Fe-Ni linear regression equations is, expressed as y = 0.0006x - 0.0007 with a correlation coefficient of 0.973, PANI-Fe-Al as y = 0.0005x - 0.0006 with a correlation coefficient of 0.9473, and PANI-Fe-Mn as y = 0.000003x - 0.00002 with a correlation coefficient of 0.9845, respectively.

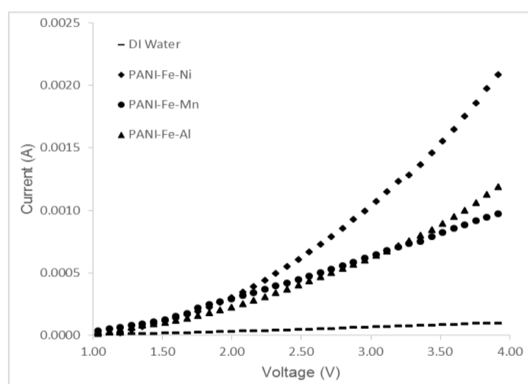

Fig. 4 : *I-V* measurement for PANI-Fe-Mn nanocomposite thin film, PANI-Fe-Al nanocomposite thin film and PANI-Fe-Ni nanocomposite thin film with Leptospira bacteria

The sensitivity of a sensor was explained by the ratio of the response volume when imposed to the bacteria (I_e) and without being imposed to the bacteria (I_o). The sensitivity (S) of Leptospira bacteria detection with PANI-Fe-Mn nanocomposite thin film, PANI-Fe-Al nanocomposite thin film and PANI-Fe-Ni nanocomposite thin film sensor with Leptospira bacteria could be estimated by using formula (1) [34]

$$S = (I_e - I_o / I_o) \times 100\% \quad (1)$$

where *(S)* is the sensitivity of PANI thin films on Leptospira, I_e is the current when the biosensor is imposed to Leptospira, and I_o is the current when the biosensor terminal is not imposed to Leptospira. Fig. 5 shows that the PANI-Fe-Ni nanocomposite thin film had a higher sensitivity performance compared to the other sample. The samples of PANI-Fe-Ni showed the highest sensitivity at around 2250 while the sample of PANI-Fe-Al nanocomposite thin film was around 1550, and amples of PANI-Fe-Mn nanocomposite thin film was around 1000. This happened because, when the thin films were immersed in the Leptospira solution, positive ion of thin film interacted with a negative charge of microbe wall [35].

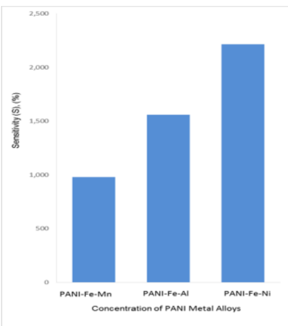

Fig. 5: Sensitivity (S) of PANI-Fe-Mn nanocomposite thin film, PANI-Fe-Al nanocomposite thin film and PANI-Fe-Ni nanocomposite thin film with Leptospira bacteria.

C. *Electrochemical Impedance Spectroscopy (EIS) Characteristics*

Electrochemical impedance was measured by applying an *AC* potential to the thin films. Electrochemical impedance spectroscopy was measured using a small excitation signal for characterizing the changes at the surface of the thin film. Fig. 6 shows the Nyquist impedance plots of the d PANI-Fe-Mn, PANI-Fe-Al, and PANI-F-Ni nanocomposites when the thin films were immersed into Leptospira bacteria. The differential impedance of the thin film was affected by the gram-negative cell wall structure of the Leptospira. The impedance was developed due to the changes in number, expansion and morphological behavior of the cell wall. When the cells of bacteria were enclosed to the electrode terminal, the electron moved among electrodes, where a rising trend of the electron resistance area showed appearance of signal from the bacteria [36].

Fig. 6 shows the total impedance drawn to a low value ranging from 0 to 2000 ohm when the PANI-Fe-Ni nanocomposite thin film was immersed into Leptospira solution. Next, the total impedance was drawn to a medium value ranging from 0 to 6000 ohm for PANI-Fe-Al nanocomposite thin film. Finally, the total impedance was later drawn to a high value ranging from 0 to 18000 ohm for PANI-Fe-Mn nanocomposite thin film. Concurrently, the readings showed that the current flow increased on thin film surfaces when the thin films were conducted in deionized water (DI). The semicircle on the Nyquist plot increased when PANI-Fe-Ni nanocomposite thin film was imposed with Leptospira. Then, the semicircle decreased while using the PANI-Fe-Al nanocomposite thin film and continues to increase a little bit when PANI-Fe-Mn nanocomposite thin film was used instead. Naturally, metabolism process causes a microbe to release gas structure at the cell walls and at te outer membrane arrangement. Due to this gas like structures, the interactions between microbe and surface of his thin film is affected in the experiment [37]

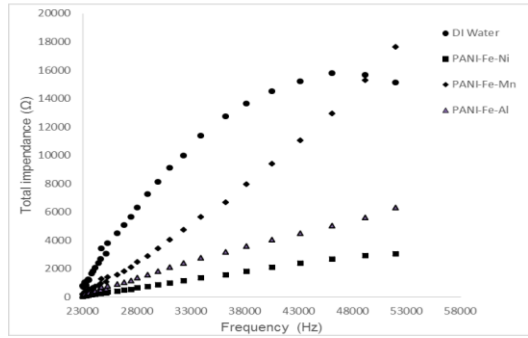

Fig. 6: Nyquist impedance plot of PANI-Fe-Mn nanocomposite thin film, PANI-Fe-Al nanocomposite thin film and PANI-Fe-Ni nanocomposite thin film with Leptospira bacteria

IV. Conclusion

In this study, PANI metal alloys nanocomposite thin films were synthesized via sol-gel method to be applied as Leptospira impedimetric sensor. The performance analysis of the sensor is implemented using *IV* and *EIS* measurements in deionized water and Leptospira bacteria solution. The metal alloys were successfully incorporated into the PANI matrix to increase the sensitivity of the thin film surfaces. In this attempt, Fe with Al, Mn, and Ni were incorporated to find the optimum condition for detecting Leptospira. PANI-Fe-Ni nanocomposite thin film performed at the highest sensitivity compared to the other samples. The FESEM image shows that the thin films surfaces were agglomerated because of the PVA effect. Besides that, the presence of the metal alloys also increases the detection capability of the sensor. AFM shows us that surface roughness decreases when the quantities of Al, Mn, and Ni increase in the PANI-Fe and these effects the detection capabilities as well. In this study, the experiment regarded to the lab-on-a-chip method has been used to detect Leptospira bacteria because this method is very fast and sensitive. It also provides a compatible platform for portable Leptospira detection. Another issue faced that must be taken into consideration is the interference from other bacteria. Thus, in future the selectivity test will be implemented to distinguish between Leptospira bacteria and other bacteria to deal with this problem. Likewise, the study to differentiate between the detection of saprophytic and pathogenic Leptospira are highly recommended as well.

Conflicts of Interests

The authors declare that there is no conflict of interests regarding publication of this paper.

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REFERENCES

[1] B. Unit, "Pathogenic and Saprophytic Leptospira Species in Water and Soils from Selected Urban Sites in Peninsular Malaysia," vol. 28, no. 1, pp. 135–140, 2013.

[2] T. Branch, "Isolation and identification of pathogenic and saprophytic Leptospira spp . from the rice fields of Tonekabon Township ...," no. November 2013, 2017.

[3] D. Benacer, S. Nursheena, M. Zain, F. Amran, R. L. Galloway, and K. L. Thong, "Isolation and Molecular Characterization of Leptospira interrogans and Leptospira borgpetersenii Isolates from the Urban Rat Populations of Kuala Lumpur , Malaysia," vol. 88, no. 4, pp. 704–709, 2013.

[4] Z. Arent, C. Frizzell, C. Gilmore, A. Allen, and W. A. Ellis, "Leptospira interrogans serovars Bratislava and Muenchen animal infections : Implications for epidemiology and control," *Vet. Microbiol.*, vol. 190, pp. 19–26, 2016. https://doi.org/10.1016/j.vetmic.2016.05.004

[5] C. Chirathaworn and S. Kongpan, "Review article Immune responses to Leptospira infection : roles as biomarkers for disease severity," vol. 8, no. 1, pp. 77–81, 2013.

[6] A. T. Slack *et al.*, "Leptospira kmetyi sp . nov ., isolated from an environmental source in Malaysia," no. 2009, pp. 705–708, 2016.

[7] S. T. Bvsc, I. D. Robertson, A. Fairuz, L. Suut, and M. T. Abdullah, "Leptospirosis , an emerging zoonotic disease in Malaysia," vol. 35, no. 2, pp. 123–132, 2013.

[8] B. Adler and A. De Pen, "Leptospira and leptospirosis," vol. 140, pp. 287–296, 2010.

[9] J. Rawlins, A. Portanova, I. Zuckerman, and A. Loftis, "Molecular Detection of Leptospiral DNA in Environmental Water on St . Kitts," pp. 7953–7960, 2014.

[10] P. Hernández-rodríguez, C. A. Díaz, E. A. Dalmau, and G. M. Quintero, "A comparison between polymerase chain reaction (PCR) and traditional techniques for the diagnosis of leptospirosis in bovines," *J. Microbiol. Methods*, vol. 84, no. 1, pp. 1–7, 2011. https://doi.org/10.1016/j.mimet.2010.10.021

[11] P. Chalayon, P. Chanket, T. Boonchawalit, and S. Chattanadee, "Transactions of the Royal Society of Tropical Medicine and Hygiene Leptospirosis serodiagnosis by ELISA based on recombinant outer membrane protein," *Trans. R. Soc. Trop. Med. Hyg.*, vol. 105, no. 5, pp. 289–297, 2011. https://doi.org/10.1016/j.trstmh.2011.01.008

[12] C. K. Murray *et al.*, "Transactions of the Royal Society of Tropical Medicine and Hygiene Use of patient-specific Leptospira isolates in the diagnosis of leptospirosis employing microscopic agglutination testing (MAT)," *Trans. R. Soc. Trop. Med. Hyg.*, vol. 105, no. 4, pp. 209–213, 2011. https://doi.org/10.1016/j.trstmh.2010.12.004

[13] M. Gremlica, R. Olejnik, D. G. Babar, and P. Slobodian, "SENSING ELEMENT BASED ON POLYANILINE COMPOSITES FOR POLAR ORGANIC VAPOURS DETECTION," pp. 4–8, 2014.

[14] A. P. Science and M. Sciences, "Ag / polyaniline nanocomposites : Synthesize , characterization , and application to the detection of dopamine and tyrosine Ternary Ag / Polyaniline / Au Nanocomposites : Preparation , Characterization and Electrochemical Properties 1," no. November, 2013.

[15] Z. Wang, E. Liu, D. Gu, and Y. Wang, "Author ' s personal copy Glassy carbon electrode coated with polyaniline-functionalized carbon nanotubes for detection of trace lead in acetate solution."

[16] S. C. K. Misra, P. Mathur, and B. K. Srivastava, "Vacuum-deposited nanocrystalline polyaniline thin film sensors for detection of carbon monoxide," vol. 114, pp. 30–35, 2004.

[17] B. Thakur, C. A. Amarnath, S. H. Mangoli, and S. N. Sawant, "Sensors and Actuators B : Chemical Polyaniline nanoparticle based colorimetric sensor for monitoring bacterial growth," *Sensors Actuators B. Chem.*, vol. 207, pp. 262–268, 2015. https://doi.org/10.1016/j.snb.2014.10.045

[18] B. Massoumi, S. Fathalipour, A. Massoudi, and M. Hassanzadeh, "Ag / Polyaniline Nanocomposites : Synthesize , Characterization , and Application to the Detection of Dopamine and Tyrosine," pp. 1–10, 2013.

[19] M. Matsuguchi and T. Asahi, "Sensors and Actuators B : Chemical Properties and stability of polyaniline nanofiber ammonia sensors fabricated by novel on-substrate method," *Sensors Actuators B. Chem.*, vol. 160, no. 1, pp. 999–1004, 2011. https://doi.org/10.1016/j.snb.2011.09.017

[20] Y. Hou, J. Feng, Y. Wang, and L. Li, "Enhanced antibacterial activity of Ag-doped ZnO / polyaniline nanocomposites," *J. Mater. Sci. Mater. Electron.*, 2016. https://doi.org/10.1007/s10854-016-4669-0

[21] P. Sciences, "Gold Nanoparticle and Conducting Polymer – Polyaniline – Based Nanocomposites for Glucose Biosensor Design," no. SEPTEMBER 2013, 2016.

[22] T. D. Centre, "Efficient ammonia sensing over zinc oxide / polyaniline nanocomposite," vol. 20, no. August, pp. 319–324, 2013.

[23] P. Sciences, "Gold Nanoparticle and Conducting Polymer – Polyaniline – Based Nanocomposites for Glucose Biosensor Design," no. SEPTEMBER 2013, 2016.

[24] D. Wei and A. Ivaska, "Electrochemical Biosensors Based on Polyaniline," vol. 839, pp. 839–852, 2006.

[25] Z. Durmus, A. Baykal, and H. Kavas, "Preparation and characterization of polyaniline (PANI)– Mn 3 O 4 nanocomposite," vol. 406, pp. 1114–1120, 2011.

[26] P. Wang, M. Liu, and J. Kan, "Sensors and Actuators B : Chemical Amperometric phenol biosensor based on polyaniline," vol. 140, pp. 577–584, 2009.

[27] L. Q. Hoa, Y. Sugano, H. Yoshikawa, M. Saito, and E. Tamiya, "Electrochimica Acta Structural assembly effects of Pt nanoparticle – carbon nanotube – polyaniline nanocomposites on the enhancement of biohydrogen fuel cell performance," *Electrochim. Acta*, vol. 56, no. 27, pp. 9875–9882, 2011. https://doi.org/10.1016/j.electacta.2011.08.071

[28] M. Malathi, V. D. V, and V. Dhayabaran, "CODEN (USA): PCHHAX Highly Sensitive Determination of Chromium (VI) Based on Reduced Graphene Oxide / Polyaniline - Nafion Composite Modified Electrode," vol. 8, no. 19, pp. 49–56, 2016.

[29] H. Abdullah, N. A. N. Azmy, N. M. Naim, A. A. Hamid, and S. Idris, "Synthesis and fabrication of ZnO-CuO doped PVA and ZnO-PbO doped PVA nanocomposite films by using γ-radiolysis and it's microbial sensor application," *J. Sol-Gel Sci. Technol.*, vol. 74, no. 1, pp. 15–23, 2015. https://doi.org/10.1007/s10971-014-3565-4

[30] R. Archana, B. D. Priya, M. Monisha, T. Vinoth, P. Raji, and R. Krishna, "Synthesis Of Al / ZnO-PANI Nanocomposites For Gas Sensing Application," pp. 319–326.

[31] A. Dutta, A. De, and C. Roy, "Sensors and Actuators B : Chemical Label free polyaniline based impedimetric biosensor for detection of E . coli O157 : H7 Bacteria," *Sensors Actuators B. Chem.*, vol. 171–172, pp. 916–923, 2012.

[32] H. Kim, S. H. Park, and H. Park, "Synthesis of a new electrically conducting nanosized Ag – polyaniline – silica complex using g -radiolysis and its biosensing application," *Radiat. Phys. Chem.*, vol. 79, no. 8, pp. 894–899, 2010. https://doi.org/10.1016/j.radphyschem.2010.02.005

[33] S. Ebrahimiasl and A. Zakaria, "Electrochemical Synthesis , Characterization and Gas Sensing Properties of Hybrid Ppy / CS Coated ZnO Nanospheres," vol. 11, pp. 9902–9916, 2016.

[34] V. Dixit, J. C. Tewari, and B. S. Sharma, "Detection of E . coli in water using semi-conducting polymeric thin film sensor," vol. 120, pp. 96–103, 2006.

[35] P. Mathur, S. C. K. Misra, M. Yadav, S. S. Bawa, and A. K. Gupta, "NANOCOMPOSITE COPOLYMER THIN FILMS SENSOR FOR DETECTION OF ESCHERICHIA COLI," vol. 6036, no. 2006, pp. 1–13.

[36] Y. Li *et al.*, "Biosensors and Bioelectronics Impedance based detection of pathogenic E . coli O157 : H7 using a ferrocene-antimicrobial peptide modi fi ed biosensor," *Biosens. Bioelectron.*, vol. 58, pp. 193–199, 2014. https://doi.org/10.1016/j.bios.2014.02.045

[37] V. Escamilla-gómez, S. Campuzano, M. Pedrero, and J. M. Pingarrón, "Biosensors and Bioelectronics Gold screen-printed-based impedimetric immunobiosensors for direct and sensitive Escherichia coli quantisation," vol. 24, pp. 3365–3371, 2009.

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