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Nano-composite sensors of polyaniline-Fe-Co for the detection of pathogenic Leptospira bacteria in environmental water

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Abstract-— **The detection of Leptospira bacteria in water using polyaniline (PANI) nanocomposites thin films doped with Fe-Co via sol-gel method is investigated in this research. In this study, the relationship between Leptospira bacteria concentration to current was measured based on current-voltage (I-V) performance. The morphologies of the thin film are characterized using FESEM, TEM and AFM. Based on the results, the developed nanocomposite thin films are compatible to detect the presence of pathogenic Leptospira bacteria. FESEM shows the irregularity of nanoparticles in terms of substrate size which was in the range of 80 nm to 120 nm. AFM image provided a specific morphology structure for Leptospira detection. Precisely, the PANI-Fe0.4-Co0.6 nanocomposite thin film has shown higher sensitivity towards pathogenic Leptospira detection. These results reaffirmed the potentials of Polyaniline-Fe-Co nanocomposite thin films for pathogenic Leptospira bacteria detection in water.**

Keywords - Pathogenic Leptospira; Polyaniline; I-V measurement; Thin Film.

I. INTRODUCTION

Leptospirosis is a zoonotic disease caused by gram-negative microbe called spirochaetes from the Leptospira species [1]. Normally, it can be found in tropical countries. The disease is spread via urine and body fluids of infected animal like rats or by indirect contact with contaminated water and soil [2]. As reported, rice field workers, farmers, veterinarians, and sewer workers is at risk of getting infected because they easily come in contact with this microbe [3]. Normally, Leptospirosis disease is detected using a microscopic agglutination test (MAT) or enzyme-linked immunosorbent assay (ELISA) method through serum antibodies, blood, and serological sample [4]. Similarly, the PCR technique can also be used to detect Leptospirosis using serum, urine, feces and various tissues samples. Culturing of blood, tissues, and serum samples for Leptospirosis diagnosis is allowed down due to time and technological constraints [5]. Development of alternative methods is needed to assess the presence of Leptospira in actual condition to prevent the massive infection. Therefore, the exploration of alternative Leptospira detection methods in water or wet sample is worthwhile to be investigated.

Microbial sensor can be considered to be a serious alternative method to detect Leptospira compared to other method currently used. Nowadays, various methods are available for detection of the pathogen according to various samples obtained like cell, pathogens, bacteria, viruses [6]. But still all the methods used have certain disadvantages like the sensitivity is insufficient for low-level detection of bacteria (≥105 CFU mL^{-1}).

The function of a biosensor is to convert biochemical reactions of bacteria into quantifying electrical response for analysis purposes. Conducting polymers have been extensively used in fabricating electrochemical biosensors because of its compatibility and stability towards biological environment. In present scenario, the polyaniline (PANI) are among the conducting polymers (CP) considered because it can be used for wide applications including biosensors owing to its stability, electrical properties and low costs [7]. The incorporation of metal alloys with conducting polymers can provide additional advantages in the form of electronic properties, unique structure, and thermal properties. Besides that, this

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combination also produces large specific surface area and high surface free energy which can be used for immobilization of biomolecules of biosensor structure. In addition, the electroactive properties of a surface area have been increased by the electron transfer kinetics between medium and the electrode sensor with a presence of metal alloys nanoparticles in the form of PANI[8,9]. The advantage of an ionic precursor infused with the polymer is that it provides a platform for better particle-polymer interaction. This is because, metal nanoparticles bind tidily into a conductive polymer to produce nanocomposites. PANI nanocomposite are widely used in various industries like electrostatic discharge system, biochemical sensors, light-emitting diodes, electronic devices, and chemical sensors.

In this research, PANI-Fe-Co nanocomposites thin films were synthesized and fabricated using a sol-gel method to be used as a microbial sensor. The performance of the thin film will be evaluated based on current-voltage (I-V). The morphology and structure of the PANI-Fe-Co were analyzed using atomic force microscopy (AFM), and field emission scanning electron microscope (FESEM). This study will help the researchers to detect Leptospira in waters which will be compared to the conventional detection methods such as MAT, ELISA, and PCR. By incorporating the metal alloys in PANI it can produce a sensor design with rapid and high sensitive for Leptospira detection.

Experimental: Method and Materials

Materials: The materials used were iron nitrate $Fe(NO_3)_3$ cobalt (II) nitrate $Co(NO_3)_2$, polyvinyl alcohol (PVA), nitric acid and aniline (C_6H_7N) which were purchased from Sigma-Aldrich Chemicals. Deionized (DI) water was provided by ELGA equipment system and was employed for the preparation of the solutions.

Preparation of PANI-Fe-Co Nanocomposites.

2.5 gram of polyvinyl alcohol (PVA) was dissolved in 40 mL of deionized (DI) water at 80 ^{0}C - 90 ^{0}C for 24 h. Next, 0.5 gram of iron nitrate and cobalt (II) nitrate $Co(NO_3)_2$ were added into the PVA solution according to the composition formula of Fe_{1-x}–Co_x where x represents the weight ratios of Fe and Co (x = 0.8, 0.6, 0.4, 0.2 and 0.0). These solutions were added with 1.25 mL of aniline and nitric acid at 1.0 M (HNO_3). The solutions were continuously stirred until the color changes to dark brown which shows that the solution has been turned into nanocomposites.

Sensor Fabrication

The solution was spread over using a spin-coater onto the substrates (20 mm × 25 mm) by using a dropper. The speed was controlled to be in between 1900 - 2000 rpm for 18-20 s. The spin coater used was from Laurell Technologies Corporation (LTC). The glass (thin films) was annealed at a temperature of 250 ˚C. The silver was sputtered onto the thin films following a comb pattern using silver magnetron sputtering model PVD 75. The pathogenic Leptospira samples used in this experiment were categorized at 10^8 CFU mL^{-1} (colony forming units per milliliter). These samples were provided from the Department of Veterinary Pathology & Microbiology, Faculty of Veterinary, and Universiti Putra Malaysia.

Characterizations and Sensitivity Performance

The morphology of the thin films was determined using field emission scanning electron microscope (FESEM). The surfaces morphology of the thin films was estimated using AFM scans. The current-voltage (I-V) was measured via A GAMRY-Physical Electrochemistry equipment. The measurement was obtained using two-electrodes as working electrode where one acts as a counter electrode and another one as a reference electrode. It was used to determine the current flowing across the films. I-V measurements were recorded when the thin films were immersed in DI water and Leptospira solution as shown in Fig. 1.

RESULT AND DISCUSSIONS

Morphological studies

FESEM images in Fig. 2 shows the surface morphologies of the film. The distributions of the nanoparticles were obtained in the range of 80 - 120 nm. Due to the combination of Fe-Co embedded into PANI, the films surfaces are very smooth with a coated spherical polymer [10]. This structure is highly beneficial for electrochemical sensing applications. The nanoparticle surfaces are derived from the agglomeration, due to the PVA characteristic. Besides that, the sensor surfaces contain small grain sizes and significant pore structures that have excellent responses during detection [11].

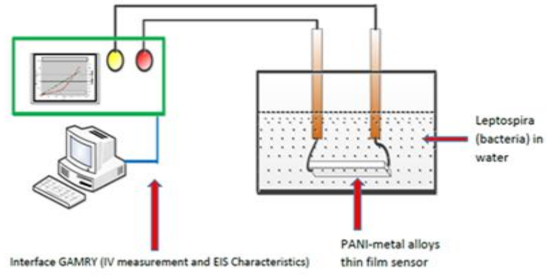

Fig. 1: Experimental setup

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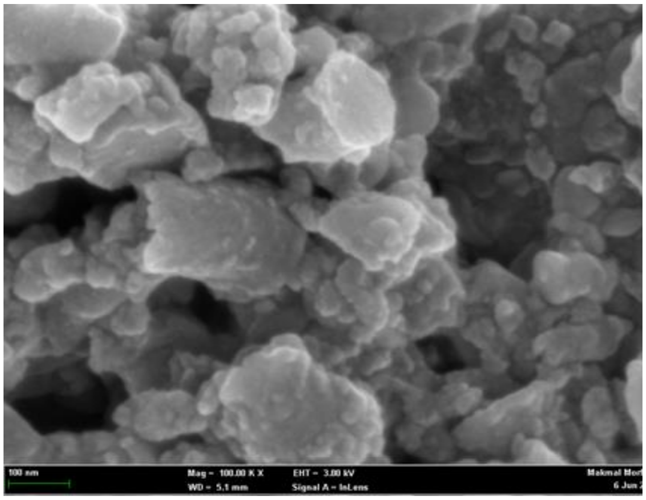

Fig. 2: FESEM image of PANI-Fe-Co nanocomposite

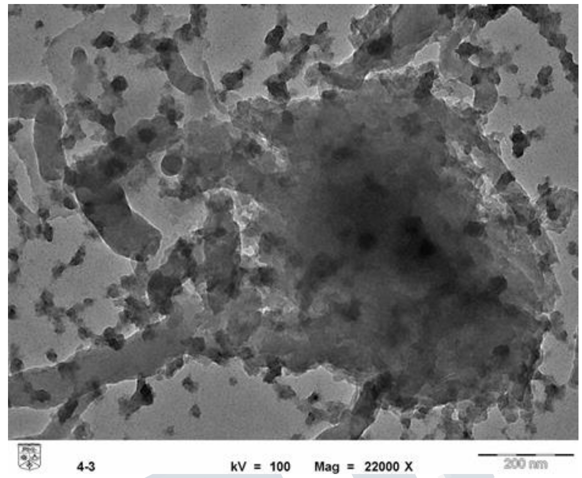

Fig. 3: TEM image of PANI-Fe-Co nanocomposite

TEM images in Fig. 3 shows the nanocomposite films of PANI-Fe-Co. The pattern of nanoparticles is spherical symmetry in shape and consists of numerous flower-like aggregates with multi-leaves. This may be due to the interaction of metal alloys (Fe-Co) infused into the PVA matrix [12]. It was observed that the metal alloys coated PANI showed a dark region as compared to a bright region of PANI. The PANI-metal alloys nanoparticles had typical diameters of 50-100 nm and lengths of 2-4 µm.
The AFM images in Fig. 4 show the morphology of the layered PANI nanosheets. The layered PANI nanosheets exhibit relatively high roughness between 30 nm to 50 nm. In fact, the electrode with high roughness surfaces affects the high electrochemical behavior. The PANI-coated metal alloys display a uniform layer, due to the spin-coated process. It represents polymer aggregates, which is important in determining the sensitivity of the sensor [13]. When the thin film is immersed into a Leptospira solution, the bacteria are attached on the thin film surface where interaction can be detected.

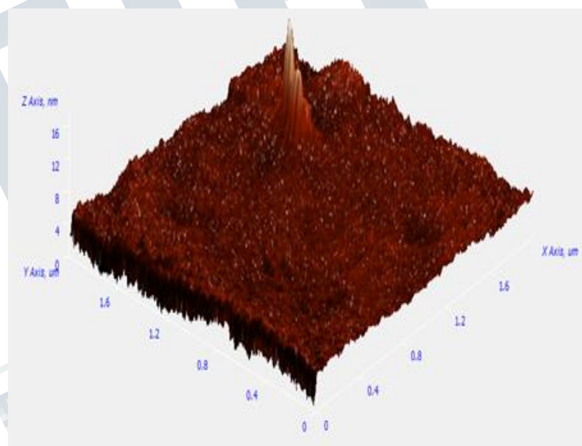

Fig. 4: AFM image of PANI-Fe-Co nanocomposite

Sensitivity (*S*) measurement

The *I-V* curve in Fig. 5 shows a response of current when the thin films are immersed into the Leptospira solution compared to the DI water. The current detected was low when thin films were immersed into DI water. However, a noticeable increase in current detection was observed after the thin films were immersed into Leptospira solution. The change in current-voltage characteristics shows the sensitivity of varied PANI-Fe-Co thin films against Leptospira bacteria [14].

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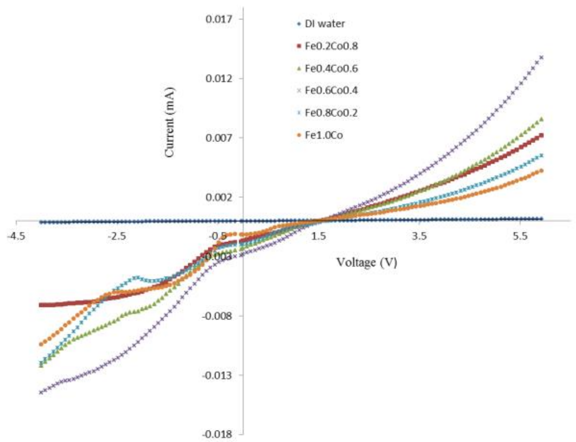

Fig. 5: *I-V* measurement of PANI-Fe-Co nanocomposite thin films with pathogenic Leptospira concentration: 10^8 CFU mL^{-1}

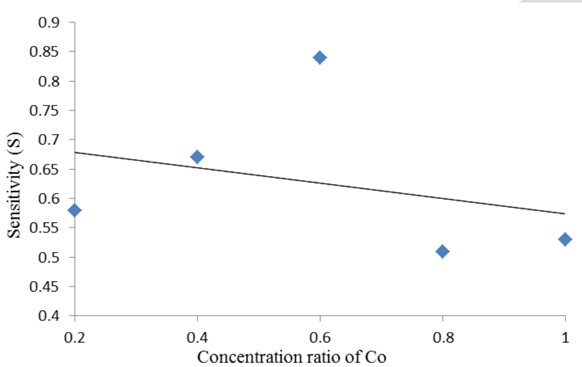

Fig. 6 Sensitivity of Fe–Co alloy thin films toward Leptospira bacteria against the molar ratio of Co

The PANI-Fe-Co nanocomposites sensitivity was analyzed through the ratio of the response volume on imposing bacteria Ie' and was compared to the values of imposing without the bacteria Io'. This was estimated from the I-V result using Equation 1.

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

where S is the sensitivity of various compositions of PANI-Fe-Co nanocomposite thin films towards the Leptospira concentration. Fig. 6 shows the sensitivity of PANI-Fe-Co nanocomposites thin films towards the pathogenic Leptospira concentration (10^8 CFU mL^{-1}). It has been found that PANI-$Fe_{0.4}$-$Co_{0.6}$ nanocomposites thin film gave a higher value. The samples of PANI-$Fe_{0.4}$-$Co_{0.6}$ performed the highest percentage of sensitivity which is 8.4%, while PANI-$Fe_{0.2}$-$Co_{0.8}$ only performed 5.0% of sensitivity towards Leptospira bacteria. The sensitivity of thin films also depends on the dopant of metals in the substrate. The interaction of Leptospira with Fe ion is compared with Co ion. When the concentration of Co decreases, both surface roughness and grain size of the thin films will decrease. Surface roughness and grain size are proportional to Fe concentration, which has a smaller radius than Co. The films surfaces become smooth because majority of Fe are on the substrates. It reduces the sensitivity towards the bacteria. Leptospira bacteria is attracted to the modified electrode because of the opposite polarity condition. This increases the current flow across the film sensor with decreased of impedance.

Conclusions

In this study, The PANI-Fe-Co nanocomposites thin films were successfully synthesized via sol-gel technique with low cost to be applied for Leptospira bacteria detection. The FESEM analysis shows the thin films coated with polymer and metal alloys produces suitable morphological characteristics of the films for Leptospira detection. The added advantage obtained was agglomeration happened on the films surfaces as well. AFM images shows that the sensitivity of the film surface has been enhanced for Leptospira detection due to addition of metal alloys such as Fe and Co. From the TEM images, the metal alloys added to the PANI have been identified to have an inner dark surface. I-V characterization indicates that the film is sensitive towards Leptospira bacteria. The sensitivity of films was observed on the I-V curves at different films. Finally, the results shows that Leptospira detection can be done instantly even under low concentration via the application of PANI-Fe-Co nanocomposites films.

Conflict of Interests

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

Acknowledgments

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