

Performance of electrochemical amperometric sensor based on annealed and non-annealed PANI-Ag-Pt nanocomposite thin films for *E. coli* detection

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ABSTRACT

Herein, we report the fabrication of polyaniline with Ag-Pt alloy nanocomposite thin films based amperometric *E. coli* sensor. The effects of annealing at 300°C during the synthesis of PANI-Ag-Pt nanocomposite thin films are studied. XRD, AFM, FESEM and FTIR spectroscopy analyses are conducted to characterize the films. Sensor performance is investigated by the *I-V* characteristic of PANI-Ag-Pt films with and without the presence of *E. coli*. From XRD analysis, annealed films have larger crystallite sizes than non-annealed films. AFM results indicate that annealed films have lower surface roughness but larger grain size than non-annealed films. FESEM images show Ag-Pt nanorods with sizes of 200-300 nm for non-annealed films and Ag-Pt nanocubes with sizes around 100-550 nm for annealed films. The composition of PANI-Ag_{0.2}-Pt_{0.8} nanocomposite thin film has higher conductivity and performed the maximum sensitivity upon *E. coli* presence. The sensitivity of annealed PANI-Ag-Pt films is higher than non-annealed PANI-Ag-Pt films.

Keywords: Polyaniline, Ag-Pt, thin films, Annealing, *E. coli*, Amperometric sensor

1. INTRODUCTION

Environmental hygiene is the most important thing to be concerned about in our daily routine, especially in our food and drinks to be free from any harmful pathogen. *Escherichia coli* (*E. coli*) O157:H7 is a zoonotic pathogen with the ability to cause human illnesses ranging from diarrheal disease to fatal hemolytic uremic syndrome [1]. This O157:H7 strain produces a large quantity of a potent toxin, in the lining of the intestine and causes severe damage resulting in fatal hemolytic uremic syndrome which may lead to death, especially in children [2]. Therefore, it is important to investigate the prevalence of *E. coli* O157:H7 in water sources especially used for drinking and to develop diagnostic methods for its early detection. Polymeric sensors are the recent ones for fabricating monitors for pathogen microorganisms [3-5]. Conducting polymers such as polyaniline (PANI) can be the most promising material for sensor devices due to its intrinsic conductivity [6, 7]. Conducting polymer nanocomposite has a much greater exposed surface area which can greatly improve diffusion and hence can enhance low detection limit in sensor application. Regarding the oriented microstructure and high surface area, conducting polymer can promote high

biomolecule loading, and hence highly sensitive detection is possible [8]. In electrochemical sensing, polyaniline is extensively used due to its electroactivity, compatibility with biological molecules, environmental stability, and ease of synthesis from low-cost aniline monomer. Polyaniline is also known as compatible with the biomolecule in a neutral liquid solution [9].

Great attention has been given to bimetallic nanoparticles due to their unique optical, electronic, magnetic and catalytic characteristics which are greatly difference from the monometallic nanoparticles [10]. When metal ions are added to a synthesized polyaniline system, the ions will react with the nitrogen atoms in the polyaniline chain and increase the electrical property which is good for sensor fabrication [11]. Ag nanoparticles are well known for their effectiveness against bacteria, viruses and fungi resistance. In antibacterial application, Ag nanoparticles were widely used against *E. coli* and other bacteria such as *S. aureus*, *P. aeruginosa*, *S. epidermidis* and *B. Subtilis* [12-16]. Pt nanoparticles are well known as catalytic, and the catalytic characteristic depends on their particle size and shape which are related to the synthesis method. Silver ion (Ag⁺) has an important role in controlling the shape of Pt

nanoparticles. In some reports, the combination of silver ion and platinum ion (Ag^+ and Pt^+) in the synthesis of silver-platinum (Ag-Pt) bimetallic nanoparticles can form various shapes of particles such as cubic, octahedral [17], nanoflowers [18], nanodendrites [19] and nanoislands [20]. The synthesis methods that influence the size and shape of particles include the annealing process. Annealing can produce uniformly spaced spherical structures of desirable sizes. It allows good control over the particle dimension and shape [21]. In this paper, the annealing temperature was set at 300°C for the synthesis of PANI-Ag-Pt thin film because at this temperature, the crystal growth, internal structure properties and sensitivity level for PANI-Ag-Pt thin film are at the optimum level [11, 22]. Temperatures above 300°C were not chosen because higher temperatures above 330°C could damage the properties and structure of polyaniline in the PANI-Ag-Pt thin film.

In sensing applications, some studies have been reported upon the use of Pt nanoparticles in various types of sensing apart from microbial sensors such as Hg^+ detection in water samples [23], determination of dopamine [24], glucose sensor [25] and pesticide detection [26]. The bimetallic Ag-Pt nanoparticles have been studied to enhance the antimicrobial properties of *E. coli* [27]. This shows that Ag-Pt possesses a potential characteristic in interaction with microorganisms to be used in microbial sensor applications.

The working principle of an amperometric is measuring the current (I) induced during the oxidation and reduction process by reaction material at a constant applied potential. The important factor which affects the amperometric sensor function is electron transfer between the catalytic molecule and the electrode surface of a conducting polymer [9]. The sensor converts biochemical reactions into a quantifying electrical response. The change in current-voltage (I - V) characteristics of the polymer thin films after exposure to various microorganisms is a measure of microbial sensitivity and their sensitivity is directly depending on the concentration of the microorganisms.

In this paper, Ag-Pt nanoparticles were embedded in polyaniline nanocomposite thin films to be fabricated as an amperometric sensor to detect *E. coli* bacteria in solution. The annealing effect during synthesis process of PANI-Ag-Pt nanocomposite thin films was being studied to obtain the best result in sensitivity performance.

2. EXPERIMENTAL METHOD

The experimental method is divided into several steps, including thin film synthesis, sensor fabrication, characterization analysis and sensor performance test.

2.1. Chemicals and Reagents

Aniline, silver nitrate (AgNO_3) and platinum (II) chloride (PtCl_2) were purchased from Sigma-Aldrich. Polyvinyl alcohol (PVA) and nitric acid (HNO_3 60%) were purchased from R&M Chemicals. The glass substrates were from

Corning 2947 and the silver target disc (for magnetron sputtering) is from Kurt J. Lesker. Sample of *E. coli* O157:H7 with a concentration of 10^8 CFU/mL were obtained from the Microbiology Laboratory, School of Bioscience and Biotechnology, Universiti Kebangsaan Malaysia.

2.2. Instruments for Film Characterizations

X-ray diffraction (XRD) patterns of PANI-Ag-Pt nanocomposite thin films were recorded using Advanced Bruker model D8 X-ray diffractometer from 2θ of 20° to 60° with step of 0.05° in 1 s using $\text{CuK}\alpha$ radiation and wavelength $1.5496 \text{ \AA}$. The surface morphology of the films was measured by atomic field microscopy (AFM) instrument model NT-MDT, NTEGRA Prima. The morphology of the films was analyzed by field emission scanning electron microscopy (FESEM) by Zeiss model LEO Supra 55 VP with magnitude 30 kx for all captures. Fourier transform infrared (FTIR) characterization was carried out from 4000 to 400 cm^{-1} wavenumber using FTIR spectrometer Nicolet 6700. For sensor performance, the sensitivity towards *E. coli* was measured via current-voltage (I - V) by electrochemical impedance spectroscopy Gamry model Series G 300.

2.3. Preparation of PANI-Ag-Pt Nanocomposite Thin Films

A total of 0.5 g of AgNO_3 and PtCl_2 powder (with molar ratio 4:1, 3:2, 2:3 & 1:4 for Ag to Pt) was dissolved in 40 mL deionized water before being added to polyvinyl alcohol (PVA) solution. The solution was stirred and heated at 70 - 80°C until homogeneous bimetallic Ag-Pt nanoparticles dispersion was produced by the appearance of dark brown colour. Aniline and 1.0 M of nitric acid were added to the Ag-Pt solution and the solution was continually stirred until the colour changed to cloudy greenish brown, indicating PANI-Ag-Pt nanocomposite solution was produced. The solution of PANI-Ag-Pt was then coated onto glass substrates by Laurell Technologies Corporation Spin-coater. The thin films of PANI-Ag-Pt nanocomposite were spin-coated at 2000 rpm for 15 s in atmosphere conditions. The films were then annealed in a tube furnace with a maximum temperature of 300°C for 24 h .

2.4. Fabrication of Sensor Films

A 100 nm layer of a silver electrode in comb-structure shape (as shown in Figure 1(a)) was sputtered on the PANI-Ag-Pt films by a magnetron sputtering method. The arrangement of these film layers can be seen in Figure 1(b). Masking was used to form the comb-structure of the silver electrode on the PANI-Ag-Pt nanocomposite film layer. The silver disc was used as the target source in the magnetron sputtering process. Argon gas was supplied in the sputtering chamber with the output power of 50 W in 454 s to sputter a 100 nm thickness of silver film. For the sensor performance experiment, copper wires were soldered to the silver electrode terminals as the connection to the measuring equipment.

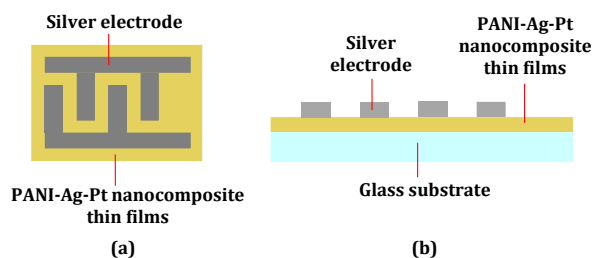


Figure 1. (a) Sensor electrode design with comb-structured silver electrode and (b) the cross-sectional view of PANI-Ag-Pt nanocomposite thin films (Patent no.: PI2015701438)

2.5. *E. coli* Detection Method

The performance of *E. coli* detection was conducted using current-voltage (I - V) measurement. The sensor device is connected to the instrument by wire clips based on a 2-electrodes system. The experiment was conducted by dipping the sensor device into deionized water and *E. coli* O157:H7 solution with concentration 10^8 CFU mL⁻¹. The data was measured from -4 V to 4 V with the scan rate 800 mVs⁻¹.

3. RESULT AND DISCUSSIONS

PANI-Ag-Pt nanocomposite thin films have been analyzed by X-ray diffraction (XRD), atomic force microscopy (AFM), field emission scanning electron microscopy (FESEM) and Fourier transforms infrared (FTIR) spectroscopy to study the crystallization, surface morphology, structure morphology and chemical bonding. To test the sensitivity performance and obtain the sensitivity value, the thin films were analyzed using I - V measurement.

3.1. X-Ray Diffraction (XRD) Analysis

Figures 2 (a) and (b) show XRD patterns of non-annealed and 300°C annealed PANI-Ag-Pt nanocomposite thin films with different compositions of Ag-Pt. The broad peak at $2\theta = 20^\circ$ to 25° was attributed to the characteristic peak of

amorphous PANI [28-30]. The low intensity of PANI peaks in Figure 2 (b) is due to the exposure to high temperatures during annealing [31]. The XRD patterns were analyzed and referred to ICDD for Pt (No. PDF: 00-004-0802) and bimetal AgPt (No. PDF: 03-065-3258) with face-centered cubic structure and space group symmetry Fm-3m (225). Three main peaks that appeared in Figures 2 (a) and (b) are attributed to AgPt (110), Pt (111) and Pt (200) which lies at $2\theta = 32.5^\circ$, 39.5° and 46.3° , respectively. From both figures, all the peaks at (1 1 0), (1 1 1) and (2 0 0) become sharper and narrower from $x = 0.2$ to $x = 0.8$. It means the crystallinity and the crystal growth in PANI-Ag-Pt increase when the composition of Pt increases.

The crystallite sizes were determined from the dominant peak in XRD patterns, and the values were calculated based on the classic equation of Scherrer [32]:

$$D = \frac{K\lambda}{\beta \cos \theta} \quad (1)$$

where D is the crystallite size, λ is X-ray wavelength, β (in radian) is full width at half maximum (FWHM), θ is Bragg's angle and K is Scherrer's constant. Table 1 shows the crystallite size of both non-annealed and annealed PANI-Ag-Pt nanocomposite thin films. From the result, it was found that the annealed films have larger crystallite sizes than the non-annealed films. The crystallite size increases after annealing due to the heat treatment effect upon the atomic structure of Ag and Pt which influence the crystal growth.

Table 1. The crystallite size of non-annealed and annealed PANI-Ag-Pt nanocomposite thin films

Sample	Crystallite size (nm)	
	Non-annealed	Annealed
(a) PANI-Ag _{0.8} -Pt _{0.2}	34.83	32.19
(b) PANI-Ag _{0.6} -Pt _{0.4}	38.91	33.79
(c) PANI-Ag _{0.4} -Pt _{0.6}	44.09	37.55
(d) PANI-Ag _{0.2} -Pt _{0.8}	47.24	39.75

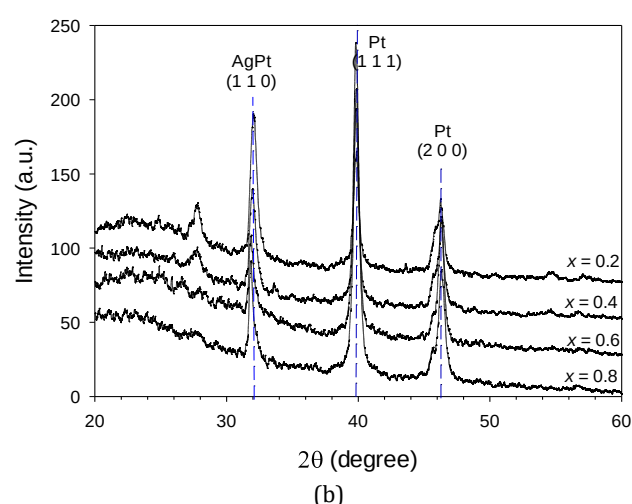
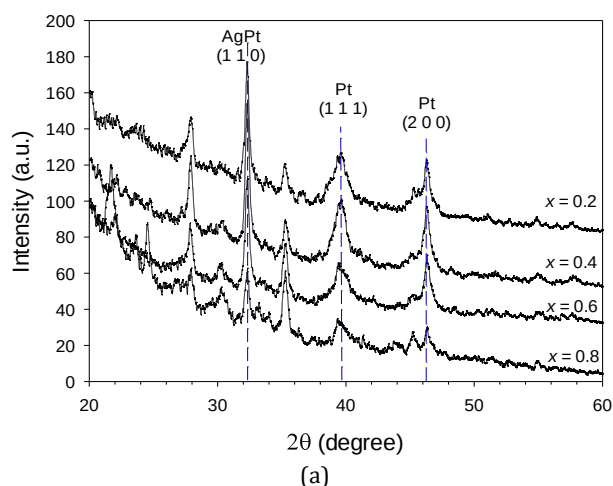


Figure 2. XRD patterns of (a) non-annealed and (b) annealed PANI-Ag_(1-x)-Pt_x nanocomposite thin films with $x = 0.2, 0.4, 0.6$ and 0.8

However, crystallite size value decreases as Pt concentration increases. This trend can be explained based on the ionic radius of Ag^+ and Pt^{2+} . Because the ionic radius of Ag^+ is larger than Pt^{2+} , thus the crystallite size of PANI-Ag-Pt is more influenced by the concentration of Ag [33]. The increasing of crystallite size with the increasing of Pt can also be related to the electronic configuration of Pt^{2+} ($4f^{14} 5d^8$) which is more likely to interact with ligands and anions of oxygen instead of Ag^+ ($4d^{10}$) which possesses a complete electronic configuration [34]. When the concentration of Pt became higher, the crystals grew larger.

3.2. Atomic Force Microscopy (AFM) Analysis

Surface morphology studies on PANI-Ag-Pt nanocomposite thin films can be conducted by AFM analysis. From the analysis, the surface roughness of the films can be determined, and the grain size can be estimated as well. Figure 3 shows AFM surface morphology image for non-annealed and annealed PANI-Ag-Pt nanocomposite thin

films, in different concentrations of Ag-Pt. The analysis data of surface roughness and grain size have been summarized in Table 2. The surface roughness of annealed PANI-Ag-Pt thin films is smaller than the non-annealed. The reduction of surface roughness after annealing can be explained by the dynamics of crystallization where PANI-Ag-Pt nanocomposite melts and coalesce into a relatively smooth surface at a higher temperature during annealing [35]. This is due to the diffusion of Ag and Pt atoms on the surface when the heat is exposed during the annealing process and surface diffusion occurs more easily than volume diffusion because of the smaller constraint on the surface [36]. The surface roughnesses increase when the Pt percentage increases for annealed and non-annealed films. The grain sizes of annealed films are larger than the non-annealed films, and the grain sizes increase when the Pt percentage increases. These results aligned with the crystallite size data from XRD, which is due to the heat treatment effect upon the atomic structure of Ag and Pt which influence the particle or grain size.

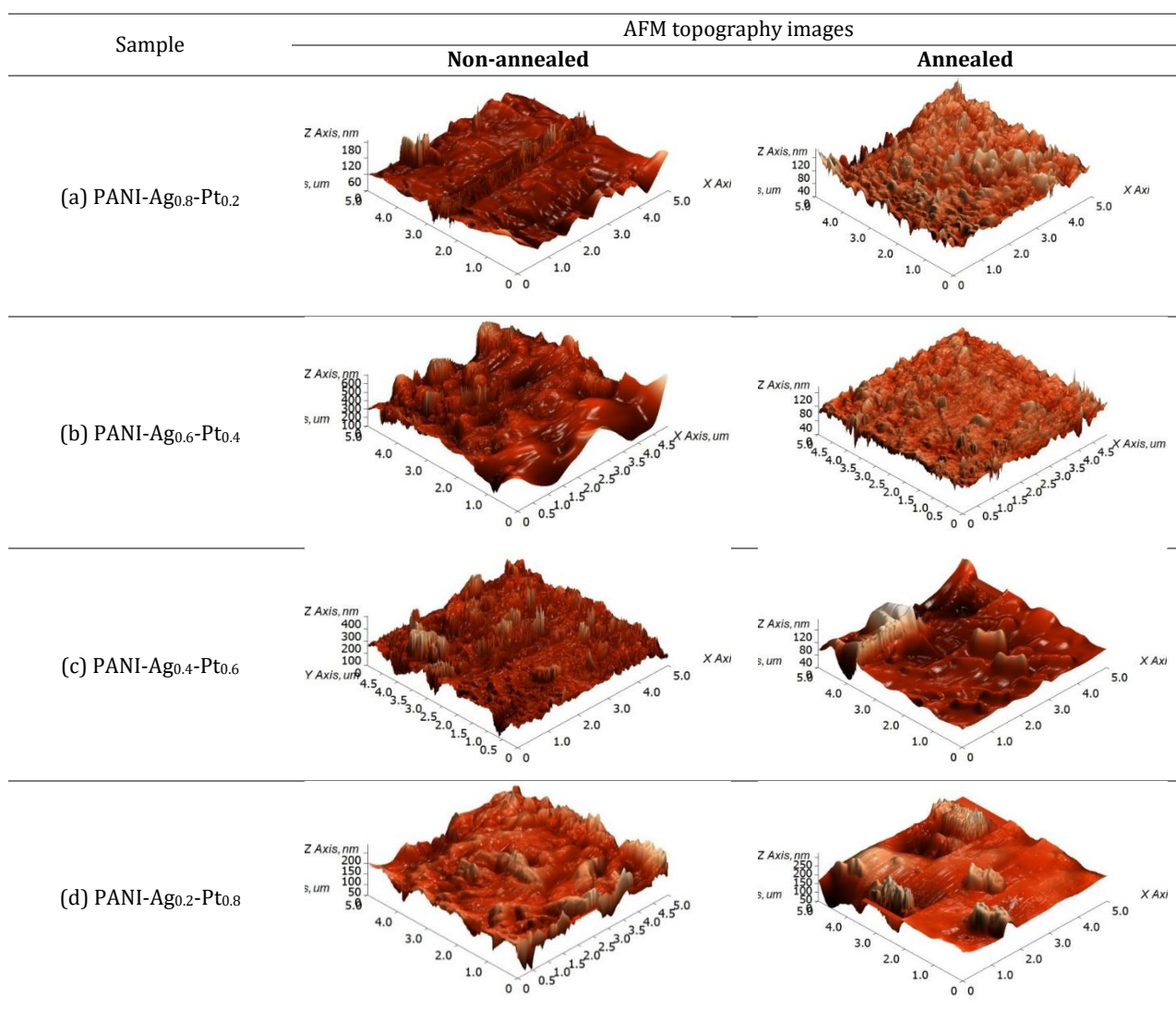


Figure 3. AFM 3D topography images of non-annealed and annealed PANI-Ag_(1-x)-Pt_x nanocomposite thin films with $x = 0.2, 0.4, 0.6$ and 0.8

Table 2. The average surface roughness and grain size of non-annealed and annealed PANI-Ag-Pt nanocomposite thin films

Sample	Surface roughness (nm)		Grain size (nm)	
	Non-annealed	Annealed	Non-annealed	Annealed
(a) PANI-Ag _{0.8} -Pt _{0.2}	21.084	6.740	43.3	49.5
(b) PANI-Ag _{0.6} -Pt _{0.4}	26.349	5.260	55.5	57.0
(c) PANI-Ag _{0.4} -Pt _{0.6}	27.949	10.007	50.0	54.5
(d) PANI-Ag _{0.2} -Pt _{0.8}	34.857	10.693	59.0	70.0

3.3. Field Emission Scanning Electron Microscopy (FESEM) Analysis

The internal and cross-sectional structure of PANI-Ag-Pt nanocomposite thin film has been studied via FESEM images. The sample of PANI-Ag_{0.5}-Pt_{0.5} nanocomposite thin film has been chosen as the representative to be analyzed. Figures 4 (a) and (b) are the internal structure image and cross-sectional image of a non-annealed PANI-Ag_{0.5}-Pt_{0.5} nanocomposite thin film. Figure 4 (a) shows a bunch of Ag-Pt nanorods in the size range around 200 – 300 nm long. Figure 4 (b) shows the thickness of the film cross-section is about 122.8 nm. Figure 4 (c) and (d) are the internal structure image and cross-sectional image of annealed

PANI-Ag_{0.5}-Pt_{0.5} nanocomposite thin film. Figure 4 (c) shows well-distributed Ag-Pt nanocubes in the size range around 100 – 550 nm and Figure 4 (d) shows the thickness of the film cross section is about 223.3 nm. The dispersion of the particles is loose but most of them form agglomeration. The shape and size of Pt particles depend on the method of synthesis and Ag plays a role as the controlling agent. The heat exposure during annealing on PANI-Ag_{0.5}-Pt_{0.5} nanocomposite thin film has promoted the particles to form a nanocubic shape and the size is larger than non-annealed film. The heat from the annealing process has increased the atom mobility of Pt. The atom mobility of Pt increased the surface diffusion rate hence increasing the size of particles and the film's thickness [37].

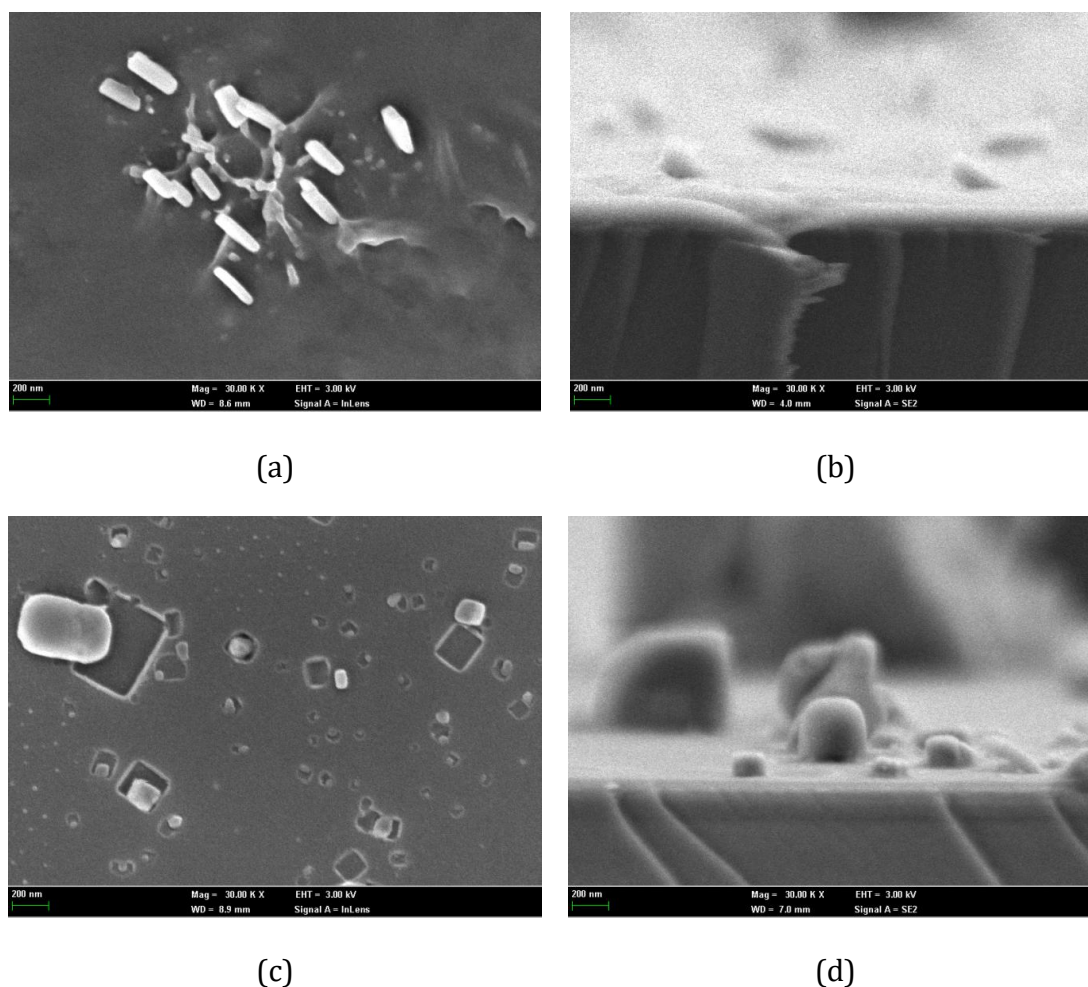


Figure 4. FESEM images of (a) internal structure and (b) cross-sectional structure of non-annealed PANI-Ag_{0.5}-Pt_{0.5} nanocomposite thin film; (c) internal structure and (d) cross-sectional structure of annealed PANI-Ag_{0.5}-Pt_{0.5} nanocomposite thin film

3.4. Fourier Transforms Infrared (FTIR) Microscopy Analysis

The FTIR spectrum of non-annealed and annealed PANI-Ag-Pt nanocomposite thin films was recorded in the wavenumber range 400-4000 cm^{-1} , using an FTIR spectrometer, is given in Figure 5. The peaks around 750 cm^{-1} in both spectra are attributed to Ag-O and Pt-O stretching vibrations. The peaks at around 880 – 900 cm^{-1} are assigned to the formation of polyaniline with 1, 4-substituted phenyl rings [38]. The bands around 1200 to 1500 cm^{-1} are ascribed to the stretching band of C-OH and C-O while the bands around 1600 to 1700 cm^{-1} are ascribed to the stretching band of C=C and C=O, where those stretching bands are from oxygen-containing functional groups [39].

3.5. Sensor Performance of Amperometric PANI-Ag-Pt Nanocomposite Thin Films

The sensor performance of the amperometric PANI-Ag-Pt nanocomposite thin films sensor was conducted by using *I-V* measurement when the sensor electrodes were immersed in deionized water and *E. coli* 10⁸ CFU mL⁻¹. Figure 6 (a) and 6 (b) show the *I-V* curve of non-annealed PANI-Ag-Pt nanocomposite thin films in deionized water and *E. coli* solution, respectively. Figures 6 (c) and (d) show the *I-V*

curve of annealed PANI-Ag-Pt nanocomposite thin films in deionized water and *E. coli* solution, respectively. Based on the equation of linear graph which is $y=mx+c$, the gradient m in the *I-V* curve is the inverted of resistivity, $1/R$ and R is inversely proportional to conductivity. From the graph of Figures 6 (a), (b), (c), and (d), PANI-Ag_{0.2}-Pt_{0.8} nanocomposite thin film has the highest gradient means it

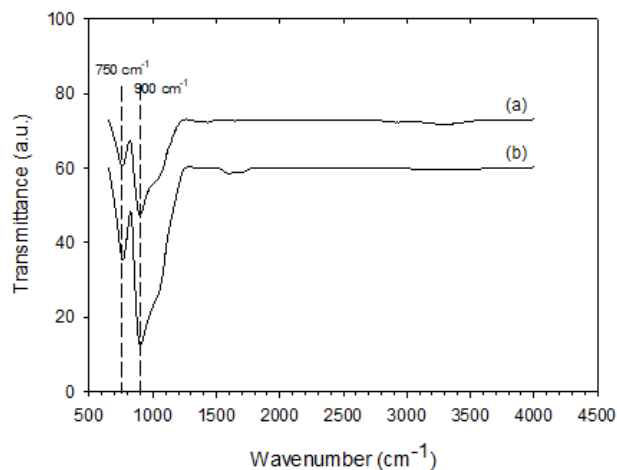


Figure 5. FTIR transmittance spectra of (a) non-annealed and (b) annealed PANI-Ag-Pt nanocomposite thin films

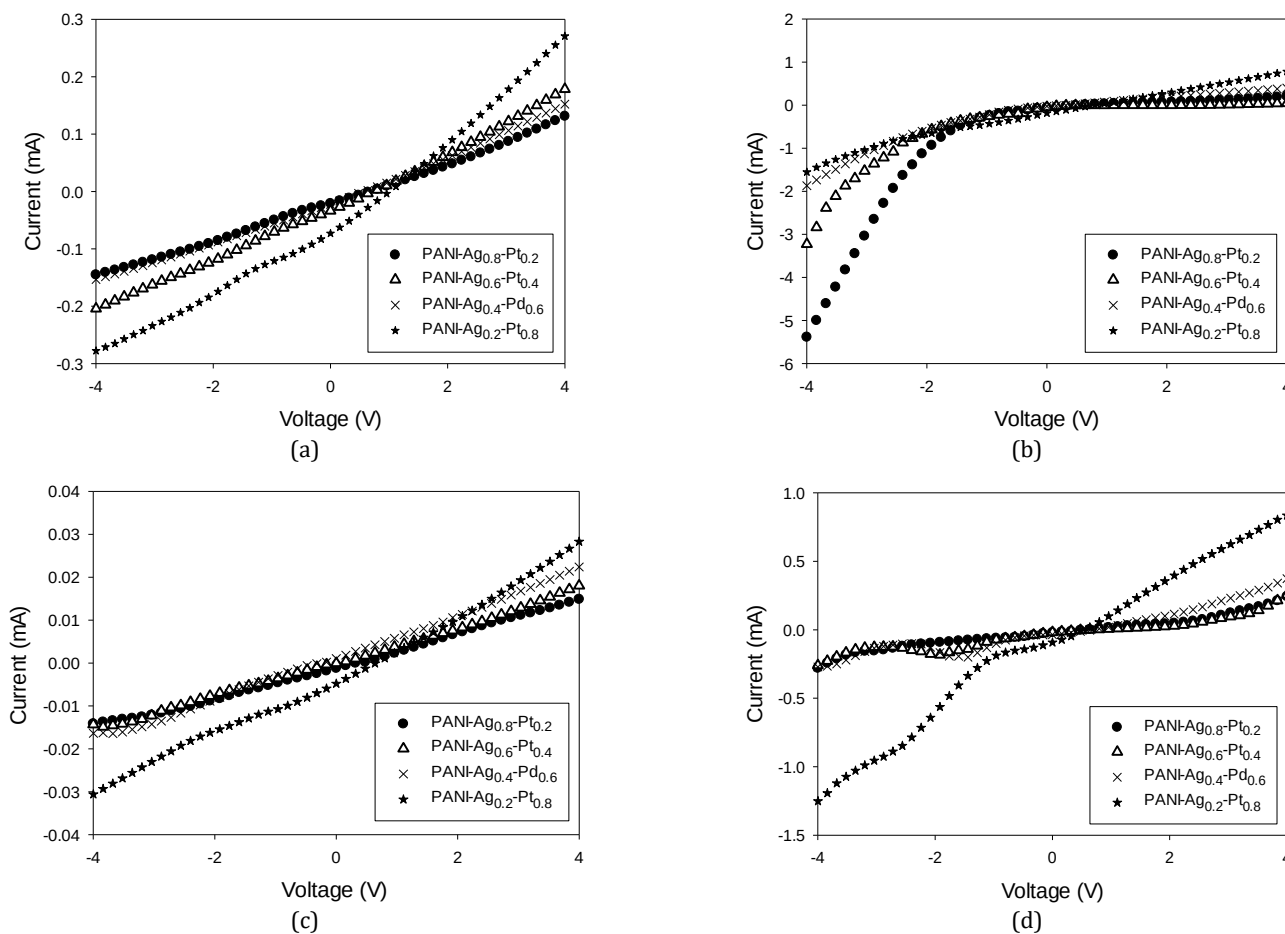


Figure 6. *I-V* measurement of non-annealed PANI-Ag-Pt thin films sensor in (a) deionized water and (b) *E. coli* solution; and annealed PANI-Ag-Pt thin films sensor in (c) deionized water and (d) *E. coli* solution

performed the highest conductivity, but PANI-Ag_{0.8}-Pt_{0.2} has the lowest conductivity. This shows that the higher percentage of Pt composition increases the conductivity of the system.

In Figure 6 (a), the current ranges for non-annealed PANI-Ag-Pt thin films in the deionized water are smaller (about -0.028 to 0.028 mA) than those in *E. coli* solution (about -5.5 to 0.7 mA). In Figure 6 (b), the current ranges for annealed PANI-Ag-Pt thin film in the deionized water are also smaller (about -0.03 to 0.03 mA) than is in *E. coli* solution (about -1.75 to 0.8 mA). The larger range of current flow in *E. coli* solution proved the presence of interaction between microbe and metal particles in the PANI-Ag-Pt matrix. The metabolism from microbes produces a more acidic environment for the metal ion release and the metal ion interacts with bacteria cell walls [40]. When the PANI-Ag-Pt thin film sensor is dipped in the *E. coli* solution, silver ion (Ag⁺) and platinum ion (Pt²⁺) on the film surface react with the *E. coli* cell. The positive charge in Ag⁺ and Pt²⁺ combine with the negative charge from *E. coli* cells, thus producing a more conducive environment. The higher the conductivity in a system, the higher the current flow.

The sensitivity of the sensor is described as the ratio of the current upon the sensor electrode exposed to the *E. coli* solution (I_e) to that of without exposure to the *E. coli* which is in deionized water (I_o). Figure 7 shows the graph of sensitivity (S) on *E. coli* against the percentage of Pt concentrations which are calculated using Equation (2) [41]:

$$S = \frac{I_e - I_o}{I_o} \quad (2)$$

where S is the sensitivity of the sensor electrode on *E. coli*, I_e is the current when the sensor electrode is exposed to *E. coli*, and I_o is the current when the sensor electrode is not exposed to *E. coli*. Figure 7 (a) shows the sensitivity (S)

towards *E. coli* for non-annealed PANI-Ag-Pt nanocomposite thin films against the concentration percentage of Pt and Figure 7 (b) shows the sensitivity (S) towards *E. coli* for annealed PANI-Ag-Pt nanocomposite thin films against concentration percentage of Pt. From both figures, it shows the maximum sensitivity is performed by PANI-Ag_{0.2}-Pt_{0.8} with sensitivity, $S = 2.55$ for Figure 7 (a) and $S = 34.62$ for Figure 7 (b). These results indicate that Pt particles interact with more cells in the microbe than Ag particles, thus increasing the conductivity in the system. As a comparison of Figures 7 (a) and (b), the annealed PANI-Ag-Pt nanocomposite thin films performed higher sensitivity than the non-annealed films. This is due to the annealing effect which enhances the crystal growth and increases the size of particles. By the result of crystallite size and particle size from XRD, AFM and FESEM, the larger size of crystallite and particle in PANI-Ag-Pt nanocomposite thin film increases the sensor performance towards *E. coli* presence.

4. CONCLUSION

Ag-Pt nanoparticles embedded in polyaniline nanocomposite thin films were fabricated as an amperometric sensor to detect *E. coli* bacteria in solution. The annealing effect during the synthesis process of PANI-Ag-Pt nanocomposite thin films had affected the growth of Ag-Pt crystal in the PANI matrix. The heat during annealing increased the crystallite size and particle size of Ag and Pt. From I - V measurement, PANI-Ag_{0.2}-Pt_{0.8} nanocomposite thin film has the highest conductivity among the others. From the sensor performance, PANI-Ag_{0.2}-Pt_{0.8} nanocomposite thin film performed the maximum sensitivity upon the *E. coli* presence. This indicates Pt nanoparticles interact with more cells in the microbe than Ag nanoparticles. The annealed PANI-Ag_{0.2}-Pt_{0.8} film performed higher sensitivity than the non-annealed PANI-Ag_{0.2}-Pt_{0.8} film. This is due to the larger size of particles in annealed films which enhances the sensitivity of the electrode film.

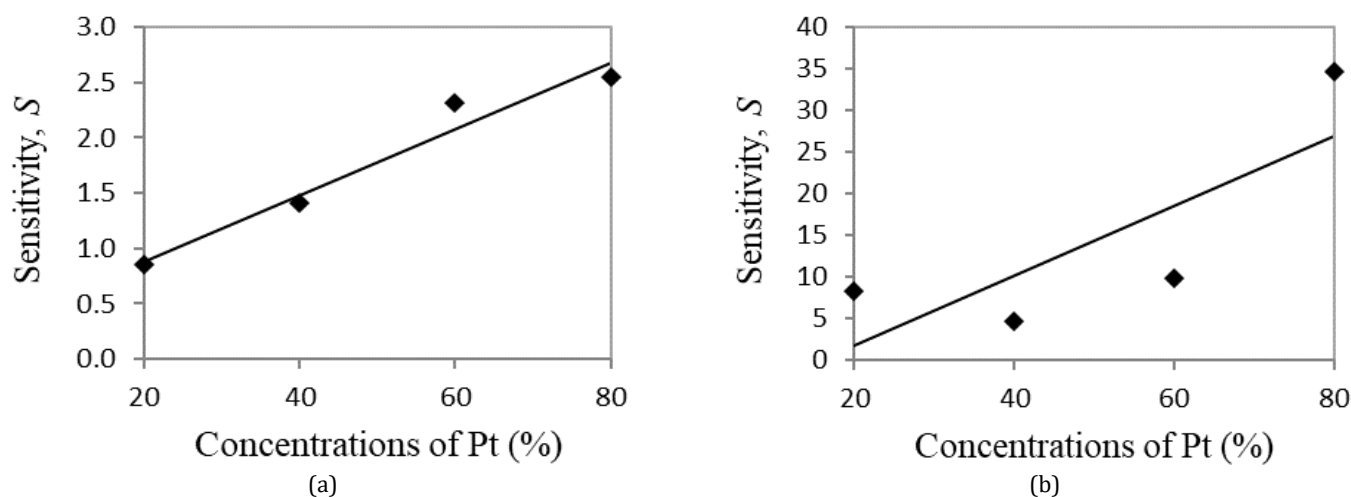


Figure 7. Sensitivity (S) upon *E. coli* for (a) non-annealed and (b) annealed PANI-Ag-Pt nanocomposite thin films against concentration percentages of Pt

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