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Synthesis and characterization of PVA-Ag doped metals (Co, Al) nanocomposite thin films biosensors for detection of *E. coli* in water

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Abstract. Polyvinyl alcohol (PVA) doped with Ag thin films were synthesized from aqueous solution via sol gel method. The nanoparticle of silver was synthesized by chemical reduction using hydrazine hydrate. PVA-Ag thin films were deposited on the glass substrate by spin coating technique. Samples were varied with different combinations of metals such as Ag-Co and Ag-Al. The solutions and the films were characterized by using XRD, UV-Visible spectroscopy, AFM and TEM. XRD analysis indicates the formation of the single crystal Ag, Co and Al nanoparticles laid on (111) lattice planes. The crystallite sizes decrease when Co and Al are added to the PVA-Ag. UV-Vis absorption spectra confirmed the formation of Ag nanoparticles in the PVA matrix and the resonance plasmon band located at 417, 421 and 429 nm. Surface roughness of PVA-Ag nanocomposite thin film increased with the addition of Co and Al. TEM images show the non-agglomerated spherical particles in all samples. The performance of the sensor has been fabricated using *I-V* measurement with and without incubated the sensor electrode into *E. coli*. The result shows PVA-Ag nanocomposite thin film performed the higher sensitivity.

Introduction

Determination and identification of the microorganisms are necessary to control the microbial infestation in contaminated food and water. Quick and simple methods to detect the type of life threatening microbial are very essential. Polymeric sensors are the recent ones for fabricating monitors for microorganisms. These sensors have been found to exhibit excellent sensitivity for monitoring microbiological activity in fermentation, living systems and medical sciences.

Nanomaterials can play an important role in antibacterial applications primarily due to their large surface area and size/shape-dependent physicochemical properties. Among various antibacterial nanomaterials nanosilver is the most promising one, which has exhibited much greater antibacterial effect as compared to bulk silver materials [1]. Silver exhibits the highest electrical and thermal conductivities among all the metals. Silver nitrate ($AgNO_3$) is used as a precursor because its decomposition temperature is very low and the product of the reaction is silver metal. Chemical reduction method has been widely used due to low preparation cost and its advantages of producing nanoparticles without aggregation. Regarding to previous report, $AgNO_3$ can be reduced from the silver salt to zero valent silver with the presence of reducing agents such as sodium borohydride ($NaBH_4$) [2], sodium formaldehyde sulfoxylate (SFS) [3] dimethyl formamide (DMF) [4], ethylene glycol and glucose [5]. It is now well established that the polymers are excellent host materials for nanoparticles of metals and semiconductors. Polymers have been frequently used as a size controlling agent and particle stabilizers in chemical synthesis of metal colloids, since they prevent agglomeration and precipitation of the particles as have been reported by Gautam *et al*., Abdul Kareem and Anu Kaliani [6, 7]. They use only PVA to promote the silver reduction reaction and stabilize the Ag particles, without any other chemical in order to avoid unwanted effects due to associates and impurities. Polyvinyl alcohol (PVA) is used as a stabilizing agent in this experiment to inhibit the growth of silver nanoparticles. When the nanoparticles are embedded or encapsulated in polymer, the polymer can acts as surface capping agent. In addition, film preparation become easier using PVA and the particle size can be controlled well within the desired regime when a thin film is formed.

The dispersions of silver nanoparticles display intense colours due to the plasmon resonances absorption. The surface of a metal is like plasma, having free electrons in the conduction band and positively charged nuclei. Surface plasmon resonance is a collective excitation of the electrons in the conduction band; near the surface of the nanoparticles. Electrons are limited to specific vibrations modes by the particle's size and shape. Therefore, metallic nanoparticles have characteristic optical absorption spectrums in the UV-Vis region [8].

In this paper, we give comprehensive research activities that concentrate on synthesis of Ag nanoparticle in polymer PVA aqueous and its fabrication as the thin film sensor. The PVA-Ag nanocomposite solution is deposited on glass substrate by spin coater. The prototype biosensor is developed from a low-cost and clean method, which also has ease of preparation to detect *E. coli* contamination in water.

Experimental details

The precursor silver nitrate ($AgNO_3$, 99.99% purity) and the size controlling agent polyvinyl alcohol (PVA, 99% hydrolysis) were purchased form Sigma-Aldrich Chemicals. 2.5 g of PVA salt was dissolved in 40 mL of deionized water and stirred at 80^o C - 90^oC. Silver nitrate solution was synthesized by dissolving 0.5 g of $AgNO_3$ in deionized water. When the PVA becomes completely dissolved, the solution transform into a transparent liquid, and then silver nitrate solution was added drop by drop into PVA solution by using pipette. Magnetic stirring continued until the solution become a brownish viscous liquid. The Ag^+ ions in the PVA/$AgNO_3$ aqueous solutions were reduced via the refluxing process without any chemical reducing agents, thus produce silver nanoparticles. 0.25 wt% of Cobalt and Aluminium are separately being added to the PVA-Ag nanocomposite solution. The nanocomposite of PVA-Ag, PVA-Ag-Co and PVA-Ag-Al solution was spin-coated on glass substrate using Laurell Technologies Corporation photoresist spinner, with the speed of 3000 rpm for 30 s. A comb structure of silver electrode was sputtered on the nanocomposite thin film for 1000 Å thickness. Copper wires were soldered to the silver electrodes as the connection between thin film and the measuring device. X-ray diffraction (XRD) analysis was conducted on Bruker model D8 Advanced X-ray diffractometer using CuKα radiation (λ = 1.5406Å) and the measurement were performed in 2θ range from 20^o to 60^o. The optical characterization of

PVA-Ag thin films was carried out using Perkin Elmer UV-Visible spectroscopy. The surface morphology of the thin films and the size of nanoparticles in PVA-Ag nanocomposite were studied from atomic force microscopy (AFM) and transmittance electron microscopy (TEM) respectively. Electrical characterization of the thin films were conducted using GAMRY-Physical Electrochemistry.

Results and discussions

Xray diffraction patterns in Fig. 1 show the formation of silver, cobalt and aluminium nanoparticles embedded in PVA matrix. All the reflection peaks can be indexed to face-centered cubic of silver, cobalt and aluminium correspond to the lattice plane (111). The average crystallite size of Ag (111) was calculated using Scherrer's equation [9]:

$$D = \frac{0.9\lambda}{\beta \cos\theta} \tag{1}$$

where λ=0.154 nm is the wavelength of X-ray for CuKα, β is FWHM (full width at half maximum intensity of the peak), θ is the diffraction angle and D is the crystallite size. The values of the calculated crystallite size have been summarized in Table 1. The average crystallite size in PVA-Ag nanocomposite is the largest among those samples which is 11.59 nm. The crystallite size decreased to 6.11 nm and 8.30 nm when Co and Al are added respectively to the PVA-Ag nanocomposite. This is due to the small lattice constant of fcc Co and Al compared to the lattice constant of Ag, thus the small addition of Co and Al contributes to the decreasing of average crystallite size in PVA-Ag-Co and PVA-Ag-Al nanocomposite.

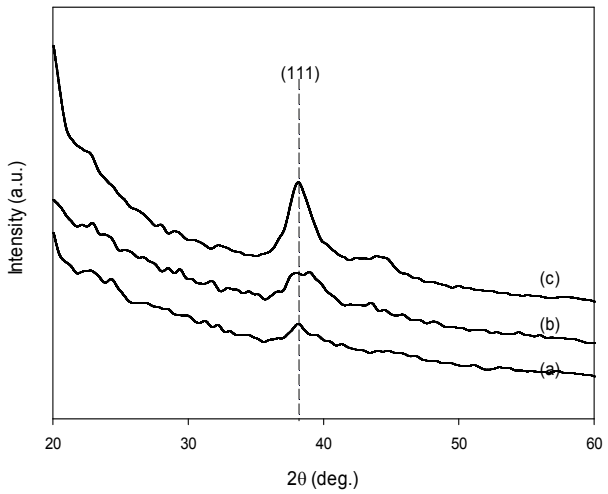

Fig. 1 XRD pattern of (a) PVA-Ag, (b) PVA-Ag-Co and (c) PVA-Ag-Al nanocomposite thin films.

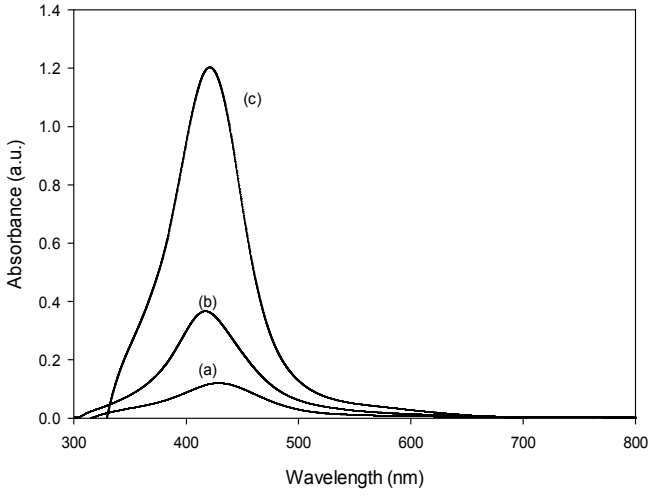

Fig. 2 UV-Vis absorption spectra of (a) PVA-Ag, (b) PVA-Ag-Co and (c) PVA-Ag-Al nanocomposite thin films.

UV-Vis absorption is performed to verify the presence of silver nanoparticles in the prepared samples of PVA-Ag, PVA-Ag-Co and PVA-Ag-Al as shown in Fig. 2. Those spectra contain strong plasmon band at 429 nm, 417 nm and 421 nm in Fig. 2(a), 2(b) and 2(c) respectively, confirmed that silver ions were reduced to Ag^o in the aqueous phase [10, 11]. Fig. 2 (c) has the higher absorption intensity. It was found that the higher absorption intensity reflects the formation of more nanoparticles. The absorption peaks shift to the longer wavelength which indicates the size of

particle become larger. This is because the larger particles require lesser energy and hence longer wavelength [12]. So, sample in Fig. 2(a) has larger particle size compared to the others.

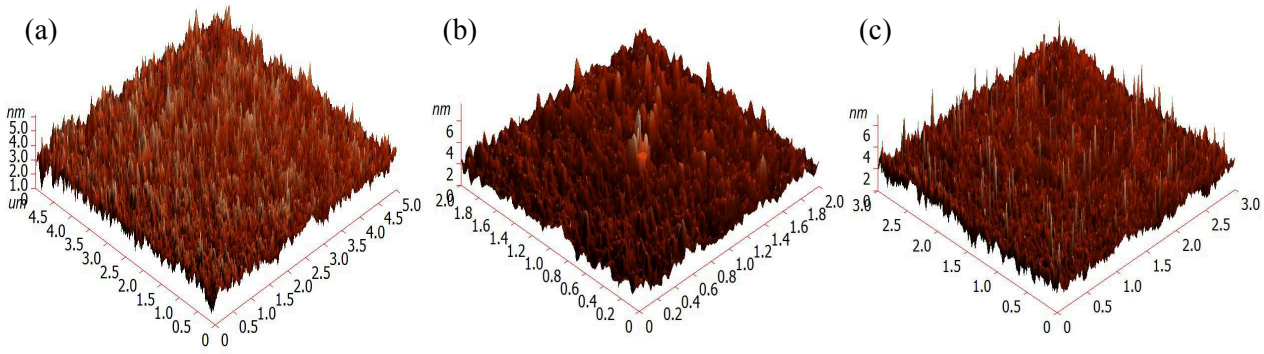

Fig. 3 AFM images of (a) PVA-Ag, (b) PVA-Ag-Co and (c) PVA-Ag-Al nanocomposite thin films.

AFM images in Fig. 3 show the morphology of the prepared nanocomposite thin films surface. The average surface roughness and grain size were being listed in Table 1. It is found that PVA-Ag nanocomposite in Fig. 3(a) has the lowest roughness thin film surface but it forms larger size of grain compared to Fig. 3(a) and 3(c) which have higher roughness and small grain size. The increasing in roughness is due to the mix of different metal grains in a PVA matrix even though the grain size is small.

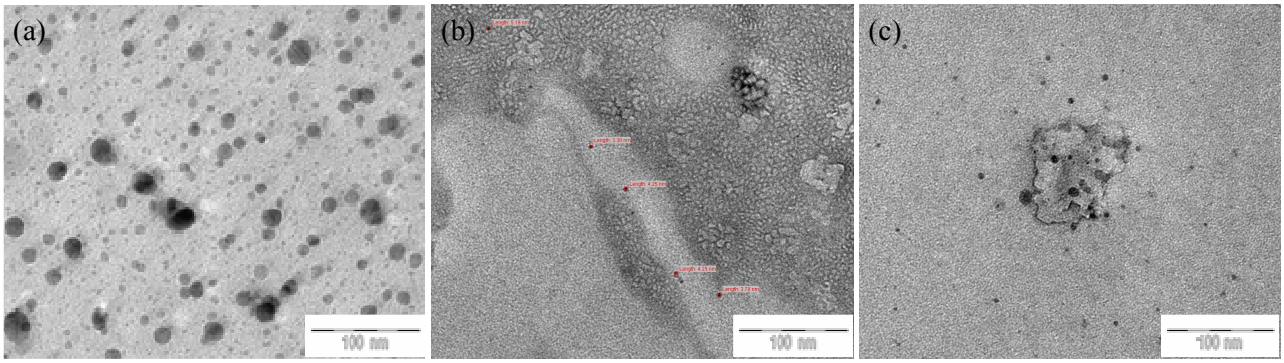

Fig. 4 TEM images of (a) PVA-Ag, (b) PVA-Ag-Co and (c) PVA-Ag-Al nanocomposite thin films.

The TEM image of PVA-Ag nanocomposite thin film as shown in Fig. 4(a), displays the growth of non-agglomerated spherical silver nanoparticles with the size range of 6 – 24 nm. In Fig. 4(b), the nanoparticle sizes are decreasing in the PVA-Ag-Co nanocomposite thin films with the size range of 3 – 6 nm. In Fig. 4(c), the particles in PVA-Ag-Al nanocomposite are larger than the particles in Fig. 4(b) with the size range of 4 – 9 nm and it formed more nanoparticles. The value of nanoparticles size in all samples has been summarized in Table 1.

Table 1. Surface roughness and internal characteristics of PVA-Ag, PVA-Ag-Co and PVA-Ag-Al nanocomposite thin films.

	Sample	Roughness (nm)	Grain size (nm)	Crystallite size (nm)	Particle size (nm)
(a)	PVA-Ag	0.434	50.7	11.59	6 – 24
(b)	PVA-Ag-Co	0.495	27.6	6.11	3 – 6
(c)	PVA-Ag-Al	0.447	33.0	8.30	4 – 9

The performance of the prototype biosensor has been measured through the *I-V* measurement to study the current change of the thin film sensor with and without incubation to *E. coli*. Fig. 5 describes the change of current for each PVA-Ag, PVA-Ag-Co and PVA-Ag-Al nanocomposite thin films in two different conditions which are in deionized water and in water with *E. coli*. The results show PVA-Ag nanocomposite performed the higher sensitivity among the other samples. The current is apparently changed between the condition when the sensor film was immersed in deionized water and *E. coli*. This proves that existence of reactions between metal and microbe. When the sensor films are immersed into water with *E. coli*, the metal ions and metal nanoparticles on the PVA-Ag thin film surface interact with the microbe. The positive charged of Ag^{+}, Co^{2+} and Al^{3+} could be attached to the negative charged *E. coli* [13]. The metabolism of the microbe creates an acid environment for the release of metal ions and it is believed that the silver ions interact with bacterial cell walls, plasma membranes, bacterial DNA and proteins, as well as ribosomes, resulting in bactericidal effects [1]. The metal and microbe interactions are mainly related to the cell wall and outer membrane arrangement. This is due to the significant differences in the outer layers of Gram-negative and Gram-positive bacteria. The cell wall of gram-negative bacteria consists of lipids, protein and lipopolysaccharides (LPS) that ensure more effective defense against biocides in comparison tp gram positive bacteria where the cell wall does not contains outer membrane of LPS [14]. Since *E. coli* are gram-negative bacteria, they possess an outer membrane and unique periplamic spaces [15], thus *E. coli* are more susceptible to the particles. From Fig. 5, we also found that PVA-Ag and PVA-Ag-Co nanocomposite thin film performs a good sensitivity as a biosensor while PVA-Ag-Al nanocomposite thin film has low of sensitivity on *E. coli*. According to silver's large surface area-to-volume ratio, it exhibits great efficiency against a broad spectrum of bacteria and is widely used in clinical applications such as catheter coating, wound dressing and antibacterial hand gels [16].

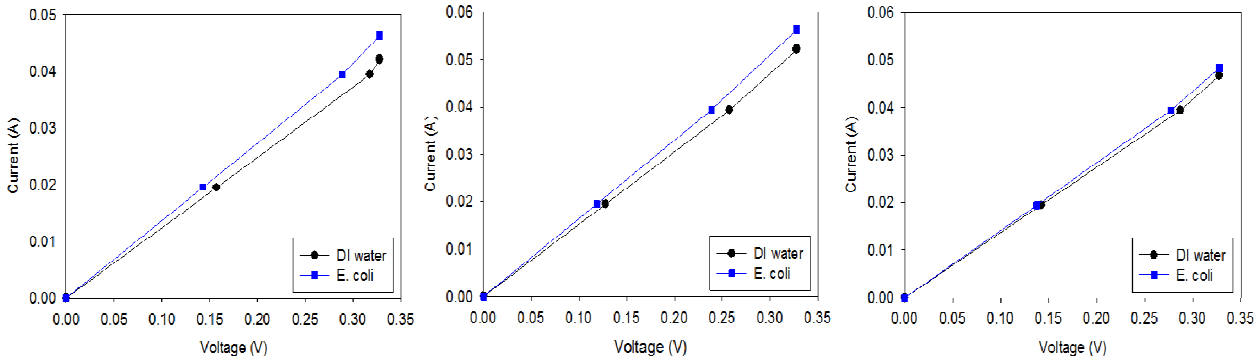

Fig. 5 *I-V* measurements of (a) PVA-Ag, (b) PVA-Ag-Co and (c) PVA-Ag-Al nanocomposite thin films in deionized (DI) water and with *E. coli*.

Summary

PVA-Ag nanocomposite thin films were synthesized by sol-gel method to be applied as *E. coli* contamination sensor. Ag-Co and Ag-Al alloys are also being added to the PVA matrix to study the internal characteristic of the nanocomposites. XRD analysis indicates the formation of the single crystal Ag, Co and Al nanoparticles laid on (111) lattice planes. The crystallite sizes decrease when Co and Al are added to the PVA-Ag. UV-Vis absorption spectra confirmed the formation of Ag nanoparticles in the PVA matrix and the resonance plasmon band located at 417, 421 and 429 nm. Surface roughness of PVA-Ag nanocomposite thin film increased with the addition of Co and Al. TEM images show the non-agglomerated spherical particles in all samples. The performance of the sensor has been conducted using *I-V* measurement with and without incubated the sensor electrode into *E. coli*. The result shows PVA-Ag nanocomposite thin film performed the higher sensitivity

among the other samples. The change in current when the sensor electrode incubated in *E. coli* proves the existence of reactions between metal and microbe.

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