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# Chemical oxidative polymerization of conductive polyaniline-iron oxide composite as an electro-transducer for electrochemical sensing applications

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**Abstract:** This study explored the preparation of conductive polyaniline-iron oxide (PANI-Fe<sub>2</sub>O<sub>3</sub>) that served as the electrical signal transducer, to convert the electrochemical interactions between the biotinylated-goat-anti-mouse IgG (b-IgG) and bovine serum albumin (BSA) into a measurable resistance signal. In this study, PANI was synthesized through the oxidative polymerization of aniline monomer (AM) in the presence of ammonium persulfate (APS) as the oxidizing agent. Concentration effects of AM and volume ratios of AM:APS were evaluated so as to obtain higher conductivity performance in an electrochemical sensing application. The synthesized PANI composites were analyzed through conductivity measurement. The conductive PANI-Fe<sub>2</sub>O<sub>3</sub>-GA-b-IgG bio-conjugates was then interacted with BSA, relays the antigen-antibody binding as a measured electrical detection in an assembled pulse-mode electrochemical biosensor. In present work, 0.2 M of AM with 1:3 volume ratio of AM:APS has contributed to the optimal ionic conductivity property of PANI, with excellent electrochemical sensing performance recorded at 3.538±0.067 MΩ.

**Keywords:** bio-conjugations; electrochemical-transmission; oxidative polymerization; polyaniline; transducer.

## 1 Introduction

Polyaniline (PANI) is among the most studied conducting polymers found in variety of applications such as rechargeable batteries, photonics, optoelectronic devices, biochemical sensing devices (1–4), membrane separation as well as modified electrodes for electro-reduction (5, 6). Scientific interest in PANI originates from the fact that this conjugated polymer is unique among conducting polymers (7–9). PANI produced by chemical oxidative polymerization of aniline (10–12), is well known to have good environmental stability (1, 3, 10, 13), controllable electrical properties (10, 14–16), easy to synthesis (13, 17), as well as its high pseudo-capacitance (5) that make them highly utilized in development of an electrochemical biosensor (11, 16, 18, 19). As for bio-sensing application, PANI performed as the transducer where this conductive polymer will integrate with the biological element, relays any antigen-antibody binding as a measured electrical quantity (20, 21).

Chemically synthesized PANI has been subjected to electrochemical characterization. Its electrical conductivity are reversibly controlled both by changes in the oxidation state of the main chain and by protonation of the imine nitrogen atoms (2, 9, 14). The potential-dynamic behavior of PANI obtained through chemical oxidation could be varies in the presence of different oxidants such as ammonium persulfate ((NH<sub>4</sub>)<sub>2</sub>S<sub>2</sub>O<sub>8</sub>), iron (III) chloride (FeCl<sub>3</sub>), hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) or dichromate (Cr<sub>2</sub>O<sub>7</sub><sup>2-</sup>) (19). Particularly, PANI exists stable in three different oxidation states: fully reduced leucoemeraldine base, partially oxidized emeraldine base and fully oxidized pernigraniline (15, 17, 22–24). Each oxidation state can exist in the protonated form after treatment with an acid. The conductivity of the PANI can increase up to ten orders of magnitude by addition of a protic solvent such as hydrochloric acid (25, 26).

Recently, there has been increasing interest in the synthesis of conductive polymer-inorganic composites with an organized structure. Physical properties of the conductive

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organic/inorganic nanocomposite can be tailored as a function of the amount of the composite constituents (27). Such polymer/inorganics composites have found a number of practical applications in molecular electronics, electronics displays, telecommunication, electrochemical storing systems and the most recently in biosensor (25, 28).

Looking more specifically to the hybrid composites with magnetic and electric properties (29), the colloidal PANI-iron oxide composite is synthesized through *in situ* oxidation polymerization in a micellar medium where the gamma iron oxide cores are embedded in a PANI matrix layer (29). Numerous research has been done by employing this conductive hybrid composite as a transducer in the development of the electrochemical sensor. For examples, the PANI-iron oxide composite has been directly applied to a direct-charge transfer sensing device for the detection of *Bacillus anthracis* endospores in contaminated food samples (29). Recent literature shows that different types of magnetic cores and doping agents have been used for the synthesis of the magnetic polyaniline nanoparticles. Typical examples of the magnetic core include iron (II, III) oxide, hydroxyl iron, Ni Zn Ferrite and Li Ni Ferrite (29). However, the structure and properties of the composite materials which depend on the protonation level and the particle-polymer interactions are still lacking access for wider development of the sensing tools.

Thus, in this work, PANI and magnetic PANI-Fe<sub>2</sub>O<sub>3</sub> composite were chemically prepared at different imine nitrogen protonation levels, that depend on the concentration of the aniline monomer (AM) and the volume ratio of AM to the oxidizing agent (ammonium peroxodisulfate, APS). An attempt was made to investigate the variation in the electrochemical properties of PANI-Fe<sub>2</sub>O<sub>3</sub>. The surface morphologies and conductivity properties of the composites were characterized using transmission electron microscopy (TEM) as well as the ionic conductivity measurement. The practicability of applying the PANI-Fe<sub>2</sub>O<sub>3</sub> composite for electrochemical-transmission were also conducted in the present work. All conductive PANI-Fe<sub>2</sub>O<sub>3</sub> composites were subjected to biological modification with the biotinylated goat anti-mouse IgG to perform the complex antigen-antibody biomolecules interactions in an assembled electrochemical sensing platform.

## 2 Methodology

### 2.1 Chemical and reagent

Methanol, monopotassium phosphate, dipotassium phosphate, glutaraldehyde 25% aqueous solutions, aniline

monomer, ammonium peroxodisulfate (APS), hydrochloric acid (HCl), lithium chloride, sodium chloride (NaCl) and *N,N*-dimethylformamide (DMF) were purchased from Merck (Darmstadt, Germany). Iron (III) oxide (Fe<sub>2</sub>O<sub>3</sub>) and biotinylated goat anti-mouse IgG were supplied by Sigma-Aldrich (St. Louis, MO, USA). All chemicals were reagent grade and were used without further purification.

### 2.2 Synthesis of conductive polyaniline-iron oxide (PANI-Fe<sub>2</sub>O<sub>3</sub>) nanocomposite

Oxidative polymerization of the aniline monomers (AM) were performed in the presence of ammonium peroxodisulfate (APS) as an oxidizing agent and HCl as protoning agent (doping agent). AM (concentration range from 0.2 M to 1.0 M) was first diluted in 1 M HCl and mixed with 0.8 mM APS at different volume ratio (AM:APS volume ratio range from 1:1 to 1:5). Subsequently, iron (III) oxide (Fe<sub>2</sub>O<sub>3</sub>) was added to the mixture for *in-situ* chemical oxidative polymerization. Magnetic nanoparticles (Fe<sub>2</sub>O<sub>3</sub>) were used as the separation agent to collect the successful conjugated PANI-Fe<sub>2</sub>O<sub>3</sub> nano-composite from the unconjugated PANI solution. A reaction was allowed in an incubated shaker (Protech, Model SI-100D, Malaysia) at 27°C for 4 h until the solution was visibly transitioned from rust brown (color for the Fe<sub>2</sub>O<sub>3</sub> suspension) to dark green, indicating conductive emeraldine salt of polyaniline was produced. The ionic conductivity property of the PANI emeraldine salt was measured by using the conductivity meter (Thermo Scientific Orion 4 Star, Singapore) at room temperature of 27°C.

Methanol was then added to quench the polymerization. The successful conjugated PANI-Fe<sub>2</sub>O<sub>3</sub> nano-composite was then separated using an external magnetic bar, where the unconjugated PANI was removed from the solution. Collected composite materials were then suspended in phosphate buffer saline (PBS) contained of 0.1% (v/v) LiCl and 10% (v/v) DMF. The chemical properties and structure of the synthesized PANI and PANI-Fe<sub>2</sub>O<sub>3</sub> composites were analyzed using UV-Vis absorption (Spectroquant Pharo 300, Merck, Darmstadt, Germany) and transmission electron microscopy (TEM, Philips/FEI CM12, Hillsboro, OR, USA). Three replications were performed for each synthesis composition for repeatability estimates. The composition of HCl-doped PANI was analyzed using a Thermo Scientific Fourier transform infrared spectrometer (ATR-FTIR, NICOLET iS10, USA) over the wavenumber range of 4000–525 cm<sup>-1</sup>. A diamond crystal was used with an incident angle of 45°. Each spectrum was taken from 16 scans with a resolution of 4 cm<sup>-1</sup>.

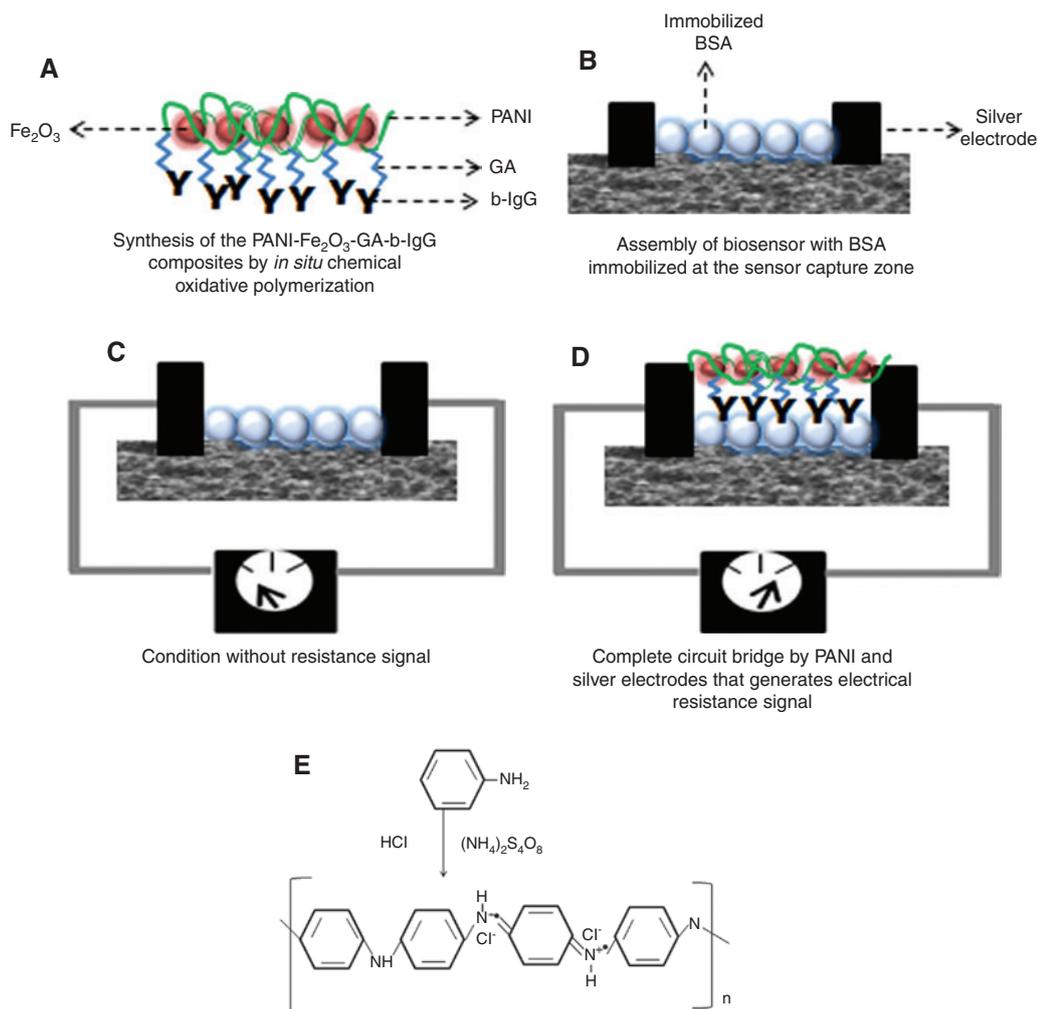
### 2.3 Biologically modification of the PANI-Fe<sub>2</sub>O<sub>3</sub> composites

Prior conjugate with the biotinylated goat anti-mouse IgG (b-IgG), glutaraldehyde (GA) was first conjugated to the PANI-Fe<sub>2</sub>O<sub>3</sub> nano-composite to increase the biomolecule's stability for later bio-sensing application. Conjugation occurs in an incubated shaker for 30 min at 100 rpm. Subsequently, the conjugated PANI-Fe<sub>2</sub>O<sub>3</sub>-GA composite was separated using an external magnetic bar to remove the excess GA. The composites were then suspended in PBS buffer and sonicated for 30 min to ensure good dispersion for better conjugation with b-IgG. A total of 200 µg/ml of b-IgG in PBS buffer (pH 7.4) was added to the suspended PANI-Fe<sub>2</sub>O<sub>3</sub>-GA composite at a weight ratio of 1:1. The suspended solution was shaken at 100 rpm and temperature of 37°C. The successful bio-conjugated

PANI-Fe<sub>2</sub>O<sub>3</sub>-GA-b-IgG (Figure 1A) were separated using the external magnetic bar to remove the unbound b-IgG. The bio-conjugates were finally suspended in the PBS buffer (containing 0.1 v/v % LiCl and 10 v/v % DMF) and stored at the temperature of 4°C. Samples before and after conjugation were analyzed using UV-Vis spectroscopy and sent for zeta potential measurement (Zetasizer Nano ZS900, Malvern Instruments Ltd, UK) to ensure the success bio-conjugation of the b-IgG to PANI-Fe<sub>2</sub>O<sub>3</sub>-GA composite.

### 2.4 Performance evaluation of PANI-Fe<sub>2</sub>O<sub>3</sub>-GA-b-IgG as an electrical signal transducer

In the present work, the PANI-Fe<sub>2</sub>O<sub>3</sub>-GA-b-IgG composite acted as an electrical signal transducer to convert the

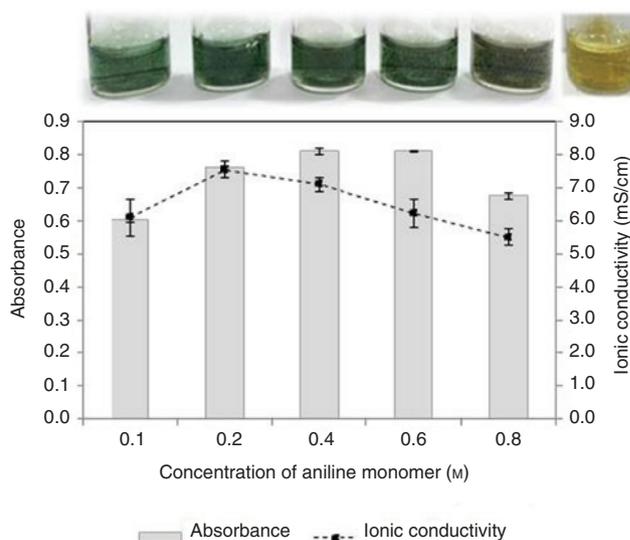


**Figure 1:** Schematic diagram of the: (A) bio-conjugates PANI-Fe<sub>2</sub>O<sub>3</sub>-GA-b-IgG (B) assembly of biosensor with BSA immobilized on the membrane capture zone (C) conductivity performance of biosensor via pulse-mode measurement (D) complete electrical circuit by PANI and silver electrodes that generates electrical resistance signal (E) the formation of the emeraldine salt.

electrochemical interactions between b-IgG and bovine serum albumin (BSA) into a measurable resistance signal. The conductivity performances of the synthesized PANI-Fe<sub>2</sub>O<sub>3</sub>-GA-b-IgG composites were evaluated through pulse-mode measurement. First, the electrochemical sensing platform was assembled schematically as shown in Figure 1B. BSA which acts as the capture analyte was immobilized at the sensing device's capture zone by using the protein dispenser (Kinematic M1600, USA) under a controlled dispense volume of 1  $\mu$ l/cm. Prior to the electrochemical sensing performance, two drops of HCl were added to the bio-conjugates solution to ensure the electrical activation of PANI. For electrochemical sensing application, 100  $\mu$ l of testing solution containing bio-conjugates (PANI-Fe<sub>2</sub>O<sub>3</sub>-GA-b-IgG) was placed in each well of a 96-well microplate. Subsequently, the assembled membrane sensor was mounted into the solution well for detection analysis. The bio-conjugates solution was moved laterally along the sensing strip towards the upper capture zone for biomolecule interactions between b-IgG and BSA. Throughout the biomolecule interactions, the PANI composites formed a bridging electrical circuit between both the silver electrodes and generated electrical resistance recorded by the multimeter (model U1253B, Agilent, Malaysia), as described in Figure 1C and D. Three replications were performed for each composition of PANI-Fe<sub>2</sub>O<sub>3</sub>-GA-b-IgG bio-conjugates for repeatability estimates.

### 3 Results and discussion

As aniline and ammonium peroxydisulfate (APS) were chosen as the monomer and oxidizing agents, respectively, for the preparation of polyaniline (PANI), their concentration and volume ratio effects on the ionic conductivity of PANI were studied. The experiments were conducted by oxidative polymerized different concentrations of aniline monomer (AM) in an acidic aqueous media with APS, the color intensities of the solutions are illustrated in Figure 2. As is known, the occurrence of the oxidative polymerization will turn the colorless AM dark green in color, which indicates the formation of the conductive emeraldine salt of PANI. The protonation level of PANI is reflected by the absorbed visible light at a given wavelength in a sample, which is described by the Beer-Lambert law. The UV-visible spectrum of the emeraldine salt PANI was first examined from wavelengths 200 to 1100 nm. Two absorption bands were observed at 365 and 748 nm. The first band is assigned to the  $\pi$ - $\pi^*$  transition of the benzenoid rings and the second band is correlated to the  $\pi$ -polaron transitions. The presence



**Figure 2:** UV-Vis absorbance intensity and ionic conductivity properties of PANI-emeraldine salt (green color) produced at different concentration of aniline monomer. Note: PANI was synthesized using a constant volume ratio of AM: APS at 1:3.

of the  $\pi$ -polaron transitions confirm the emeraldine doped state of PANI (24, 30, 31), that depends on the transition of the oligoanilines which rendered to different detection wavelength (365 nm and 748 nm) of the produced oligomer chain. Thus, the wavelength of 748 nm was used to analyze the produced emeraldine salt PANI.

A change in AM concentration causes a change in its capacity that eventually affects the ionic form of the substance in the solution. A more concentrated AM solution is assumed to extend the induction and propagation chain steps that anticipate higher detected absorbance values. As expected, in Figure 2, the absorbance value was shown to be increased when more concentrated AM (from 0.1 M to 0.6 M) was added. This is due to the greater amount of AM being oxidized to form a larger oligomer. However, a contrary observation was detected where a reduced absorbance value was found when the highest concentrated AM (0.8 M) was added to the oxidizing APS solution. The decreases of absorbance value reflect the lower amount of the emeraldine base PANI that was produced. This phenomenon could be explained by the excess conversion of quionoid rings to benzoid rings during oxidation of a bulk quantity of AM that caused lack of significant polaron in the solution. Thus, fewer protoning sites were available for the subsequent protonation or radical coupling process. Consequently, shorter length oligomer chains of polyaniline were produced. Photographs in Figure 2 showed the appearance of green color solutions after the complete polymerization process (aniline concentration

of 0.1 M–0.8 M), indicates the formation of the conductive emeraldine salt of PANI. In contrast, the colorless solution with aniline concentration of 1.0 M had changed to a yellowish color at the end of the oxidation process; which suggests the polymerization of aniline occurred in a non-conducting state.

The color and absorbance values in Figure 2 indicate the presence of PANI in the emeraldine form, but we were unable to determine the ionic conductivity properties of PANI which is crucial to apply as an electro-transducer in a sensing device. In this work, an attempt was made to investigate the effects of the protonation level of PANI on its ionic conductivity responses (Figure 2). As shown in Figure 2, higher ionic conductivity response was measured when a more concentrated AM was added, from 0.1 M to 0.2 M. This is due to the formation of higher amounts of the oligomer chains emeraldine salts in the solution, as discussed earlier. However, when over-dosage of AM was added for oxidation polymerization, it will lead to the imperfection of the PANI band structure. The protonation of AM with concentrations higher than 0.2 M has shown a significant lack of protonating sites for the subsequent chain propagation step. Thus, shorter lengths of the PANI oligomer chains were produced. In terms of the ionic conductivity properties, distortion in the chain symmetry may develop shorter oligomer chains, which further affect the continuous charge delocalization process. This answered the lower ionic conductivity responses which were found when AM with a concentration more than 0.2 M was used. Although the highest UV-Vis absorbance value was detected for PANI synthesized using 0.6 M of AM, however, a lower ionic conductivity response was measured, due to the imperfection length of the oligomer chains. Undetectable ionic conductivity response was found for PANI synthesized at 1.0 M of AM (Figure 2). Obviously, the yellowish solution of the PANI produced using 1.0 M of AM was actually presented in the leucoemeraldine state. Thus, a non-conductivity response was detected. Herein, it could be simplified that the formation of the green color in the solution led to the PANI in the conducting emeraldine salt state while formation of leucoemeraldine state from colorless to yellowish solution rendered the aniline polymerization in a non-conducting state.

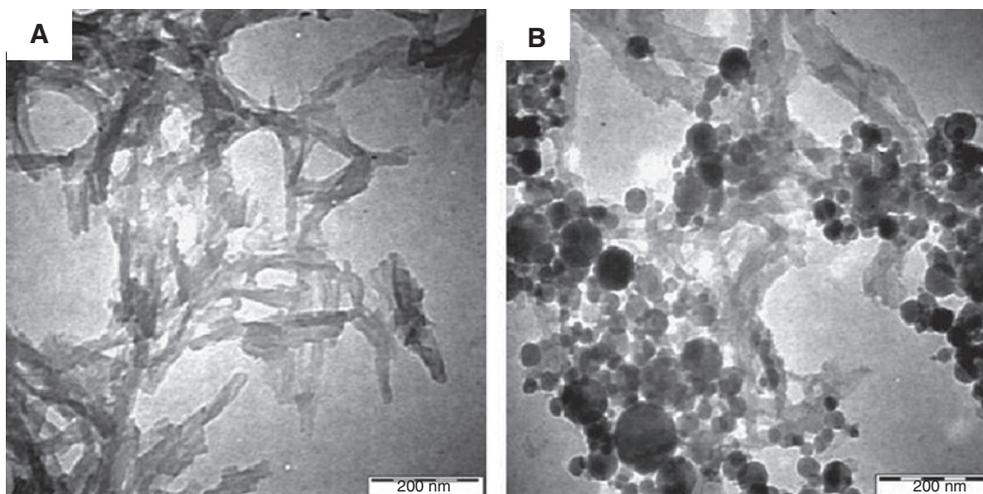
The principal mechanism of the oxidative polymerization process is illustrated in Figure 1E. The protonation and de-protonation as well as various physical-chemical properties of PANI were mainly due to the -NH- group of the aniline monomer. Indeed, the nitrogen atoms of PANI which served as the oxidation centres, involved the use of HCl as the doping agent in the presence of APS as the oxidizing agent in the aqueous medium. The oxidant will

withdraw a proton from the aniline monomer, without forming the strong co-ordination bond either with the substrate/intermediate or with the final product. The growth of the polymer chain proceeds by a redox process with the addition of monomeric aniline molecules to the active chain ends.

The FTIR spectra of the HCl-dope PANI (Figure S1 in the Supplementary material) shows the strong stretching vibrations of the quinoid and benzenoid rings at 1576 and 1491  $\text{cm}^{-1}$ , respectively. The band at 1292  $\text{cm}^{-1}$  was attributed to the stretching of C-N bonds of the secondary aromatic amines or displacement of  $\pi$  electrons, induced by acid doping of the polymer (23). The conductive form of the doped PANI was observed at 1197  $\text{cm}^{-1}$ , which was attributed to the C-N<sup>+</sup> polaron structure which resulted from the acid doping of the emeraldine base. Besides, the sharp peak at 1128  $\text{cm}^{-1}$  was ascertained to the stretching frequency of the NH<sup>+</sup> structure which occurred in the acid doping process of PANI (23). These results indicate the successful synthesis of the dope PANI with conductive properties for latter sensing applications.

The successful development of the electro-chemical sensing tool is highly dependent on the structure of PANI that was used to form the complete electric circuit between both silver electrodes. Morphology of the synthesized PANI was further analyzed using TEM (Figure 3A), where the “fibrous-rod” shaped structures were observed. The formation of PANI involves chain reaction between the aniline monomers through the connection of “nitrogen-carbon”. During the chain reaction, the oxidized terminal imino group as electrophiles will replace the hydrogen atom in the benzene ring of the neutral aniline monomer at the ortho or para position (32). The monomer units were linked in a head-to-tail mode, therefore growing polymer chains with “fibrous-rod” structures. In fact, this “fibrous-rod” shape structure of PANI contributes a positive prospect to the biosensing application, where the “fibrous-rod” polymeric wire can easily bridge between both silver electrodes along the analyte capture zone. Besides, the long chain rod shape of PANI was also able to expose stronger affinity attraction to the b-IgG molecules due to its high surface area. Thus, it generates higher biochemical interactions with BSA for better sensing of the device’s sensitivity.

After conjugation with  $\text{Fe}_2\text{O}_3$ , the PANI- $\text{Fe}_2\text{O}_3$  nanocomposite demonstrates some cross-linked fibrous, as illustrated in Figure 3B. The cross-linked structure was formed by the electrostatic interaction between PANI and  $\text{Fe}_2\text{O}_3$  whereby PANI served as the positive charged polymer and iron oxide as the negative charged. Zeta charges of PANI and  $\text{Fe}_2\text{O}_3$  colloids were found to be +29.8 mV and



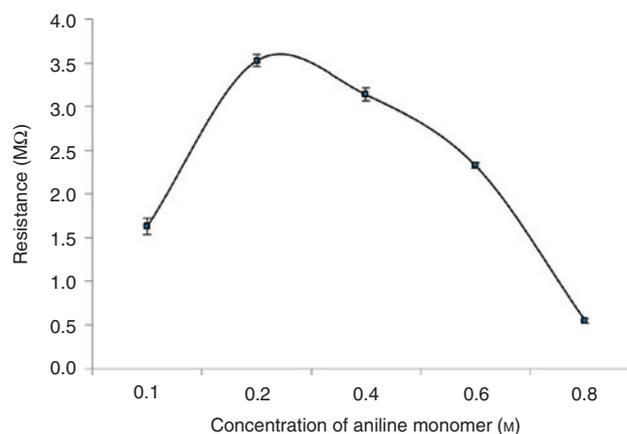
**Figure 3:** TEM image of (A) PANI (B) PANI-Fe<sub>2</sub>O<sub>3</sub> composite synthesized at an aniline concentration of 0.2 M and an AM:APS volume ratio at 1:3.

-21.4 mV, respectively. After PANI was conjugated with Fe<sub>2</sub>O<sub>3</sub>, the zeta charge of the PANI-Fe<sub>2</sub>O<sub>3</sub> was reduced to +18.0 mV, which demonstrated the occurrence of charge neutralization. This charge reduction has proved the successful conjugation of PANI to Fe<sub>2</sub>O<sub>3</sub>, whereby a portion of the positive charged PANI was neutralized by the negative charged Fe<sub>2</sub>O<sub>3</sub> through the ionic interaction. The iron oxide cores served as adsorption sites for the counter-ions (aniline monomers) during the oxidative polymerization process. The negatively charged aniline monomer accumulated around the surface of iron oxide and created bonding between the two components.

The further bio-conjugation was done by conjugating the b-IgG to the PANI-Fe<sub>2</sub>O<sub>3</sub> nano-composites prior to sensing tests. In the physical adsorption process, the negatively charged b-IgG was conjugated to the positively charged PANI-Fe<sub>2</sub>O<sub>3</sub> nano-composite through electrostatic interactions. In order to confirm the bio-conjugation, the UV-Vis absorbances values of PANI, PANI-Fe<sub>2</sub>O<sub>3</sub> and PANI-Fe<sub>2</sub>O<sub>3</sub>-IgG were measured based on the existing wavelength of the b-IgG ( $\lambda_{\max}$  = 220 nm). The suspended solution was expected to show the highest absorption value if the b-IgG has been successfully conjugated to the PANI-Fe<sub>2</sub>O<sub>3</sub> composites. The suspended solution of PANI-Fe<sub>2</sub>O<sub>3</sub>-IgG showed the highest absorbance value (1.498) as compared to the pure PANI (0.761) and the PANI-Fe<sub>2</sub>O<sub>3</sub> composite (1.084). This indicates the existence of the elongated bio-conjugate (PANI-Fe<sub>2</sub>O<sub>3</sub>-IgG) and is ready to be used as the testing analyte in pulse-mode electrochemical bio-sensing measurement.

The electrochemical sensor was assembled, as described in Figure 1 and was ready to test with the PANI-Fe<sub>2</sub>O<sub>3</sub>-GA-b-IgG bio-conjugate. One hundred microliters of

testing solution containing the PANI-Fe<sub>2</sub>O<sub>3</sub>-GA-b-IgG bio-conjugates flowed laterally along the assembled membrane sensing strip with BSA immobilized in the capture zone. The interactions between the bio-conjugates (PANI-Fe<sub>2</sub>O<sub>3</sub>-GA-b-IgG) and the capture analyte (BSA) induced the formation of the PANI nanowire-bridge, which eventually formed a complete electrical circuit between both silver electrodes at the capture zone. PANI performed as the transducer where this conductive polymer will integrate with the biochemical interaction between b-IgG and BSA, relays an antigen-antibody binding as a measured resistance response, as demonstrated in Figure 4. The generated resistance by the biochemical reaction between the b-IgG and BSA is hereafter called an electrochemical reaction. Higher resistance responses are anticipated for



**Figure 4:** Resistance responses of biosensor performance at varying concentrations of AM. Note: PANI was synthesized using a constant volume ratio of AM:APS at 1:3.

electro-transducer (PANI-Fe<sub>2</sub>O<sub>3</sub>-GA-b-IgG bio-conjugates) with higher ionic conductivity.

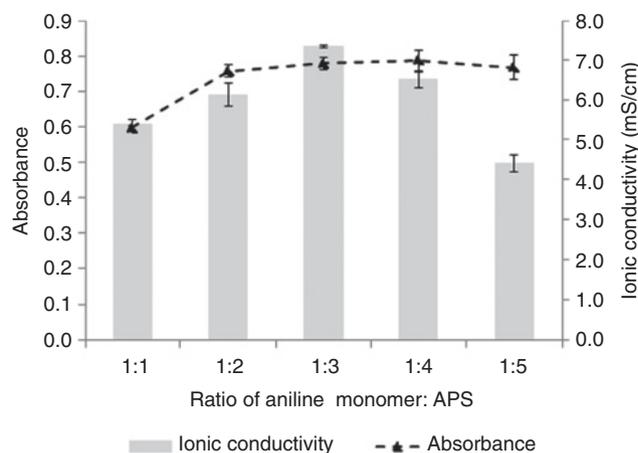
PANI with better ionic conductivity properties (Figure 2) reflects higher electrical resistance responses (Figure 4) when the membrane sensor was mounted in the testing solution. Indeed, the sensitivity of a sensor relies on the compatibility of the conductive PANI polymer to the biomolecules (b-IgG) which has efficiently transferred the biochemical reaction (b-IgG and BSA) to the electronic charged circuit (PANI nanowire-bridge between silver electrodes) (33). The high conductive PANI is desired to accommodate the interaction of the target analyte (b-IgG) towards the immobilized BSA on the capture region (20). In a testing solution, the high conductive PANI will initiate better interactions with the targeted b-IgG biomolecules. Conductive PANI that successfully interacts with the b-IgG will be moved laterally along the membrane strip, to accommodate the final binding of b-IgG towards the immobilized BSA on the capture zone, and eventually induces better electrical resistance responses. In the present work, PANI with highest ionic conductivity (Figure 2, produced using 0.2 M of aniline monomer) induced better interactions with b-IgG and subsequently BSA, rendered to a higher resistance response in the membrane sensing platform, as shown in Figure 4. The formation of the PANI nano-bridge assists in direct charge transfer across the silver electrodes, as it initiates as an “ON” switch for the generation of the electrical resistance signals. In other words, the high conductive PANI will cause an increment of conductance across the silver electrodes that generates better electrical resistance signals for a biosensor (28).

As discussed, the synthesized bio-conjugates PANI-Fe<sub>2</sub>O<sub>3</sub>-GA-b-IgG need to have a good compatibility with the capture reagent (BSA in the present work). With the increasing of AM concentration, the interactive activities with b-IgG will also increase, hence, resulting in better resistance responses. However, the result in Figure 4 seems to be counterintuitive where a high concentration of AM (range of 0.4 M–0.8 M) contributes to a lower resistance response. This finding can be explained by the steric hindrance caused from the interaction between the b-IgG and BSA bonded to a bulky label (33). At a high concentration of AM, the conductive composites may be over occupied by the b-IgG, thus restricts the charge transfer within the conductive PANI structure (34) which eventually lowers the electro-resistance performance of the sensing device.

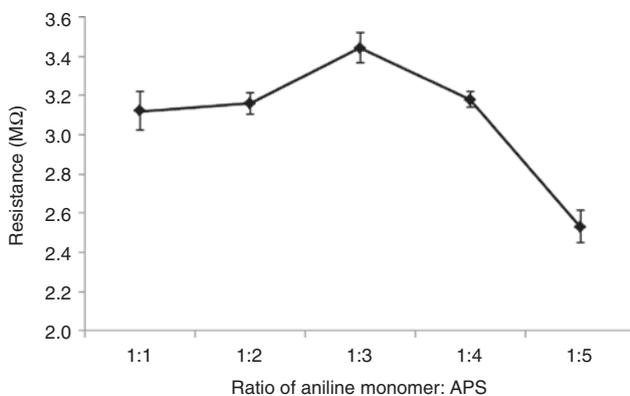
Besides the concentration of AM that affect the final chain structure of PANI, ammonium persulfate (APS) also plays an important role as radical initiators throughout the polymerization process. The volume ratios AM to APS

may affect the produced bond structure of the PANI-emeraldine, as high protonation level of AM at the initial oxidation step may cause the lack of protonating sites for the later chain propagation step (radical coupling process). Based on Figure 5, the UV-Vis absorbance value of PANI was increased when a higher volume of APS was applied (from the volume ratio of 1:1–1:3), showing the more conductive PANI was produced. This could be simply due to more oxidizing agent (APS) that increased the conversion rate of the monomers to the oligomers. However, a decreasing trend was observed when a higher volume of APS (volume ratio of 1:4 and 1:5) was utilized. Oxidation of emeraldine-base PANI leads to the pernigraniline state of PANI structure with complete oxidized nitrogen atoms. The higher formation rate of the aniline cation radical at the initial oxidation step may eventually produce a small portion of the pernigraniline. Thus, constant color absorbance intensity was observed in Figure 5 although higher APS volume was added to the solution. Pernigraniline is known as a poor conductor. By examining the ionic conductivity properties of the synthesized PANI, the decreased ionic conductivity were also observed when a high APS volume (ratio of 1:4 and above) was applied for oxidation. Again, it proves the existence of the pernigraniline state of PANI with an excessive oxidation.

As for electro-chemical sensing performances, the highest electrical resistance signal was detected when a volume ratio of AM:APS at 1:3 was used to synthesis PANI (Figure 6). At higher volume of APS, the conversion possibility of the monomer to the oligomers will also be higher. The high formation rate of oligomers leads to a better interconnection network, thus, it increase the production



**Figure 5:** UV-Vis absorbance intensity and ionic conductivity properties of PANI prepared at different volume ratio of AM:APS. Note: AM concentration was at 0.2 M.



**Figure 6:** Resistance responses of biosensor performance with varying concentrations of aniline monomer at 1:3 ratio of AM:APS.

of the PANI emeraldine-salt with a greater resistance signal (35). Similar to the PANI ionic conductivity properties in Figure 5, the higher volume of APS contributes to the formation of the non-conductive pernigraniline state PANI, eventually reducing the electro-chemical resistance signal of the sensing device (Figure 6). By combining the findings of electric resistance responses in Figure 4 and 6, PANI synthesized by using AM concentration of 0.2 M and at 1:3 volume ratio of AM:APS has demonstrated the highest resistance at  $3.538 \pm 0.067$  MΩ. These results reflected the importance of the PANI's protonation level and its particle-polymer interactions in determining the sensitivity of a sensing device.

## 4 Conclusion

Conductive bio-conjugates PANI-Fe<sub>2</sub>O<sub>3</sub>-GA-b-IgG have been successful synthesized through oxidative polymerization and steric interaction. Morphology and ionic conductivity properties of PANI and its conjugates were confirmed under TEM micrographs and the highest logged ionic conductivity response was found at  $7565 \pm 0.262$  mS/cm. Fibrous-rod shaped PANI emeraldine-salt were successfully prepared using 0.2 M concentration of aniline monomer and 1:3 volume ratio of AM:APS with excellent electro-bio-chemical sensing performance recorded at  $3.538 \pm 0.067$  MΩ. The present work revealed the important roles of the PANI's protonation level and its particle-polymer interactions that should be taken into consideration for the development of a electrochemical sensing tool.

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