Synthesis of a nano electro-magnetic polymer and its application in a DCT biosensor

Sudeshna Pal, Emma B. Hummel and Evangelyn C. Alocilja

Biosystems and Agricultural Engineering, Michigan State University, East Lansing, MI, USA.
Correspondence to: alocilja@msu.edu

ABSTRACT

A nano electro-magnetic conductive polyaniline was synthesized for application in a direct-charge transfer biosensor for the detection of *Bacillus species.* The polymer was synthesized in the presence of ?-Fe_2O_3 nanoparticles with a monomer to ?-Fe_2O_3 nanoparticle ratio of 1:0.4 and had diameter between 50 and 200 nm. The room temperature hysteresis measurements showed that the magnetic polymer had a saturation magnetization value of 43.0 emu/g and approached super paramagnetic behavior. The synthesized nanomagnetic polyaniline was coated with anti *Bacillus cereus* antibodies and used for separation of the target antigens by applying a magnetic field which was followed by its application into a direct charge transfer (DCT) biosensor. The detection was based on the capillary flow of the nanomagnetic polymer-antigen conjugates which enabled a direct charge transfer in the capture membrane region of the biosensor. Signal generation and data recording was completed in 6 min in a reagentless process. The sensitivity of detection of the biosensor was found to be 10^1 CFU/ml. This easy to use, portable reagent-less biosensor has the potential to serve as a rapid detection tool for countering bioterrorism agents.

Keywords: electro-magnetic, polyaniline, direct-charge transfer, *Bacillus*, biosensor.

1 INTRODUCTION

Conducting polymers such as polyacetylene, polypyrrole, polyaniline, and polythiophene have attracted much interest among the researchers because of their unusual electrical, optical and magnetic properties [1, 2]. Polyaniline has been extensively studied due to its simple synthetic procedure, good environmental stability and special doping mechanism which allow it to be switched between an insulator and a conductor. This versatility makes polyaniline suitable for numerous applications like batteries, anticorrosion coatings, electrodes, and more recently, in biosensors and fuel cells [3-5]. Present research interests are directed toward the synthesis and characterization of polyaniline nanostructures. Several approaches have been developed in the synthesis of magnetic polyaniline nanoclusters using iron-oxide, as the core material [6, 7]. Although magnetic nanomaterials have potential applications in magnetic recording media and as carrier for drug delivery, there is hardly any application of magnetic nanostructures of polyaniline [8, 9].

This paper deals with the novel application of nano-magnetic polyaniline as an immunomagnetic separator and a transducer in biosensors. A biosensor is an analytical device that integrates a biological sensing element with an electronic transducer to quantify a biological event into an electrical output. Biosensors using magnetic beads as immunomagnetic separators have been well reported in the literature. Such separation techniques offer advantages as low interference, reduced background signal and no sample pretreatments [10, 11]. In this study, polyaniline nanostructures with magnetic properties close to super paramagnetic behavior have been synthesized and coated with antibodies specific to a target antigen. The antibody coated polyaniline nanoparticles are used for immunomagnetic separation of antigens from a liquid sample and directly applied to a conductometric biosensor. The polyaniline nanostructures act as a transducer in the biosensor and are responsible for signal generation.

2 MATERIALS AND METHODS

The biosensor used in this study was composed of three distinct, disposable, membrane regions: the sample application region, the capture region, and the absorption region. The membranes were arranged in the order as shown in Figure 1 and the biosensor had an overall dimension of (60mm × 5mm). The sample application and the absorption regions were made up of cellulose membranes (flow rate 180 ml/min) and the capture region was made up of nitrocellulose membrane (flow rate 135 sec/4cm). The prepared biosensor was attached to an etched copper wafer and two silver electrodes were fabricated along the sides of the capture region 0.5 mm apart before connecting it to the data collection system. The data collection system consisted of an ohmmeter with RS-232 interface and software for connecting to a computer.

2.1 Reagents

Iron (III) oxide nanopowder (?-Fe_2O_3), aniline, glutaraldehyde, polysorbate 20 (Tween 20), perchloric acid, ammonium peroxy disulfate, tris buffer, sodium phosphate (dibasic and monobasic) and lithium chloride were purchased from Sigma-Aldrich (St. Louis, MS). Nitrocellulose and cellulose membranes were purchased from Millipore (Bedford, Massachusetts). Micro-Tip silver

pen was supplied from (Chemtronics, GA). All chemicals and diluents were prepared with reagent grade double deionized water.

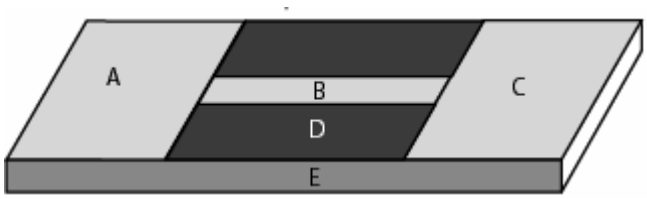


Figure 1: Biosensor schematic, A. sample region, B. capture region, C. absorption region, D. electrode, E. platform.

2.2 Bacterial isolates and antibodies

Strains of *Bacillus cereus* were obtained from the collection of the Biosensors Laboratory at Michigan State University. Tryptic soy broth (BD Biosciences) was used for bacterial enrichment. A 10 µl loop of the bacterial isolate was incubated in 10 ml of the media for 24 hours at 37°C to prepare stock cultures. The stock cultures were serially diluted in 0.1% peptone water (Sigma-Aldrich, St Louis, MS) to prepare different concentrations of the bacteria ranging from 10^0 to 10^7 colony forming units per milliliter (CFU/ml). Rabbit polyclonal antibodies (IgG) to *Bacillus cereus* were obtained from Biodesign International (Saco, ME). The antibodies were diluted in phosphate buffer (pH 7.4) according to the desired concentration. All experiments were performed in a certified Biological Safety Level II (BSL II) Laboratory

2.3 Synthesis and characterization of the electro-magnetic polymer

The conductive polymer, polyaniline, was synthesized in the presence of iron (III) oxide nanoparticles (?-Fe_2O_3) [12], where, the ?-Fe_2O_3 to the aniline monomer weight ratio was maintained at 1:0.4. The ?-Fe_2O_3 nanoparticles were first dispersed in a mixture of 50 ml 1M HCl, 10 ml de-ionized water and 0.4ml of aniline. The above mixture was sonicated for 1 h in an ice bath in order to disintegrate the agglomerated nanoparticles. This was followed by a slow drop-wise addition of the oxidant (ammonium persulfate) to the above mixture with continuous stirring. The ice bath reaction was then continued for an additional 4 h. The final product obtained was filtered, washed repeatedly with 1M HCl and diethylether, and allowed to dry for 24 h at room temperature. The above procedure yielded a dark green colored powder.

A Quantum Design MPMS SQUID (Superconducting quantum information device) magnetometer was used for magnetic characterization and room temperature hysteresis measurements of the synthesized electromagnetic polyaniline. Conductivity of the magnetic polyaniline at room temperature was determined in a solid pellet form using a four point probe (Lucas/Signaton Corporation, Pro4; CA, USA). The structural morphology of the synthesized polymer was studied by a Transmission Electron Microscope (JEOL 100CX).

2.4 Preparation of polymer-antibody conjugates and biosensor membranes

The polyaniline-antibody conjugates were prepared by incubating the *Bacillus cereus* antibodies in a suspension of polyaniline in phosphate buffer (pH-7.4) containing dimethylformamide and lithium chloride. The incubation was carried out for 1h. Various concentrations of the antibody and polyaniline were tested to determine the optimum concentrations for both. The antibody concentration was 0.15 mg/ml while the polyaniline concentration was maintained at 0.1g/ml. After the incubation, the conjugates were treated with a blocking solution (tris buffer containing 0.1% casein) for 30 min. The antibody conjugated polyaniline was collected by centrifugation at 13000 rpm for 5min (×3) and was resuspended in phosphate buffer (pH-7.4) containing dimethylformamide and lithium chloride.

The cellulose sample application and absorption membranes were washed thoroughly with deionized water and air-dried before assembling into the biosensor. The nitrocellulose capture membrane was washed with deionized water and treated with the crosslinking agent glutaraldehyde (0.1%) for 1h. The capture membrane surface was then incubated with *Bacillus cereus* antibodies at a concentration of 0.5 mg/ml at 37°C in the incubator for 1h. This allowed attachment of the antibodies on the surface of the capture membrane. The above procedure was followed by treatment of the membrane surface with a blocking solution (tris buffer with 0.1% Tween 20) to block the nonspecific binding sites. Finally, the capture membrane was dried before being assembled as part of biosensor.

2.5 Immunomagnetic separation and biosensor detection

The antibody coated polyaniline conjugates were used to capture *Bacillus cereus* cells from varying concentrations of the pure cultures. Two ml of the polymer-antibody conjugates, suspended in phosphate buffer was added to 8 ml of each cell concentration and the mixture was incubated for 15 min. The polymer–antibody-antigen complex formed after this reaction was concentrated by a magnetic separator (Spherotech, IL, USA) and the supernatant was discarded. The polymer bacteria pellets were washed using the magnetic separator and re-suspended in 10 ml of phosphate buffer (pH 7.4).

After preparation of the individual membrane pads, the biosensor was assembled and connected to the data collection system as mentioned before. To begin the signal measurement process, 0.1ml of the polymer bacteria complex solution was added to the sample application region of the biosensor. As the sample flowed through the capture region of the biosensor by capillary action, the electronic signal generated was recorded in terms of electrical resistance by the data collection system for 6 min. The sensitivity of the prepared biosensor was assessed in pure cultures of *Bacillus cereus.* For each cell concentration, testing was done in triplicate. Polymer-antibody conjugates suspended in phosphate buffer (ph 7.4) and without the bacterial incubation step was used as control for these experiments.

After each testing, the concentration of *Bacillus cereus* was determined by plating the bacteria in blood agar plates (BD Biosciences).The plates were incubated at 37°C and the suspected microorganism was confirmed by the guidelines mentioned in the Bacteriological Analytical Manual (2001) of the Food and Drug Administration.

3 RESULTS AND DISCUSSION

This experimental study was carried out to synthesize an electromagnetic polyaniline and to evaluate its performance as a transducer in a direct charge transfer biosensor. Polyaniline was synthesized in the presence of ?-Fe_2O_3 nanoparticles obtained from a commercial source (Sigma Aldrich, MS, USA) and consisted mostly of ? iron oxide particles according to the manufacturer's description. The ?-Fe_2O_3: aniline monomer ratio was maintained at 1:0.4 as mentioned earlier. Figure 2 shows the hysteresis measurements of the polymer and iron oxide nanomaterials carried out at 300 K. The electromagnetic polyaniline showed a saturation magnetization value of 43.0 emu/g and a retentivity of 9.5 emu/gm which was about the same as mentioned in the published protocol [12]. The ?-Fe_2O_3 had a saturation magnetization value of 63.7 emu/g. Both the polymer and ironoxide showed a tendency to saturate at 15 kOe and the electromagnetic polyaniline had a coercivity value of 200 Oe. The small values of the coercivity and retentivity show that the synthesized magnetic polyaniline is close to superparamagnetic behavior [12, 13].

The transmission electron microscopy (TEM) images of both the ?-Fe_2O_3 particles and the synthesized electro-magnetic polyaniline are presented in figure 3 showing their shapes and sizes. The iron oxide nanoparticles are between 5 and 25 nm in diameter and confirms the manufacturer's specification (Sigma Aldrich, MS, USA). They show a distinct spherical shape and exhibit an association of several spherical particles [14]. As evident from figure 3, the electro-magnetic polymer is between 50 to 200 nm in diameter and also exhibited tendency to agglomerate [12].

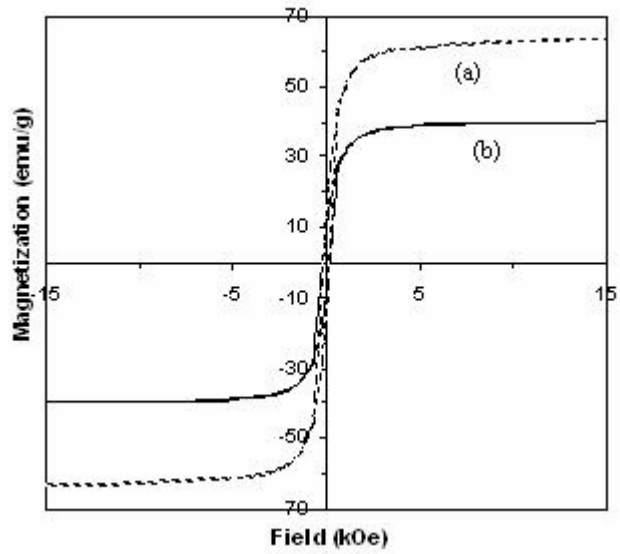
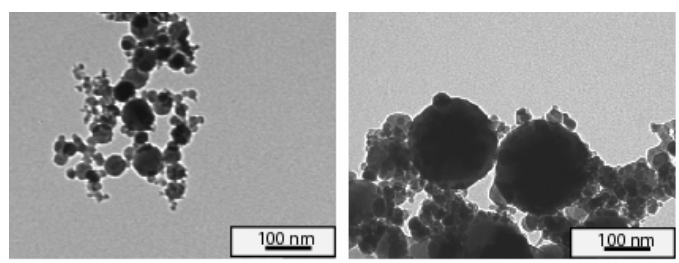


Figure 2: Experimental hysteresis measurements for (a) pure ?-Fe_2O_3, and (b) ?-Fe_2O_3 –polyaniline.



(a) (b)

Figure 3: TEM Images of (a) pure ?-Fe_2O_3, and (b) ?-Fe_2O_3 –polyaniline.

The conductivity of the iron oxide and the electro-magnetic polymer was evaluated after drying the samples for 48 h to evaporate any solvent present in the polymer. The bare iron oxide particles showed a conductivity of 5.0 × 10^{-5} S cm^{-1} while the electro-magnetic polyaniline showed a conductivity of 2.4 × 10^{-2} S cm^{-1}. The increase in conductivity is due to the presence of polyaniline.

The biosensor prepared was a modified design of the conductometric biosensor that was previously developed by the authors [15]. The sensor used lateral flow technique for movement of liquid sample from one membrane to another. Magnetic polyaniline coated primary antibodies were used to concentrate the target antigens from a liquid sample, and applied directly to the biosensor. Before sample application, the capture membrane was an open channel with secondary antibodies to *Bacillus cereus* functionalized on its surface. At that time, the resistance across the electrodes on the biosensor was infinite. Upon addition of the polymer-antibody-antigen complex to the sample application region, the complex flowed to the capture

region of the biosensor by capillary action. The secondary antibodies on the capture membrane served as an anchor for capturing the polymer-antibody-antigen complex and forming a sandwich complex. The electromagnetic polyaniline nanoparticles in the sandwich complex acted as the charge transfer agent between the electrodes and initiated a voltage-controlled 'ON' switch [15]. This direct charge transfer across the electrodes generated an electrical signal which was being recorded in terms of resistance. The flow of the sandwich complex initially generated a transient electric signal which stabilized within 1 min. The signal was recorded for about 6min after which a fluctuation was observed. The dissociation of the antigen-antibody complex from the membrane surfaces may be a possible reason for the signal fluctuation.

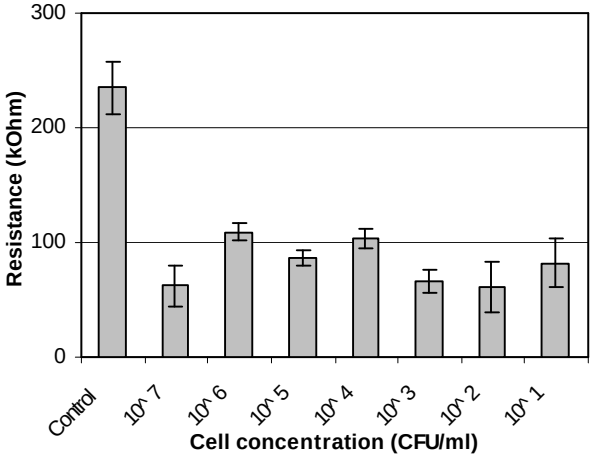


Figure 4: Biosensor sensitivity performance

The mean resistance values obtained from the three experimental trials were plotted against the respective cell concentrations. Figure 4 shows the sensitivity results of the biosensor in pure cultures of *Bacillus cereus*. The different cell concentrations showed lower resistance due to the formation of a sandwich complex in the capture region of the biosensor, which is facilitated by the electromagnetic polyaniline. This complex formation was absent in control solution. However, the resistance values are not linearly dependent to the cell concentrations indicating that the biosensor is only suitable for quantitative evaluation. Statistical analysis of the data showed that the average resistance values obtained for different cell concentrations were significantly different from the control. The data indicated that the developed biosensor was sensitive to the presence of *Bacillus cereus* at concentrations as low as 26.5 CFU/ml and had similar range as the biosensors previously developed by the authors [15, 16].

CONCLUSION

The biosensor presented above shows a novel application of an electromagnetic conductive polyaniline for simultaneous immunomagnetic separation of antigens and direct detection. The biosensor shows a sensitivity of 10^1 CFU/ml in pure cultures of *Bacillus cereus*. Current research is focused on improving the performance of the nano-magnetic conductive polyaniline, optimizing various parameters in the assay conditions, and exploring the possibility of this biosensor in detecting other organisms of interest. This biosensor is fast and sensitive and has the potential to be used as a field based device.

REFERENCES

[1] T. Ahuja, I. A. Mir, D. Kumar and Rajesh, Biomaterials, 28, 791-805, 2007.

[2] L. Zuppiroli, S. Paschen and M. N. Bussac, Proceedings of the International Conference on Science and Technology of Synthetic Metals Part 1, July1994, Seoul, South Korea

[3] H. Devendrappa, U. V. S. Rao and M. V. N. A. Prasad, Journal of Power Sources, 155, 368-374, 2006.

[4] S. Kuwabata, H. Nakano and Y. Tachibana, 207th Meeting of the Electrochemical Society, May 2005, Quebec, Canada

[5] M. Tiitu, A. Talo, O. Forsen and O. Ikkala, Polymer, 46, 6855-6861, 2005.

[6] J. L. Wilson, P. Poddar, N. A. Frey, H. Srikanth, K. Mohomed, J. P. Harmon, S. Kotha and J. Wachsmuth, Journal of Applied Physics, 95, 1439-1443, 2004.

[7] Z. Zhang and M. Wan, Synthetic Metals, 132, 205-212, 2003.

[8] C. M. Hangarter and N. V. Myung, 2004 Joint International Meeting - 206th Meeting of the Electrochemical Society/2004 Fall Meeting of the Electrochemical Society of Japan,, Oct 2004, Honolulu, HI, United States

[9] J. Chatterjee, Y. Haik and C.-J. Chen, Journal of Magnetism and Magnetic Materials, 246, 382-391, 2002.

[10] K. Kriz, J. Gehrke and D. Kriz, Biosensors and Bioelectronics, 13, 817-823, 1998.

[11] D. Abrahamsson, K. Kriz, M. Lu and D. Kriz, Biosensors and Bioelectronics, 19, 1549-1557, 2004.

[12] R. Sharma, S. Lamba, S. Annapoorni, P. Sharma and A. Inoue, Journal of Applied Physics, 97, 014311-014316, 2005.

[13] M. Kryszewski and J. K. Jeszka, Synthetic Metals, 94, 99-104, 1998.

[14] S. R. R. Tannenbaum, C.L. Flenniken, E.P. Goldberg,, Advanced Materials, 14, 1402-1405, 2002.

[15] Z. Muhammad-Tahir and E. C. Alocilja, Biosensors and Bioelectronic,18, 813-819, 2003.

[16] Z. Muhammad-Tahir, E. C. Alocilja and D. L. Grooms, IEEE Sensors Journal, 5, 757-762, 2005.