Nanobiosensor designing with molecular framework polymer method compared with agent-linked nanosilica biosensor for *Staphylococcus aureus* exotoxin detection

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Abstract

Considering the ever increasing population and industrialization leading to developmental trend of humankind's life, we are hardly able to detect the toxins produced in food products using traditional techniques. In this technique, the production of molecular framework and polymer is done using meta acrylic acid monomers, which are formed via covalence connection between meta acrylic acid monomers (MAA) of white polymer. Here also hydrogenised connection between exotoxin amino acid and meta acrylic acid is made that would function as the selective absorption for that. Then in the second stage, based on the bacterial antibody connection to nanoparticle, a sensor was used. In this part of the research, as the basis for absorption for the recognition of bacterial toxin, medium sized silica nanoparticles of 10 nanometer in the form of solid powder were utilized with Notrino brand. Then the suspension produced from agent-linked nanosilica which was connected to bacterial antibody was positioned near the samples of distilled water, that were contaminated with *Staphylococcus aureus* bacterial toxin with the density of 10^{-3}, so that in case any toxin exists in the sample, a connection between toxin antigen and antibody would be formed. Finally, the light absorption related to the connection of antigen to the particle attached antibody was measured using spectrophotometers. The results indicate that the molecular framework polymer sensor is capable of detecting up to the density of 10^{-3}, but not lower than that, whereas using the second sensor, up to 10^{-4} of density is detectable. Additionally, the sensitivity of the sensors were examined after 60 days and the first sensor by the day of 28 and the second sensor by the 56 day had confirmatory results and started to decrease after those time periods.

Keywords: Nanobiosensor, *Staphylococcus aureus*, Exotoxin, Molecular Framework Polymer Nanosensor.

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Introduction

Annually, billions of dollars is spent on throwing out and exclusion of rotten food stuff from the food industry due to various reasons such as long haul air and road transportation, or late or inconclusive quality control test results from accredited and reference laboratories. This delay is caused by the use of standard conventional methods and also due to false negative results, when in many cases bacteria are destroyed but their toxin still remains in the sample (Turkoglu *et al*., 2012; Panneer Selvam and Prasad, 2013).This becomes extremely important in such cases as natural disasters, and in cases of import and exports of tons of dairy and meat products, to and from countries, in normal or in unusual circumstances like wars, and sometimes in deliberate acts of bioterrorism that involves millions of defenseless people, sacrificed for sinister intentions.

The huge volume of food stuff imported into the country is not properly tested, due to lack or absence of test facilities, or even due to insufficient waiting time. This food is then consumed, causing a planned poisoning and mortality of a defenseless population. Thus, with advancement and use of nano-technology in food industry, especially in quality control of food products, and also given the research results, it is time for this technology to be commercially mass produced. Both, in terms of time, which is a decisive factor in food products, exchanged at customs still in containers (sometimes with no time for quality control assessment like at time of disasters such as earthquake, flood, etc.), and more importantly in terms of accuracy and sensitivity of food safety and security which are the two critical points in standards of control.

Given the time required to obtain microbial culture test results in quality control of food materials (no less than 48 hours), and in some strains that require pre-enrichment and enrichment such as salmonella that needs at least a week to obtain initial results, use of biosensors and nano-biosensors is extremely valuable. In many research and development, and quality control departments of food manufacturers, it takes a long time to declare test results and approve or disapprove a marketing product. This delay shortens shelf life, and also indirectly hurts the manufacturer's products.

Some food industries such as dairy and meat are fundamentally different from others like grains, pulses, oil, canned food, etc. Thus, detection time is highly important in quality control systems and in payback period for the producer (Cuero *etal*., 2012; Gerasimova and Kolpashchikov, 2013).

Healthy food production is a major concern in food industry. Today, consumers seek least processed foods, free from microorganisms, additives, and preservatives, and yet with long shelf life. This has become possible with the help of biosensors (Suehiro *et al*., 2009).

The most important consideration of industry sector in every country where food is concerned is food safety. A highly considered new approach in this sector is utilization of new technologies. Convergence of nano-technology and food

science has led to many great capabilities, with nearly 200 companies hugely investing in this area worldwide and already marketing new products. Given the extraordinary potential of nano-technology applications in food industries, a great revolution in food and agricultural products is expected, in a way that its repercussions will extend far beyond mechanized agriculture and the green revolution (Hakalehto *et al*., 2009).

The molecularly imprinted polymer method is the latest design method in construction of biosensors for microbial detection in food. However, this is more used in food chemistry applications (enzymatic follow up, organoleptic properties, etc) and less used in microbial detection (Cuero *et al*., 2012).

This study aimed to detect exotoxin excreted by *S. aureus*, which is one of the most common causes of food poisoning using two methods of potentiometric and spectroscopy with the aid of targeted modified nano-particles. The nano-technology application in sensor design makes it highly sensitive and accurate in detecting the toxin excreted by *S. aureus* that is the most common cause of food poisoning.

Materials and Methods

- Staphylococcal bacterial toxin- Sigma®
- *S. aureus* toxin antibody,
- Nano-silica particles- Neutrino® size 10 nm
- Nano-silical – Neutrino® size 10nm
- Acetic acid obtained from Merck® Company
- DMF solvent from Merck® Company
- Ethoxylated tetra Silone from Sigma® Company
- Ammonium hydroxide from Merck® Company
- APTES reactor from Sigma® Company
- Succinic anhydride
- Tri-ethyl amine
- (All materials used in this study were of analytical purity, and all solutions were prepared by double distilled water)

Potentiometry based on Molecularly Imprinted Polymerto produce physically and chemically resistant patterns for staphylococcal exotoxin, first, a right solution of this sensor was prepared from the pure solution. The pure solution procured from Sigma Company was diluted by double distilled water to produce a solution with concentration of 1×10^{-5}mol, under standard temperature conditions (25°C). These solutions were prepared on daily basis at different stages of the test (Ozdemir *et al*., 2013; Kou *etal*., 2013).

First, 10^{-5}mol concentration of *S. aureus* type Aexotoxin was prepared from Sigma® Company solution, diluted with the double distilled water. The toxin solution was produced daily. In the Molecularly Imprinted Polymer method, various doses of Meta-Acrylic Acid Monomer were used. The monomers to toxins ratios were 2, 4, 6, 8, 10, 12, and ultimately the 10 to 1 ratio was chosen as the optimum ratio for producing the best imprint (Abbasi *et al*., 2012; Xie and Bakker, 2013,).

To form a suitable polymer pattern around exotoxin, and for better distribution of the coating solvent, more solvent volume was used.

As opposed to the mass method, in the sedimentary polymerization method more solvent is used, thereby creating a nucleus regeneration opportunity that leads to production of particles on the nano-scale. In this method, 38 ml of the solvent was used, and gently stirred with 11.32 ml of the transverse binding agent, ethylene glycol methacrylate. Also, 10 mg azobisisobutyronitrile was used as the initiator of polymerization reaction. Soon after adding the initiator, ultra-violet irradiation was used to speed polymerization reaction by forming free radicals followed by the onset of polymerization.

Once the reaction was completed, covalent bond was formed between Meta Acrylic Acid (MAA) monomers, and a spectrum of white polymer particles resulted. Hydrogen bonds are made between exotoxin amino acid and meta acrylic acid, which will be its elective absorption factor.

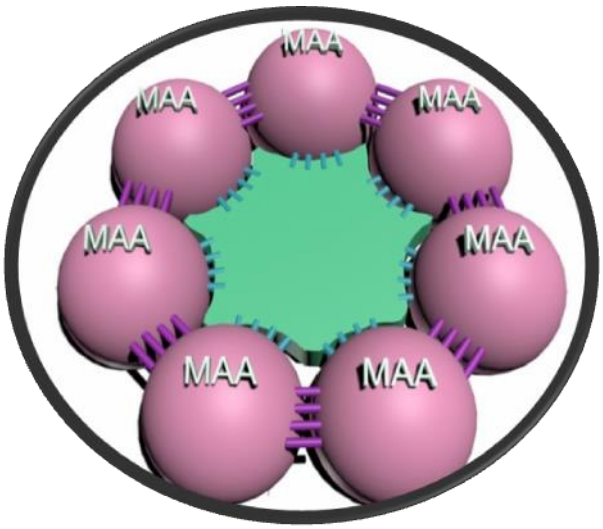

Figure 1: Covalent bonds between meta acrylic acid monomers and exotoxin Interaction with molecular imprint.

The polymer pattern was formed around exotoxin molecules and made hydrogen bonds with amino acid agents present in the toxin structure. Therefore, to remove toxin molecules, diluted acid solution was used, and the remained polymer pattern e was used in potentiometry sensor in the next stage of experiment (Chen *et al*., 2012; Gurtova *et al*., 2013). A 1 to 10 ratio of methanol and acetic acid (as alcohol-acid solution) was used to extract exotoxin molecules from the formed polymer. The mechanism of action is shown through elimination of hydrogen bonds between amino acid and meta acrylic acid as monomer units in the polymer (Kozyra *et al*., 2012).

To determine the produced polymer nano particles and morphology and size of these particles, Scanning Electron Microscope (SEM) was used.

Therefore, first polymer particles of Molecularly Imprinted Polymer (MIP) and Non-Imprinted Polymer (NIP) were prepared through producing suspension in acetonitrile solvent in falcons. 3 ml of this solution was placed on a base to evaporate, and then it was transferred to an argon spotter coater to stabilize gold coating on the samples on the base. After 10 minutes, the samples coated with gold were ready, and transferred to the SEM microscope, at ×10 magnification. The images are presented in the results section.

Construction of membrane microelectrode with molecularly imprinted polymer modifier:

Both Figureite and gold can be used for building the body of microelectrode. Considering the limitations in this experiment, Figureite electrode was used. This Figureite microelectrode was confined in a capillary tube sheath in the shape of a micro-wire, and cut cross-sectionally, revealing a small cross section of the Figureite, which was used to prepare the thin polymer membrane on the cut face of the electrode (Jia *et al.*, 2011; Jia *et al*., 2012; Ozdemir *et al.*, 2013).

Preparing thin polymer membrane on the cross section of electrode:

50 mg polyvinylchloride powder, 50 mg ionophore, and a certain amount of the additive KTPCIPB were mixed with 75 mg plasticizer. The resulting mixture was then dissolved in 5 ml tetrahydrophoran in a 25 ml glass beaker. This then remained in the laboratory for 20 minutes to evaporate, resulting in a homogeneous dense oily solution. To accelerate this process indirect heat was used (without boiling the solution) (Kou *et al*., 2013). To produce thin polymer membrane on the surface of the electrode: tip of the cut membrane was dipped into the dense oily solution. The membrane formed on the tip of electrode was left at room temperature in the laboratory for 24 hours to dry. It was then placed in 10^{-3}mol toxin *S. aureus* solution for 48 hours so that exotoxin could connect to the previously designed position. It was then analyzed using potentiometric method and Nernst slope (Abounassif *et al*., 2011; Sanan and Mahajan, 2013,).

This method is based on electrochemical mechanism, in which the molecularly imprinted polymer reaction is used as a modifier to improve electrochemical properties of polyvinylchloride membrane to detect the associated bacteria toxin. To detect the potential difference due to presence or non-presence of bacteria toxin, the Swiss made pH/mV meter model 827 was used, for measurement of the designed ion selective electrode potential. This device contains an Ag/Ag Cl, 222 volt reference electrode, saturated with 3 mol potassium chloride (the 222 volt is a function of the potassium chloride concentration in the electrode). The potential difference between Ag/Ag Cl electrode and PVC electrode indicates the sensor's response to presence or non-presence of bacteria exotoxin (Mashhadizadeh and Talemi, 2011).

Sensor sensitivity analysis:

According to the Sigma® Company analysis note on exotoxin type A *S. aureus* bacterium, every µl contains 2 mg toxin, and a vial volume of 200 µl should contain 400 mg of toxin. To calculate the

molecular mass of the commercial toxin according to the analysis note, each mmol of toxin has a molecular mass of 202 mg, and there is 2 mmol in the previously calculated vial mass. According to the definition of molar (mol/l or mmol/ml), the 1 molar toxin was prepared by dissolving 2 mmol of the solution in 1 ml. The 1 molar toxin was used for the minimum concentration detectable by the sensor.

1 mg of the 1 molar toxin was dissolved in 9 ml of double distilled water to produce 0.1 dilution of toxin, and again 1 ml of the 0.1 toxin dilution was added to 9 ml of double distilled water to produce 0.01 toxin dilution. This was repeated until a toxin dilution of 1×10^{-6} was reached. For preparation of these dilutions, ELISA plate was used to facilitate use of sensor and entering the electrode into the chamber.

Using the potentiometer, potential of each dilution was measured and recorded. According to the definition, the Nernst relationship applies when potential difference between one dilution and the next is 59 mV, and the electrode reading in the next dilution will be acceptable and higher. If the potential difference between a dilution and the next more diluted dilution is 59 mV/decade, then the standard Nernst slope applies, and the sensor has registered presence of exotoxin in that dilution. But if the potential difference between any dilution and the previous less diluted dilution is less than 59 mV, then the sensor has not been sensitive to that dilution and could not detect exotoxin presence (Mashhadizadeh and Talemi, 2011).

Design of nano-biosensor with spectrometry method

In this study, silica nano-particles with average size of 10 nm were used for detection of bacterial toxins. In order to detect exotoxin, the bond formed between nanoparticles and antibodies was used as the detection basis. The reason for using nano-silica particles was presence of hydroxyl agents in this particle, making it more prone to modification. After modification in Strober method, the hydroxyl can be converted to amine factor, and subsequently to carboxyl. The nano-silica in solid powder form turns to liquid after modification; it is then dried to be used in bonding with antibody.

For the bonding of nano-particles with antibody, surface modification of nano-silica particles was used, in which antibody was directly stabilized on the carboxylic acid silica nano-particles, providing a series of modified nano-particles bonds with antibody. This biosensor has the benefit of short analysis time and sensitivity in toxin detection. Also, a modified Strober method was used for preparation of modified silica nano-particles.

In this method, silica nano particles containing amine agent was used as precursor and production of nano particles was carried out with a group of carboxylic acid agent in DMF solvent (Di-Methyl Formamide). Investigations revealed that nano-particles have unique properties in bioanalysis and biotechnology applications. The new modified nano-particles described in this study lead to an amide bond between nano-particles and protein antibody. This bond is formed through an active ester reaction between the antibody chain amino group and

carboxylic acid agent. Relevant stages are shown in Figure 2 (Hasanzadeh *et al*., 2013).

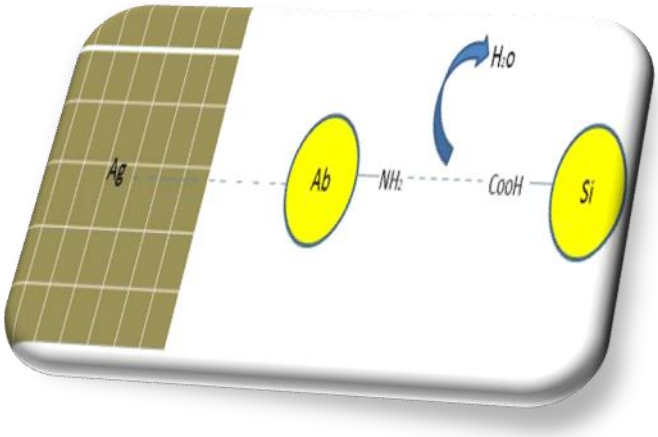

Figure 2: Schematic diagram of antibody bond with modified silica nano-particles.

Modified Strober method:

As seen in the schematic diagram in Fig. 3, first, 4 ml of tetra-ethoxylated silane was added to a mixture of 3.3 ml ammonium hydroxide and 47 ml of ethanol, and stirred by a magnetic stirrer at room temperature for 24 hours, so that the silica colloidal mix was revealed by the end of stirring. Then, 0.3 ml of APTES reagent was added to the reaction medium, and stirred for 24 hours at 25°C. In this way, the surface of the produced silica nano-particles was transformed into amine group, that is, they become'aminized'. To change amine group to carboxylic acid group, 1 gram aminized silica nanoparticles with 50 ml of Di-Methyl Formamide solvent were placed in a dispersion flask. Then, ½ gram succinic anhydride and 5 ml Tri-Ethyl-Amine were added to the flask. This mixture was placed in a stirrer at 70°C and stirred for 20 hours. In this process, the silica nano-particles surfaces were modified with carboxylic acid agent. After several times dispersion (exposed to sonicator with ultra-sound waves) in Di-Methyl-Formamide, and centrifuge, these particles were fully rinsed (Hasanzadeh *et al.*, 2013).

(TEOS) (4 ml) + mixture [ammonium hydroxide (3.3 ml) +ethanol (47 ml)]

stirring
reaction was continued 24 h **silica colloidal dispersion**
vigorously stirring
+APTES (0.3 ml) **mixture was stirred overnight.**
nanoparticle was purified by centrifugation and redispersion in ethanol.
(replicated three times).
.

Figure 3: Strober method for modification of silica nano-particles used in the sensor.

APTES: 3_Amino Propyl Tri-Ethoxyl Silane

Sensitivity of the sensor:

To assess sensitivity of the sensor, the amount of nano-silica, density of antibody, and finally modified antigen were determined and increased or decreased

sensitivity of the sensor was found for each stage. First, the factorized nano-particles produced in the previous stage, prone to bonding with antibody, were exposed to different dilutions of the antibody. The bonding of carboxylic group of nano-particles with amine group of antibody is to be considered at this stage. Thus, 5 mg of modified nano-particles was placed in 5 ml of different dilutions of antibody, and gently mixed for 10 minutes. The antibody dilutions used were; 1:5, 1:10, 1:20, and 1:40. Once bonding was completed, 10^{-3} molar toxin was added to each of the above solutions, and after mixing gently for 1 minute in a test tube, chromogen solution was added, and intensity of spectrum change of each solution was assessed in wavelength of 520 nm (given the seven color spectrum, red and orange colors of this wavelength were investigated).

To increase sensitivity of the sensor, the amount of nano-silica powder was increased. 6, 8, 10 milligrams of nano-silica with agent were produced in Strober method and made ready for bonding with antibody (as in stages described above). Then, the resulting suspension of nano-silica with agent bonded with bacterial antibody was placed in contact with distilled water samples contaminated with toxin of *S. aureus* with a density of 10^{-3}, so that, with presence of toxin in the sample, the toxin antigen could bond with antibody. Then, the results of light absorption due to chromogen material in the sensor containing different grams of nano-silica were assessed by using spectrophotometer (Rastogi *et al*., 2011; Hasanzadeh *et al*., 2013).

In order to assess the last possible parameter in design of this sensor, different dilutions of toxin were investigated. Therefore, optimal grams of nano-silica bonded with ideal amounts of antibody (found in previous section) were exposed to different dilutions of bacteria toxin, so that, the minimum detectable amount by the sensor with optimal nano-silica and antibody amounts could be determined.

Determining the lifetime of the sensor:

What is meant by the sensor lifetime is to determine how long each molecularly imprinted polymer sensor and the sensor based on antibody bacteria bond with nano-silica can maintain its diagnostic properties. In other words, can they detect the toxin? This was investigated on 4, 8, 12, 16, 20, 24, 28, 32, 36, 40, 44, 48, 52, and 60 days after design of the sensor (Wu *et al*., 2012).

Results

Sensitivity of molecularly imprinted polymer sensor:

Table 1: Potential differences from different dilutions of bacteria toxin *S. aureus.*

Order	Voltage 107 mV	Dilutions 10^{-1}
1	166 mV	10^{-2}
2	226 mV	10^{-3}
3	269 mV	10^{-4}
4	338 mV	10^{-5}
5	365 mV	10^{-6}

As can be seen in Table 1, in 10^{-2} and 10^{-3} dilutions, the numerical difference of 59 mV was evident, but thereafter the difference in potential voltages was not 59 mV, and the sensor was unable to distinguish between this dilution and more diluted solutions. Thus, 10^{-3} was the minimum dilution in which nano-biosensor in distilled water can detect the toxin.

Microscopic magnification images

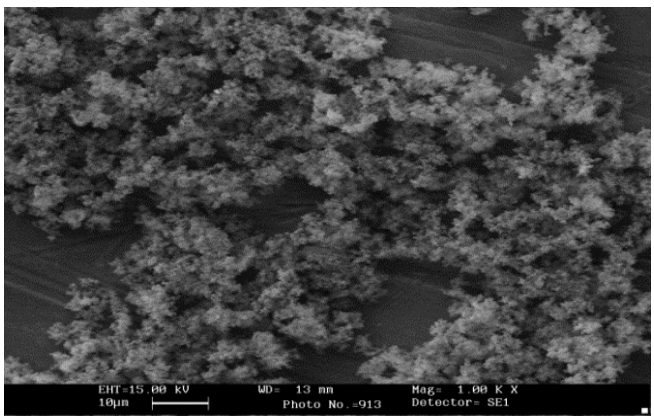

Figure1: Electron-microscopic images of molecularly imprinted polymer particles-magnification 1.00 KX, with particle diameter 10 µm.

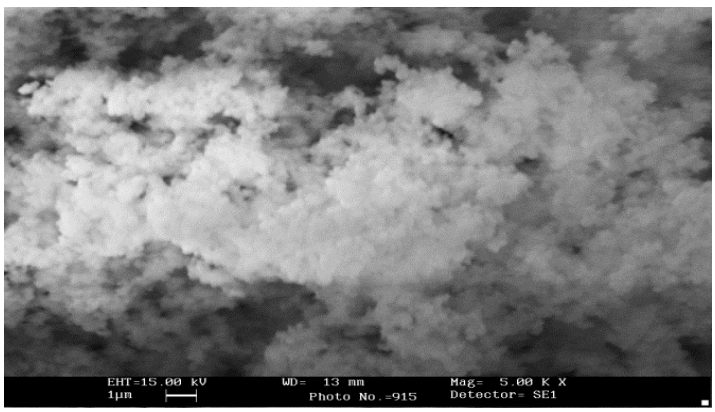

Figure2: Molecularly imprinted polymer (MIP)- magnification 5.00 KX, with particle diameter 1 µm.

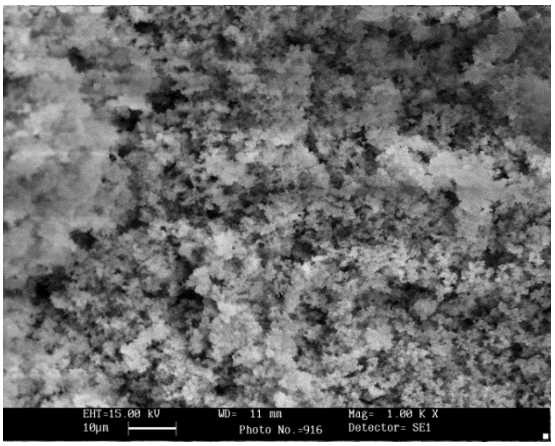

Figure3:Electron-microscopic images of non-molecularly imprinted polymer (NIP)-magnification 5.00 KX, with particle diameter 2 µm.

Stability of sensors' sensitivity:

The analysis of stability of sensor sensitivity with time in the second part of the project showed that after the 30thday, sensitivity of the first sensor in responding to antigen bond declined, whilst the second sensor (with higher percentage of meta acrylic acid monomers) responded positively in detecting toxins until the 52ndday, after which time, its response gradually declined. Therefore, the second sensor performed better for longer time as compared to the first one.

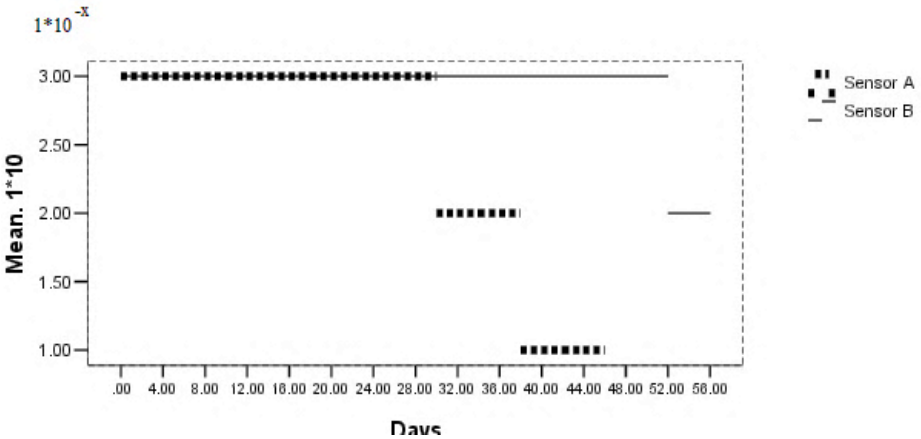

Figure 4: Comparison of the least separation of sensor according to lifetime.

Spectroscopic sensor sensitivity based on the optimal amount of nano-silica parameter

According to the results, 8 mg of nano-silica powder has more bonding with antibody amine group. Production of water molecules from ester reaction between amine group and carboxyl group indicated this reaction. Therefore, based on the 8 ml of water produced in this reaction, the optimal amount of powder was 8 gram, and any more than this amount did not have any effect on the reaction.

Second sensor sensitivity based on the antibody parameter

As can be seen, the results of this section revealed that the light absorbance on the vertical axis declined after 1:20 dilution to $1X10^{-2}$. Reduction in absorbance means lack of bonding to antibody in thinner dilutions. Fig. 5 shows the variations in absorbance intensity with different antibody dilutions. As can be seen in this Fig., up to 1:20 dilution, the absorbance is linear, and the maximum bonding capacity of modified nano-particles to antibody is at 1:20 dilution.

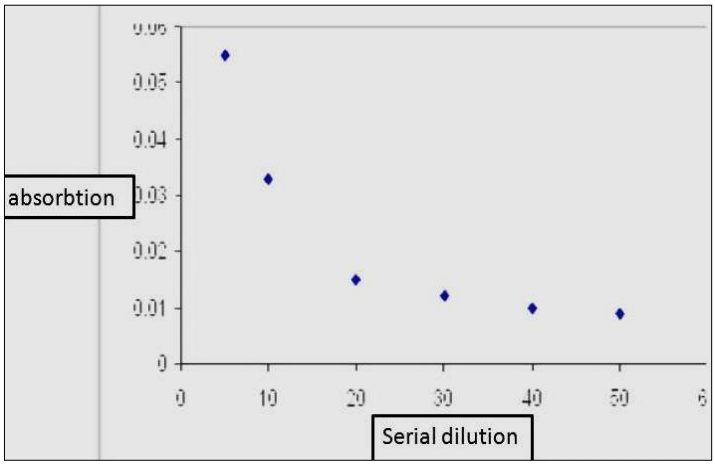

Figure 5: Variations in absorbance of modified nano-particle toxin detection system with antibody dilution coefficient.

Second sensor sensitivity based on antigen parameter

According to the results of the toxin detection experiments at constant dilution of 1:20, the bonding between antibody and nano-particles continued, and different toxin dilutions including: 1:5, 1:10, 1:25, 1:50, 1:75, 1:100 in contact with the medium were also investigated. In other words, different dilutions of exotoxin were analyzed with the modified nano-silica at 8 grams and the bond with 1:20 antibody dilution maintained. It can be seen in the Fig.6 that the sensor detection system is able to seek toxin up to 1:78 dilution with high accuracy, and at higher dilutions no significant absorbance is found.

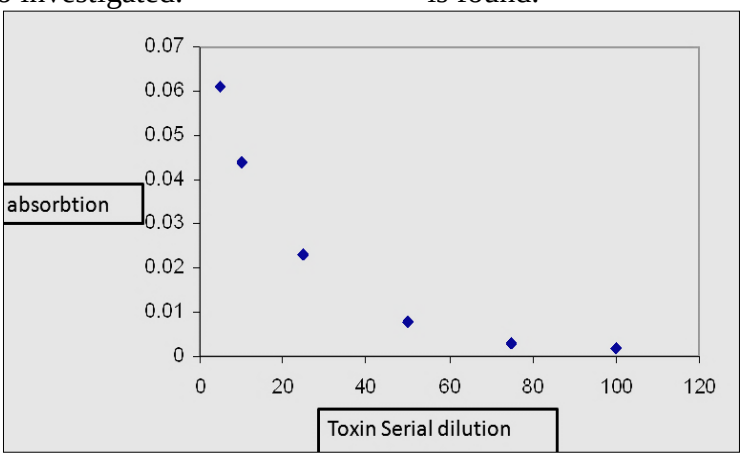

Figure 6: Variations in absorbance of modified nano-particle toxin detection system with toxin dilution coefficient.

Discussion

Today, use of rapid and sensitive techniques for detection of food-borne pathogens is important. Since many food-borne pathogens are in very low concentrations, therefore, to ensure food safety, rapid and sensitive methods are required (Won *et al*., 2012).

The food-borne toxin detection methods require preliminary preparations of samples and extraction of toxin, which is very lengthy and cumbersome, while using biosensors increases sensitivity, saves detection time, and is economical (Lee *et al*., 2012).

An important benefit of using this method for food quality control management systems is direct, on-line monitoring of results. Thus, detection time is highly important in quality control systems and also in payback period for the producer.

In quarantine and even in military and strategic conditions, this detection time is applicable, covering a wider spectrum. For example, in ISO systems, in addition to the quality control tests, raw material production must also be analyzed and examined because this food cycle is interdependent and complementary. Thus, for import and export of food products, or even the raw materials imported for a manufacturer, the customs discharge time and diagnostic results are highly important. Here, the accuracy and sensitivity of the detection tests affect the cycle, as well.

In many cases, the exotoxin of bacterial that is detectable by traditional methods is destroyed due to competition with other pathogens and environmental factors such as temperature and acidic medium, and the bacterial toxin that may be resistant to temperature remains intact. Biosensors have the ability to detect such cases and thus greatly help researchers and food quality control specialists in food safety management systems.

As a result, speed, accuracy, and conditions of detection are the sensors' important superiority over traditional methods in quality control in the food industry (Cuero *et al*., 2012;Gerasimova and Kolpashchikov, 2013).

In total, of the 32 samples of distilled water contaminated with *S. aureus* bacteria toxin type A, 27 were detected by molecularly imprinted polymer sensor. But, with the sensor based on nano-silica bonding with antibody, 30 samples with minimum detectable concentration of 10^{-3} molar out of 32 were detected in terms of quality and quantity.

Table 2: Descriptive statistics on the two potentiometry and spectrometry sensors.

Descriptive Statistics

GROUP	N	Mean	Std. Deviation	Minimum	Maximum
Sensor A S	32	.8438	.36890	.00	1.00
Sensor B S	32	.9375	.24593	.00	1.00

Table 3: Binominal test results of the two sensors

Binomial Test

GROUP		Category	N	Observed Prop.	Test Prop.	Asymp. Sig. (1-tailed)
Sensor A S	Group 1	YES	27	.8	.9	.211
	Group 2	NO	5	.2		
	Total		32	1.0		
Sensor B S	Group 1	NO	2	.1	.9	.000
	Group 2	YES	30	.9		
	Total		32	1.0		

According to Table 1, it seems that toxin detection of potentiometer sensor of 84.38±36.89 is less than that of spectrometer sensor of 93.75±24.59 .

Based on the results in Table 2, and since the toxin positive samples separated in the potentiometry and spectrometry sensors were 27 and 30 out of 32 respectively, the significant level for the first sensor is p=0.211 that confirms the likelihood of toxin detection by potentiometer sensor is less than the assumed 90%. Null hypothesis showed this being small compared to 0.9. As for the spectrometer sensor, the significant level is P= % and hypothesis zero means this ratio is bigger than or equal to 0.9. So, it can be concluded that spectrometer sensor has the detection rate of at least 90%.

Generally, this study showed that nano-particles have unique characteristics in bio-analysis and biotechnology applications. New methods in modification of nano-particles described in bonding between antibody and nano-particles have led to formation of an amide bond between nano-particles and protein exotoxin. This bond is formed through an active ester reaction between amine group of exotoxin chain and carboxylic acid group.

This nano-biosensor has the benefits of short analysis time and higher sensitivity in toxin detection. However, the flaws in this method include some cases where the ability of some antibodies to bond with antigens have diminished due to involvement with silica nano-particles, thereby restricting the sensitivity of the detection system. Here, the determining factor in sensitivity of the sensor is the

antibody itself, rather than the concentration of exotoxin. When the level of antibody is less than the optimal amount, it bonds with modified nano-silica, but the ability to bond with the antigen or the same toxin will be reduced due to involvement with nano-silica (Hasanzadeh *et al*., 2013) causing a reduction in sensitivity of the sensor. In most cases when there is no difficulty with presence of antibody, the sensitivity of this type of sensor is higher and it is more accurate compared to molecularly imprint polymer sensor. In the second part, the cost and microbial properties of sensors are compared.

The molecularly imprint polymer sensor requires a large outlay of expenditure for initial design, but these shortcomings can be overcome in the simulation and final mass production. However, the finished cost of antibody bond with nano-particle sensor is much more ideal, and it is a function of the nano-particle, type of antigen and antibody (of any pathogen strain) used.

Producing potentiometer sensors based on molecularly imprint polymeris cost effective. Once the sensor is manufactured, tests can be conducted with a simple potentiometer. In other words, the compositions required to build this sensor are expensive, but the equipment needed for detection and obtaining results are simple, inexpensive and available. However, the chemicals used in making this sensor are mostly dangerous and considered environmental pollutants. According to the Environmental Protection Agency (EPA), the chemicals used in the antibody connected to nano-particle sensor, unlike the molecularly imprint polymer sensor, score two environmental stars, and less chemicals are used compared to the first sensor. Also, expensive materials are used in the first type of sensors, making them highly costly overall. Other flaws of this type include short lifetime and loss of sensitivity. This fault applies to both sensors, but it is much more evident in type 1 sensor with elapsing time. Therefore, based on the research conducted and review of literature, the sensor designed in accordance with antibody connection with nano-particle is highly sensitive, costs less, lasts longer, and has less harmful chemicals to the environment and human health. However, the amount and purity of antibody are important factors in sensitivity and lifetime of the sensor (Rastogi *et al*., 2011).

Generally, producing biosensors based on antibody connection with nano-particle requires technical know-how and expert hands for targeted connection with antibody. Production of nano-particles is highly technical. In this study, the sensors used were commercially procured at a great expense (according to the type).

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References

Abbasi, M.A., Ibupoto, Z.H., Hussain, M., Khan, Y., Khan, A., Nur, O. and Willander, M., 2012. Potentiometric zinc ion sensor based on honeycomb-like NiO nanostructures. *Sensors (Basel),* 12**,** 15424-37.

Abounassif, M.A., Al-omar, M.A., Amr, A.G. and Mostafa, G.A., 2011. PVC membrane sensor for potentiometric determination of iron (II) in some pharmaceutical formulations based on a new neutral ionophore. *Drug Testing and Analysis,* 3**,** 373-9.

Chen, Y., Chen, L., Bi, R., Xu, L. and Liu, Y., 2012. A potentiometric chiral sensor for L-Phenylalanine based on crosslinked polymethylacrylic acid-polycarbazole hybrid molecularly imprinted polymer. *Analytica Chimica Acta,* 754**,** 83-90.

Cuero, R., Lilly, J. and Mckay, D.S., 2012. Constructed molecular sensor to enhance metal detection by bacterial ribosomal switch-ion channel protein interaction. *Journal of Biotechnology,* 158**,** 1-7.

Gerasimova, Y.V. and Kolpashchikov, D. M., 2013. Detection of bacterial 16S rRNA using a molecular beacon-based X sensor. *Biosens Bioelectron,* 41**,** 386-90.

Gurtova, O., Ye, L. and Chmilenko, F., 2013. Potentiometric propranolol-selective sensor based on molecularly imprinted polymer. *Analytical and Bioanalytical Chemistry Research,* 405**,**287-95.

Hakalehto, E., Pesola, J., Heitto, A., Deo, B.B., Rissanen, K., Sanilampi, U., Humppi, T. and Paakkanen, H., 2009. Fast detection of bacterial growth by using Portable Microbe Enrichment Unit (PMEU) and ChemPro100i((R)) gas sensor. *Pathophysiology,* 16**,** 57-62.

Hasanzadeh, M., Shadjou, N. and Omidinia, E. ,2013. Mesoporous silica (MCM-41)-FeO as a novel magnetic nanosensor for determination of trace amounts of amino acids. *Colloids and Surfaces B: Biointerfaces,* 108C**,** 52-59.

Jia, Y., Gao, C., Feng, D., Wu, M., Liu, Y., Chen, X., Xing, K. and Feng, X., 2011. Bio-initiated light addressable potentiometric sensor for unlabeled biodetection and its MEDICI simulation. *Analyst,* 136**,** 4533-8.

Jia, Y.F., Gao, C.Y., He, J., Feng, D.F., Xing, K.L., Wu, M., Liu, Y., Cai, W. S. and Feng, X.Z., 2012. Unlabeled multi tumor marker detection system based on bioinitiated light addressable potentiometric sensor. *Analyst,* 137**,** 3806-13.

Kou, L.J., Liang, R.N., Wang, X.W., Chen, Y. and QIN, W., 2013. Potentiometric sensor for determination of neutral bisphenol A using a molecularly imprinted

polymer as a receptor. *Analytical and Bioanalytical Chemistry Research,*405(**14**), 4931-4936

Kozyra, A., Wiora, J. and Wiora, A., 2012. Calibration of potentiometric sensor arrays with a reduced number of standards. *Talanta,* 98**,** 28-33.

Lee, H.R., Seo, J.W., Kim, M.J., Song, S.H., Park, K.U., Song, J. and Han, K.S., 2012. Rapid detection of bacterial contamination of platelet-rich plasma-derived platelet concentrates using flow cytometry. *Annals of Clinical* & *Laboratory Science*, 42**,** 174-81.

Mashhadizadeh, M.H. and Talemi, R.P., 2011. Used gold nano-particles as an on/off switch for response of a potentiometric sensor to Al(III) or Cu(II) metal ions. *Analytica Chimica Acta*, 692**,** 109-15.

Ozdemir, M.S., Marczak, M., Bohets, H., Bonroy, K., Roymans, D., Stuyver, L., Vanhoutte, K., Pawlak, M. and Bakker, E., 2013. A label-free potentiometric sensor principle for the detection of antibody-antigen interactions. *Analytical Chemistry*, 85(**9**), 4770-4776.

Panneer Selvam, A. and Prasad, S., 2013. Nanosensor electrical immunoassay for quantitative detection of NT-pro brain natriuretic peptide. *Future Cardiol,* 9**,** 137-47.

Rastogi, S.K., Pal, P., Aston, D.E., Bitterwolf, T.E. and Branen, A.L. ,2011.8-aminoquinoline functionalized silica nanoparticles: a fluorescent nanosensor for detection of divalent zinc in aqueous and in yeast cell suspension. *ACS Applied Materials and Interfaces,* 3**,** 1731-9.

Sanan, R. and Mahajan, R.K. ,2013. Micellar and analytical implications of a new potentiometric PVC sensor based on neutral ion-pair complexes of dodecylmethylimidazolium bromide-sodium dodecylsulfate. *Journal of Colloid and Interface Science,* 394**,** 346-52.

Sowri Babu, K., Ramachandra Reddy, A., Sujatha, C. and Venugopal Reddy, K. ,**2013**. Effects of precursor, temperature, surface area and excitation wavelength on photoluminescence of ZnO/mesoporous silica nanocomposite. *Ceramics International*, 39(**3**), 3055-3064.

Suehiro, J., Ikeda, N., Ohtsubo, A. & Imasaka, K., 2009. Bacterial detection using a carbon nanotube gas sensor coupled with a microheater for ammonia synthesis by aerobic oxidisation of organic components. *IET Nanobiotechnol,* 3**,** 15-22.

Turkoglu, E.A., Yavuz, H., Uzun, L., Akgol, S. and Denizli, A., 2012. The

fabrication of nanosensor-based surface plasmon resonance for IgG detection. *Artificial Cells, Nanomedicine and Biotechnology* 41(**3**), 213-21

Won, H., Yang, S., Gaydos, C., Hardick, J., Ramachandran, P., Hsieh, Y.H., Kecojevic, A., Njanpop-Lafourcade, B.M., Mueller, J.E., Tameklo, T.A., Badziklou, K., Gessner, B.D. and Rothman, R.E., 2012. A broad range assay for rapid detection and etiologic characterization of bacterial meningitis: performance testing in samples from sub-Sahara. *Diagnostic Microbiology and Infectious Disease*, 74**,** 22-7.

Wu, W., Sun, Z., Zhang, Y., Xu, J., Yu, H., Liu, X., Wang, Q., Liu, W. and Tang, Y., 2012. A multifunctional nanosensor based on silica nanoparticles and biological applications in living cells. *Chemical Communications*, 48**,** 11017-9.

Xie, X. and Bakker, E. ,2013. Non-Severinghaus potentiometric dissolved CO_2 sensor with improved characteristics. *Analytical chemistry*, 85**,** 1332-6.