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DETECTION OF *SALMONELLA TYPHI* IN AQUATIC ENVIRONMENT USING NANO BIOSENSOR BASED ON *FLUORESCENCE*

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ABSTRACT

It takes about 10 days to detect the bacterium in water and food using traditional methods and there is an essential need for the introduction and manufacturing of nano biosensor kits for a fast detection in few minutes, so an attempt in determining the mass of *Salmonella typhi* by means of a sensor design was made to obtain this shortened.

In this research, by means of a micro contact imprinted polymer scheme, molecularly imprinted polymer was achieved. First, Meta acrylic acid (MAA) with covalent bond among its monomers formed a white polymer and hydrogen bond between antibody and MAA. They were used to prepare imprinted molecule and polymer. It was noticed that with the aid of fluorescence converter and its connection with the antibody of *Salmonella typhi* bacterium, it would be possible to detect the *Salmonella typhi* antigen.

The results indicate that detecting the concentration of *Salmonella typhi* bacterium is possible with a minimum mass of 10 colonies per ml in polluted waters. The achieved results were quantified by a spectrofluorometer device. Additionally,the *Ecoli* bacteria were inseminated in an aquatic environment containing *Salmonella typhi* and it was detected that there was no interference with function of sensor. Besides, sensitivity of the sensor up to 60 days was examined and it was agreed that performance of the sensor could be verified up to 56 days and then would start to diminish. In conclusion the role of sensor at $10^{-1\ to}10^{9}$ concentration of *Salmonella typhi* antigen are exclusively precise and sensitive.

Keywords: Biological nano-sensor, Microcontact, *Salmonella Typhi*, Spectrofluorimetery

INTRODUCTION

Unstable and spoiled product causing foodborn infection, toxicity and morbidity produced by microorganism like *Salmonella* are one of the major concerns for human life. It is estimated that roughly 76 million of food poisoning cases are annually caused by *Salmonella* in the United States , hence it is indispensable to find a fast and precise technique to detect *Salmonella typhi* to preserve food products safe and sound **[1]**. *Salmonella typhi(the cause of Typhoid fever)* is transferred either from person to person or through contaminated water in sewages, in contrast, most of the non-typhoid *Salmonellas are* entered the body from contaminated food **[2]**. The prevention of *Salmonella* as a food-born element relies on how the principles of food hygiene are followed **[3]**.

In 2013, Deng and his colleagues employed molecular imprinted polymer to measure the uranium level in urban areas and sea water to evaluate the biocontaminators. The results of detection level and linear range were 10^{-8}, 10^{-2}, and 2×10^{-8} respectively.

In 2012, Kryscio and colleagues measured melanin in milk by a film sensor on a polymer ground modified with molecular imprinting. The results calculated for detection level and linear range were 10^{-6}, 10^{-2} and 5×10^{-6} respectively.

In 2007, Lin and colleagues made a modified sensor with molecular imprinting to measure chlortetracycline in urine and blood serum. The linear range for this project was achieved respectively 10^{-3}, 4.93×10^{-5} colonies per mlcolonies per ml.

In 2012, Ahari and colleagues used potentiometer with molecular imprinted polymer to molecularly detect the exotoxin of *Staphylococcus aureus*. The results indicated that the dilution up to 10^{-3} colonies per ml could be detected with sensor and sensitivity was assessed up to 60 days that it was approved for 28 days.

In 2008, Javanbakht and colleagues applied a modified carbon pulp electrode with molecular imprinted polymer to measure cetirizine in blood serum. The results indicated the linear range was 10^{-8}, 10^{-2} and 7×10^{-7}.

The increase in microbial polluters of environment as typhoid, *Para typhoid* in stale water, the residues of factories and litters of municipalities and hospitals has highlighted the essential role of biosensors in detecting the pathogens, infectious factors and toxicant. In this research, the simulation of biosensor is the area of concern to recognize the most dangerous bioterrorist among the environmental polluters.

MATERIALS

- PTCC-1609 *Salmonella typhi*

- Biospes (poly Anti-*Salmonella typhi* antibody)
- Acetic acid from Merck company
- Polyclonal Anti-*Salmonella typhi* antibody (HRP conjugated)
- Broth BHI
- PBS (Phosphate buffer salin)
- Stop solution
- 5% skim milk
- Cover glass
- Support glass
- 3- methoxy propyl silyl metacrilat (Sigma company)
- THB (tetra methyl Benzaldehyde)
- APTES(3-Aminopropyltriethoxysilane) activator prepared from Sigma
- 4-Alil oxy coumarin, network builder (EGDMA) and AIBN initiator
- Sodium dodecil solphate (SDS) 5% w/w (Sigma company)
- BSA (bovine serum albumin)

All the materials used in this research have pure analytic degree and twice distilled water is used for the solutions.

METHODOLOGY

The anti serum of *Salmonella typhi* is in the category of IgG antibody .This group of antibodies are Y-shape with molecular weight of 150 kilo Dalton. In the structure of IgG, carboxyl and amino acid groups are abundantly seen. The amino acids as well as tryptophan, tyrosine, phenylalanine in the ending part of this protein have fluorescent property, yet being positioned in the structure of the protein, the fluorescence intensity is sharply diminished pertaining to conformation change and complicated structure of antibody.

In this project, the antibody could be detected with Molecular Imprinted Polymer (MIP) and fluorescence convertor. Ultimately, having the reaction finished, covalent bond between Meta Acrylic Acid (MAA) monomers was formed and white spectrum polymer particles were achieved; moreover, hydrogen bond formed between its amino acid and Meta acrylic acid was the reason for its selective absorption.

Sensor simulation

The reason for making this sensor is to prepare a thin film of molecular imprinted polymer with a fluorophore as the fluorescence monomer of cover glass.

The process of making the sensor is as follows:

1. Preparing the surface at cover and support glass,
2. Activating the support glass,
3. Marinating the cover glass in antibody solution,
4. Preparing MIP solution including MMA and fluorescence monomer,
5. Alyl oxy coumarin, GDMA network builder, AIBN initiator,

6. Pouring MIP solution over support glass and lying the cover glass containing antibody upon it and finally polymerization reaction under UV [**4**].

To prepare the support glass: piranha solution including (H_2SO_4: H_2O_2, 3:1 $^{v}/_{v}$) was provided and glasses were laid in the solution, then they are washed and dried. Support glass should be noticed to have smeared with sailan derivatives just prior to the preparation of MIP film. It is needed to use 0.5 ml 3-methoxy propyl silyl metacrilat in 20 ml of ethanol, 1.5 ml water, 72 ml acetic acid, next the glasses must be dried in nitrogen gas.

To prepare the cover glass, they should, in turn, be sonikated in Sodium Dodesyl Solfate (SDS) (H_2SO_4: 5% $^{w}/_{w}$, distilled water, propanol, ethanol, and distilled water (30 minutes for every section), then dried with Nitrogen gas; after that, the glass should be lain in antibody solution for two hours at the temperature of the environment and again is dried with Nitrogen gas.

MIP solution prepared with methyl metacrilat, 50% multi ethylene glycol, de metacrilate EGDMA and 10% of multi AIBN initiator and 4-Alyl Oxy coumarin is poured on support glass approximately 10-20 µl and glass containing antibody is stamped on support glass. The polymerization lasts in UV cabin under 350 nm for 20 hours; next two glasses are separated. All the above mentioned process should be repeated without antibody to make NIP (control) **[5]**.

Removing the antibody

To remove the antibody, MIP film is rinsed in SDS solutions 5% w/w and NaOH 0.6% w/w and 85˚C for 30 min and then in one colonies per ml NaCl solution and finally with distilled water. To ensure the removal of antibody from the surface of the film, there should be no absorption in washing solution examined with spectrophotometer at 280 nm.

Preparing microbial culture: first, *Salmonella typhi* strain from Iranian Scientific and Industrial Investigating Organization with PTCC (Persian type culture collection) number 1609 is provided, then it is transferred from solid to liquid broth (BHI) and preserved for 24 hours in incubator at 37˚C to obtain the dilution of half McFarland determining 10^8colony per ml in spectrophotometer set. After that the liquid broth containing bacterium mass was centrifuged at 3300 for 9 minutes. Having this period passed, the upper layer of the liquid is thrown out and the bacterium mass will be washed with saline and adequately shaken again in order to gather the mass for having them rinsed well. It should be blended with saline at the end of Falcon tube. The centrifuge was performed again

and this washing act was repeated for three times. Afterward, it is put in Benmary for 20 min to deactivate any capsules causing failure in recognition **[6]**.

Microbial Dilutions

Having extracted the content of Falcon including bacterium, the following process is done for dilution:

1. First, the Spectrophotometer should be calibrated for reading at 620 nm. Distilled water should be put at the cuvette which zero should be read in OD indicating calibration of the set.
2. Should Falkon including bacterium mass followed by the vortex be transferred to cuvette, the 0.08 to 0.1degree must be read by the set at 600nm. In our case, it was very thick and was read at higher range, so we diluted it to see 0.09 which was considered ideal for indicating 10^8 dilutions. We made the other dilutions based upon that.
3. There is poured 20ml saline into Falcon tubes (according to $C_1*V_1=C_2*V_2$ formula). At first C_1 was 10^8, we wonder how much of it should be poured to the next Falcon to make 10^5 dilutions. The other side of equilibrium is 10^5 C2 which should be multiplied by 20 ml. $V1*10_8=10^5*20$ $V_1=0/02ml=20$ µl. First, 20 µl should be taken from the Falcon containing 20 ml serum. Then 20 µl should also be taken from the Falcon 10^8 and poured into the next Falcon from which 20 µl has been taken, in order to make 10^5 dilution [2].
4. To make 10^3 dilution, the Falcon containing 10^5 dilution should be shaken (vortex), then we should take 200 µl from the Falcon specified for 10^3 to be replaced with 200 µl from 10^5 dilution, therefore, 10^3 dilution is achieved.
5. To make 10^1 dilutions, the Falcon 10^3 is shaken (vortex) and acted out exactly like section 4. Now dilutions are ready to approach the nano-kit [7].

Intensity of fluorescence

The primary study on distribution of fluorescence on MIP and NIP sensors was assessed with and without antibody. The rinsed NIP and MIP sensors were marinated in 10 ppm antibody solution (pH=7.4 phosphate buffer) for 1 hour. In the presence of analyte, the florescent spectra scattering (distribution) proved an increase in the intensity of MIP in comparison with NIP.

The lifetime of Sensor

The study on life time of the electrode in designed sensor has shown how long the sensor can keep detecting properties based upon molecular imprinted polymer attached (bound) to the bacterium. In other words, how much it can detect the germ. This was done at 0, 4, 8, 12, 16, 20, 24, 28, 32, 36,

40, 44, 48, 52, 60 days after the design of sensor **[8]**.

Particle Size Analyzer

The size and the way formed capsules are distributed have been evaluated with particles size analyzer. The nano-particles are dispersed in ionized water and having calibrated the set with ionized water, 2 ml of solution containing nano particles was added to the set and the results were reported based on the average volume and diameter of nano particles (0.1 d or diameter of 10% nano particles), (0.5 d or diameter of 50 % nano particles), (.9 d or 10% of diameter of nano particle). The diameter of nano particle was also examined by light microscope (leica DHLB) for 10 samples analysed with software leica Qwin 550 **[8]**.

RESULTS

The results of magnified pictures achieved by electron microscope:

As it can be seen in electron microscope images, the distribution of particles with 15 kv and 1.00 kx is in the limit between 1-10 micrometer that illustrates the average of molecular imprinted polymer is 20-45 nanometer and NIP particles are equal to 80-100 nanometer.

The reason for the accumulation of dark matters which are seen in the pictures is the MIP and NIP samples were not dissolved in Acetonitrile.

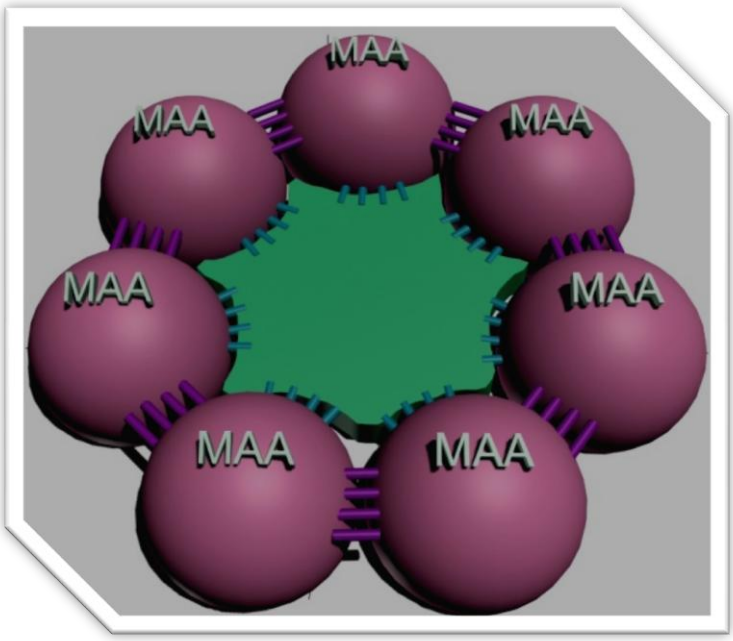


Figure 1: Display of covalent bonds between Meta acrylic acid monomers and interaction of molecular imprinted with bacterium

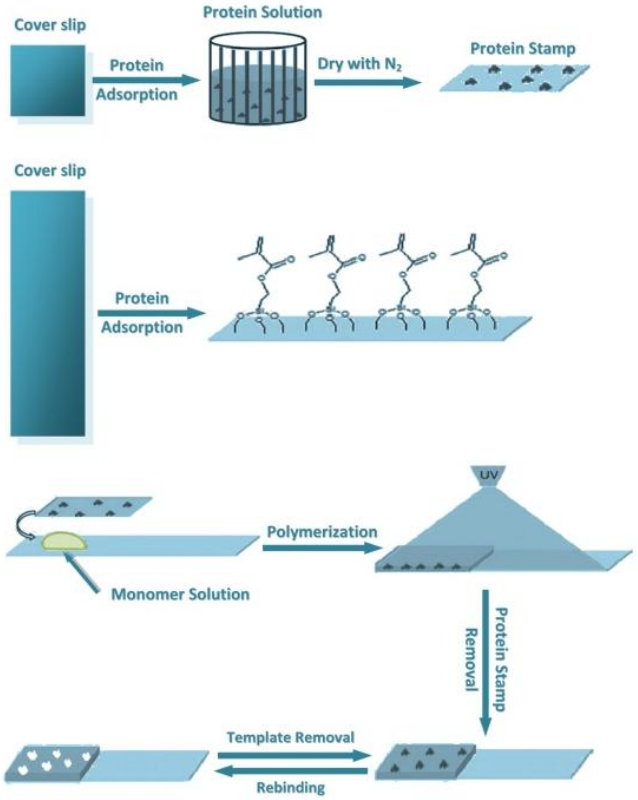


Figure 2: The process of making nano-sensor based on molecular fluorescence for antiserum of Salmonella typhi [4].

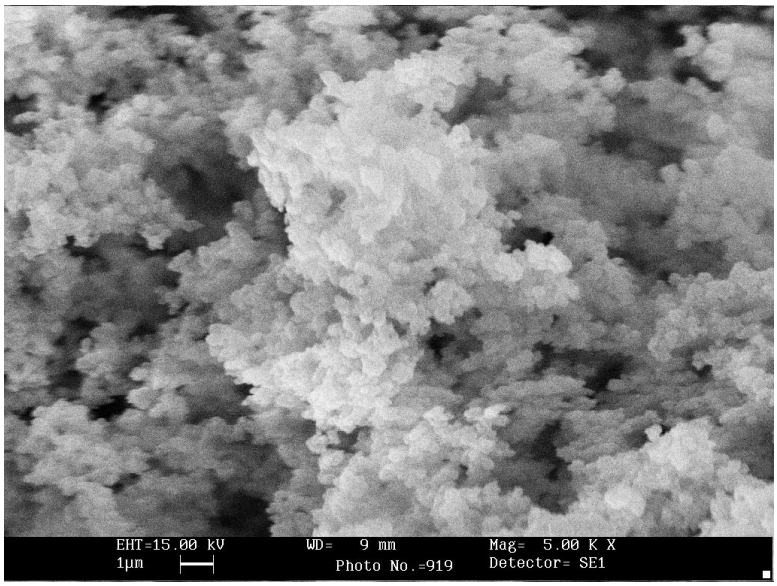


Figure 3-A: Electron microscope pictures of molecular imprinted polymer particles with 1.00 kx magnifying with 10 mm diameters of particles

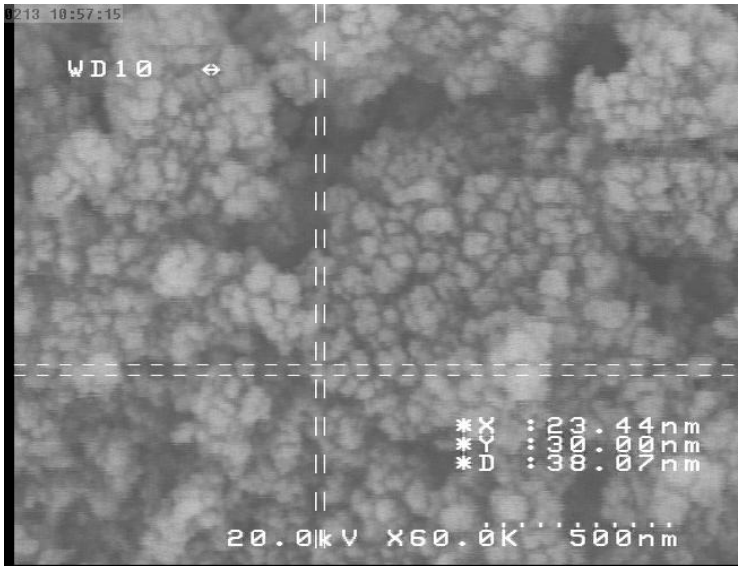


Figure 3-B: magnified 5 kx with 2 mm diameter of particles

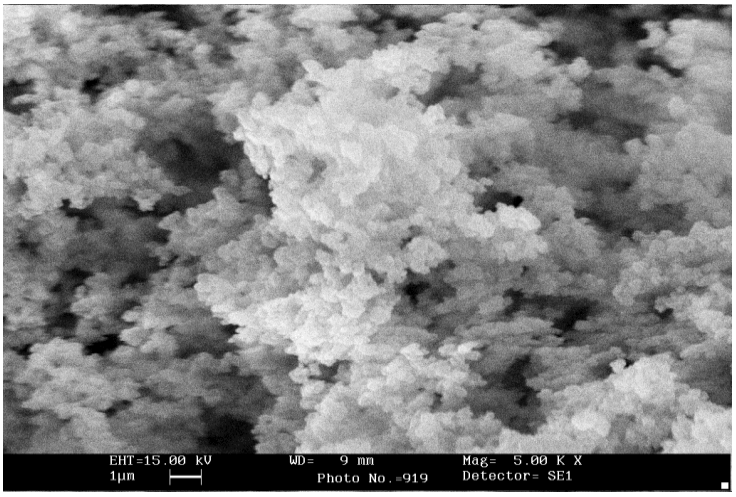


Figure 3-C: Molecularly imprinted polymer MIP. Magnified 5kx with1 mm diameter of particles

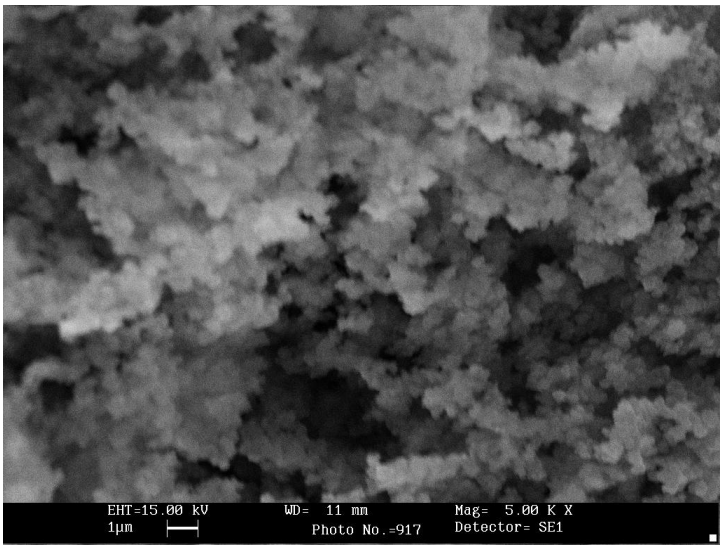


Figure 3-D: magnified 5.00 kx with 1 mm diameter of particles

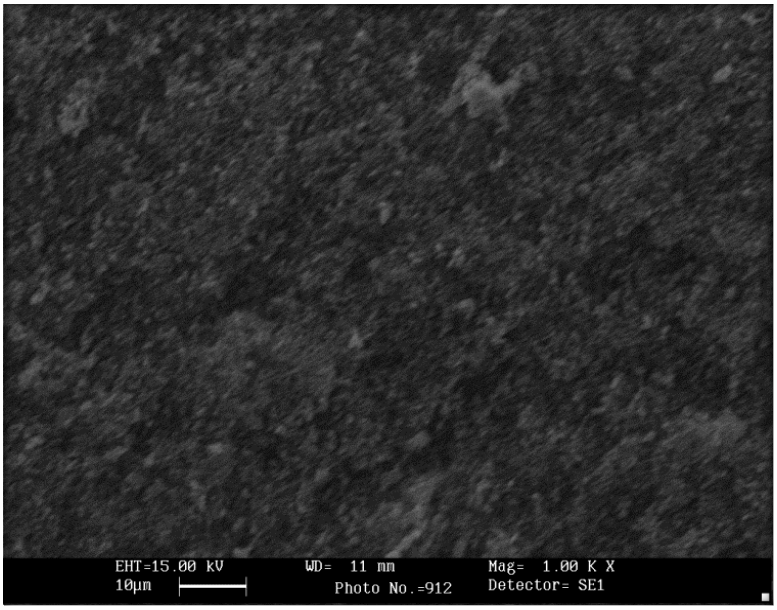


Figure 3-E: Pictures of non-imprinted polymer (NIP) with electron microscope Magnified 1.00 kx with 10 mm diameter of particles

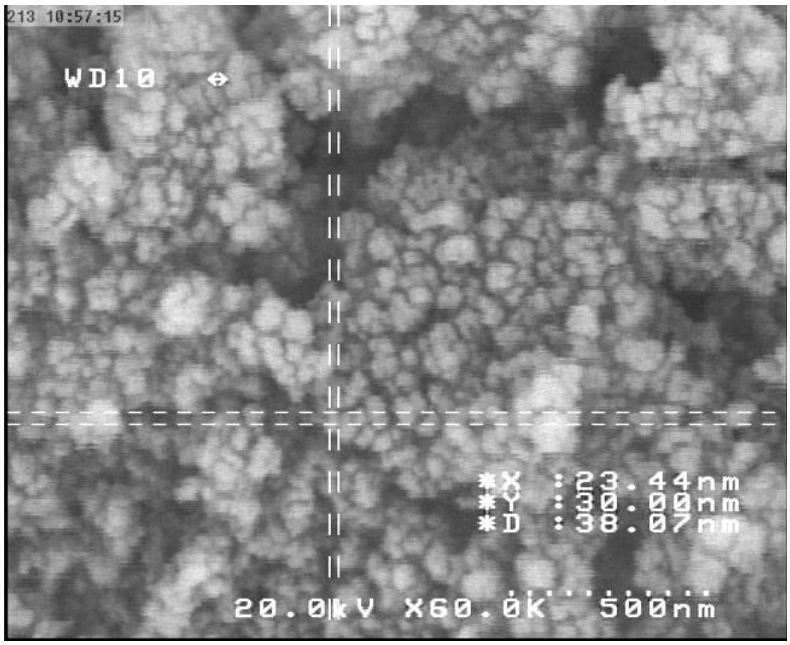


Figure 3-F: Particles with diameter of 500 nm

Results of TEM

As it can be seen in electron scanning with TEM microscope, the distribution of nano particles are entirely homogenous.

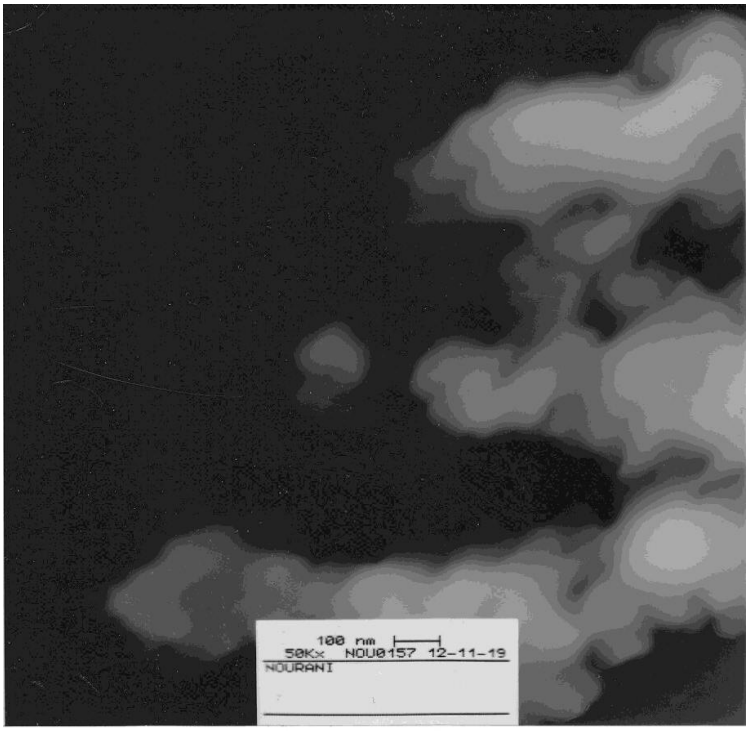


Figure 4-A: TEM picture, magnified 50kx and particles distribution (100 nm)

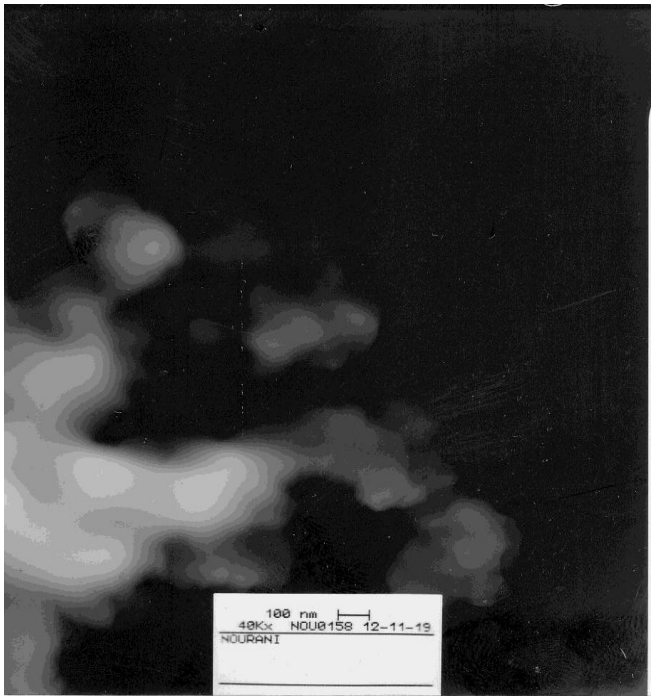


Figure 4-B: TEM picture, magnified 40kx and particles distribution (100 nm)

The results of life time

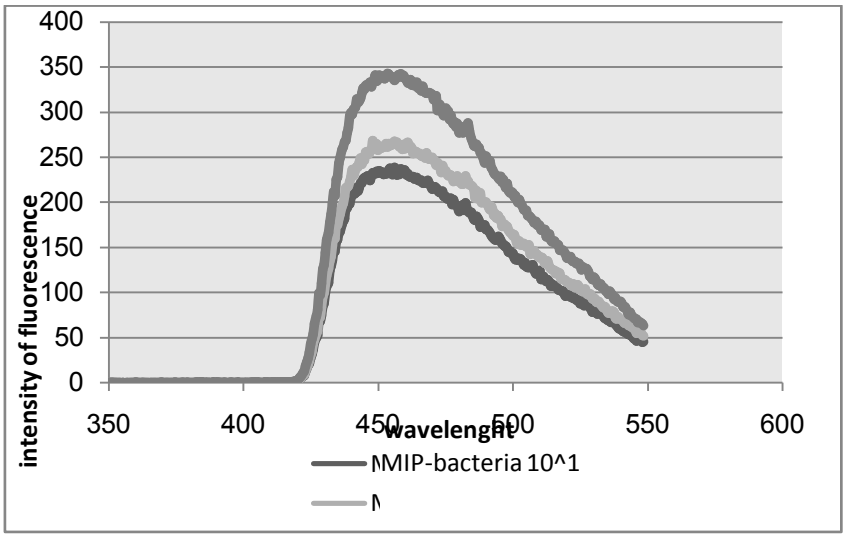
The analysis of the sensor sensitivity indicates that its response to bind with antigen is declined after 52 days.

Results of Sensor Sensitivity

As it can be seen in **Table-1**, antibody bind to MIP in the absence of antigen has scattering intensity of 273 while antibody bind to MIP in the presence of antigen at 10^1 dilution is 325 nanometer and at 10^5 dilution shows 448nm and consecutively at higher dilutions such as 10^8, the scatter of fluorescence is enhanced to meet a peak of 745nm, therefore fluorescent scattering level will go up due to increase in dilution. According to the table, there is also a definite link seen between the increase in fluorescent scatter and surge in antigen concentration as at $10^{1,}$ the intensity of fluorescence scatter was 234, at 10^3 , 263, at 10^5 , 341 and at 10^8 it was 658. This also has been borne out by the attached graphs.

Table 1- the changes in sensor response in form of the intensity of fluorescence based on antigen dilution on the first day of confrontation

Intensity of Fluorescence Scatter	Sample Name
273	Antibody bind to MIP in the absence of antigen
325	Antibody bind to MIP in the presence of antigen 10^1
350	Antibody bind to MIP in the presence of antigen 10^3
448	Antibody bind to MIP in the presence of antigen 10^5
745	Antibody bind to MIP in the presence of antigen 10^8



Graph 2- the survey of the intensity of fluorescence based on light absorption

Table 2- the changes of sensor response to the intensity of fluorescence based on antigen dilution on day 52

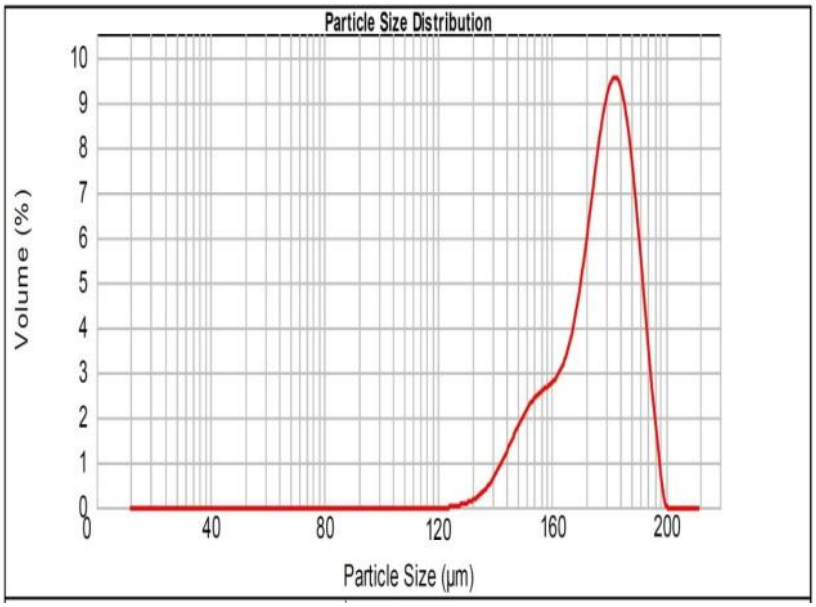
Intensity of Fluorescence Scatter	Sample Name
182	**Antibody bind to MIP in the absence of antigen**
234	**Antibody bind to MIP in the presence of antigen 10^1**
263	**Antibody bind to MIP in the presence of antigen 10^3**
341	**Antibody bind to MIP in the presence of antigen 10^5**
658	**Antibody bind to MIP in the presence of antigen 10^8**

Sensitivity and Specificity of Sensor

The sensitivity of sensor starts from 10^1 to higher levels, the highest of which is 10^8. *Ecoli* (PTCC2216) is also mixed with *Salmonella typhi* in aquatic environment that did not make any changes in the performance of the kit. It should be mentioned that the results achieved from **Table 1 and 2** indicate the fact that there is an increasing trend for the intensity of fluorescent scatter from the lowest to highest level while kit meets with the designed Nano biosensor; however, there has been a reduction in table-2 pertinent to day 52, so it can be concluded that the efficiency of the sensor in terms of sensitivity is not acceptable and the results of recognition are no longer desirable after 52 days.

As far as there is no other similar kit available in the market since this kit is newly designed, the verification of that cannot be assessed.

According to the results achieved from **Table 3**, the more the concentration of bacterium in meeting with the Nano sensor, the less is the reduction of the intensity percentage of fluorescence compared between day 1 and day 52 and that states an optimum function of our designed sensor. Due to the fact that in most of the cases higher than 10^3 dilution, which is assumed as threatening dosage of this bacterium, and as more approaching to 10^8, the reduction in intensity of fluorescent scatter will be less diminished proving the sensor proper function.



Graph 3- the distribution of nano particles based on the data of the set (particle size analyzer)

The evaluation of reduction in response of fluorescent scattering intensity in sensor

Table 3- The results of reduction in the sensitivity of the sensor indicated by the intensity of fluorescence for the first day compared with the day 52

The Percentage of Reduction in Fluorescence Scatter	Sample Name
%33.33	Antibody bind to MIP in the absence of antigen
%28	Antibody bind to MIP in the presence of antigen 10^1
%24.85	Antibody bind to MIP in the presence of antigen 10^3
%23.88	Antibody bind to MIP in the presence of antigen 10^5
%11.67	Antibody bind to MIP in the presence of antigen 10^8

DISCUSSION

- The traditional methods of using medium of the culture for detection of bacteria mass are very time-consuming and boring. Today, food pathogen detection techniques, which are rapid and sensitive, are of paramount importance for the recognition of the transferred pathogens in food products. As long as, we are encountered with very low concentrations of pathogens in food, a sensitive and fast recognition is needed to ensure the healthiness of food. The common methods for detection of toxin and bacteria are generally based on the medium of the culture and biochemical tests, lasting at least 4-7 days [9].
- Considering the fact that the time needed to see the primary results of microbial culture in quality control

of food product is absolutely 48 hours and in some strains requiring pro-enriching and enriching, like *Salmonella*, it takes even 1 week to ten days to obtain the primary results, it is invaluable using biosensors and Nano biosensors. This is because, in many food industries in both the research and development as well as quality control section, the time to preserve goods and announce the test results of the supply to be confirmed or rejected for being handed to the consumer is too long causing reduction in maintenance time and indirectly inflict great losses on producers. This is while, for dairy products and meat products which are largely different from cereal, grain, oil, canned foods and the like, time is a very critical factor in both return of investment and quality control systems [10].

- ✓ The highly important point of these detecting methods is their capability of detection in the initial phase of infection where the antigen concentration is low and lower than infectious level; whereas the Nano biosensor kit designed for this study is capable of detection farther below the infectious dosage. One of the advantages of this approach is on line direct procession of the results in food quality control systems; therefore, it saves time as an outstanding factor in the quality of product. To sum up, high sensitivity, low price, broad frequency range, small size, repeatability, zero temperature quotient and liner function are of their advantages [10].

In another study by Ahari and colleagues in 2012, molecular imprinted polymer sensor was designed to detect exotoxin of *Staphylococcus aureus*. The life time for this sensor was 52 days and separated the 10^{-4} molar dilution, while the life time of this sensor is also 52 days with 10^{1} to 10^{8} colon in milliliter separation ability and with a much less detection time [8].

Grigoriev and colleagues in a study in 2010 created a biosensor with molecularly imprinted polymer for measuring chlortetracycline in blood serum. In their research the tendency of polymers to mixture was investigated among which tetra-ethylene glycol di meta acrylat (TEGDMA) had the lowest tendency for the sample types and the tendency of methyl meta acrylat (MMA) was selected as the function monomer. When being mixed with TEGDMA, it demonstrated the highest tendency with the proportion of 1 to 3 in comparison with the other polymers

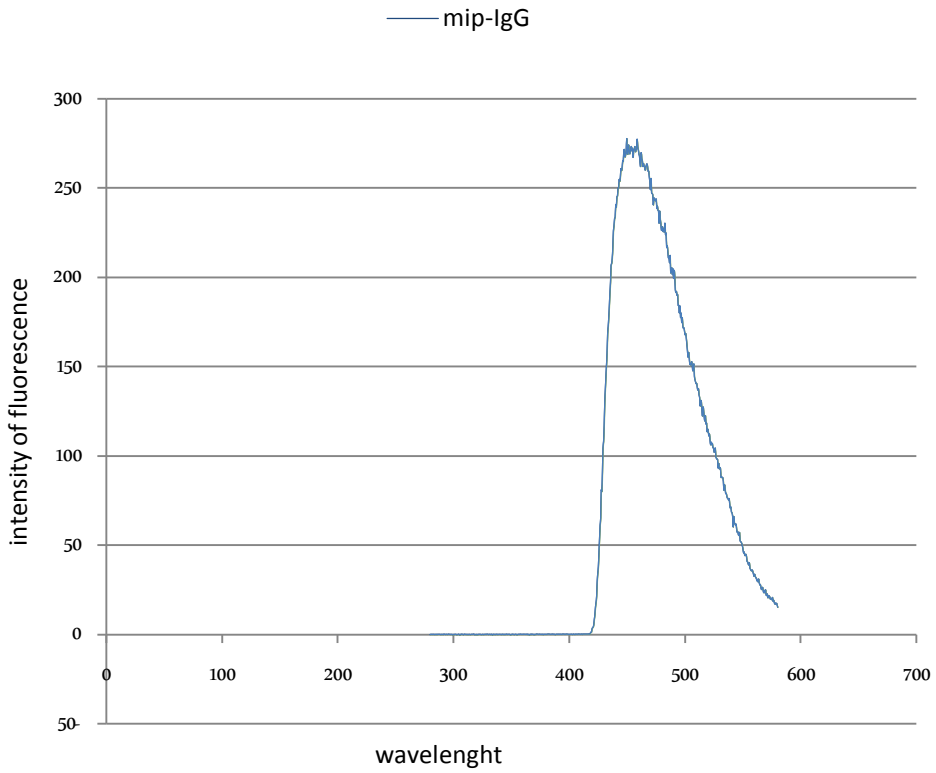
produced using the other function polymers. It should be noted that the type of sensor was exactly compatible with our designed sensor and our researches perfectly approved this fact. However, the detection time was 15 minutes and the linear slope was 62.5 volt which proved the significant sensitivity difference of the designed sensor. The research by Kryscio and colleagues (2012) resulted in production of a biosensor for specifically detecting the cow serum albumin in blood serum through the micro-contact molecularly imprinted polymer. In this method, there is no need to place the protein in the monomers dilution before polymerization which is totally in line with the method used in our study. In addition, the outcomes of these studies indicated renewability as a paramount part of the results not evident in the previous works. Also the thin films optimized against sample proteins as compared with the similar rival proteins enjoy a very high selectivity. All results obtained in the present study are compatible with the stated outcomes and substantiate them.

There are various new approaches employed for producing the imprinted proteins. The present method has applied the micro contact imprinted polymer method which enhances the efficiency of imprinting due to the absence of solvent and stabilization of the protein on the surface. In the recent years, novel methods for molecular imprinting for proteins have flourished. In the traditional methods of imprinting, a solvent is made which entails the pattern molecule, function molecule, network builder and initiator and then through either alluvial or massive polymerization, polymer is formed [11].

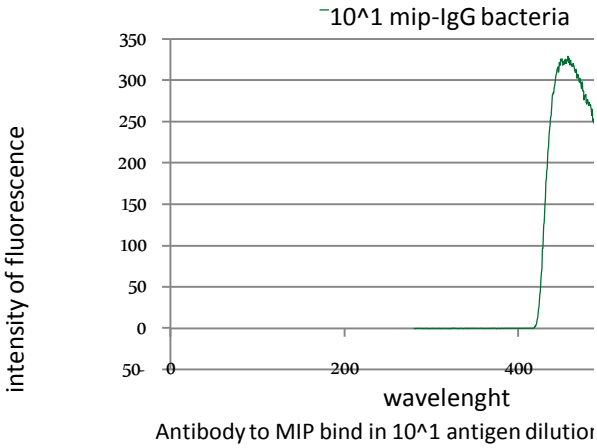
- In the recent years, molecular imprinting is utilized as a useful separation technique in producing detection elements as an imitation of the biological receptors. Molecularly imprinting polymer has diverse uses in the organic solvents due to its low ingredient costs as compared with enzymes, high heat and mechanical resistance in high temperatures, and resistance in environments with high or low acidity, as well as their long lifetime. One of the advantages of the molecularly imprinting polymer is that the fashioned polymer can be obtained from opposite mixtures which are employed for utilizing antibodies. In the past two decades, molecularly imprinting polymers have attracted many scientists who work on developing chemical and biological sensors [12, 13].
- Molecularly imprinting polymers are used in designing and producing different chemical and biochemical

sensors based on various electrochemical, photoelectric, piezoelectric and other transudes. In electrochemical methods, different voltmeter, transductometer, potentiometer and Amperometer sensors were made based upon molecular imprinted polymer. In transductometer approach, a film with molecular mould is prepared to function as a screen between two electrolyte solvents. Piezoelectric crystals are other transducers for which molecular mould functions as a chemical interval, where the molecular mould is made and placed as a thin layer lying on the surface of piezoelectric crystal. Numerous types of these sensors were made to bind the molecular imprinted polymer with other various kinds of piezoelectric tools to analyze in gas and liquid phase. Nowadays, the optical sensor has also taken the privilege of molecular imprinted polymer to enhance selectivity. There are namely, fluorescence sensors, low luminescence ones and those based on absorption of infrared. In fluorescence approach which is used in the present research, the function monomer or network builder with fluorescence properties is used for molecular imprinted polymer synthesis and should the target molecular have fluorescence property, optic sensor also can be made based on molecular imprinted polymer [11, 13].

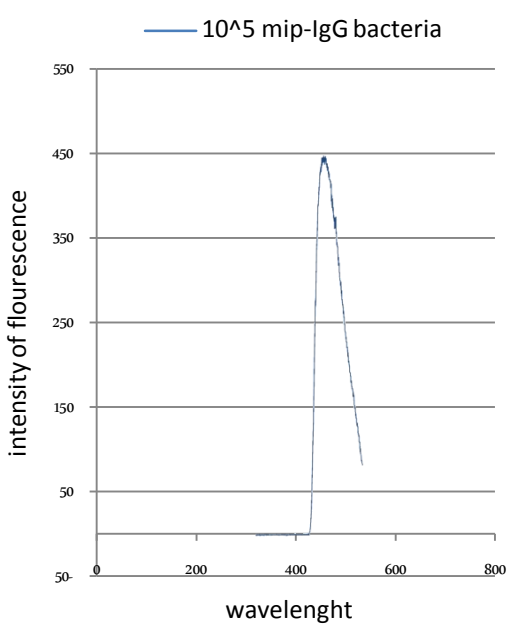
- As shown in table 1 and 2, the changes in sensor response based on antigen dilution in form of florescence intensity on the first day of encounter and the day 52, the other conditions of antigen dilution and the environment temperature being equal, the higher the antigen dilution, the higher the intensity of florescence scatter. Moreover, from results in table 2, we understand that on the day 52 of the sensor lifetime the sensitivity is reduced while before it was satisfactory. The following charts approve this fact.



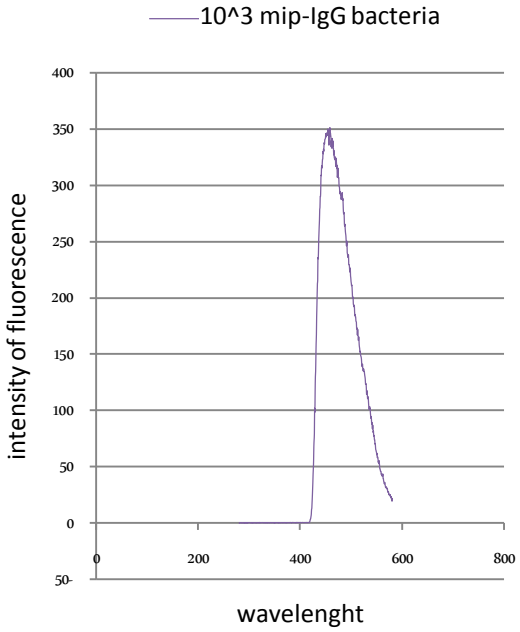
Antibody to MIP bound in absence of antigen



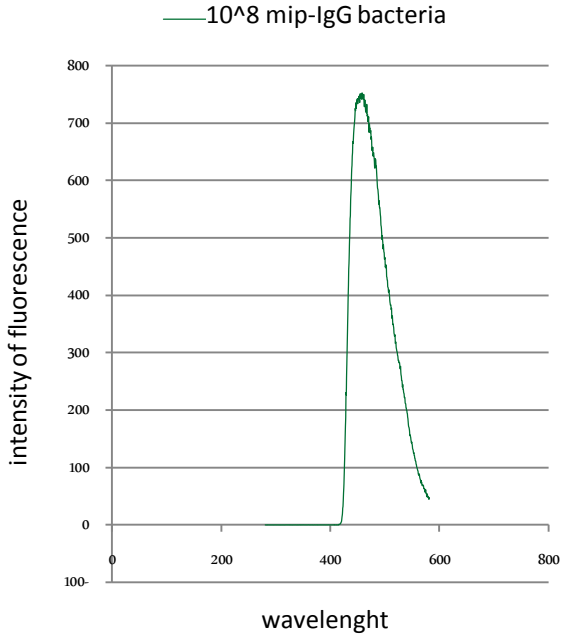
Antibody to MIP bind in 10^1 antigen dilution



Antibody to MIP bind in 10^5 dilution of antigen

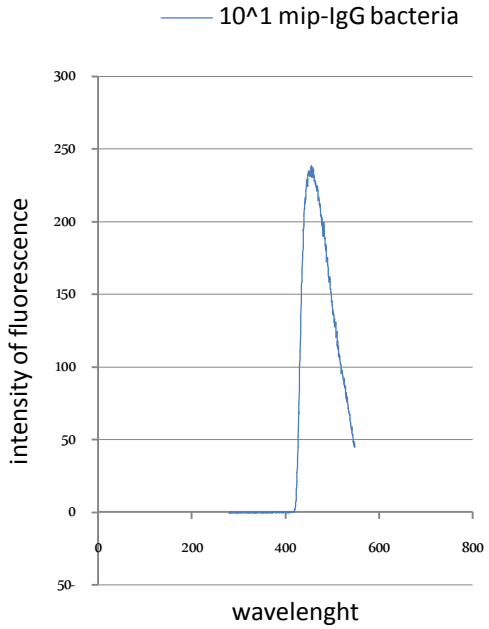


Antibody to MIP bind in the 10^3 antigen of dilution

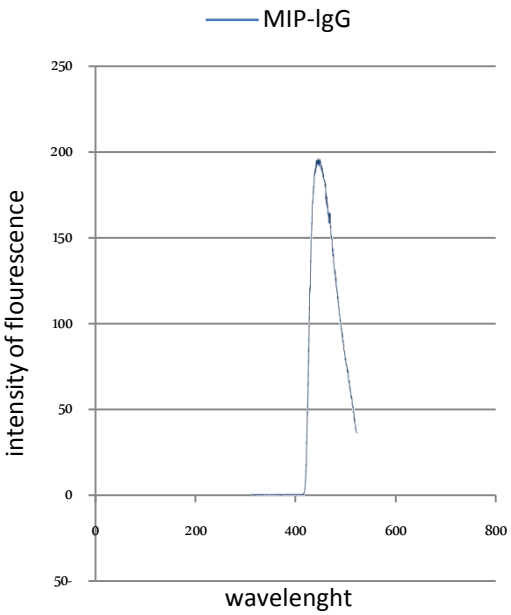


Antibody to MIP bind in 10^8 antigen of dilution

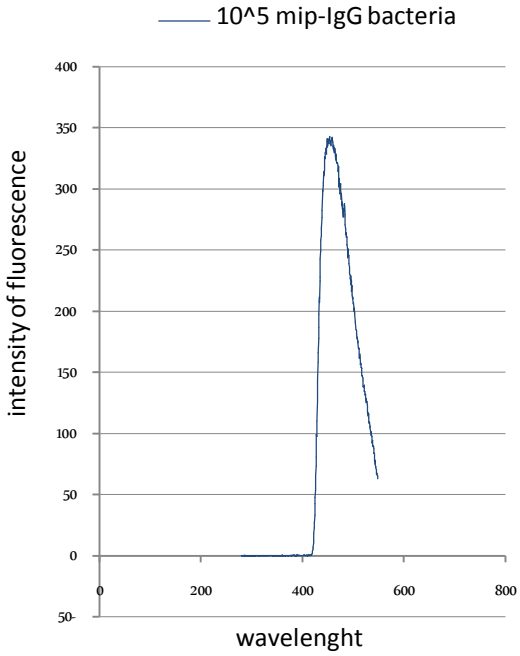
- The designed sensor has undergone the drop in the intensity of florescence scatter while the increase of antigen dilution in meeting the sensor causes boost in the intensity of florescence scatter. The following charts depict this fact.



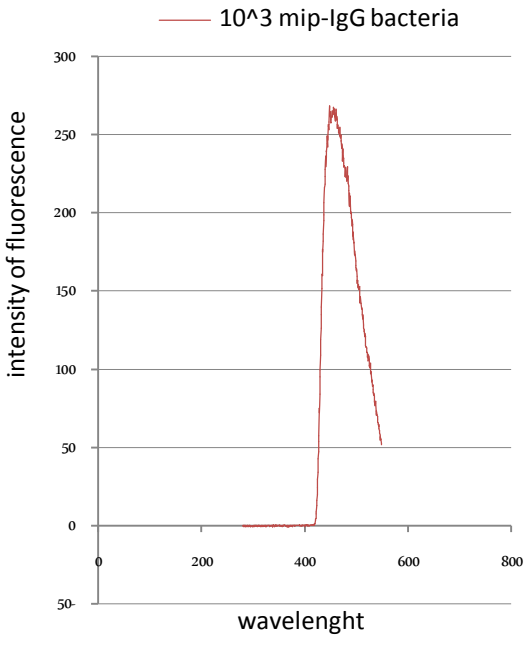
Antibody to MIP bind in 10^1 antigen of dilution



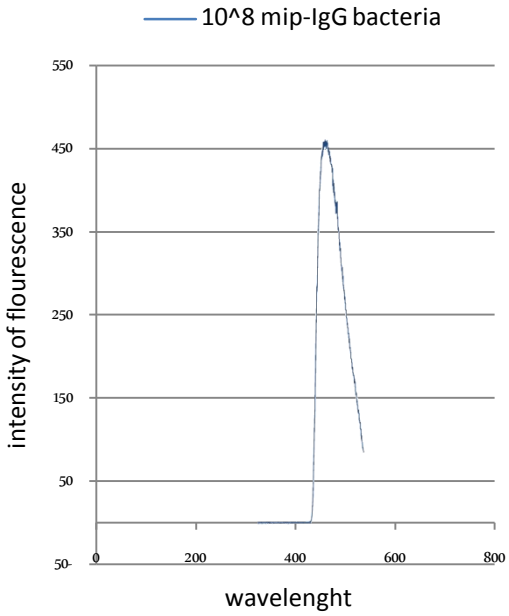
Antibody to MIP bind in the absence of antigen



Antibody to MIP bind in 10^5 antigen of dilution



Antibody to MIP bind in 10^3 antigen of dilution



Antibody to MIP bind in 10^8 antigen of dilution

Overall, from the total of 40 aquatic environment samples which are infected with different dilutions of Salmonella typhi bacteria, 34 samples are precisely evaluated qualitatively and qualitatively in terms of the intensity of florescence scatter. The sensitivity level of Nano biosensor kit designed ranges from 10^1 to 10^8 in a milliliter.

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REFERENCES:

[1] Marotta, G., M. Simeone, and C. Nazzaro, *Product reformulation in the food system to improve food safety. Evaluation of policy interventions.* Appetite, 2014. **74**: p. 107-15.

[2] Kumar, S., V. Tripathi, and S. Garg, *Antibiotic resistance and genetic diversity in water-borne Enterobacteriaceae isolates from recreational and drinking water sources.* International Journal of Environmental Science and Technology, 2013. **10**(4): p. 789-798.

[3] Yoshida, W., et al., *Automatic polymerase chain reaction product detection system for food safety monitoring using zinc finger protein fused to luciferase.* Anal Chim Acta, 2013. **801**: p. 78-83.

[4] Kryscio, D.R. and N.A. Peppas, *Surface imprinted thin polymer film systems with selective recognition for bovine serum albumin.* Anal Chim Acta, 2012. **718**: p. 109-15.

[5] Lin, H.Y., J. Rick, and T.C. Chou, *Optimizing the formulation of a myoglobin molecularly imprinted thin-film polymer--formed using a micro-contact imprinting method.* Biosens Bioelectron, 2007. **22**(12): p. 3293-301.

[6] Safari Foroshani, N., A. Karami, and F. Pourali, *Simultaneous and Rapid Detection of Salmonella typhi, Bacillus anthracis, and Yersinia pestis by Using Multiplex Polymerase Chain Reaction (PCR).* Iran Red Crescent Med J, 2013. **15**(11): p. e9208.

[7] Sankar, S., et al., *A multiplex nested PCR for the simultaneous detection of Salmonella typhi, Mycobacterium tuberculosis, and Burkholderia pseudomallei in patients with pyrexia of unknown origin (PUO) in Vellore, South India.* Mol Diagn Ther, 2014. **18**(3): p. 315-21.

[8] Ahari, H., et al., *Comparison of potentiometry and spectroscopy-based modified nanoparticles linked to antibodies for detection of Staphylococcus aureus exotoxin.* Iranian Journal of Nutrition Sciences & Food Technology, 2014. **8**(4): p. 247-258.

[9] Rani, N., et al., *Quantification of Salmonella Typhi in water and sediments by molecular-beacon based qPCR.* Ecotoxicol Environ Saf, 2014. **108C**: p. 58-64.

[10] Meemken, D., et al., *Establishment of serological herd profiles for zoonoses and production diseases in pigs by "meat juice multi-serology".* Prev Vet Med, 2014. **113**(4): p. 589-98.

[11] Frimat, J.P., et al., *Make it spin: individual trapping of sperm for analysis and recovery using micro-contact printing.* Lab Chip, 2014. **14**(15): p. 2635-41.

[12] Moradi, S., P. Englezos, and S.G. Hatzikiriakos, *Contact angle hysteresis of non-flattened-top micro/nanostructures.* Langmuir, 2014. **30**(11): p. 3274-84.

[13] Shin, H.S., et al., *The effect of thermal annealing on pentacene thin film transistor with micro contact printing.* J Nanosci Nanotechnol, 2012. **12**(7): p. 5325-9.