

## A novel optical DNA biosensor for detection of trace concentration of Methylene blue using Gold nano-particles and Guanine rich single strand DNA

*Rasoul Pourtaghavi Talemi<sup>\*</sup>; Mohammad Hossein Mashhadizade*

*Faculty of Chemistry, Kharazmi University, Tehran, Iran*

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### Abstract

The glass surface modification with 3-(mercaptopropyl) trimethoxysilane (MPTS), gold nano-particles (GN) and guanine rich single strand DNA (ss-DNA) was utilized as a novel and efficient platform for sensing trace concentration of methylene blue (MB) by an inexpensive spectrophotometric method. Methylene Blue (MB) can interact with the guanine base of single strand DNA and absorbed onto glass surface and its absorbance can be determined at  $\lambda_{\max}$  of MB. Increase in methylene blue absorbance was linear with an increase in MB concentration and can be viewed by the naked eye and detected by spectrophotometric method. The linear range of the developed biosensor for determination of MB was from 10 to 100 nmol L<sup>-1</sup> with a detection limit of 4 nmol L<sup>-1</sup>. The fabricated spectrophotometric MB biosensor possessed excellent selectivity and good sensitivity.

**Keywords:** *Glass Surface; Gold nano-particles; Guanine; Methylene blue; Optical DNA biosensor; Single strand DNA.*

### How to cite this article

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### INTRODUCTION

Methylene Blue (MB), (3, 7-bis(dimethylamino) phenothiazin-5-ium chloride trihydrate) has found many applications in microbiology, surgery and in the diagnostic field [1, 2]. MB is an example of a redox active non-metal molecule characterized by having a high affinity for nucleic acids. Recent research suggests that MB, toluidine blue, and other 3, 7-diaminophenothiazinium-based redox cyclers induce selective cancer cell apoptosis by NADPH: Quinone oxidoreductase (NQO1)-dependent bio-reductive generation of cellular oxidative stress [3]. Although MB is not regulated for use with edible aquatic animals, sometimes be used as a chemotherapeutic agent in the aquaculture industry [4]. In humans, it activates a normally dormant reductase enzyme system which reduces MB to leucomethylene blue, which in turn is able to reduce methaemoglobin to hemoglobin. The release of azo dyes such as MB into the environment is a concern due to

\* Corresponding Author Email: [rasoulpourtaghavi@gmail.com](mailto:rasoulpourtaghavi@gmail.com)

coloration of natural waters and due to the toxicity, mutagenicity and carcinogenicity of the dyes and their biotransformation products [5]. MB is one of the most commonly used substances for dyeing cotton, wood, and silk. Though methylene blue is not strongly hazardous, it can cause some harmful effects where acute exposure to methylene blue may cause increased heart rate, vomiting, shock, cyanosis, jaundice and quadriplegia and tissue necrosis in human [6].

Because MB is used as a replacement for other antifungal dyes in aquaculture nowadays, Japan has established a maximum residue limit (MRL) of 10 mg/kg for aquatic products. It is important to have a practical method to monitor MB in edible aquatic product tissues. Numerous approaches have been reported for the determination of MB's concentration, such as spectrophotometry [7], ion-exchange chromatography [8], reductimetric titration [9], polarography [10] and potentiometric titration [11] and so on [12-17]. Unfortunately,

most of these methods are either time consuming, involving multiple sample manipulations, or too expensive for most analytical laboratories. On the other hand, the as described spectrophotometric or potentiometric methods suffer from poor sensitivity or selectivity.

DNA biosensors can theoretically be used for medical diagnostics, forensic science, agriculture, or even environmental clean-up efforts. The DNA immobilization procedure is a very important aspect since it influences the characterization of the DNA probe, the sensor response and its performance [18]. Thus, a key issue with a DNA biosensor is the accessibility and molecular orientation of the probe DNA, which requires a high degree of control over the immobilization of the probe oligonucleotides.

Self-assembled monolayers (SAMs) of alkane thiols modified DNA, which have shown to provide molecular level control over the immobilization of several types of biomolecules, have been used as active films on which DNA segments can be attached using covalent linkers for example gold nano-particles [19, 20]. Gold nano-particles (GNPs) are the most extensively investigated nano-materials, due to their distinct physical and chemical attributes, that have encouraged researchers to explore novel sensing strategies with improved sensitivity, stability and selectivity [21-23].

In previous works [24-30] well-known interaction between the MB and the free guanine bases in the single stranded DNA has been utilized in many electrochemical biosensor applications [30]. However, in spite of high absorption of MB in the visible region and its strong and selective interaction with the guanine base of ss-DNA, to our knowledge, there is no report on the use of single strand DNA for the spectrophotometric sensing of MB. In our previous work [31], we immobilized S-H labeled ss-DNA onto a glass surface that had been modified with MPTS and gold nano-particles for construction a mercuric biosensor based on different interaction of MB with ss and ds-DNA and we observed that ss-DNA concentration can affect dramatically on MB absorption that emphasized the interaction of MB with ss-DNA. The described work in ref [31] used from hybridization of the two oligonucleotides through T-Hg<sup>2+</sup>-T coordination chemistry for constructing a mercuric biosensor. In the present work we used a guanine rich oligonucleotide for design a DNA biosensor for

determination trace amount of MB concentration based on specific interaction between guanine bases of immobilized DNA and methylene blue molecules. As a consequence, the detection strategy and optimization of some parameter of the present biosensor is different from ref. 31. Accordingly, the present work is not similar to ref 31 and can be regarded as a new work.

Based on above description, in this paper, we demonstrated a novel optical DNA biosensor based on a guanine rich ss-DNA immobilized onto modified glass surface for sensing trace concentration of MB. Firstly, glass surface was functionalized with MPTS and then gold nano-particles was attached to a S-H group of MTPS through Au-S covalent binding and then thiolated probe DNA, was immobilized onto gold nano-particles. MB can be absorbed through intercalative and electrostatic ways onto the free guanine base of probe DNA and approximately a large absorption peak for MB can be observed. The experimental conditions for determining of MB such as probe DNA concentration, pH of MB solution, accumulation time, and NaCl concentration were optimized. Under the optimum experimental conditions, the molar absorptivity of MB was  $5.80 \times 10^5 \text{ L mol}^{-1} \text{ cm}^{-1}$ . Calibration graph was linear in the range from 10 to 100 nmol L<sup>-1</sup> with a detection limit of 4 nmol L<sup>-1</sup>. The interfering effects of some organic dyes were also studied. Finally, the proposed biosensor was successfully applied for the determination of MB in some water samples.

## EXPERIMENTAL

### *Reagents and materials*

Analytical reagent grade chemicals and double distilled water (DDW) were used for preparing all aqueous solutions. Hydrogen tetrachloroaurate (HAuCl<sub>4</sub>·3H<sub>2</sub>O), trisodium citrate, potassium monohydrogen phosphate, potassium dihydrogen phosphate, citric acid, hydrogen peroxide, sodium chloride, methylene blue (MB), Erio chrome Black T (ECBT), Congo Red (CoR), Methyl Orange (MO), Thymol Blue (TB), Phenol Phetalein (PP), Cresol Red (CrR), Phenol Red (PR), and Bromothymol Blue (BTB) were obtained from Merck (Germany). Mercaptohexanol (MCH) and 3-(mercaptopropyl) trimethoxysilane (MPTS) were purchased from Sigma (USA).

S-H labeled poly-G oligonucleotide (5'-S-H-GGGGGGGGGGGGGGGG-3') was purchased from

the Amins Company (Iran) and oligonucleotide stock solution was prepared in phosphate-citrate buffer solution (pH 3.5).

#### *Apparatus and instrumentations*

The pH values were controlled by a pH/mV meter (Metrohm-827, Switzerland), UV-Vis absorption spectrums were recorded using a double-beam in-time Lambda 25 UV-Vis spectrophotometer (Perkin Elmer, Netherlands). Scanning electron microscopy (SEM) and transmittance electron microscopy (TEM) experiments were carried out with a TESCAN scanning electron microscope and Hitachi H-8100 transmission electron microscope respectively.

#### *Preparation of gold nano-particles*

Colloidal gold nano-particles (GNPs) were prepared according to the literature [32] briefly by adding 1.5 mL of 1% (w/v) sodium citrate solution to 25 mL of 0.01% (w/v)  $\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$  at  $80^\circ\text{C}$ . The final mixture of red color was boiled for 15 min. The prepared gold nanoparticles were stored in dark glass bottles at  $4^\circ\text{C}$ .

#### *Biosensor fabrication*

A commercially available glass slide (50× 24 mm, Citoglass) was used as the template for constructing MB biosensor. After cleaning glass surfaces with a piranha solution (7 : 3 mixtures of concentrated sulfuric acid and 30% hydrogen peroxide) and a diluted alkaline detergent, the glass surface was washed with DDW three times. Then, the cleaned glass substrate was immersed in a solution of 290 mmol  $\text{L}^{-1}$  solution of MPTS in toluene for 5 min, rinsed five times in ethanol with sonication for 5 min, and was rinsed two times in water for 5 min. Both sides of the glass slide were coated with an organization using this procedure and named Glass/MPTS. The prepared Glass/MPTS surfaces were rinsed with DDW and were immersed in a colloidal gold suspension to fabricate gold nano-particle layers on both sides of the substrate for 3 h. In this step, gold nanoparticles interacted covalently with S-H groups of MPTS and were introduced onto glass surface. Afterward, the glass slide was washed gently with DDW and named Glass/MPTS/GN.

For Glass/MPTS/GN/S-H-DNA construction, Glass/MPTS/GN was immersed in a 10 nmol  $\text{L}^{-1}$  phosphate-citrate buffer solution of S-H-DNA (pH 3.5) for 6h. Then, the glass surface was incubated

in the MCH solution for 30 min for producing a self-assembly well-aligned monolayer (SAM) of thiolated oligonucleotide. Finally the glass surface was rinsed thoroughly with DDW to remove physically adsorbed S-H-DNA to obtain a modified glass surface denoted as Glass/MPTS/GN/S-H-DNA and was stored in DDW until the SEM or UV-Vis measurement carried out.

#### *Spectrophotometric determination of MB*

All modified glass slides were stored in DDW until the SEM or UV-Vis measurement carried out. For spectrophotometric characterization of gold nano-particles, colloidal gold nano-particles were poured in a spectrophotometer cell and absorption spectra were obtained against distilled water as reference. For gain calibration curve, Glass/MPTS/GN/S-H-DNA was immersed in solutions with different concentrations of MB and 10 mmol  $\text{L}^{-1}$  of NaCl for 10 min with mild stirring and after washing with DDW its absorbance versus Glass/MPTS/GN/S-H-DNA as blank was recorded by spectrophotometer.

## **RESULT AND DISCUSSION**

#### *Characterization of prepared gold nano-particles*

GNPs was synthesized by the above method yielded spherical particles with an average diameter of  $39 \pm 3$  nm by the particle size analysis. The surface plasmon resonance peak of unmodified gold nano-particles is centered on 525 nm (Fig. 1a) as reported earlier indicating that the nanoparticles were not aggregated but well dispersed as individual particles. The results were further confirmed by TEM imaging. The generation of spherical nano-particles with nearly identical sizes was substantiated from the TEM image shown in Fig. 1b.

#### *Characterization of different modification on the glass surface*

As our previous work [31], electronic characterization of step by step modification of glass surface was investigated by scanning electron microscopy (SEM). Functionalization of the glass surface with MPTS led to occasional hemispherical features of about 100 nm diameter that were not self-associating (Fig. 2a). After introducing gold nano-particles onto MPTS functionalized glass surface, accumulation of spherical gold nano-particles onto MPTS surface was observed (Fig. 2b), whereas immobilization of guanine rich ss-

DNA, produced a non-uniform coverage onto glass surface (Fig. 2c). Finally, incubation of Glass/MPTS/GN/S-H-DNA with 50 nmol L<sup>-1</sup> MB (Fig. 2d) resulted in a more dense and homogeneous coverage.

*Preliminary studies*

The optical MB biosensor was prepared with the step by step modifying of glass surface by MPTS, gold nano-particles and S-H-labeled guanine rich ss-DNA. The whole biosensor fabrication process was schematically demonstrated in Fig. 3.

Preliminary studies were carried out in order to select the optimum conditions for the detection of MB at Glass/MPTS/GN/S-H-DNA. Best results were obtained with these conditions: concentration of MPTS and gold nano-particles were 290 mmol L<sup>-1</sup> and 0.58 nmol L<sup>-1</sup>, respectively and optimum immersion time of MPTS and gold nano-particles were 5 min and 3 h respectively. These results were in complete agreement with [33] and hence next experiments were done with these concentrations and immersion times.

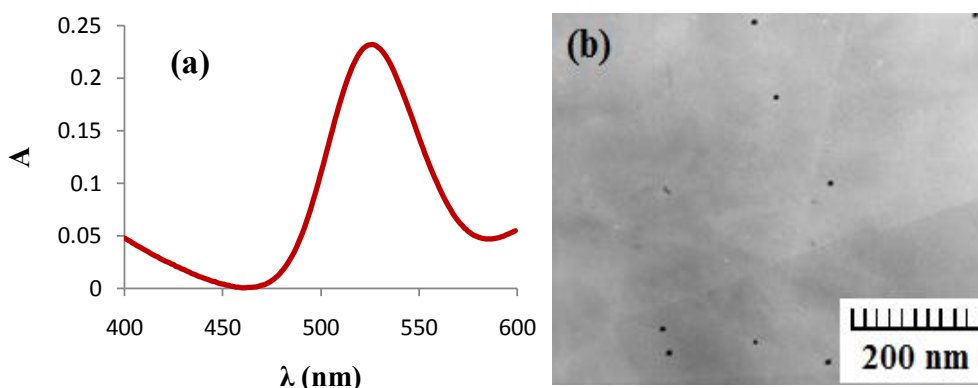


Fig. 1: Absorption spectra (a) and TEM image (b) of the synthesized gold nano-particles.

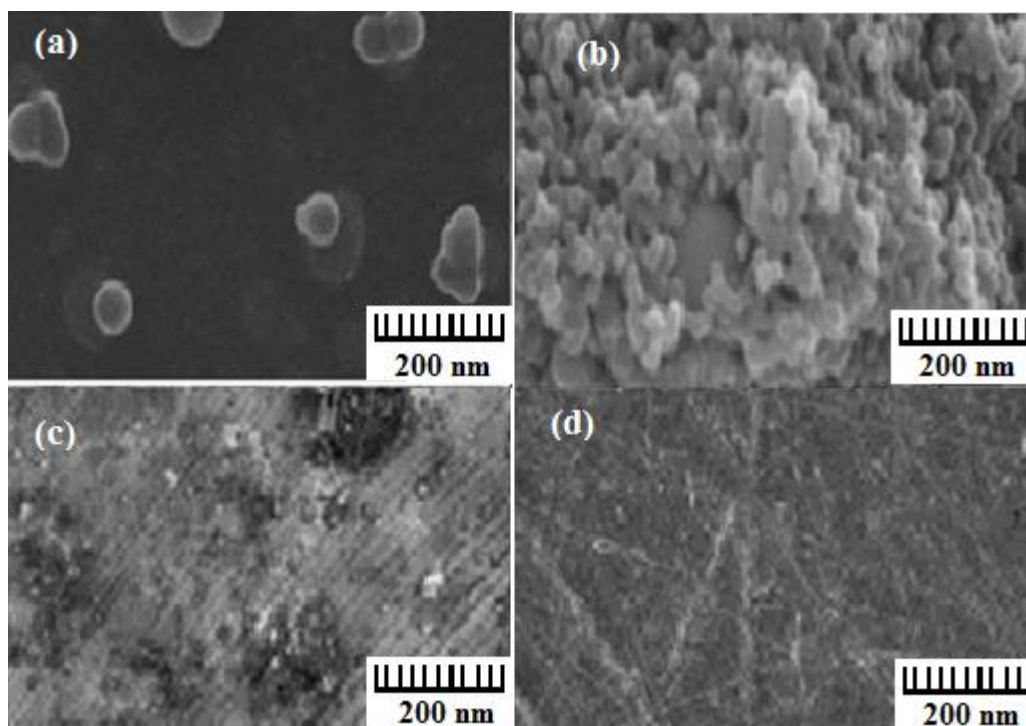


Fig. 2: SEM images of Glass/MPTS (a), Glass/MPTS/GN (b), Glass/MPTS/GN/S-H-DNA (c) and Glass/MPTS/GN/S-H-DNA-MB (d).

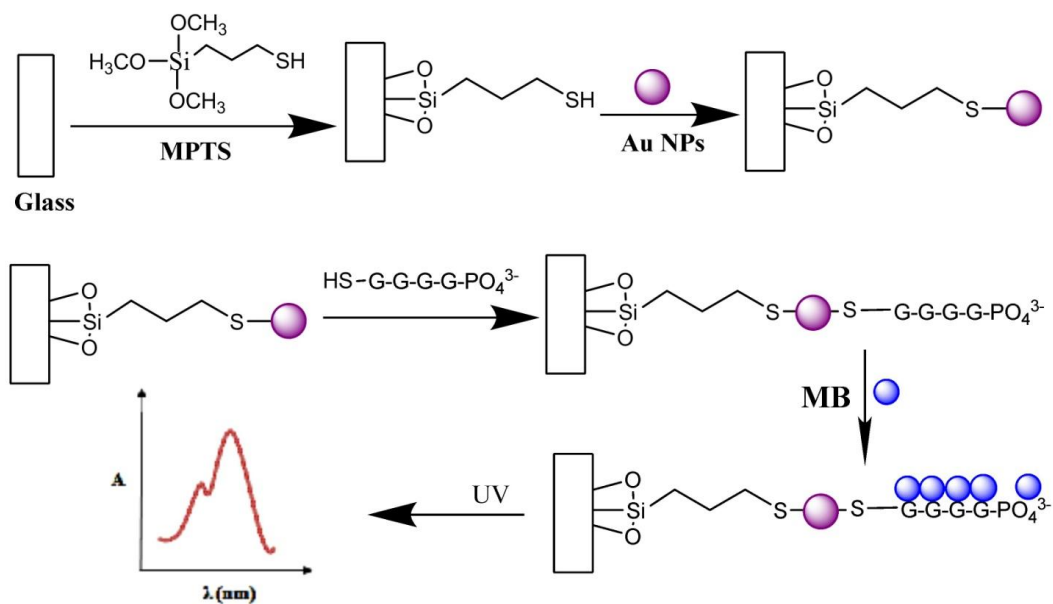


Fig. 3: Schematic illustration of the fabrication steps of the MB biosensor.

The effect of probe concentration based on absorption peaks of MB was also explored in different probe concentrations from 1 to 100 nmol L<sup>-1</sup> (Fig. 4a) and constant value of MB. This figure illustrates the absorption peak of MB vs. different concentration of guanine rich DNA probe. As shown, the absorption peak rapidly increases with the probe DNA concentration from 1 to 10 nmol L<sup>-1</sup>, then reaches to the maximum value at 10 nmol L<sup>-1</sup>. Therefore, 10 nmol L<sup>-1</sup> of probe DNA was chosen as an optimum value in order to obtain a high sensitivity in the subsequent experiment.

In general, pH is one of the variables which commonly and strongly influence the response of the biosensor. It is important to investigate the effects of pH on the proposed spectrophotometric biosensor. The absorption responses for 50 nmol L<sup>-1</sup> of MB were examined in phosphate-citrate buffers (pH 2.5-8). The absorption peak of MB was found to be markedly dependent on the pH. As can be seen from (Fig. 4b), when the pH of MB solution was increased, the absorption of MB increased, and it achieved a maximum at about pH 3.5, then decreased. With respect to guanine pKa (pKa=3.3) at very low pH (pH < 3.0), the phosphodiester backbone of DNA can be hydrolyzed and some guanine base can be expected to be protonated. On the other hand, with respect to the pKa of MB (pKa=3.8), at a pH higher than 3.8 MB is in its anionic form. Therefore, at pH between 3.3 and

3.8, MB can be observed in positive and DNA must be in their negative forms and highest electrostatic interaction between ss-DNA and MB can be observed. However, at pH above 3.8, MB was in its anionic form and less electrostatic interaction with DNA was occurred. Therefore, pH 3.5 was selected as optimum value of pH for construction of DNA sensor.

Accumulation time was varied between 1 and 40 min (Fig. 4c) and a higher absorbance for MB was obtained for 10 min, which was chosen as accumulation time for further measurements. The decrease observed in accumulation times higher than 10 min indicates that this is the interval of time necessary to reach the saturation of the binding sites on the guanine base of ss-DNA.

The effect of NaCl concentration in the MB solution onto the biosensor response was also studied (data not shown). The absorption peak of MB was observed to augment with the increase in NaCl concentration until a concentration of 10 mmol L<sup>-1</sup> was reached; for higher salt concentrations (up to 0.2 mmol L<sup>-1</sup>) no further variations were recorded. This result seems to indicate that the electrostatic or weak base interaction contributions were present and important at NaCl concentrations lower than 10 mmol L<sup>-1</sup>. For NaCl concentrations higher than this value the MB response leveled off becoming more or less constant; this is consistent with the

presence of a second contribution, independent of the electrostatic properties of the DNA. This contribution can be associated with the presence of the free guanine bases in the DNA sequence. Notably a residual electrostatic contribution can be still identified even at very high NaCl concentrations.

#### Analytical data

The prepared DNA biosensor system was sensitive for determination trace concentration of MB. Three different mechanisms of MB-DNA interaction have been recognized: (i) electrostatic interaction with the negatively charged DNA backbone [34], (ii) intercalation within the DNA double helix [35] and (iii) preferential binding to free guanine bases present in single stranded DNA (ss DNA) [24]. In this work, MB can bind into the free guanine bases of ss-DNA and electrostatically absorbed onto the DNA at pH 3.5. The ranges of linearity (Table 1) of absorbance as a function of MB concentration, obeys the Beer's law, provide a satisfactory measure of the sensitivity of the

method (Fig. 5a). Under the optimum conditions the absorbance of the MB obeys Beer's law in concentration range of 10-100 nmol L<sup>-1</sup> (Fig. 5b). The molar absorptivity value is 5.80×10<sup>6</sup> Lmol<sup>-1</sup> cm<sup>-1</sup> in mentioned condition that indicates high sensitivity of method. Limit of detection, limit of quantification, standard deviation (S.D), and the correlation coefficient were also calculated and summarized in Table 1. It was found that the proposed MB DNA biosensor was applicable for the spectrophotometric determination of MB as a sensitive, simple and accurate method. Large amounts of MB can be absorbed onto the glass surface through its electrostatic and specific interaction with immobilized DNA and respect to high absorption of MB in the visible region, high sensitivity for biosensor was achieved. On the other hands, self-assembly of colloidal gold onto the modified glass surface because of their high surface-to-volume ratio and excellent biological compatibility, enlarged the glass surface area and enhanced greatly the amount of immobilized ss-DNA and hence the sensitivity of MB biosensor.

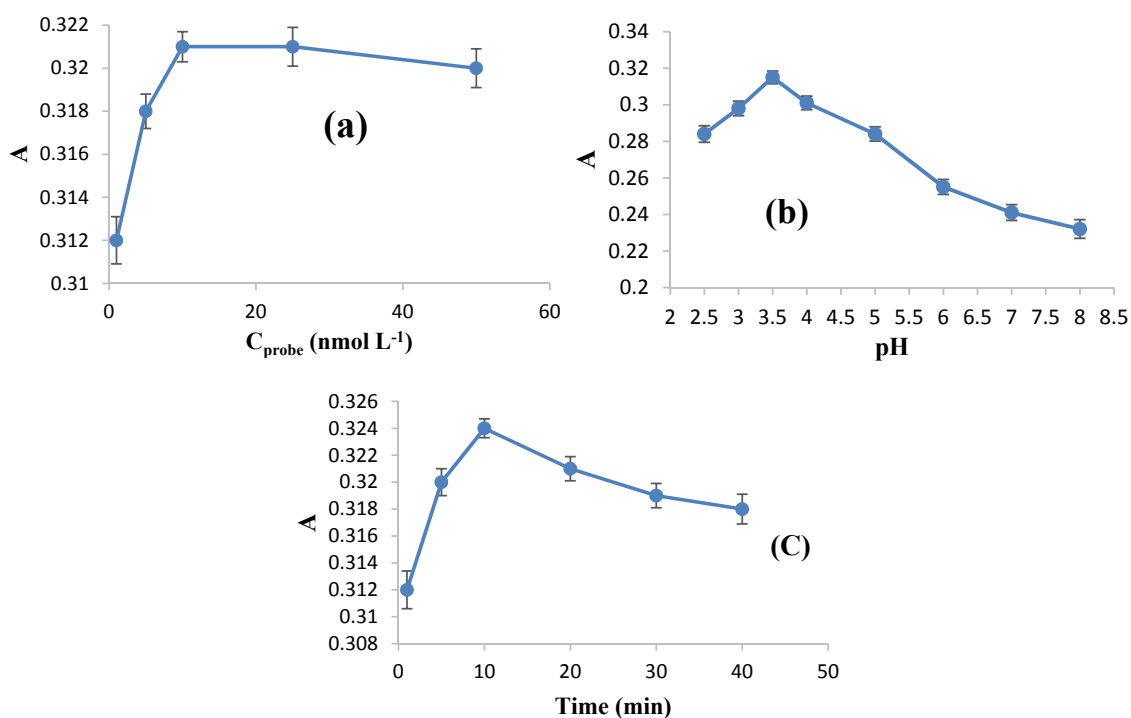


Fig. 4: Absorption peak of 50 nmol L<sup>-1</sup> MB at  $\lambda_{Max}$  measured in (a) Various concentration of DNA immobilized onto the surface of Glass/MPTS/GN at pH 3.5. DNA concentrations are 1, 5, 10, 25, and 50 nmol L<sup>-1</sup> respectively. (b) Various pH from 2.5 to 8 at Glass/MPTS/GN/S-H DNA and 10 nmol L<sup>-1</sup> DNA. (c) Various accumulation time of MB from 1 to 40 min at Glass/MPTS/GN/S-H DNA where DNA concentration was 10 nmol L<sup>-1</sup>. Error bars show the standard deviations of the measurements taken from three independent experiments.

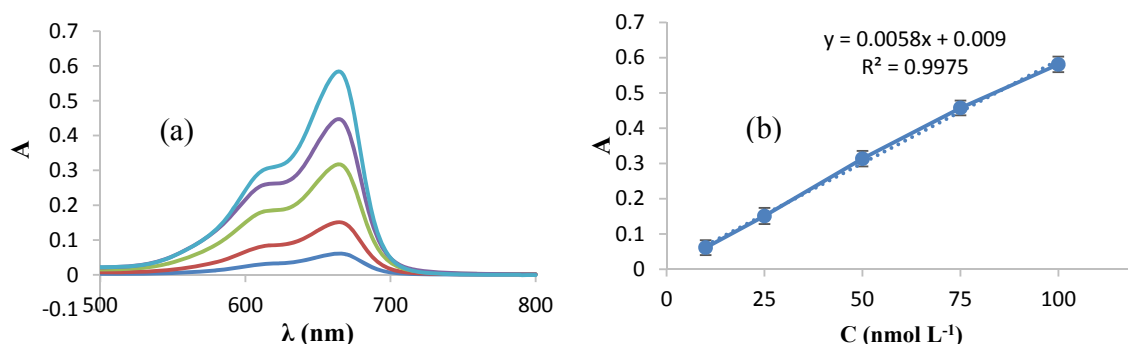


Fig. 5: (a) The UV–Vis spectrums measured by proposed biosensor upon addition of different concentrations of MB at optimum conditions. MB concentrations are 10, 25, 50, 75, and 100 nmol L<sup>-1</sup> respectively. (b) Absorption of MB against different concentration of MB (Beer's law plot).

Table 1: Optical parameters for the determination of MB with proposed DNA biosensor.

Parameters	Characteristic
Beer's law range (nmol L <sup>-1</sup> )	10-100
Molar absorptivity (L mol <sup>-1</sup> cm <sup>-1</sup> )	$5.80 \times 10^6$
Limit of detection (nmol L <sup>-1</sup> )	4
Limit of quantification (nmol L <sup>-1</sup> )	13
Regression equation <sup>a</sup>	$Y = 0.0058 x + 0.009$
Correlation coefficient	0.9975
Slope	0.0058
Intercept <sup>b</sup>	0.0090
Standard deviation <sup>b</sup>	0.0040

<sup>a</sup>  $Y = ax + b$  where  $x$  is the concentration of MB in nmol L<sup>-1</sup> and  $Y$  is the absorbance.

<sup>b</sup> Three replicates measurements.

### Selectivity

The specificity of the biosensor was also evaluated by substituting 50 nmol L<sup>-1</sup> MB in the buffer with various anionic organic dyes such as Erio chrome Black T (ECBT), Congo Red (CoR), Methyl Orange (MO), Thymol Blue (TB) and cationic dyes such as Phenolphthalein (PP), Cresol Red (CrR), Phenol Red (PR) and Bromothymol Blue (BTB) at 1 μmol L<sup>-1</sup> concentration, respectively. As shown in Fig. 6, the biosensor exhibits a remarkable response to MB, but hardly responds to other organic dyes.

As it is evident, most of the anionic organic dyes have a weak absorption peak in biosensor surface and respond to cationic dyes are very less than MB response. The response of biosensor to cationic dyes is greater than anionic dyes because cationic dyes can be absorbed on biosensor surfaces by electrostatic interaction. However, their absorption peak is less than the absorption peak of MB, because MB possesses

both electrostatic and intercalative interaction with the immobilized ss-DNA. On the other hand, anionic dyes that absorbed neither via electrostatic interaction nor interacted with a guanine base of ss-DNA do not absorb on biosensor surface and mostly a weak absorption peak for them has been seen. Likewise, the sensor's response to MB (25 nmol L<sup>-1</sup>, Fig. 6a) is not significantly affected by the presence of mixed Interferential organic dyes (all at 1 μmol L<sup>-1</sup>, Fig. 6b), which indicates an excellent selectivity for MB detection.

### Analytical application

From the preceding observation, it is apparent that the proposed method possesses a high sensitivity, selectivity, and a very good detection limit for the determination of MB in an ideal laboratory sample.

To evaluate possible matrix effects, we tested the proposed biosensor with water samples using

lab tap water and spring water without sample pretreatment or time-consuming extraction or evaporation steps prior to analysis of the MB. These water samples were spiked with MB from its stock solutions at concentrations of 25, 50 and 75 nmol L<sup>-1</sup>, followed by measuring the MB content as described above. The results of analysis are listed in Table 2. The recovery percentage values are about 97.6-104.8% indicated good accuracy of results.

**Comparative study**

For comparative purposes, Table 3 lists the linear range and detection limit of some previously published MB sensors [37-39, 13-14] against the proposed sensor. It is noteworthy that the limit of detection and linear range of the proposed sensor are also considerably improved with respect to most of the reported MB sensors and is comparable with ref [39] that utilizes an expensive ultra-performance liquid chromatography and mass spectrometry system.

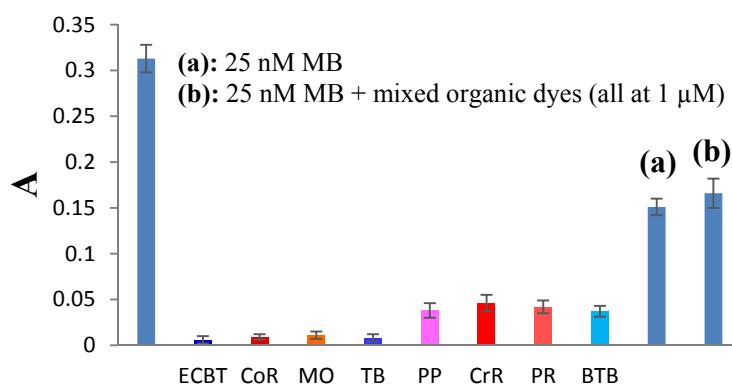


Fig. 6: The biosensor responses to 50 nmol L<sup>-1</sup> of MB and some anionic and cationic organic dyes with 1 µmol L<sup>-1</sup> concentration. Response of biosensor to 25 nmol L<sup>-1</sup> MB (a) and a solution containing 25 nmol L<sup>-1</sup> MB and 1 µmol L<sup>-1</sup> mixture of other organic dyes (b). All measurements carried out at optimum conditions.

Table 2: Detection results of water spiked with MB.

Origin <sup>a</sup>	MB added to the samples (nmol L <sup>-1</sup> )	MB determined by biosensor (nmol L <sup>-1</sup> ) <sup>b</sup>	Recovery (%)
Lab tap water	25	25.5 (±0.2)	102.0
	50	49.2 (±0.1)	99.2
	75	74.1 (±0.2)	98.9
Spring water	25	26.3 (±0.3)	104.8
	50	50.6 (±0.2)	101.2
	75	73.2 (±0.3)	97.6

<sup>a</sup> All samples were collected and analyzed on 8<sup>th</sup> June 2016 and the initial concentration of MB in these water samples was undetectable (<4 nmol L<sup>-1</sup>), <sup>b</sup> Values in parentheses are SDs based on four replicate analyses.

Table 3: Comparison of the proposed methylene blue DNA biosensor with the previously reported sensor.

Ref no.	Detection method	Linear range	Detection limit
37	Spectrophotometric determination after ion-pair extraction	0.3-28µM	-
38	Potentiometric sensor based on methylene blue-silicotungstate ion association	1µm-1 mM	0.7 µM
14	Potentiometric sensor using Methylene Blue-Phosphotungstate as Electroactive Material	1µm-1 mM	0.7 µM
13	Liquid chromatography tandem mass spectrometry	13-62 nM	6 nM
39	solid-phase extraction and ultra-performance liquid chromatography tandem mass spectrometry:	3-15	0.3 nM
This work	Spectrophotometric method using DNA biosensor strategy	10-100 nM	4 nM



## CONCLUSIONS

In this paper, we used electrostatic and specific interaction of guanine bases of immobilized ss-DNA onto modified glass surface with MB for constructing a highly sensitive and selective novel spectrophotometric DNA biosensor for determination trace amount of MB. The proposed biosensor developed in this paper has been demonstrated an efficient way for MB determination. The proposed method for determination of MB does not require costly equipment and sophisticated sample pretreatment, and had many salient advantages such as simple, cost-effective and rapid. The results from validation indicate that the proposed method successfully used for the determination of MB in some water samples. However, it is inevitable to design more sensitive and selective sensors based on this strategy to monitor MB and the promising performance of the developed DNA spectrophotometric MB biosensor makes this methodological study and application attractive in the analysis of the other organic dyes.

## CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

## REFERENCES

- [1] Wheeler M. M., Wade J. S. H., (1982), Intraoperative identification of parathyroid glands: Appraisal of methylene blue staining. *Am. J. Surg.* 143: 713-716.
- [2] Milani A., Ciammella A. M., Degen C., Siciliano M., Rossi L., (1992), Ascites dynamics in cirrhosis. Proposal and validation of a methylene blue dilution test. *J. Hepatol.* 16: 369-375.
- [3] Noodt B. B., Rodal G. H., Wainwright M., Peng Q., Horobin R., Nesland J. M., Berg K., (1998), Apoptosis induction by different pathways with methylene blue derivative and light from mitochondrial sites in V9 cells. *Int. J. Cancer.* 75: 941-948.
- [4] Turnipseed S. B., Roybal J. E., Plakas S. M., Pfenning A. P., Hurlbut J. A., Long A. R., (1997), Determination of methylene blue in channel catfish tissue by liquid chromatography with visible detection. *J. AOAC Int.* 80: 31-35.
- [5] Borwitzky H., Haefeli W. E., Burhenne J., (2005), Analysis of methylene blue in human urine by capillary electrophoresis. *J. Chromatogr. B.* 826: 244-251.
- [6] Kolekar Y. M., Pawar S. P., Gawai K. R., Lokhande P. D., Shouche Y. S., Kodam K. M., (2008), Decolorization and degradation of Disperse Blue 79 and Acid Orange 10, by *Bacillus fusiformis* KMK5 isolated from the textile dye contaminated soil. *Bioresour. Technol.* 99: 8999-9003.
- [7] Muthuraman G., Teng T. T., (2009), Extraction and recovery of rhodamine B, methyl violet and methylene blue from industrial wastewater using D<sub>2</sub>EHPA as an extractant. *J. Ind. Eng. Chem.* 15: 841-846.
- [8] Burhenne J., Riedel K. D., Rengelshausen J., Meissner P., Müller Q., Mikus G., Haefeli W. E., Walter-Sack I., (2008), Quantification of cationic anti-malaria agent methylene blue in different human biological matrices using cation exchange chromatography coupled to tandem mass spectrometry. *J. Chromatogr. B.* 863: 273-282.
- [9] Rukmini N., Kavitha V. S., (1978), Reductimetric titration of methylene blue, thionine, nitroso-R-salt, 1-nitroso-2-naphthol and 2-nitroso-1-naphthol. *J. Indian. Chem. Soc.* 55: 660-661.
- [10] Pamfilov A. V., Ya S., Mazurkevitch X., Pakhomova E. P., (1968), Mechanism of reduction of methylene blue and thionine. *Ukr. Khim. Zh.* 34: 276-280.
- [11] Selig, W., (1981), Potentiometric titration of organic cations precipitated by tetraphenylborate. *Fresenius Z. Anal. Chem.* 308: 21-23.
- [12] Orwitzky H., Haefel W. F., Burhenne J., (2005), Analysis of methylene blue in human urine by capillary electrophoresis. *J. Chromatogr. B.* 826: 244-251.
- [13] Xu J., Dai L., Wu B., Ding T., Zhu J. J., Lin H., (2009), Determination of methylene blue residues in aquatic products by liquid chromatography-tandem mass spectrometry. *J. Sep. Sci.* 32: 4193-4199.
- [14] Zhao Y., Chen X., Wang C. Y., (1998), A methylene blue-selective membrane electrode using methylene blue-phosphotungstate as electroactive material and its pharmaceutical applications. *Croat. Chim. Acta.* 71: 757-764.
- [15] Lachheb H., Puzinat E., Houas A., Ksibi M., Elaloubi E., Gyllard C., Herrmann J. M., (2002), Photocatalytic degradation of various types of dyes (Alizarin S, Crocein Orange G, Methyl Red, Congo Red, Methylene Blue) in water by UV-irradiated titania. *Appl. Catal. B.* 39: 75-90.
- [16] Chitikela S., Dentel S. K., Aleen H., (1995), Modified method for the analysis of anionic surfactants as Methylene Blue active substances. *Analyst.* 120: 2001-2004.
- [17] Yang F., Xia S., Liu Z., Chen J., Lin Y., Qiu B., Chen G., (2011), Analysis of methylene blue and its metabolites in blood by capillary electrophoresis/electrospray ionization mass spectrometry. *Electrophoresis.* 32: 659-664.
- [18] Chiorcea Paquim A. M., Diculescu V. C., Oretskaya T. S., Oliveira Brett A. M., (2004), AFM and electroanalytical studies of synthetic oligonucleotide hybridization. *Biosens. Bioelectron.* 20: 933-944.
- [19] Kerman K., Ozhan D., Kara P., Meric B., Gooding J. J., Ozsoz M., (2002), Voltammetric determination of DNA hybridization using methylene blue and self-assembled alkanethiol monolayer on gold electrodes. *Anal. Chim. Acta.* 462: 39-47.
- [20] Mashhadizadeh M. H., Talemi R. P., (2013), A new methodology for electrostatic immobilization of a non-labeled single strand DNA onto a self-assembled diazonium modified gold electrode and detection of its hybridization by differential pulse voltammetry. *Talanta.* 103: 344-348.
- [21] Sanchez-martinez M. L., Aguilar-Caballeros A., Gomez-hens A., (2009), Homogeneous immunoassay for soy protein determination in food samples using gold nanoparticles as labels and light scattering detection. *Anal. Chim. Acta.* 63: 58-62.
- [22] De M., Ghosh P. S., Rotello V. M., (2008), Applications of nanoparticles in biology. *Adv. Mater.* 20: 4225-4241.
- [23] Mannelli I., Marco M. P., (2010), Recent advances in analytical and bioanalysis application of noble metal

- nanorods. *Anal. Bioanal. Chem.* 398: 2451-2469.
- [24] Erdem A., Kerman K., Meric B., Akarca U. S., Ozsoz M., (2000), Novel hybridization indicator methylene blue for the electrochemical detection of short DNA sequences related to the hepatitis B virus. *Anal. Chim. Acta.* 422: 139-149.
- [25] Meric B., Kerman K., Ozkan D., Kara P., Erensoy S., Akarca U. S., Mascini M., Ozsoz M., (2002), Electrochemical DNA biosensor for the detection of TT and Hepatitis B virus from PCR amplified real samples by using methylene blue. *Talanta.* 56: 837-846.
- [26] The H. F., Gong H., Dong X. D., Zeng X., Lai Kuan Tan A., Yang X., Tan S. N., (2005), Electrochemical biosensing of DNA with capture probe covalently immobilized onto glassy carbon surface. *Anal. Chim. Acta.* 551: 23-29.
- [27] Boon E. M., Ceres D. M., Drummond T. G., Hill M. G., Barton J. K., (2000), Mutation detection by electrocatalysis at DNA-modified electrodes. *Nat. Biotech.* 18: 1096-1100.
- [28] Kelley S. O., Barton J. K., Jackson N. M., Hill M. G., (1997), Electrochemistry of methylene blue bound to a DNA-modified electrode. *Bioconj. Chem.* 8: 31-37.
- [29] Wang J., Wang F., Dong S., (2009), Methylene blue as an indicator for sensitive electrochemical detection of adenosine based on aptamer switch. *J. Electroanal. Chem.* 626: 1-5.
- [30] Arzum E., Kagan K., Burcu M., Mehmet O., (2001), Methylene blue as a novel electrochemical hybridization indicator. *Electroanalysis.* 13: 219-223.
- [31] Christine B., Per S., Jan B., Gillis J., (1999), A feasibility study of a capacitive biosensor for direct detection of DNA hybridization. *Electroanalysis.* 11: 156-160.
- [32] Mashhadizadeh M. H., Talemi R. P., (2014), A novel optical DNA biosensor for detection of trace amounts of mercuric ions using gold nanoparticles introduced onto modified glass surface. *Spectrochim. Acta A.* 132: 403-409.
- [33] Mashhadizadeh M. H., 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. *Anal. Chim. Acta.* 692: 109-115.
- [34] Fujiwara K., Kasaya H., Ogawa N., (2009), Gold nanoparticle monolayer formation on a chemically modified Glass Surface. *Anal. Sci.* 25: 241-248.
- [35] Zhu L., Zhao R., Wang K., Xiang H., Shang Z., Sun W., (2008), Electrochemical behaviors of methylene blue on DNA modified electrode and its application to the detection of PCR product from NOS sequence. *Sensors.* 8: 5649-5660.
- [36] Nafisi S., Saboury A. A., Keramat N., Neault J. F., Tajmir-Riahi H. A., (2007), Stability and structural features of DNA intercalation with ethidium bromide, acridine orange and methylene blue. *J. Mol. Struct.* 827: 35-43.
- [37] Belaz-Davld N., Decosterd L. A., Appenzeller M., Ruetsch Y., Rhiolro H., Buchn T., Biollaz J., (1997), Spectrophotometric determination of methylene blue in biological fluids after ion-pair extraction and evidence of its adsorption on plastic polymers. *European J. Pharm. Sci.* 5: 335-345.
- [38] Wen M. L., Zhao Y. B., Chen X., Wang C. Y., (1999), Potentiometric sensor for methylene blue based on methylene blue-silicotung state ion association and its pharmaceutical applications. *J. Pharm. Biomed. Analysis.* 18: 957-961.
- [39] Rizwan Khan M., Ali Khan M., Alothman Z. A., Alshaimi A. H., Naushad M., Al-Shaalan N. H., (2014), Quantitative determination of methylene blue in environmental samples by solid-phase extraction and ultra-performance liquid chromatography and mass spectrometry: A green approach. *RSC Adv.* 4: 34037-34044.