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ORIGINAL ARTICLE

SiO_2-based nanobiosensor monitoring toxicological behavior of Mitoxantrone in vitro

Amitkumar N. Lad · Y. K. Agrawal

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Abstract The present study involves the development of nanobiosensor to determine toxicological behavior of Mitoxantrone (MTX). Mitoxantrone intercalates with DNA and produces MTX–DNA adduct, resulting in blockade of protein synthesis and excessive production of free radicals in the myocardium eventually leads to cardiac toxicity. Potentiometry was applied to develop an electroanalytical procedure for the determination of MTX and its interaction with DNA immobilized on the electrode surface modified with Silicon dioxide (SiO_2) nanoparticles. The nanobiosensor immersed in MTX solution to monitor MTX–DNA interaction with respect to time and alters the resistance of the nanobiosensor. It was observed that MTX–DNA interaction is fast initially and as time elapses, the change in interaction gets slow due to formation of MTX–DNA adduct. Determination limit of the nanobiosensor is 100–10 ng/ml. This study suggests that the nanobiosensor allows real-time monitoring of the drug–DNA interaction changes by measuring the potential at sensor interface which can prove to be an important tool in drug discovery pipelines and molecular toxicology.

Keywords Nanobiosensor · DNA · Mitoxantrone (MTX) · MTX–DNA interaction · Silicon dioxide (SiO_2)

A. N. Lad (✉) · Y. K. Agrawal
Institute of Research and Development, Gujarat Forensic Sciences University, Near Police Bhavan, Sector 18A, Gandhinagar, India
e-mail: lad_amit2@yahoo.co.in

Introduction

Nanobiosensors and nanobiochips are gaining importance in the field of life science because of its faster, direct, more accurate, more selective detection at very low concentrations. Enormous research has been carried out for the development of nanobiosensor which can be useful in life science fields such as clinical diagnosis, genomics, proteomics and toxicology. But, until now, a very few nanodevices have been developed which can monitor or detect toxicity at nano gram level. Nanoparticles play a key role in adsorption of biomolecules due to their large specific surface area and high surface free energy (Lad and Agrawal 2012c). The combination of nanomaterials and biomolecules is of considerable interest in the field of nanobiotechnology. Recently, many kinds of nanometer materials such as gold (Maxwell et al. 2002; Xiao et al. 1999; Jia et al. 2002), platinum (Ningning et al. 2005) and silicon dioxide (He and Hu 2004; Qhobosheane et al. 2001) nanoparticles are widely applied for electrochemical-based nanobiosensor due to their conducting and semiconducting properties. Also, these nanoparticles have been used to catalyze biochemical reactions, improving coverage and binding ability of the functional components and this capability can be usefully employed in biosensor design (Martin et al. 2007). Recently, we have developed multiwalled carbon nanotube-based DNA nanosensor and platinum nanoparticle-based nanobiosensor for monitoring drug–DNA interaction. Also, we developed optical nanobiosensor for determining drug–DNA interaction. (Lad and Agrawal 2012a, b).

SiO_2 nanoparticles have been used to construct biosensor due to its biocompatibility as well as good electron transfer properties. In the work of Luo et al. (2004), SiO_2 nanoparticles were introduced in the construction of

field-effect transistors (ENFETs) biosensor which could provide a biocompatible environment and improve the enzyme activity. Chen and his team reported the effect of SiO_2 nanoparticles on the adsorbability and enzymatic activity of glucose oxidase (Chen et al. 1996). The oligonucleotide-modified silica nanoparticles were prepared by Lisa R. Hilliard and her coworkers which provide an efficient substrate for hybridization and used in the development of DNA biosensors and biochips (Hilliard et al. 2002). Ningning et al. (2005) developed electrochemical DNA nanobiosensor which consists of platinum nanoparticles combined with Nafion-solubilized Multi-walled carbon nanotubes. M. Yousef Elahi and his team developed polypyrrole (PPy) nanofiber modified electrode to monitor DNA–salicylic acid/Aspirin interaction. A Platinum electrode was electrochemically modified by the polymerization of pyrrole to obtain a nanofiber PPy film using a pulse potential method. The reaction rate of Aspirin with DNA was lower than that of Salicylic Acid with DNA, potentially due to the steric hindrance of the acetyl group when binding to the minor groove (Yousef et al. 2011). Many research papers have been reported on silicon dioxide nanowire-based nanosensor to study DNA interaction and DNA hybridization studies (Zhang et al. 2011; Ryu et al. 2010). Overall, these studies suggest that silicon dioxide shows good biocompatibility as well as good electron transfer properties. Hence, it can be useful for construction of biosensor to improve its functionality.

The electromotive force (EMF) is the maximum potential difference or charge between two electrodes. This causes electrons to move so that there is an excess of electrons at one point and a deficiency of electrons at a second point (Robinson et al. 2005). The electrochemical signals are usually generated by redox reactions and changes in ionic composition. Potentiometric sensors measure the potential of an electrode at equilibrium (i.e., in the absence of the appreciable currents) by measuring the electrochemical cell potential versus a reference electrode potential (Wang et al. 2010). MTX allows extensive stabilization of the intercalated adduct by hydrogen-bonding interactions with DNA (Thurston 2008). Thus, this potentiometric nanobiosensor has been designed to monitor interaction of MTX with DNA.

In this report, we developed a real-time potentiometric nanobiosensor by modifying the electrode with SiO_2 nanoparticle and DNA. This nanobiosensor was immersed in the solution containing MTX to monitor MTX–DNA interaction (Fig. 1). Mitoxantrone, an anti-cancer agent, has a planar heterocyclic ring structure and the basic side groups are critical for intercalation into DNA. Binding of MTX to DNA inhibits both DNA replication and RNA transcription and leads to excessive production of free

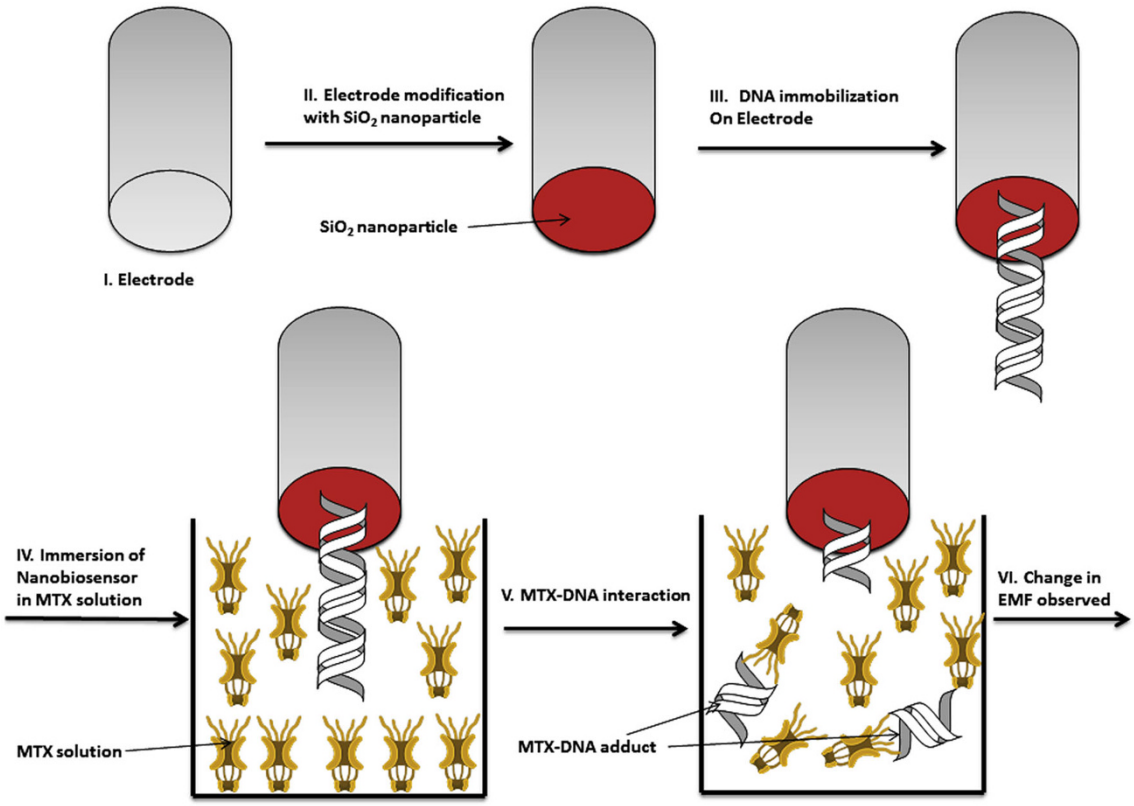

Fig. 1 Schematic representation of development of SiO_2 based nanobiosensor for monitoring MTX–DNA interaction

radicals in myocardium responsible for producing cardiotoxicity (Hajihassan and Rabbani-Chadegani 2011; Oliveira Brett et al. 1998). Therefore, the change in MTX–DNA interaction was observed by measuring changes in EMF (mV). All experiments were carried out at neutral pH and at room temperature since double—strand of DNA breaks at neutral pH and single—strand breaks at high pH (Smart and Hodgson 2008).

Experimental section

Chemicals

Highly polymerized calf thymus DNA (MP Biomedicals, US) was used in this study. DNA dilutions were prepared in phosphate buffer pH 7. Phosphate buffer was prepared by dissolving 0.1 M disodium hydrogen phosphate in water and adjusting the pH by adding 0.1 M HCl. Tetraethylorthosilicate (TEOS), ammonium hydroxide, and ethanol were used to prepare SiO_2 nanoparticles. All chemicals were purchased from E-Merck (India, Mumbai) and were all of analytical reagent grade. Mitoxantrone was obtained from Cipla Ltd (India, Mumbai) and used without purification. All aqueous solutions were prepared in Milli-Q water from a Millipore purification system and all experiments were done at room temperature.

Apparatus

The potential measurements were carried out at 25.0 ± 0.1 °C with a digital pH meter (Model LI120, ELICO, India). A saturated calomel electrode (SCE) was used. Particle size of SiO_2 was measured by Malvern Zetasizer (Model—The Zetasizer Nano ZS, UK). The morphology of SiO_2 was studied using a scanning electron microscope (SEM) EVO-18, special edition, Carl-Zeiss.

Preparation of SiO_2 nanoparticles

SiO_2 nanoparticles were prepared according to the literature (Stöber et al. 1968; Rossi et al. 2005). To 20 ml of ethanol, 2 ml of TEOS was added followed by 4 ml of concentrated NH_4OH. After that it was stirred for 12–14 h at 200–300 rpm. Then the mixture obtained was centrifuged at 3,000 rpm for 30–50 min. Finally a white color powder was formed which was named as silica nanoparticle.

Fabrication of electrode by SiO_2 nanoparticles

The surface of calomel electrode was modified with SiO_2 nanopaticles. An amount of 2.0 mg of SiO_2 were dispersed in a 10 ml of ethanol solution. After about 10 min of sonication, uniformly dispersed SiO_2 nanoparticles were formed. Before modifying the electrode with SiO_2, the electrode was cleaned by washing it with distilled water and was allowed to dry. Then the dry electrode was immersed in solution containing SiO_2 nanoparticles for 30 min with stirring at room temperature. The electrode was removed and was left for drying for about 15 min.

Immobilization of DNA on SiO_2 modified electrode

10 ppm DNA solution was prepared in phosphate buffer pH 7. The electrode was immobilized by drop casting technique. A 10 μL (100 ng) drop of DNA was delivered on the modified SiO_2 surface of electrode by micropipette and allowed to dry in air. After drying, this nanobiosensor was used for monitoring toxicological behavior of MTX. Potentiometric measurement was performed at working calomel electrode versus reference calomel electrode.

Results and discussion

Characterization of SiO_2

The morphology of SiO_2 was observed by SEM. Figure 2 illustrates that the particles are predominantly spherical in shape with diameter ranging from 20 to 25 nm. Larger and uneven shaped particles with diameter 35–70 nm were also obtained.

Particle sizes of SiO_2 were determined by Malvern zetasizer which was found as an average of 20 nm (Fig. 3). Particles were ranging from 46.98 nm (81.3 %), 0.6549 nm (7.3 %), and 2,210 nm (5.8 %).

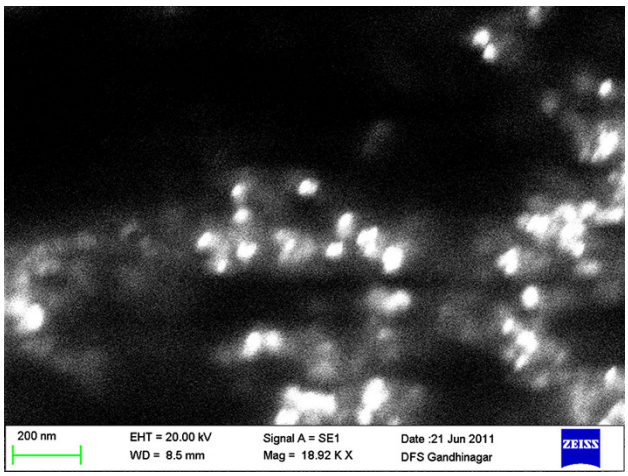

Fig. 2 Morphology of SiO_2

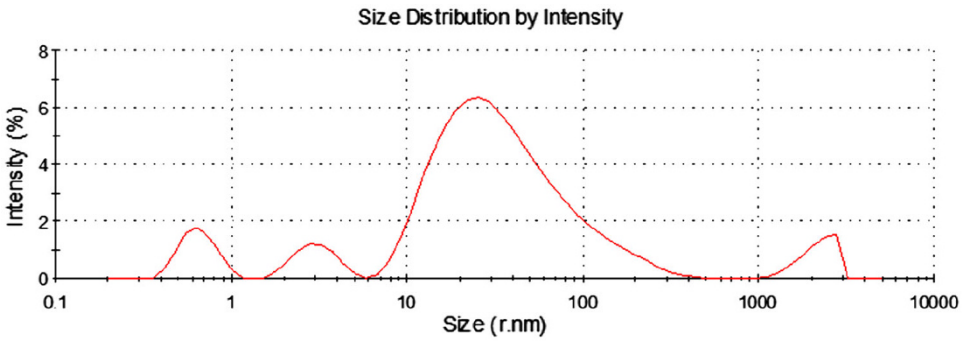

Fig. 3 Particle size distribution of SiO_2

MTX–DNA interaction in solution

MTX solution of varying concentration (100, 75, 50, 25, and 10 ng/ml) was prepared in distilled water. The MTX–DNA interaction in solution was carried out at room temperature. 1 ml of 100 ng/ml of MTX and 10 µL of 10 µg/ml of DNA was taken to perform interaction study by potentiometry. As shown in Fig. 4, the electrode potential shifted to negative direction steadily. But, at one point, the change in EMF was not increased. This indicates the formation of MTX–DNA adduct. Initially the change in EMF was very fast, but as time elapse, the change in EMF gets slow. In all MTX concentration series, potential shifted steadily but at one stage it gets stopped due to the formation of MTX–DNA adduct.

MTX interacts preferentially with DNA binding with guanine, cytosine base pairs (Oliveira Brett et al. 1998). The sensor measures the two-electron oxidation process of 5,8- hydroxyl substituents on MTX while interacting with DNA. No more hydrogen from guanine and cytosine base can be liberated and oxidized which could lead to the stopped EMF change. The change in EMF with respect to time indicates the interacting behavior of MTX with DNA.

In case of 100 ng/ml of MTX, the interaction of MTX with DNA showed more potential difference (Fig. 4) as compared to 75, 50 and 25 ng/ml of MTX because higher amount of MTX was available to interact with DNA. Thus, this study suggests that concentration of drug is directly proportional to the sensitivity of sensor and shows significant EMF changes. It was also observed that no measurable change was found in EMF at 10 ng/ml of MTX concentration. Thus, this study suggests that concentration of drug is directly proportional to the sensitivity of sensor and at very low concentration no EMF changes are observed.

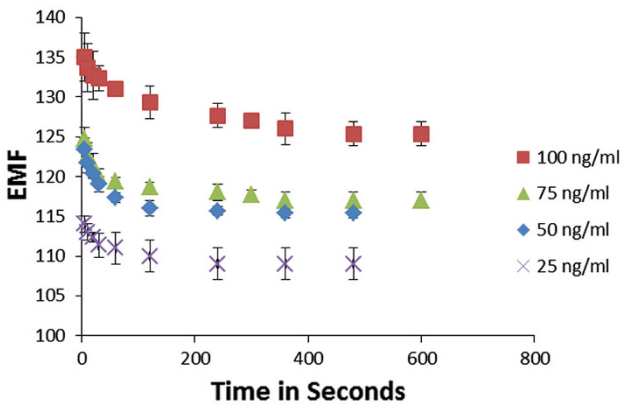

Fig. 4 MTX–DNA interaction in solution at various concentration of MTX

Nanobiosensor monitoring MTX–DNA interaction

MTX (100, 75, 50, 25 and 10 ng/ml) and DNA interaction was performed by developed nanobiosensor. The results obtained from developed nanobiosensor were significant from without modified sensor (Fig. 5). In all MTX concentration series, the MTX–DNA interaction shows more change in electrode potential. The electrode potential decreases steadily until all the amount of MTX gets interacted with DNA. At one stage, no change in EMF was observed due to formation of MTX–DNA adduct. In case of 100 ng/ml of MTX, more EMF changes were observed by nanobiosensor as compared to without nanobiosensor. This suggests that the sensitivity of sensor improves much better due to SiO_2. The biocompatibility of SiO_2 nanoparticles provides a suitable environment for DNA to keep its bioactivity and prevent DNA leakage. Moreover, signals from sensor improve much better due to conducting properties of SiO_2, which provide a faster pathway for electrons to be transferred between the active sites of the DNA and the surface of the SiO_2. Thus, nanobiosensor reveals high sensitivity.

The linearity and reproducibility of the nanobiosensor were investigated by performing three experiments using the same working calomel electrode. It has been observed that in all MTX concentration series, a significant change in EMF was reported with nanobiosensor. Also, the change in EMF was remarkable at lower concentration, i.e., 10 ng/ml of MTX determined by nanobiosensor, while without nanobiosensor did not show any change in EMF. So, sensitivity was also improved at lower concentration. Thus, the developed nanobiosensor allows real-time monitoring

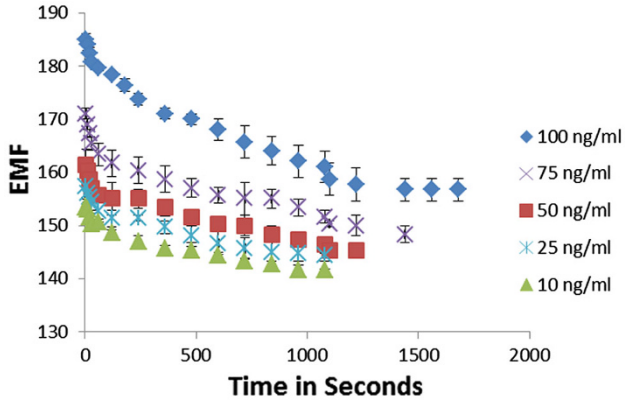

Fig. 5 Nanobiosensor monitoring MTX–DNA interaction at various concentration of MTX

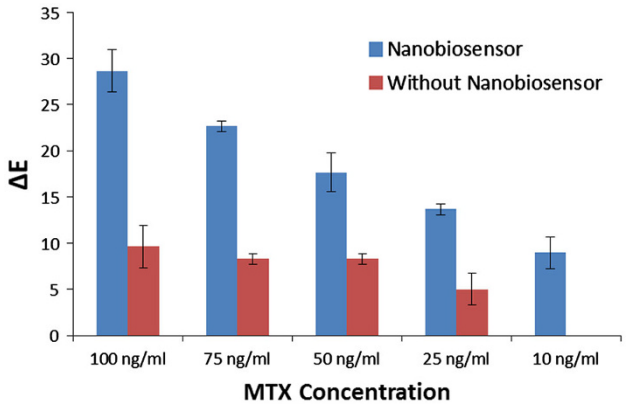

Fig. 6 Potential difference between nanobiosensor and without nanobiosensor at various MTX concentration series

Table 1 Comparison of the analytical performance for nanobiosensor and without nanobiosensor

Parameters	Nanobiosensor	Without nanobiosensor
Regression equation (*Y*)		
Slope (*b*)	4.83	2.26
Intercept (*c*)	32.82	13.06
Correlation coefficient (*r*)	0.994	0.842
Limit of detection (μg/mL)[a]	1.42	2.51
Limit of quantitation (μg/mL)[b]	4.36	7.63

[a] Limit of detection = 3.3× SD/slope

[b] Limit of quantitation = 10× SD/slope

of MTX–DNA interaction, which can play a pivotal role in screening of drugs while developing series of new drugs.

Sensitivity and selectivity of nanobiosensor

To evaluate the performance of the nanobiosensor, potential shift ΔE was calculated, i.e., the potential change from equilibrium time to the end of the experiment. From Fig. 6, it is clearly seen that the potential shift is directly proportional to MTX concentration. If the concentration of MTX is higher, more potential shift was observed. Nanobiosensor showed more ΔE than without modification of sensor at all MTX concentration series. At 10 ng/ml of MTX concentration series, nanobiosensor showed remarkable change in potential shift ΔE, while without modified sensor does not show any change in potential shift ΔE. This confirms the improvements of electrical signals and thus, nanobiosensor reveals high sensitivity.

On addition of incremental concentrations of MTX to DNA, potential difference increases in all concentration series. It is evident from the experiment that interacting behavior of DNA with the stock concentrations of MTX are as follows:

$100 > 75 > 50 > 25 > 10$ ng/ml.

Analytical performance of nanobiosensor

The linearity and reproducibility of the nanobiosensor were investigated by performing three different experiments using the same working electrode. It was observed that the nanobiosensor showed good reproducibility for all three measurements. The working electrode was water-washed to take away the DNA residuals from the surface of the electrode after each measurement. The stability of the nanobiosensor was tested by performing the experiments daily for a period of 15 days while storing in a suitable environment when not in use. Almost 90 % of the initial sensitivity was retained at the end of the period and the biosensor half-life is estimated to almost 1 month.

The comparison of analytical performances for determining MTX–DNA interaction by nanobiosensor and without nanobiosensor is given in Table 1 For nanobiosensor, the values of correlation coefficient (R2), slope, and intercept were found as 0.994, 32.83, and 4.83, respectively. Limit of detection (LOD) and limit of quantification (LOQ) were found as 1.42 and 4.36 μg/mL, respectively. In case of without nanobiosensor, the values of correlation coefficient (R2), slope, and intercept were found as 0.842, 13.067, and 2.267, respectively; limit of detection (LOD) and limit of quantification (LOQ) were found as 2.51 and 7.63 μg/mL, respectively.

Comparative study of MTX–DNA interaction by nanobiosensor

In order to compare the results and hence detect systematic errors between the two methods, a student *t* test was employed to check whether the standard deviations for the same sample differ significantly (Table 2). Since the experimental value of *t* test is higher than the critical, it is

Table 2 Statistical comparison between two methods

MTX concentration (ng/ml)	Nanobiosensor		Without nanobiosensor		*t* test	
	ΔE	SD	ΔE	SD	$t_{Experimental}$	$t_{Critical}$
100	28.67	2.3	9.67	2.3	10.10	2.132
75	22.66	0.58	8.33	0.58	30.48	2.132
50	17.66	2.08	8.33	0.58	7.46	2.132
25	13.67	0.58	5	1.73	8.25	2.132

concluded that the proposed nanobiosensor technique is more precise than without nanobiosensor. Table 2 shows the statistical comparison between two methods at various concentration of MTX. Thus, the results obtained from both the methods were not in agreement, indicating significant difference between two.

Conclusion

This work has shown experimental evidence of interaction of MTX with DNA and may contribute to the understanding of the mechanism of action of this drug with DNA. It was observed that drug–DNA interaction occurring with time which suggests that MTX intercalates with DNA and slowly interacts with it causing some breaking of the hydrogen bonds. It is interesting to note that the nanobiosensor experiments suggest preferential interaction of MTX with DNA at very low concentration. Without surface modified electrode does not seem to be suitable for the monitoring MTX–DNA interaction at low concentrations of MTX. The sensor revealed high sensitivity and selectivity. Overall, we developed nanobiosensor which allows real-time monitoring of the drug–DNA interaction changes by measuring potential at sensor interface which can be crucial biosensor in molecular toxicology and drug discovery pipelines.

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