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# Impedimetric detection of in situ interaction between anti-cancer drug bleomycin and DNA

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ABSTRACT

Surface confined interaction of anti-cancer drug bleomycin (BLM) with nucleic acids: single stranded and double stranded DNA was investigated herein by using electrochemical impedance spectroscopy (EIS) technique in combination with a graphite sensor technology. The experimental conditions were optimized: such as, dsDNA concentration, BLM concentration and interaction time. The main features of impedimetric DNA biosensor, such as its detection limit and the repeatability, were also discussed. The in situ interaction of BLM with dsDNA was also tested impedimetrically in the absence or presence of other chemotherapeutic agents, such as mitomycin C (MC) and *cis*-platin (*cis*-DDP) for testing the selectivity.



## 1. Introduction

Bleomycin (BLM, shown in Fig. S1) is an clastogenic and radiomimetic antibiotic [1] isolated from *Streptomyces verticillus* [2] which has been widely used for treatment of squamous cell carcinomas, testicular cancer, and some lymphomas, head and neck cancers [3,4]. It shows its antitumor activity on the behalf of DNA damage [3,5], both single stranded (ssDNA) and double stranded DNA (dsDNA) [1,3]. It is known that this glycopeptide can cause DNA damage through by intercalation between guanine, cytosine, DNA base pairs, and the end of the peptide binds Fe(II), which is capable to catalyze molecular oxygen reduction to superoxide or hydroxyl radicals, which causes DNA strand scission due to oxidative stress. BLM is preferentially attacking of pyrimidine nucleotides that can joint with the guanosyl-3′ phosphate at BLM–DNA binding site [6,7]. In addition, BLM has been used in many drug delivery systems as a chemotherapeutic agent [8–10] due to the fact that it has a hydrophilic and relatively large structure [9].

BLM has been widely activated by its co-factor agents, like Fe(III), Co(II), $HO_2$ and $O_2$. BLM can constitute complex form with these agents, and it affects DNA structure. The agents effectiveness could be different, as an example while Fe(III). BLM can bind preferentially to helical dsDNA, $HO_2$–Fe(III) BLM can effect both ssDNA and dsDNA cleavage at specific 5′-GC/T-3′ sites [11,12].

Rajani et al. worked on effect of the BLM complexes on DNA, and also the binding of $O_2$–Co(II) BLM to a native DNA polymer, calf thymus DNA by using conventional Raman spectroscopy [10]. It was reported that the changes observed at DNA secondary structure could be attributed to perturbation of structural water resulting from binding of $O_2$–Co(II) BLM within the minor groove [13]. Jałoszynski et al. [14] investigated the BLM-induced DNA damage by using single cell gel electrophoresis (comet) assay. They focused on breast cancer since the reports are indicating frequently an increased sensitivity towards γ-radiation. To evaluate a cellular sensitivity to BLM, and also the efficiency of repairment on the BLM induced DNA damage of breast cancer patients was explored in their study.

In another study, Gatignol et al. [15] evaluated the resistance of BLM on cells by using agarose gel electrophoresis method. Their results showed that 1 μM BLM degraded 0.2 μg/mL chromosomal, linear or covalently closed circle DNA in 5 min interaction time, i.e., indicated that BLM could affect on DNA even at its low concentrations. Tian et al. [16] worked on DNA–BLM interaction kinetic studies by using piezoelectric quartz crystal (PQC) impedance technique by modifying gold electrode using 100 μg/mL ctDNA before the interaction of DNA with BLM.

Additionally, many types of approaches [17–31] have been developed in order to determine BLM over the past three decade, including high-performance liquid chromatography (HPLC) [17–19], radioimmunoassay (RIA) [20–22], enzyme immunoassay

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(EIA) [23,24], microbiological assay [25,26], fluorescence resonance energy transfer (FRET) [27] and piezoelectric quartz crystal impedance technique [30]. However, all these methods are not so practical to be used, and they are time-consuming, labour-intensive, and in some cases performed by using some chemical products, that are harmful for health.

There have also been many efforts to develop efficient technologies for monitoring of BLM, including electrochemical methods [28,29,31]. A novel method for BLM detection based on interaction of ferrocene-linked stem loop structured probe-modified gold electrode in combination with cyclic voltammetry (CV), square wave voltammetry (SWV) and electrochemical impedance spectrometry (EIS) by Yin et al. [28]. Ghirlanda et al. [29] investigated the influence of micellization on the redox potential with BLM–Cu complexes. The CV and Osteryoung square wave voltammograms (OSWV) were carried out to analyze different complexes by using glassy carbon electrode (GCE).

BLM has been used in a mixture of *cis*-platin (*cis*-DDP) and vinblastine for post-operative chemotherapy in granulosa cell tumours [32], but its toxicity was reported as significant [33]. Similarly, Smid et al. [33] tested postoperative irradiation and MC plus BLM-based chemotherapy on head-and-neck cancer patients. Because of this reason, the development of efficient tools for recognizing the possible interaction of DNA with BLM even while its being in a mixture of other anticancer agents, has a key importance.

Electrochemical biosensor systems have also the ability to detect DNA strand breakage caused by interaction of some drugs with nucleic acids [34,35]. Since their detection scheme is easy to handle, cost effective and less time-consuming by resulting with a sensitive and selective results in comparison to other conventional methods, electrochemical sensor platforms could be preferrentially examined for monitoring of different biorecognition processes [34–46].

Electrochemical impedance spectroscopy (EIS) technique can provide information on the impedance changes before and after biointeraction process at the electrode surface, which is mainly based on the change of the charge transfer resistance ($R_{ct}$) using a redox couple. Due to its high sensitivity and label-free characteristics, there have been a growing attention on impedimetric detection of various types of biointeractions including drug–DNA interactions [28,36,37,39].

To the best of our knowledge, an impedimetric DNA sensor was developed as the first time herein for monitoring of in situ interaction of BLM with nucleic acids; double stranded DNA (dsDNA) and single stranded DNA (ssDNA) at the surface of pencil graphite electrode (PeGE). BLM–DNA interaction process was performed without using any extra co-factor agents; such as, Co(II), or Fe (III), that are used for activation of BLM while its interaction with nucleic acids. The analytical conditions; such as, dsDNA concentration, BLM concentration and interaction time were firstly optimized by using electrochemical impedance spectroscopy (EIS) technique. The possible surface confined interaction of BLM with dsDNA was also tested via EIS in the absence or presence of other chemotherapeutic agents, such as mitomycin C (MC) and *cis*-platin (*cis*-DDP). The repeatability, sensitivity and selectivity of the impedimetric method in combination with DNA biosensor technology used for biointeraction process were also discussed in order to discover their future prospects.

## 2. Experimental

### 2.1. Apparatus and chemicals

All impedimetric measurements were carried by using AUTOLAB – PGSTAT 302 electrochemical analysis system supplied with a FRA 2.0 module (Eco Chemie, The Netherlands). The three electrode system was consisted of the pencil graphite electrode (PeGE), an Ag/AgCl/KCl reference electrode and a platinum wire as the auxiliary electrode. The electrochemical impedance spectroscopy (EIS) measurements were performed in the Faraday cage (Eco Chemie, The Netherlands). BLM, *cis*-diamminedichloroplatinum (II) (*cis*-DDP), mitomycin C (MC) and calf thymus dsDNA were purchased from Sigma (Germany).

The stock solutions of calf thymus DNA (dsDNA and ssDNA) (1000 μg/mL) were prepared in Tris–EDTA buffer solution (10 mM Tris–HCl, 1 mM EDTA, pH: 8.00; TE) and kept frozen. More dilute solutions of DNA and *cis*-DDP were prepared in 0.5 M acetate buffer containing 20 mM NaCl (ABS; pH 4.80).

BLM solutions were prepared daily in 0.05 M phosphate buffer solution (PBS; pH 7.40) and its diluted solutions were prepared in PBS. MC stock solution (1000 μg/mL) was prepared in water and the diluted MC solution was prepared in 20 mM Tris–HCl buffer solution (TBS; pH 7.00). *cis*-DDP stock solution (1000 μg/mL) was prepared daily in dimethyl sulfoxide (DMSO, the purity is 99.8%). More diluted *cis*-DDP solutions were prepared in PBS (pH 7.4)

Other chemicals were in analytical reagent grade and they were supplied from Sigma and Merck. All stock solutions were prepared using deionized and autoclaved water.

### 2.2. Preparation of DNA modified PeGE

Firstly, the PeGEs were electrochemically pretreated by applying +1.40 V for 30 s in ABS [37,40]. Each pretreated graphite lead was immersed into vials containing 110 μL of DNA solution for immobilization process via passive adsorption [47] onto PEGE surface during 30 min. Each of the graphite electrodes was then rinsed with ABS for 10 s to remove unbound DNA. Impedimetric transduction was performed in the following section given below.

### 2.3. Surface confined interaction of drug with DNA at PeGE

DNA immobilized PeGEs were immersed into the vials containing 110 μL solution of drugs; BLM/*cis*-DDP/MC for surface confined interaction process [37–40] and then, the electrodes were rinsed respectively using a proper buffer solution; PBS/ABS/TBS for 10 s.

#### 2.3.1. Interaction of the mixture of BLM–cis-DDP with DNA modified PeGE

In order to examine the selectivity of impedimetric sensor, DNA immobilized electrodes were immersed into the vials containing 110 μL mixture solution of 20 μg/mL BLM and 5 μg/mL *cis*-DDP for interaction process, and the electrodes were then rinsed with PBS for 10 s.

#### 2.3.2. Interaction of the mixture of BLM–MC with DNA modified PeGE

In order to examine the selectivity of impedimetric sensor, DNA immobilized electrodes were immersed into the vials containing 110 μL mixture solution of 20 μg/mL BLM and 5 μg/mL MC for interaction process, and the electrodes were then rinsed with PBS for 10 s.

### 2.4. Preparation of drug-modified PeGE

For control experiment, the pretreated graphite electrode was immersed into vials containing 110 μL of any of these drugs; BLM/*cis*-DDP/MC for their immobilization by passive adsorption [47] onto the electrode surface. Each drug modified PeGE was then rinsed respectively using a proper buffer solution; PBS/ABS/TBS for 10 s.

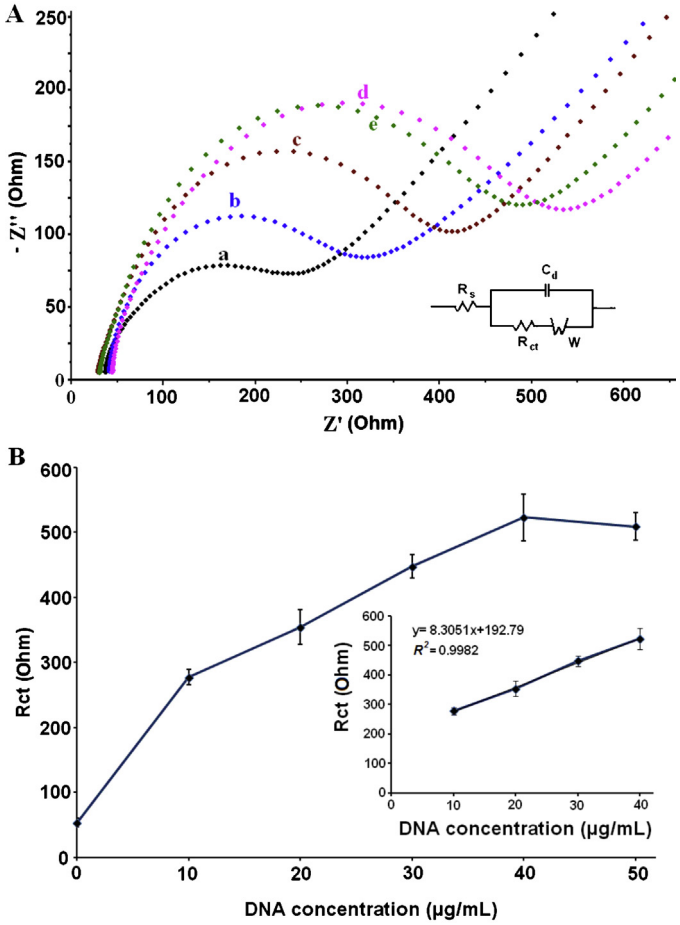



**Fig. 1.** The effect of dsDNA immobilization onto PeGE surface in different DNA concentrations varying between 10 and 50 μg/mL. (A) Nyquist diagrams recorded at PeGE before and after immobilization of (a) 10 μg/mL, (b) 20 μg/mL, (c) 30 μg/mL, (d) 40 μg/mL, (e) 50 μg/mL dsDNA by passive adsorption. Inset: the equivalent circuit model used to fit the impedance data, the parameters of which are listed in the text; $R_s$ is the solution resistance. The constant phase element $C_d$ is then related to the space charge capacitance at the electrode/electrolyte interface. $R_{ct}$ is the charge transfer resistance at the electrode/electrolyte interface. The constant phase element $W$ is the Warburg impedance due to mass transfer to the electrode surface. (B) Calibration plot representing the $R_{ct}$ values obtained in the presence of dsDNA immobilization at varying concentration of 10–40 μg/mL.

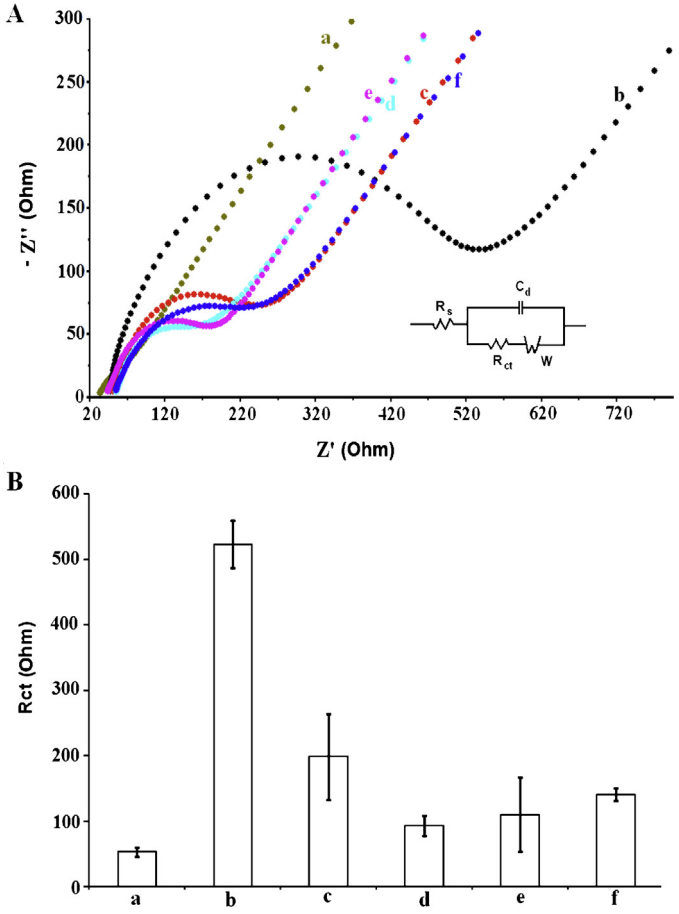



**Fig. 2.** The effect of BLM concentrations on the interaction process with dsDNA. (A) Nyquist diagram, (B) Histograms representing $R_{ct}$ values obtained before/after DNA immobilization onto PeGE surface and BLM–DNA interaction; (a) unmodified PeGE, (b) 40 μg/mL dsDNA modified PeGE and after DNA interactions with different BLM concentrations, (c) 10 μg/mL, (d) 20 μg/mL, (e) 40 μg/mL and (f) 80 μg/mL BLM.

### 2.5. Impedance measurements

Before and after each experimental step carried out as above, EIS measurement was performed in the presence of a 2.5 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ (1:1) mixture as a redox probe prepared in 0.1 M KCl. The impedance was measured in the frequency range from 100 mHz to 100 kHz in an open-circuit potential value of +0.23 V, versus Ag/AgCl with a sinusoidal signal of 10 mV. The frequency interval was divided into 98 logarithmically equidistant measure points. The respective semicircle diameter corresponds to the charge-transfer resistance, $R_{ct}$, the values of which are calculated using the fitting programme AUTOLAB 302 (FRA, version 4.9 Eco Chemie, The Netherlands).

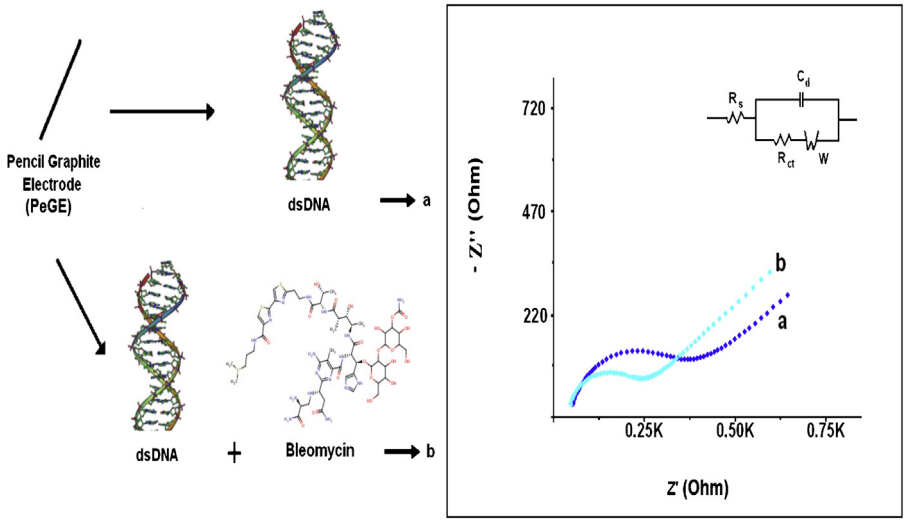
The representative impedimetric detection before/after interaction between BLM and dsDNA at PeGE surface was briefly shown in Scheme 1.

## 3. Results and discussion

The Figs. 1–6 show the changes at the impedance of the graphite electrodes associated with the stepwise of DNA immobilization and BLM–DNA surface confined interaction process. The complex impedance is consisted of the real and imaginary components ($Z'$ and $-Z''$). The charge transfer resistance "$R_{ct}$" could be defined as the resistance related to the dielectric and insulating characteristics at the electrode/electrolyte interface [36]. The impedimetric results were fitted by using an equivalent circuit model (i.e., inset in each figure). The electron transfer was limited at higher frequencies; the linear section seen at lower frequencies may be attributed to the diffusion [36].

The Nyquist diagram (Fig. 1A) and related histograms (Fig. 1B) were presented, that were concerning to the effect of dsDNA concentration varying in the range of 10–50 μg/mL immobilized onto the PeGE surface. The equivalent circuit model (Randles circuit) was used to fit the impedance data in our study. The $R_s$ represents the solution resistance. The constant phase element $C_d$ is then related to the space charge capacitance at the electrode/electrolyte interface. $R_{ct}$ is the charge transfer resistance at the electrode/electrolyte interface. The constant phase element $W$ is the Warburg impedance due to mass transfer to the electrode surface. The values of $R_{ct}$, $W$, $R_s$ and $C$ were recorded for unmodified (bare) PeGE, dsDNA modified PeGE and after interaction process between BLM and dsDNA (the representative one shown in Table S1).

The DNA prevented redox couple, $[Fe(CN)_6]^{3-/4-}$ from reaching the electrode surface due to the charge repulsion between the negatively charged phosphate backbone of dsDNA and $[Fe(CN)_6]^{3-/4-}$. Thus, it can be resulted with a reduced ability for electron transfer at the electrode surface (illustrated in the histograms of Fig. 1B). The




**Scheme 1.** The representative impedimetric detection before/after interaction of BLM and dsDNA at PeGE surface.

$R_{ct}$ value increased gradually when dsDNA concentration increased from 10 to 40 μg/mL of dsDNA. The average $R_{ct}$ value was calculated ($n = 3$) as $522.92 \pm 36.37\ \Omega$ at 40 μg/mL concentration of dsDNA which was approximately 10 times higher than the one obtained at unmodified PeGE. An increase at $R_{ct}$ value means that the electrical conductivity of electrode surface decreased in consequence of resistance occurred against the charge transfer, when while dsDNA was immobilized progressively onto electrode surface by increasing the concentration up to 40 μg/mL of dsDNA. For our further impedimetric studies, the optimum DNA concentration value was chosen as 40 μg/mL with a good repeatability

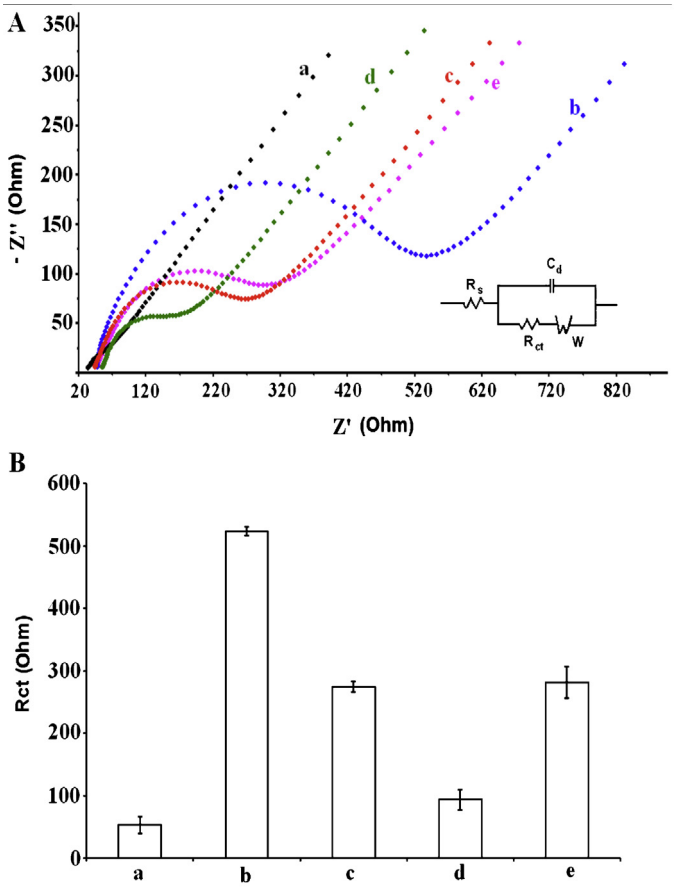



**Fig. 3.** The effect of BLM interaction times with dsDNA modified onto PeGE. (A) Nyquist diagram, (B) histograms representing $R_{ct}$ values obtained before/after DNA immobilization onto PeGE surface and BLM–DNA interaction; (a) unmodified PeGE, (b) dsDNA modified PeGE and after 40 μg/mL DNA interactions with 20 μg/mL BLM in (c) 2.5 min, (d) 5 min and (e) 10 min interaction time.

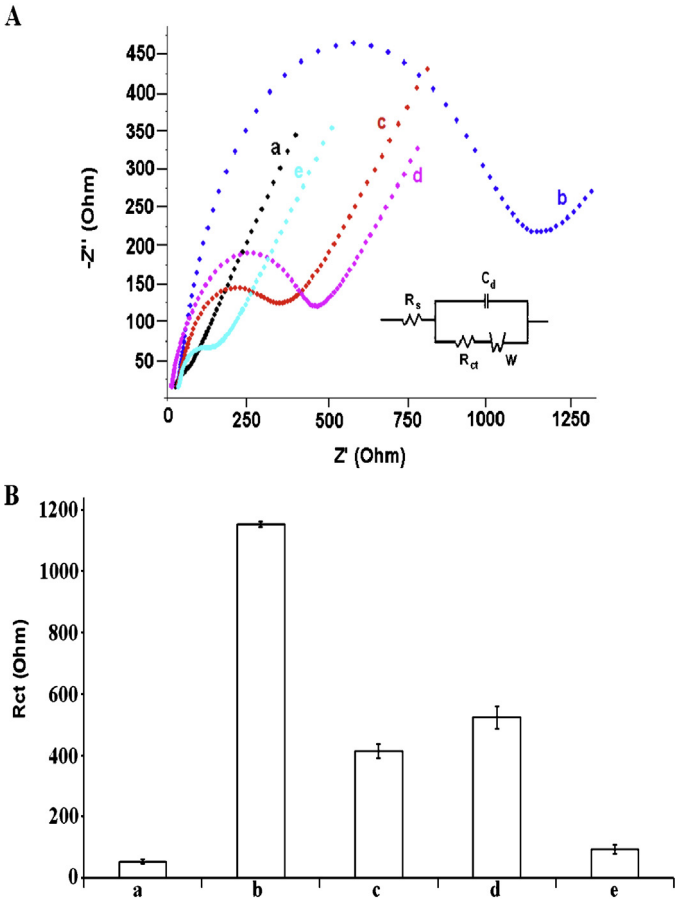



**Fig. 4.** Interaction of 20 μg/mL BLM with 40 μg/mL ssDNA, or dsDNA. (A) Nyquist diagram recorded at PeGE surface before and after interaction process. (B) Histograms representing $R_{ct}$ value of interaction processes; (a) unmodified PeGE, (b) ssDNA modified PeGE, (c) Interaction of BLM with ssDNA modified PeGE, (d) dsDNA modified PeGE, and (e) interaction of BLM with dsDNA modified PeGE.

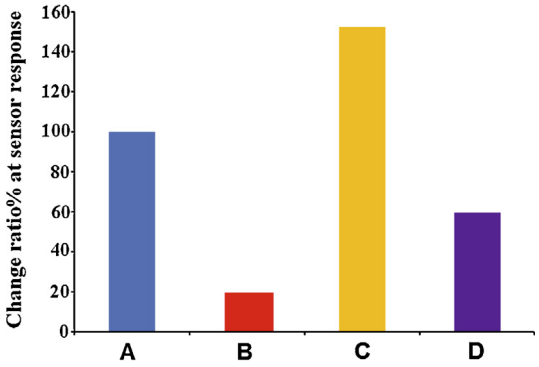



**Fig. 5.** The changes ratio % at sensor response based on $R_{ct}$ values observed before/after surface confined interaction process between dsDNA and drugs; BLM or *cis*-DDP, or in the mixture sample containing BLM and *cis*-DDP by using dsDNA modified electrode. (A) Response % calculated at dsDNA modified electrode in the absence of interaction with a drug; (B) Response % with a decrease calculated at dsDNA modified electrode in the presence of interaction with 20 μg/mL BLM; (C) Response % with an increase calculated at dsDNA modified electrode in the presence of interaction with 5 μg/mL *cis*-DDP; (D) Response % with a decrease calculated at dsDNA modified electrode in the presence of interaction with BLM in the mixture sample containing both 20 μg/mL BLM and 5 μg/mL *cis*-DDP.

(RSD% = 6.96%; $n = 3$), which is comparable lower to the optimum DNA concentration reported in earlier studies [16,36–39]. In addition to the impedimetric studies, the electrochemical behaviour of PeGE was also explored before and after the immobilization of 40 μg/mL of dsDNA onto PeGE surface by using cyclic voltammetry (CV) technique (Fig. S2). A significant decrease % was recorded at anodic ($I_a$) and cathodic ($I_c$) currents of $Fe(CN)_6^{3-/4-}$ after DNA immobilization onto PeGE surface, respectively as almost 40% and 45% (shown in Table S2).

According to the Eq. (1) described by Janek et al. [48], the appearent fractional coverage ($\theta_{IS}^{R}$) of dsDNA modified PeGE was calculated.

$$\theta_{IS}^{R} = 1 - \frac{R_{ct}^{PeGE}}{R_{ct}^{dsDNA-PeGE}} \quad (1)$$

The following order related to the calculated $\theta_{IS}^{R}$ for the surface coverage of PeGE with ctdsDNA in its different concentrations from 10 to 50 μg/mL was given in Table S3, and also as follows: $\theta_{IS}^{R}$ (40 μg/mL dsDNA modified PeGE) > $\theta_{IS}^{R}$ (50 μg/mL dsDNA modified PeGE) > $\theta_{IS}^{R}$ (30 μg/mL dsDNA modified PeGE) > $\theta_{IS}^{R}$ (20 μg/mL dsDNA modified PeGE) > $\theta_{IS}^{R}$ (10 μg/mL dsDNA modified PeGE). The highest appearent fractional coverage ($\theta_{IS}^{R}$) was found in the presence of 40 μg/mL concentration level of dsDNA in paralel to our impedimetric results by indicating the most full coverage of PeGE surface with 40 μg/mL DNA.

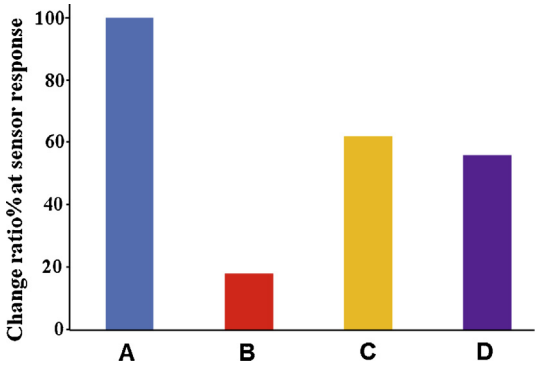



**Fig. 6.** Sensor response % based on the changes at $R_{ct}$ values observed before/after surface confined interaction process between dsDNA and drugs; BLM or MC, or in the mixture sample containing BLM and MC by using dsDNA modified electrode. (A) Response % calculated at dsDNA modified electrode in the absence of interaction with drugs; (B) Response % with a decrease calculated at dsDNA modified electrode in the presence of interaction with 20 μg/mL BLM; (C) Response % with a decrease calculated at dsDNA modified electrode in the presence of interaction with 5 μg/mL MC; (D) Response % with a decrease calculated at dsDNA modified electrode in the presence of interaction with BLM in the mixture sample containing both 20 μg/mL BLM and 5 μg/mL MC.

According to method described by Miller and Miller [49], the detection limit (DL) was calculated for impedimetric DNA detection onto PeGE surface. The DL of DNA was calculated in a linear curve by using the regression equation $y = 8.3051x + 192.79$ (the coefficient of determination ($R^2$) of the linear curve: 0.9982, shown in Fig. 1B as inset figure) as 1.63 μg/mL.

The Fig. 2 presented the Nyquist diagram and also histogram expressing the results related to surface-confined interaction between dsDNA and BLM in different concentrations varying from 10 to 80 μg/mL at PeGE surface. The decrease ratio at $R_{ct}$ values were calculated before and after surface confined interaction of BLM with dsDNA, and found respectively as 62%, 82%, 79% and 73% (respectively shown in Fig 2B from c to f).

It was earlier reported that BLM can interact more with DNA structure after the activation of BLM by the helpf of its co-factors agents; Fe(III) and Co(II) [6,7]. None of these co-factors were additionally supplemented into the BLM solutions while processing in situ interaction between BLM and DNA in our study. However of this fact, BLM could be more activated in the presence of redox probe, $[Fe(CN)_6]^{3-/4-}$, since the EIS measurements in our study were already carried out in the redox probe solution containing Fe(III) ions as the medium of EIS measurement.

There was a slightly decrease at $R_{ct}$ values after interaction in comparison to before interaction process as seen in Nyquist diagram (Fig. 2A). The average $R_{ct}$ value ($n = 3$) was calculated as 93.13 Ω in the case of DNA interaction with 20 μg/mL BLM, which was approximately five folds less than the $R_{ct}$ obtained before interaction process with DNA. The most decrease at $R_{ct}$ with a ratio of 82% was obtained after interaction of 20 μg/mL BLM with dsDNA at the PeGE surface since BLM intercalated into the base pairs of double stranded DNA. It could be concluded that BLM in 20 μg/mL concentration value may cause the strand breakage at dsDNA.

Fig. 3 represents the results based on the effect of BLM interaction times with dsDNA modified onto PeGE. The Nyquist diagram of BLM interaction with dsDNA obtained in different interaction times was shown in Fig. 3A. It was found that the $R_{ct}$ values were sharply decreased till 5 min (presented in Fig. 3B from b to d), and then the decrease was levelled off while interaction time increased to 10 min (shown in Fig. 3B-e). The average $R_{ct}$ value was calculated as 93.13 Ω for 5 min interaction time, which was approximately three times lower than the $R_{ct}$ obtained before interaction process with DNA. The ratio of decrease at $R_{ct}$ was also calculated for each experimental condition, and it was found respectively, as 47.6%, 82% and 46.2% while increasing the interaction time of BLM with dsDNA.

After interaction between 20 μg/mL BLM and 40 μg/mL ctdsDNA at the surface of PeGE in different interaction times varying from 2.5 to 10 min, the appearent fractional coverage ($\theta_{IS}^{R}$) of dsDNA modified PeGE was also calculated, and shown in Table S4. The lowest $\theta_{IS}^{R}$ was recorded as 0.386 at 5 min interaction time in the presence of surface confined interaction of BLM with ctdsDNA at PeGE in paralel to our impedimetric results. Thus, 5 min was chosen as the optimum interaction time for our further experiments.

Next, the interactions of BLM with different types of DNA were examined by the effect of BLM on single stranded (ssDNA) and dsDNA. As seen in the Nyquist diagram of Fig. 4A, the $R_{ct}$ obtained by ssDNA modified PeGE was considerably higher than the one

of dsDNA modified PeGE. The net negative charges of dsDNA prevented redox couple, $[Fe(CN)_6]^{3-/4-}$ from reaching the electrode surface, leading to obtain 522.92 Ω, which was almost 2 times lower $R_{ct}$ value (Fig. 4B-b) in comparison to the one (i.e., 1152.5 Ω) obtained with ssDNA modified PeGE (in Fig. 4B-d). This decrease at $R_{ct}$ value may be attributed to the increased capacitance value of solution/graphite interface due to the presence of dsDNA that may have a higher negative charge density than ssDNA, similiarly to the results in earlier literatures [36,50,51].

The average $R_{ct}$ values ($n = 3$) recorded after interaction between 40 μg/mL DNA and 20 μg/mL BLM were also shown in Fig. 4B, and calculated as 93.13 Ω and 412.67 Ω respectively for the interaction case between BLM and dsDNA, or BLM and ssDNA. Since the most decrease % (as 70%) at $R_{ct}$ value was obtained in BLM–dsDNA interaction process at PeGE surface, it could be concluded that BLM may be giving more damage to dsDNA due to intercalation compared to the one (as 60%) recorded in BLM–ssDNA interaction. Considering the fact that BLM intercalates preferentially G-rich sites of double stranded DNA by inducing the strand breakage dsDNA [15,16], BLM can disturb to dsDNA structure more effectively and strongly due to the fact that dsDNA has more G-rich sites in comparison to ssDNA.

The medicine treatment based on the mixture of BLM including other chemotherapy agents, such as *cis*-DDP [32] and MC [33] has been preferentially used for treatment of advanced granulosa cell tumours [32], head and neck carcinoma [33]. In order to understand electrochemically how BLM interacts with dsDNA in the presence of other anticancer agents; *cis*-DDP and MC, the set of experiments was also performed as the first time herein in the literature by using EIS technique for electrochemical monitoring of DNA interaction with BLM plus *cis*-DDP, or BLM plus MC. Consequently, the results were shown respectively in Figs. 5 and 6.

Figs. 5 and 6 show the changes ratio % at sensor response before/after surface confined interaction between dsDNA and drugs; BLM, *cis*-DDP, MC, or in the mixture sample containing BLM and other drugs, *cis*-DDP, or MC by using DNA modified electrode. After dsDNA interaction with BLM, 82% decrease was calculated at $R_{ct}$ (shown in Figs. 5 and 6 – column B). The control experiments were done according to the possible interaction of *cis*-DDP with dsDNA (Fig. 5 – column C), or DNA interaction with BLM in the mixture sample of *cis*-DDP (Fig. 5 – column D). After the interaction processed in the mixture sample containing both BLM and *cis*-DDP, there was 40.72% decrease observed at $R_{ct}$ comparison to the one measured in the absence of interaction with these drugs (shown in Fig. 5 – column B). The same experimental procedure was then followed in the case of interaction of BLM with dsDNA in the sample containing another anticancer agent, MC. Similiarly to earlier results shown in Fig. 5, there was 80% decrease obtained at the $R_{ct}$ value after the surface confined interaction process between BLM and dsDNA in contrast to the one measured in the absence of interaction (Fig. 6, blue column). After DNA interaction with MC, the sensor response % decreased about as 38% (Fig. 6 – column C). Regarding to the interaction of BLM with dsDNA in the presence of MC, 44% decrease was recorded at $R_{ct}$ (Fig. 6 – column D) compared to the one obtained in the absence of interaction (Fig. 6 – Column A).

Concerning to the preparation of DNA modified sensor and its application on biointercation process, a simpler and faster DNA modification step was used herein for development of impedimetric DNA sensor by following passive adsorption using a small amount of nucleic acids onto PeGE surface. The detection protocol on BLM–DNA interaction was also completed in a short time. In contrast to the study of Yin et al. [28] developed a ferrocene-labelled oligonucleotide modified gold electrode for BLM detection (i.e., resulting in the period of nine hours), and the study of Li et al. [36] performed impedimetric detection of DNA binding drugs at gold nanoparticles modified gold disc electrode (i.e., resulting in a longer period than one day), our impedimetric DNA sensor system based on single-use graphite electrode resulted only in one hour for spesific recognition of BLM–DNA interaction, since there is no time consuming chemical treatment steps for PeGE, and also the nucleic acid modification.

## 4. Conclusion

An impedimetric DNA sensor was introduced as the first time herein for monitoring of in situ interaction of BLM with nucleic acids, single stranded and double stranded DNA at the surface of pencil graphite electrode (PeGE). The one of the most important advantages in our assay is that BLM–DNA interaction could be performed without using any extra co-factor agents; such as, Co(II), or Fe (III), that are used for activation of BLM while its interaction with nucleic acids. For understanding of whether other chemotherapeutic agents, MC and *cis*-platin affect the characteristic properties of BLM, the interactions were also performed selectively in the mixture samples containing BLM and MC, or BLM and *cis*-platin under optimum experimental conditions.

In consideration of the fact that PeGEs brings some important advantages to the impedimetric sensor technology; such as, being cost-effective and portable, and also presenting a higher sensitivity with a better repeatability, which are imperative properties of devices designed for further chip technology. Besides, the easy surface modification with nucleic acids onto the graphite electrodes can facilitate us to provide the faster and more selective detection protocols resulting with a good repeatability (RSD% = 6.96%, $n = 3$) by the advantages of EIS technique in contrast to earlier studies [13,15–19,28,30] performed by using other methods, such as, Raman spectroscopy, HPLC, PQC, and SWV.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ijbiomac.2013.07.012.

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