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ORIGINAL PAPER

Disposable DNA biosensor with the carbon nanotubes–polyethyleneimine interface at a screen-printed carbon electrode for tests of DNA layer damage by quinazolines

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Abstract A screen-printed carbon working electrode within a commercially available screen-printed three-electrode assembly was modified by using a composite of multiwalled carbon nanotubes (MWCNT) dispersed in polyethylenimine (PEI) followed by covering with the calf thymus dsDNA layer. Several electrochemical methods were used to characterize the biosensor and to evaluate damage to the surface-attached DNA: square wave voltammetry of the $[Ru(bpy)_3]^{2+}$ redox indicator and mediator of the guanine moiety oxidation, cyclic voltammetry and electrochemical impedance spectroscopy in the presence of the $[Fe(CN)_6]^{3-/4-}$ indicator in solution. Due to high electroconductivity and large surface area of MWCNT and positive charge of PEI, the MWCNT–PEI composite is an advantageous platform for the DNA immobilization by the polyelectrolyte complexation and its voltammetric and impedimetric detection. In this respect, the MWCNT–PEI interface exhibited better properties than the MWCNT–chitosan one reported from our laboratory previously. A deep DNA layer damage at incubation of the biosensor in quinazoline solution was found, which depends on the quinazoline concentration and incubation time.

Keywords Screen-printed carbon electrode · DNA biosensor · Carbon nanotubes · Polyethylenimine · Quinazoline

Dedicated to Professor Jan Garaj on the occasion of his 75th birthday

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Introduction

In recent years, there is a huge increase in the use of nucleic acids (NA) as tools of biorecognition of many species of human health and, therefore, also analytical chemistry interests. Nucleic acid layer combined with the signal transducer represents a kind of affinity biosensor for study of molecular interactions of the surface-attached NA with drugs and various pollutants. Investigations of NA bases sequence and NA damage are of particular interest [1]. Procedures have been also published to test water, food, soil, and plant samples for the presence of risk chemicals and pathogenic microorganisms [2]. Regarding the main feature of the biosensors, i.e., specificity or high degree of selectivity [3], the devices based on NA fulfill it by possessing specific response to an analyte (e.g., oligonucleotide with a given nucleic bases sequence, aptamer, and protein) and/or the NA itself. With respect to the classification of the biosensors according to the analytes and reactions that they monitor [3], the ratio of investigations seems to be shifted to NA reactions comparing to the enzyme and immunobiosensors used mostly for the direct determination of analytes.

DNA damage is a rather complex term, which includes various changes from subtle damage to strand breaks and disintegration of DNA molecules into small fragments, 8-oxoguanine formation, base deamination, etc. [1]. In our laboratory, we deal with the development of biosensors as simple screening devices and warning systems for a deep DNA damage detection by one analyte of particular interest (e.g., potential drug) or a group of species within a complex sample. Such a biosensor could/even should be nonspecific regarding the analyte; however, it should indicate a change

of the surface-attached DNA. Then, having such information, conventional and much more sophisticated analytical methods can be used to analyze/determine the cleavage effect and cleavage agent. These biosensors can be also used in other areas such as food chemistry to evaluate antioxidants protecting DNA at it oxidative damage [4, 5].

To improve the properties of the electrochemical/electrical NA biosensors, particularly the ratio of specific to nonspecific signals, by an enhancement of electrical conductivity and active area of the transducer, nanomaterials such as carbon nanotubes (CNT) are now frequently used at their construction [revieved in 6–8]. DNA can be immobilized onto the CNT-modified electrode CNT composite electrode by simple physical adsorption [9] or by covalent bonds [10]. CNT-enhanced signals have been used for the detection DNA hybridization and interactions with chlorpromazine [11] and ethidium bromide [10] as well as DNA damage by nitric oxide [12].

As nonderivatized CNT are insoluble in common solvents, it is difficult to get them as a homogeneous mixture. They can be dispersed in water after the preoxidation [9, 10], concentrated acids solutions [13], composite matrixes using binder liquids (teflon, bromoform, mineral oil) [14], nonpolar organic solvents like *N*,*N*′-dimethylformamide [15], surfactants like sodium dodecyl sulfate [5, 16], and in polymers (nafion [17, 18]), chitosan [19–21], or even in DNA [22]. Cationic polymer, polyethylenimine (PEI), was also tested for this aim [23, 24]. PEI is a branched polymeric amine with a high positive charge density, which allows its interaction with negatively charged substrates. It is commonly used as a precursor base layer for creating multilayer films [25]. PEI was shown to be an excellent scaffold making stable and homogenous multilayer films for treatment at various pH values [26]. CNT dispersed in PEI showed an enhanced electroactivity of analytes when immobilized on the surface of glassy carbon electrode. Such a modified electrode was successfully used as the detector in flow systems for the determination of neurotransmitters, phenolic compounds, and herbicides [27]. PEI was also used as an anchoring film for the bacterial proteins at the pyrolytic graphite electrode [28].

Quinazolines—1,3-benzodiazines—are biological active compounds used in the pharmaceutical industry, agriculture, and medicine. As reported previously, many quinazoline derivatives exhibit both DNA damaging [29] and protective effects [30]. These properties depend on both quinazoline derivative structure and conditions of the DNA incubation. Here, we report the preparation and characterization of a DNA biosensor based on the screen-printed carbon working electrode (SPCE) with an interface formed by multiwalled carbon nanotubes (MWCNT) dispersed in PEI. The prepared biosensor DNA/MWCNT–PEI/SPCE was used for the detection of damage to the DNA layer caused by two quinazoline derivatives tested as chemical individual (Fig. 1). To characterize the DNA-modified electrodes voltammetrically, intrinsic signals of DNA bases (e.g., direct or mediated oxidation of guanine and adenine), redox indicators bound to DNA (metal complexes with aromatic ligands and organic dyes) or present in the solution phase (e.g., hexacyanoferrate), and covalently bound DNA labels are typically used [31]. Electrochemical impedance spectroscopy (EIS) is also utilized widely at the evaluation of DNA biosensors [32]. In this work, simple and fast detection schemes are proposed, which do not need time-consuming indicator binding and allow repeat the measurement without a sensor renewal. Square wave voltammetry (SWV) with the $[Ru(bpy)_3]^{2+}$ indicator and mediator [29] as well as coupled cyclic voltammetry (CV) and EIS with the $[Fe(CN)_6]^{3-/4-}$ redox pair [5] have been performed.

Experimental

Apparatus

Electrochemical and impedimetric measurements were performed with a potentiostat Autolab (Eco Chemie B.V., Netherlands) equipped with an impedance module. Screen-printed electrode assembly was purchased from the Food Research Institute, Bratislava, Slovakia. It consists of the carbon working electrode (SPCE, 21 mm^2 geometric surface area), a silver/silver chloride reference electrode (Ag/AgCl/SPCE with the potential of 0.284 V vs conventional Ag/AgCl/sat KCl electrode), and carbon counter electrode and was fabricated using a typical screen-printing equipment. Silver wires as electric contacts on a plastic support are covered by a carbon paste ink on the place of

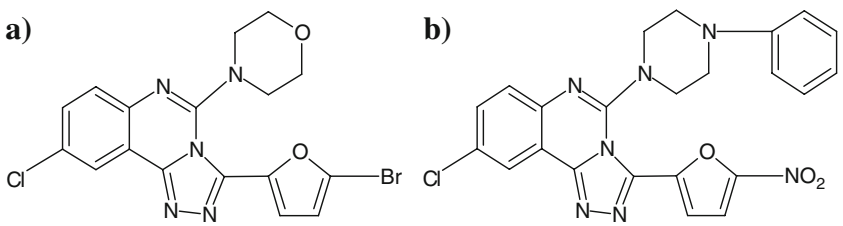

Fig. 1 Chemical formulae of the quinazolines under study: **a** 3-(5-bromo-2-furyl)-9-chloro-5-morpholin-4-yl[1, 2, 4]triazolo [4,3-*c*] quinazoline (QI). **b** 3-(5-Nitro-2-furyl)-9-chloro-5-phenylpiprazine-4-yl[1, 2, 4]triazolo [4,3-*c*] quinazoline (QII)

working and counter electrodes and silver chloride ink on the place of reference electrode. The contacts are covered by an insulating film.

Reagents

MWCNT (OD 40–60 nm, ID 5–10 nm, length 0.5–500 μm) were obtained from Aldrich and used as received. High molecular mass PEI (50% (m/m) in water, average M 750,000) was purchased from Steinheim, Germany. Its solutions were prepared in 98% ethanol (EtOH) of analytical grade purity. Phosphate buffer solutions (PBS; 1 and 100 mM) of pH7.0 were used as supporting electrolytes. Calf thymus dsDNA was obtained from Merck and used as received. Its stock solution (0.1 mg mL^{-1}) was prepared in 10 mM Tris–HCl and 1 mM EDTA solution of pH8.0 and stored at –4 °C. Quinazoline derivatives were prepared as described elsewhere [33, 34] and dissolved in dimethyl sulfoxide (Biocom, Bratislava). The test solutions of quinazolines in the concentrations of 10, 50, 100, and 200 μg mL^{-1} were prepared by adding corresponding aliquots to 1 or 100 mM PBS (pH7.0), and the final content of DMSO never exceeded 1%.

Preparation of the biosensors

DNA/SPCE DNA stock solution (5 μL; 0.1 mg mL^{-1}) were dropped onto a bare SPCE and allowed to evaporate to dryness (overnight) in order to obtain the DNA layer stable at a subsequent use of biosensor in solution [35].

The polyelectrolyte DNA–PEI films were prepared by two ways: layer-by-layer coverage (DNA/PEI/SPCE) and mixed coverage (DNA–PEI/SPCE).

DNA/PEI/SPCE PEI solution (5 μL; 1 mg PEI stock aqueous solution and 1.000, 0.500, 0.250, or 0.125 mL EtOH) were dropped onto a bare SPCE and allowed to evaporate to dryness. PEI/SPCEs were then modified by dropping 5 μL of the DNA stock solution (0.1 mg mL^{-1}) and evaporating overnight.

DNA–PEI/SPCE The PEI solution (1 mg PEI stock solution and 1.000, 0.500, 0.250 or 0.125 mL EtOH) was mixed with the DNA stock solution (0.1 mg mL^{-1}) in the volume ratio 1:1. Then, 5 μL of the resulting mixture was dropped onto a bare SPCE and allowed to evaporate to dryness.

DNA/MWCNT–PEI/SPCE MWCNT (2.0, 1.0, or 0.5 mg) was dispersed in 1 mg PEI stock solution with 0.250 mL EtOH by using sonification, and 5 μL of the MWCNT–PEI suspension was dropped onto a bare SPCE and allowed to evaporate to dryness. MWCNT–PEI/SPCE was then modified by dropping 5 μL of the DNA stock solution (0.1 mg mL^{-1}) and evaporating overnight.

Procedures

The DNA biosensors as well as other modified SPCEs described here were used as disposable sensors. In order to remove any weakly fixed electrode modifiers, including DNA, the prepared sensors were preconditioned by immersing in 1 mM PBS pH7.0 for 5 min under stirring prior to measurements. SWV was performed in 20 mM solution of $[Ru(bpy)_3]^{2+}$ in 1 mM PBS in the potential range from 200 to 1,500 mV using pulse amplitude 100 mV, scan rate 10 mV s^{-1}, and frequency 25 Hz. The peak current in the region 0.88 to 0.98 V depending on the electrode modifier was evaluated against a base-line using the standard software and corrected to blank.

CV was performed in $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ (1/1 mM) in 100 mM PBS in the potential range from 1,000 to −800 mV using a scan rate of 50 mV s^{-1} and was evaluated against the blank. EIS measurements were carried out in the same solution as CV at the potential of 0 V, the frequency range was $12–1\times10^4$ Hz, and the potential amplitude was 10 mV. The optimized equivalent circuit is depicted in Fig. 2. All measurements were carried out at ambient temperature.

For the detection of DNA damage, the DNA/MWCNT–PEI/SPCE biosensor was incubated in the quinazoline solution for a given time at ambient temperature under stirring. After rinsing with water, the voltammetric and impedimetric measurements were performed. The signals values obtained at the biosensor with survived DNA have been corrected by subtraction of the signal measured at MWCNT–PEI/SPCE.

Results and discussion

Voltammetric characterization of the sensors

In order to optimize the composition of PEI solution used to cover the SPCE surface, 1 mg of PEI stock solution was mixed with 1, 0.5, 0.25, or 0.125 mL 98% EtOH. SWV of the $[Ru(bpy)_3]^{2+}$ complex and CV of the $[Fe(CN)_6]^{3-}$ indicator were used to evaluate the quality of the biosensors

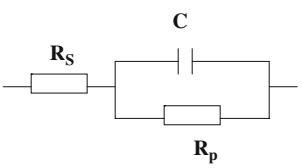

Fig. 2 The equivalent circuit for EIS. R_s solution resistance, R_p polarization resistance, C capacitance

without MWCNT (Table 1). From the comparison of SWV data for the electrodes with and without DNA, it is evident that the layer-by-layer coverage (DNA/PEI/SPCE) is much more effective than the mixed coverage (DNA–PEI/SPCE), and PEI dissolved in 0.250 mL of EtOH provide the highest peak current signal enhancement. With respect to the electrodes without PEI (SPCE and DNA/SPCE), the presence of PEI polymer has a negative influence on the absolute $[Ru(bpy)_3]^{2+}$ signal evidently due to the repulsion of positively charged PEI and the ruthenium(II) complex. In the case of the mixed coverage, the wrapping of DNA by PEI polymer evidently results in poor access of $[Ru(bpy)_3]^{2+}$ to DNA and to the electrode surface. In the case of layer-by-layer coverage, the DNA covers the PEI matrix and electrostatic interactions between DNA and PEI take part [36]. Moreover, the DNA is accessible for the interaction with the Ru(II) complex [37], which results in the high current signal.

The CV scans of $[Fe(CN)_6]^{3-/4-}$ indicator show significant decrease in the ΔE_p values in the presence of PEI comparing to bare SPCE evidently due to electrostatic interaction between the cationic PEI and the anionic iron complex. Again, the highest ΔE_p difference was obtained for PEI dissolved in 0.250 mL of EtOH. Distribution of negative charge in the DNA layer causes a repulsion of $[Fe(CN)_6]^{3-/4-}$ ions and, consequently, an increase in ΔE_p. The DNA biosensor with PEI mixed with 0.250 mL EtOH and prepared by the layer-by-layer DNA coverage was chosen for further experiments.

In order to improve the electrical properties of the transducer, the MWCNT were introduced onto SPCE to form an electroconductive interface. The effect of MWCNT to PEI mass ratio on the voltammetric behavior of $[Ru(bpy)_3]^{2+}$ and $[Fe(CN)_6]^{3-/4-}$ is presented in Table 2. In fact, the reversibility of $[Fe(CN)_6]^{3-/4-}$ redox change was enhanced when compared to that observed at the sensors without MWCNT. The best results were obtained for DNA/MWCNT–PEI/SPCE with the MWCNT to PEI mass ratio of 1:1, where the highest SWV peak current and the largest ΔE_p values were obtained comparing to electrodes without MWCNT. The MWCNT–PEI interface possesses significant enhancement of the biosensor sensitivity regarding the presence of DNA.

Impedimetric characterization of the sensors

The Nyquist plots obtained at the electrochemical impedance spectroscopy measurements are shown in Fig. 3. They represent semicircles characterizing the electron transfer controlled process at high frequencies and the linear part at low frequencies, which is typical for a diffusion controlled process. The DNA layer on the electrode increases the diameter of the semicircles. Measured data were evaluated using the simple Randles circuit shown in Fig. 2. The values of solution resistance R_s, charge capacitance C, and polarization resistance R_p obtained by fitting the experimental data to the circuit model are presented in Table 3. The lowest R_p value for MWCNT–PEI/SPCE shows the impact of carbon nanotubes. In the case of the DNA immobilization on the MWCNT–PEI platform, it acts as an insulator layer, and the highest R_p value is obtained. These results are in agreement with those found in literature [10].

Table 1 The effect of PEI on the SWV peak current of 20 μM $[Ru(bpy)_3]^{2+}$ in 1 mM PBS, pH=7.0 and the CV anodic to cathodic peak potential separation of 1 mM $[Fe(CN)_6]^{3-/4-}$ in 100 mM PBS, pH=7.0

PEI solution	Type of electrode	SWV of $[Ru(bpy)_3]^{2+}$ I_p (μA)	CV of $[Fe(CN)_6]^{3-/4-}$ ΔE_p (V)
1 mg PEI stock solution+1.0 mL EtOH	PEI/SPCE	0.43±0.08	0.15±0.01
	DNA–PEI/SPCE	0.41±0.02	0.15±0.01
	DNA/PEI/SPCE	1.00±0.02	0.19±0.01
1 mg PEI stock solution+0.500 mL EtOH	PEI/SPCE	0.58±0.06	0.20±0.02
	DNA–PEI/SPCE	0.56±0.02	0.18±0.02
	DNA/PEI/SPCE	1.05±0.04	0.24±0.02
1 mg PEI stock solution+0.250 mL EtOH	PEI/SPCE	0.36±0.03	0.12±0.02
	DNA–PEI/SPCE	0.29±0.02	0.18±0.02
	DNA/PEI/SPCE	1.51±0.09	0.19±0.01
1 mg PEI stock solution+0.125 mL EtOH	PEI/SPCE	0.56±0.01	0.19±0.01
	DNA–PEI/SPCE	0.52±0.03	0.18±0.01
	DNA/PEI/SPCE	1.04±0.03	0.22±0.02
–	SPCE	3.32±0.07	0.64±0.08
	DNA/SPCE	11.02±0.15	0.82±0.11

The standard deviations (α=0.05) are for n=3 electrodes

Table 2 The effect of MWCNT on the SWV peak current of 20 μM $[Ru(bpy)_3]^{2+}$ in 1 mM PBS, pH=7.0 and the CV anodic to cathodic peak potential separation of 1 mM $[Fe(CN)_6]^{3-/4-}$ in 100 mM PBS, pH=7.0

MWCNT/PEI mass ratio	Type of electrode	SWV of $[Ru(bpy)_3]^{2+}$ I_p (μA)	CV of $[Fe(CN)_6]^{3-/4-}$ ΔE_p (V)
1:2	MWCNT–PEI/SPCE	0.50±0.01	0.25±0.02
	DNA/MWCNT–PEI/SPCE	2.28±0.11	0.50±0.01
1:1	MWCNT–PEI/SPCE	0.65±0.02	0.19±0.01
	DNA/MWCNT–PEI/SPCE	4.82±0.10	0.49±0.01
2:1	MWCNT–PEI/SPCE	0.42±0.01	0.25±0.01
	DNA/MWCNT–PEI/SPCE	2.06±0.06	0.42±0.01

The standard deviations (α= 0.05) are for n=3 electrodes

The n value represents the properties of the diffuse zone near to ideal capacitor (n~1). Thus, the n value means that the deviation from the Randle's model [38] and the lack of surface homogeneity is modeled with a constant phase element CPE. When n=1, the CPE reduces to a capacitance C [39]. Considering the parameter n as the "roughness coefficient", the PEI/SPCE surface is smoother than those of MWCNT–PEI/SPCE and DNA/MWCNT–PEI/SPCE. The difference between EIS parameters, particularly in R_p, obtained for the biosensor (DNA/MWCNT–PEI/SPCE) and the electrode without DNA (i.e., MWCNT–PEI/SPCE), is larger than that observed at DNA/MWCNT/SPCE and MWCNT/SPCE without the polymer interface [29] as well as at DNA/MWCNT–chitosan/SPCE and MWCNT–chitosan/SPCE [19]. It indicates again good DNA immobilization and the advantage of the use of MWCNT–PEI interface at the SPCE transducer.

Tests of quinazolines

An effect of quinazolines on the surface-attached DNA was tested using an incubation of the biosensor DNA/MWCNT–PEI/SPCE in the quinazoline solution for 10 min, 1 h, and 10 h. DNA damage was found by a decrease of the Ru(II) SWV peak current as well as a decrease in ΔE_p at CV of $[Fe(CN)_6]^{3-/4-}$. The biosensor incubation in blank without quinazolines for given time intervals had no effect on the analytical signals. The portion of survived DNA was expressed in the terms of normalized signals as follows:

$$I_{rel} = \left(I - I_{MWCNT-PEI/SPCE}\right)/\left(I_0 - I_{MWCNT-PEI/SPCE}\right),$$
$$\Delta E_{p,rel} = \left(\Delta E_p - \Delta E_{p,MWCNT-PEI/SPCE}\right)/\left(\Delta E_{p0} - \Delta E_{p,MWCNT-PEI/SPCE}\right),$$
$$R_{p,rel} = \left(R_p - R_{p,MWCNT-PEI/SPCE}\right)/\left(R_{p0} - R_{p,MWCNT-PEI/SPCE}\right),$$

where $I_{MWCNT-PEI/SPCE}$, I_0, and I are the SWV peak currents of $[Ru(bpy)_3]^{2+}$, $\Delta E_{p,MWCNT-PEI/SPCE}$, $\Delta E_{p0,}$ and ΔE_p are the CV peak potential separation values for $[Fe(CN)_6]^{3-/4-}$, and $R_{p,MWCNT-PEI/SPCE}$, $R_{p0,}$ and R_p are polarization resistance values at MWCNT–PEI/SPCE and DNA/MWCNT–PEI/SPCE before and after the incubation in quinazoline, respectively.

In Figs. 4 and 5, a damage of the DNA layer already after the first 10 min of the biosensor incubation in the cleavage agent can be seen depending on the concentration of the quinazoline derivatives. While the I_{rel} and $\Delta E_{p,rel}$ signal-concentration profiles are quite similar for the Q(I) derivative, they somewhat differ for Q(II). With respect to rather limited precision of the response of the disposable biosensors used, the detection limits for the concentration of individual quinazolines can be only estimated to be within mg mL^{-1} range. This detection limit characterizes the ability of given system to detect damage to DNA. It was a purpose of this study to investigate such effect of the quinazolines and not their analytical determination. The biosensor also does not allow to differentiate quinazolines one from the other.

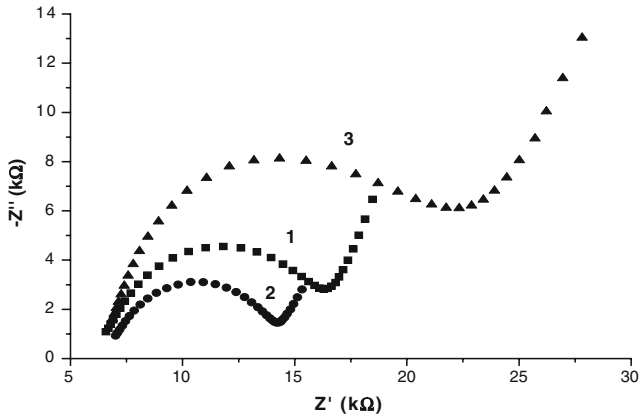

Fig. 3 Impedance spectra of 1 mM $[Fe(CN)_6]^{3-/4-}$ in 0.1 M PBS (pH= 7.0) obtained at: (*1*) PEI/SPCE; (*2*) MWCNT–PEI/SPCE; (*3*) DNA/MWCNT–PEI/SPCE. Conditions: potential amplitude 10 mV, frequency range 12–1×10^4 Hz

Table 3 Parameters of the equivalent circuit simulating the impedance spectra for various electrodes

Type of electrode	R_s (Ω)	R_p (kΩ)	C (μF)	n
PEI/SPCE	60±4	13.1±0.3	4.0±0.2	0.94
MWCNT–PEI/SPCE	68±5	10.9±0.6	6.3±0.3	0.89
DNA/MWCNT–PEI/SPCE	130±7	22.6±0.8	9.5±0.3	0.80

Measurements were performed in 1 mM $[Fe(CN)_6]^{3-/4-}$ in 100 mM PBS (pH=7.0). The standard deviation values (α=0.05) are for n=3 electrodes

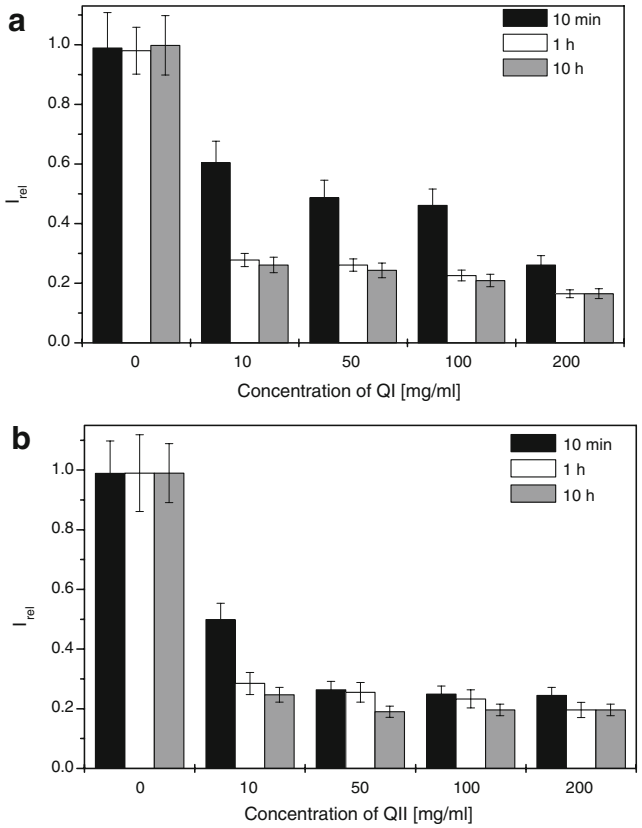

Fig. 4 Normalized SWV peak current signal of $[Ru(bpy)_3]^{2+}$ after the incubation of DNA/MWCNT–PEI/SPCE in QI (**a**) and QII (**b**) at 25 °C. Conditions: 20 μM $[Ru(bpy)_3]^{2+}$ in 1 mM PBS, the potential range 200–1,500 mV, pulse amplitude 100 mV, scan rate 10 mV s^{-1}, and frequency 25 Hz

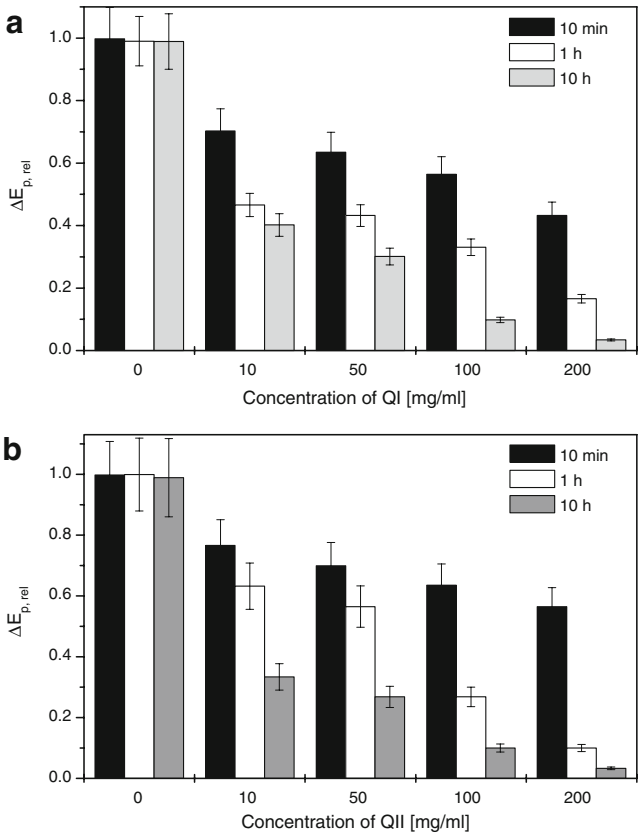

Fig. 5 Normalized ΔE_p values for CV of $[Fe(CN)_6]^{3-/4-}$ after the incubation of DNA/MWCNT–PEI/SPCE in QI (**a**) and QII (**b**) at 25 °C. Conditions: 1 mM $[Fe(CN)_6]^{3-/4-}$ in 100 mM PBS, the potential range 1,000 to −800 mV, scan rate of 50 mV s^{-1}

Rather similar effects were found on another quinazoline derivative, 3-(5-nitro-2-furyl)-9-chloro-5-morpholin-4-yl[1, 2, 4]triazolo[4,3-*c*] quinazoline, using these two detection techniques and simple DNA/SPCE biosensor [29]. For this derivative, the deep DNA damage was verified by independent conventional electrophoretic experiment, which has showed typical picture of DNA strand breaks.

The R_p parameters (Table 4) obtained from EIS performed in parallel with CV also indicate the DNA layer damage connected with a large change of the electrode surface coverage and negatively charged DNA moieties distribution. The influence of the quinazoline concentration and incubation time on the EIS profile agrees mostly with that obtained by SWV of the Ru(II) indicator.

Thus, a potential anticancer activity can be expected for both types of quinazoline derivatives under study.

Conclusions

In summary, we propose here the composite interface of MWCNT in PEI for the construction of the DNA biosensor based on the conventional SPCE transducer modified by a DNA layer. This approach combines the advantages of the highly electroconductive nanostructured material of large surface area, MWCNT, and the layer-by-layer self-

Table 4 R_p values of the equivalent circuit simulating the impedance spectra for DNA/MWCNT–PEI/SPCE after the incubation in quinazoline solution at 25 °C

Quinazoline	Concentrations (μg/mL)	$R_{p,rel}$ 10min	$R_{p,rel}$ 10h
(I)	0	1.00±0.03	1.00±0.03
	10	0.85±0.03	0.73±0.02
	50	0.78±0.05	0.69±0.04
	100	0.74±0.07	0.62±0.08
	200	0.69±0.08	0.65±0.07
(II)	0	1.00±0.03	1.00±0.03
	10	0.56±0.02	0.56±0.03
	50	0.39±0.09	0.39±0.06
	100	0.35±0.10	0.36±0.10
	200	0.31±0.09	0.31±0.10

Conditions: 1 mM $[Fe(CN)_6]^{3-/4-}$ in 0.1 M PBS, amplitude 10 mV, f= 12–10^4 Hz

assembling of polyelectrolytes like positively charged synthetic polymer PEI and negatively charge DNA backbone. It was shown that the MWCNT–PEI composite is the convenient platform for both the DNA immobilization and electrochemical/impedimetric DNA detection. In this respect, the MWCNT–PEI composite interface exhibited better properties than the MWCNT–chitosan interface reported previously [19].

Using this way, the DNA–low molecular mass species interactions leading to deep structural changes and degradation of the DNA layer are supposed to be investigated by using the disposable DNA biosensor and fast and simple testing procedures with the redox indicator in solution. In fact, damage of the DNA layer at the biosensor (not exactly the DNA macromolecule itself) was detected here, and the DNA strand breaks by quinazolines were verified by the independent electrophoretic experiments. Type of DNA damage could be estimated by using measurements with the biosensor in the absence and the presence of DNA intercalators specific for the dsDNA [10] and comparing the signals obtained. Such investigations with the DNA/MWCNT–PEI/SPCE and other nanostructured biosensors are currently in progress.

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