Int. J. Electrochem. Sci., 3 **(2008)** 223 - 235

International Journal of ELECTROCHEMICAL SCIENCE
www.electrochemsci.org

Impedimetric Nanostructured Disposable DNA-based Biosensors for the Detection of Deep DNA Damage and Effect of Antioxidants[#]

Guzel Ziyatdinova[1,2], Julia Galandova[1], Jan Labuda[1,]*

[1]Institute of Analytical Chemistry, Faculty of Chemical and Food Technology, Slovak University of Technology in Bratislava, Radlinského 9, 812 37 Bratislava, Slovakia
[2]Analytical Chemistry Department, A.M. Butlerov Institute of Chemistry, Kazan State, University, Kremlyevskaya, 18, 420008, Kazan, Russia, mail: ziyatdinovag@mail.ru
[*]E-mail: jan.labuda@stuba.sk

[#]*Dedicated to Professor Dušan Bustin on the occasion of his seventhies birthday*

Received: 2 January 2008 / *Accepted:* 8 January 2008 / *Online published:* 20 January 2008

Novel impedimetric nanostructured disposable DNA-biosensors have been created using a layer of multiwalled carbon nanotubes (MWNT) and double stranded calf thymus or herring sperm DNA deposited on the surface of a screen-printed carbon electrode (SPCE) by layer-by-layer and mixed coverage. The presence of DNA significantly decreases the electroconductivity of the MWNT/SPCE interface and represents a charge barrier for the transport of the $[Fe(CN_6)]^{3-}$ redox probe ions. Hence, electrochemical impedimetric procedure performed with DNA/MWNT/SPCE sensor in 0.1 M phosphate buffer solution (PBS) pH 7.0 using 1 mM $[Fe(CN_6)]^{3-}$ was developed for the evaluation of deep DNA damage caused by reactive oxygen species formed *in situ* as well as antioxidative effects of rutin and tea extracts. Good correlation has been found between the charge transfer resistance change obtained as a parameter of the impedimetric equivalent circuit and the voltammetric current response change of the $[Fe(CN_6)]^{3-}$ / $[Fe(CN_6)]^{4-}$ redox couple measured at the DNA modified and bare SPCEs.

Keywords: electrochemical impedance spectroscopy, multiwalled carbon nanotubes, DNA electrochemical biosensor, DNA damage, antioxidants

1. INTRODUCTION

The use of nucleic acids as recognition elements represents a rapidly growing and exciting area at the biosensors development. The DNA biosensors are used to detect binding interactions of the surface attached DNA with drugs as well as health-risk chemicals, the presence of pathogenic

microorganisms and subtle as well as deep damage to DNA [1]. Electrochemical biosensors are fabricated using the approach of chemically modified electrodes [2]. Among them, carbon electrodes including screen printed carbon electrodes (SPCE) are of general interest as mass-produced, inexpensive, non-toxic and widely accessible analytical probes.

In order to achieve good specific and analytical parameters of the biosensors, it is necessary to study electrical processes that occur at the surface of the sensor or inside the sensor membrane [3]. There are various techniques suitable for this purpose, e.g. voltammetry, scanning electrochemical microscopy and others [4, 5]. A majority of the methods probe the surface/electrolyte interface by using a large perturbation which is designed to provide mechanistic information by driving the reaction to conditions far from the equilibrium. Another approach is the use of a perturbation of a system at equilibrium by a small amplitude sinusoidal excitation signal to ensure that the kinetic information pertaining to the surface/electrolyte interface [6, 7]. Electrochemical impedance spectroscopy (EIS) is a non-destructive steady-state technique that is capable to probe the relaxation phenomena over a range of alternative current frequencies from 10^6 to 10^{-4} Hz. EIS is a tool for identifying and separation of different contributions to the electric and dielectric response of material. Traditionally, it has provided information about corrosion processes of metals and metal-coated surfaces. Nowadays, there is an increased use of EIS for the investigation of adsorption processes, mechanism of electrochemical reactions, the dielectric and transport properties of materials used for the creation of sensors and biosensors [3, 8].

Recently, the probing of biomolecular interactions at conductive and semiconductive surfaces by EIS has been outlined including impedimetric immunosensors, enzyme biosensors and DNA-sensors [9]. EIS gives a possibility to study the process of immobilization of a biocomponents, including DNA, and to characterize electric features of a biocomponent/electrode interface. It has been employed for the evaluation of various carbon-based electrodes used as the signal transducers for the detection of DNA hybridization [10], investigation of interaction of redox probes on a glassy carbon DNA-modified electrode [11], and the study of the DNA adsorption/hybridization onto a gold surface [12-14]. The dendrimer-based electrochemical impedimetric DNA-biosensor shows high sensitivity and selectivity for the DNA hybridization assay [15]. DNA impedimetric biosensor based on colloidal Ag and bilayer two-dimensional sol-gel as matrixes has been proposed for the DNA hybridization detection [16].

With respect to rather simple measurement procedure and commercially available equipments, EIS seems also to be an advantageous method for the evaluation of deep DNA degradation by reactive chemical agents [17]. Comparing to the traditional use of DNA redox indicators [18], time consuming steps of their accumulation and desorption can be omitted here. The detection window is given by an impedance difference between the electrode surface without and with DNA. Good electric conductivity of the surface without DNA can be achieved by the arrangement of an interface. Application of conductive nanomaterials such as carbon nanotubes could significantly improve electric properties of bare electrodes such as carbon paste electrode and screen printed carbon electrode [19].

The purpose of this work is to use EIS in a combination with cyclic voltammetry for the analytical characterization of DNA-nanoctructured disposable biosensors and to develop a procedure for the evaluation of DNA damage by reactive oxygen species formed *in situ* as well as DNA

protection by standard chemicals and tee antioxidants. A commercially available SPCE was used as a signal transducer which was covered by a layer of multiwalled carbon nanotubes.

2. EXPERIMENTAL PART

2.1 Reagents

Multiwalled carbon nanotubes (MWNT) (OD 40-60 nm, ID 5-10 nm, length 0.5-500 μm) were obtained from Aldrich, Germany. Their suspension (0.5 mg mL^{-1}) was prepared in a 1 % aqueous solution of sodium dodecyl sulfate (SDS) from Sigma, Germany. Herring sperm dsDNA was purchased from Aldrich Germany. Its stock solution (0.1 $mg.mL^{-1}$) was prepared in 0.1 M phosphate buffer solution (PBS) pH 7.0 and stored at -4 °C. Calf thymus dsDNA was obtained from Merck, Germany and used as received. Its stock solution of 0.1 $mg.mL^{-1}$ was prepared in 10 mmol L^{-1} Tris-HCl and 1 mmol L^{-1} EDTA solution of pH 8.0 and stored at –4 °C.

Rutin (glycoside of polyphenol quercetin) was purchased from Aldrich, Germany. Its stock solution of $1x10^{-4}$ M was prepared in PBS, pH 7.0 and diluted in the range $5x10^{-5}$ – $5x10^{-9}$ M. Other chemicals used were of analytical reagent grade purity and used as received. Deionized and double distilled water was used for measurements. Teas under investigation were commercial products obtained from a local market. Tea extracts were prepared by soaking one tea bag in 250 mL of boiled (98 ^{0}C) or hot (70 ^{0}C) water for the time recommended by the producer, i.e. 5 min for both green and black tea. Tea extracts were used after cooling to laboratory temperature and filtration through filter paper without any other pretreatment.

2.2 Apparatus

The SPCE (Food Research Institute, Bratislava, Slovakia) was a three electrode assembly consisting of working carbon electrode (25 mm^2 geometric surface area), a silver/silver chloride reference electrode Ag/AgCl/SPE (potential of 0.284 V vs conventional Ag/AgCl/saturated KCl electrode) and the same counter electrode. EIS measurements were carried out using the Autolab/FRA system with the potentiostat PGSTAT 12 and FRA-DSG, FRA-ADS modules (Eco Chemie B.V., Netherland), version 4.9.006. CV measurements were carried out on Autolab potentiostat/galvanostat with the software GPES-General Purpose Electrochemical System, version 4.9.005 (Eco Chemie B.V., Netherland). All measurements were performed in 10 ml glass voltammetric cell.

2.3 Preparation of the modified electrodes

The nanostructured MWNT interface was prepared using layer-by-layer or mixed coverage on the surface of screen-printed carbon electrode (SPCE) without any electrochemical precondition of the working electrode surface. The layer-by-layer coverage was used to create a DNA/MWNT/SPCE biosensor as follows: the working electrode was covered with 5 μl of MWNT suspension and allowed to dry. Then the DNA layer was formed by the application of 5 μl of its stock solution and evaporation to dryness. To prepare the mixed coverage, the MWNT suspension was mixed with the DNA stock

solution in the volume ratio 1:1. The resulting suspension (5 μL) was then applied as a drop to the bare SPCE surface and left to evaporate to dryness.

For a comparison, an electrode modification with single modifiers (MWNT or DNA only) was also tested using the DNA/SPCE and MWNT/SPCE sensors. They were prepared by the application of 5 μl of DNA stock solution or MWNT suspension on the surface of bare SPCE and evaporation to dryness. Prior to the measurement, the bare and modified SPCE were immersed into 5 mM PBS for 5 min under stirring to achieve an equilibrum.

2.4. Procedures

Electrochemical impedance spectroscopy

The EIS measurements were carried out in 1 mM $K_3[Fe(CN)_6]$ in the 0.1 M PBS pH 7.0 at ambient temperature, at the polarization potential of 0 V in the frequency range 0.1 – 10^4 Hz (in 51 frequency steps) and with the amplitude of 10 mV.

Cyclic voltammetry of $K_3[Fe(CN)_6]$

The biosensor was immersed into 1 mM $K_3[Fe(CN)_6]$ in the 0.1 M PBS pH 7.0. The CV scans were recorded within the potential range from 1.0 V to –0.8 V at scan rate 50 mV/s. .

DNA damage and evaluation of antioxidants

Prior to the DNA damage, the EIS and CV signals of DNA/MWNT/SPCE were obtained as described above. Then, after rinsing with distilled water, the same electrode was incubated at ambient temperature in corresponding cleavage mixture without or with the addition of antioxidant substance solution (5 mL of the stock rutin solution or tea extract in 1:1 volume ratio) for 5 min under stirring.

Subsequently, the EIS and CV measurements after this exposure were carried out. With respect to differences between individual SPCE strips, the and R_{ct} and CV parameters are expressed as the normalized (i.e. relative) values compared regarding to the parameters obtained for the original biosensor before its incubation in cleavage agent. A survived DNA (denoted as surv DNA) after damage was calculated from the change of the signals obtained at electrodes with and without DNA related to the difference of signals corresponding to original undamaged DNA according to the formulaes:

$$\Delta R_{ct(rel)} = (R_{ct(surv\ DNA)} - R_{ct(MWNT\text{-}CHIT)}) / (R_{ct(DNA)} - R_{ct(MWNT\text{-}CHIT)}) \quad (1)$$

$$\Delta I_{rel} = (I_{surv\ DNA} - I_{MWNT\text{-}CHIT}) / (I_{DNA} - I_{MWNT\text{-}CHIT}) \quad (2)$$

where R_{ct} is electron transfer resistance at EIS measured at the peak potential obtained for 1 mM $K_3Fe(CN)_6$ at the MWNT-CHIT/SPCE without DNA (about 0 V) and I is the CV cathodic current response of 1 mM $K_3Fe(CN)_6$ measured at the peak potential obtained for 1 mM $K_3Fe(CN)_6$ at the MWNT-CHIT/SPCE without DNA (about 0 V). The indexes used characterize the chemical modifiers of SPCE.

3. RESULTS AND DISCUSSION

Various biosensors with layers of double stranded calf thymus or herring sperm DNA attached to the MWNT interface at the SPCE surface have been investigated. Modification of the SPCE surface was performed using the layer-by-layer or mixed films obtained via physical adsorption of the electrode modifiers at open circuit conditions. Amount and concentration of the MWNT suspension were optimized using the biosensor response as the criterion.

3.1 Electrochemical impedance spectroscopy (EIS)

The EIS measurements have been performed using the electroactive ferricyanide anion as a standard redox probe in the solution phase. Fig. 1 shows typical impedance responses of SPCE covered with various layers of modifiers.

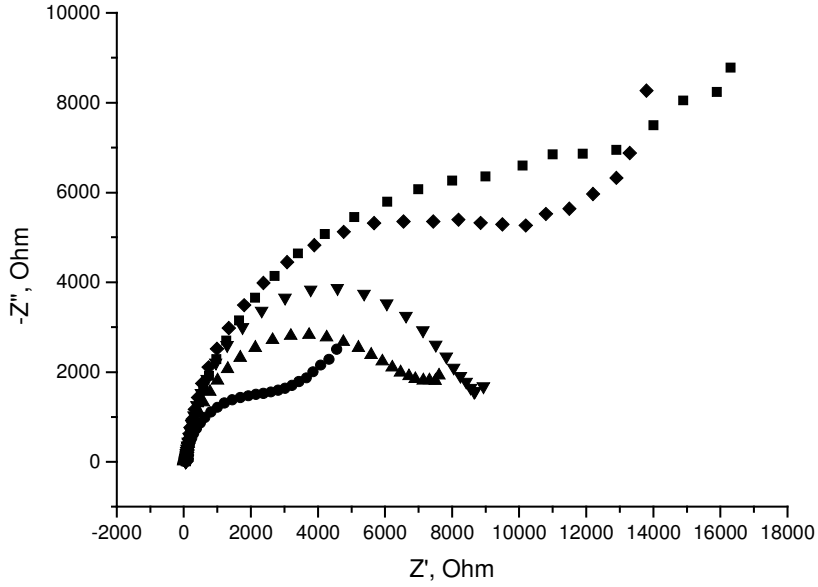

Figure 1. Niquist plots in the presence of 1 mM $K_3[Fe(CN)_6]$ in 0.1 M PBS pH 7.0 for various modified electrodes: SPCE (■), herring sperm DNA/SPCE (♦), herring sperm DNA-MWNT/SPCE (▼), herring sperm DNA/MWNT/SPCE (▲), MWNT/SPCE (●). Conditions: frequency range (0.1-10^4) Hz, polarization potential 0 V and amplitude 10 mV.

Complex impedance spectra as the Niquist plots (the dependence of an imaginary part of the impedance Z'' *vs* a real part of the impedance Z') represent semicircles at high frequencies corresponding to the electron transfer limiting process. For bare SPCE and DNA/SPCE, there are short linear parts at low frequencies resulting from the diffusion limiting step of the electrochemical process [20]. It should be noted that this part of the spectrum represents the properties of the electrolyte solution and the diffusion of the redox probe and, thus, are not affected by the modification of the electrode surface [21]. The respective semicircles diameters at the high frequencies corresponding to

the charge transfer resistance at the electrode surface increase in the presence of DNA on the electrode surface. Thus, the charge transfer resistance was used as a sensor signal.

The impedance data were simulated using the Randles equivalent circuit (Fig. 2) consisting of a parallel combination of the capacitance (C) and charge transfer resistance by redox reactions (R_{ct}) in series with the supporting electrolyte resistance (R_{sol}). The fitting of spectras to the equivalent circuit has indicated a good agreement between the circuit model and the real experimental data, especially at the high frequency values.

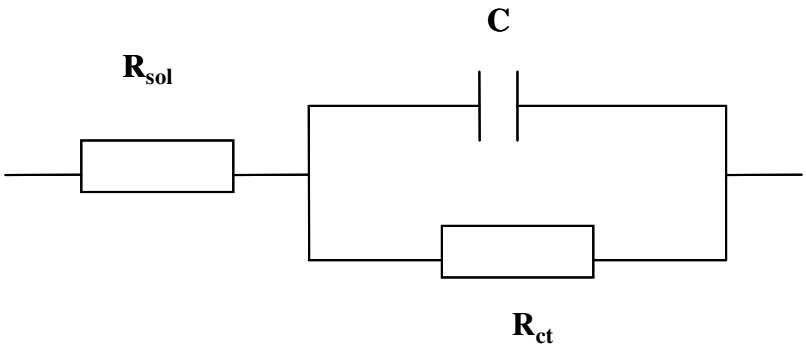

Figure 2. The scheme of equivalent circuit simulating the impedance spectra. R_{sol} – solution (supporting electrolyte) resistance, R_{ct} – charge transfer resistance, C – capacitance.

The impedance plots for the individual electrodes have shown a significant difference in the impedance values that reflect the properties of the electrodes surfaces. The presence of MWNT at the electrode surface significantly decreases the impedance, as one can expect due to the high MWNT conductivity. For the electrodes with nanostructured interface the impedance values are lower than those for the SPCE and SPCE electrodes modified with DNA layer only, independently of the DNA kind and modification way (the mixture with MWNT or the layer-by-layer coverage). The parameters obtained by fitting analysis are presented in Table 1.

For the bare SPCE, the value of R_{ct} is 19.16 kΩ and it reflects the semicircle part with very big diameter. The introduction of DNA on the electrode surface leads to a slight decrease of the semicircle diameter in the spectra with corresponding decrease of the R_{ct} values till 16.90 kΩ and 15.24 kΩ for calf thymus and herring sperm DNA, respectively. A remarkable decrease in the R_{ct} value (to 3.59 kΩ) was observed after the modification of SPCE by MWNT and the Nyquist plot exhibits very small semicircle. In the cases of DNA/MWNT/SPCE and DNA-MWNT/SPCE sensors, the R_{ct} values increase in comparison with MWNT/SPCE but they do not differ significantly between each other. So, in this sense the way of modification (mixture or layer-by-layer coverage) does not play an important role.

The increase or decrease of the R_{ct} value reflecting the increase or decrease in the diameter of the semicircle at high frequencies in the impedance spectra is associated with the blocking behavior of the electrode surface for the charge transfer to the redox probe [22]. MWNT immobilized on the SPCE play an important role similar to an electron conducting tunnel making electron transfer to the

electrode surface easier (in analogy with nano-sized Au colloids [23] or Ag nanoparticles [24]). In the presence of DNA, the electron transfer of to the redox probe is blocked by the formation of highly organized layer of the biocomponent on the electrode surface and the redox species do not penetrate this layer [25]. The "roughness coefficient" *n* reflecting the deflection from ideal smooth surface [26] is increased in all cases of the modification in comparison to unmodified SPCE which can be attributed to the enhanced homogenity of the surface, especially when MWNT are present. The small difference in *n* between DNA immobilized on simple SPCE and DNA on the MWNT interface is due to a good distribution of DNA chains on such interface.

Table 1. Parameters of the equivalent circuit simulating the complex impedance spectra of the electrodes in the presence of 1 mM $K_3[Fe(CN)_6]$ in 0.1 M PBS. R_{sol} – solution (supporting electrolyte) resistance, R_{ct} – charge transfer resistance, C – capacitance, n – „roughness" coefficient.

Electrode	R_{sol}, Ω	R_{ct}, kΩ	C, μF	n
SPCE	34±1	19.16±0.06	18±1	0.769
MWNT/SPCE	44±3	3.59±0.02	29±2	0.886
Calf thymus DNA-MWNT/SPCE	63±5	6.15±0.03	12±1	0.861
Calf thymus DNA/MWNT/SPCE	42±4	6.30±0.05	15±2	0.827
Calf thymus DNA/SPCE	50±3	16.90±0.05	13±2	0.819
Herring sperm DNA-MWNT/SPCE	52±3	9.18±0.04	12±1	0.891
Herring sperm DNA/MWNT/SPCE	56±5	7.71±0.03	17±2	0.814
Herring sperm DNA/SPCE	67±5	15.24±0.05	12±2	0.815

The capacitance can be generally defined as the amount of charge stored between two layers (a capacitor aproximation) for a potential difference or voltage existing across the layers (a double layer capacitance). The larger the area of the plate/surface the more charge can be accumulated and, consequently, the higher is the capacitance. That is why the capacitance value is increased in the case of the MWNT interface. However, there is only a small difference in the C values at the DNA modified electrodes. Therefore, the capacitance should not be considered here as a decisive sensor parameter.

3.2 Cyclic voltammetry

To confirm the EIS data, the CV scans were used performed advantageously in the same solution. The mechanism of the DNA detection using $[Fe(CN)_6]^{3-}$ resides in a barrier effect of the negativelly charged DNA backbone towards the redox probe anions. This effect leads to a decrease of the $[Fe(CN)_6]^{3-}$ current signal evaluated against the baseline [17]. The CV records of the redox couple $[Fe(CN)_6]^{3-}/[Fe(CN)_6]^{4-}$ at the bare SPCE and various modified SPCE are presented in Fig. 3.

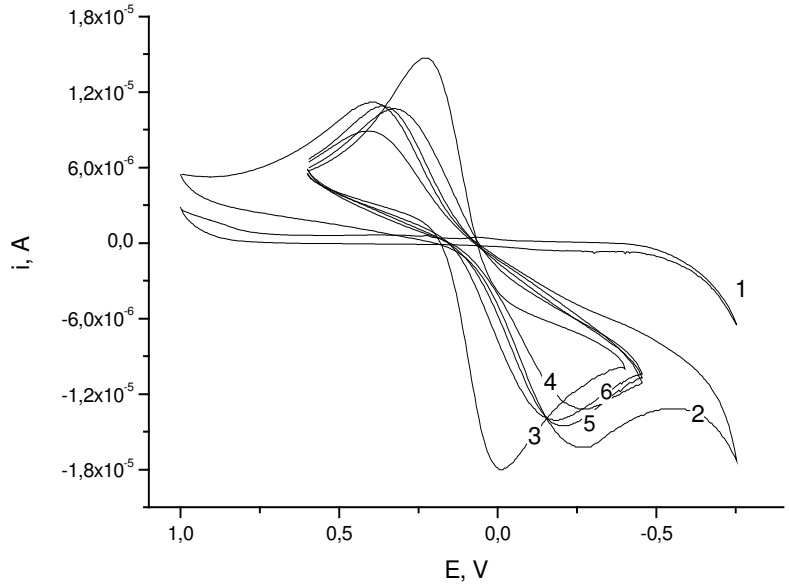

Figure 3. Cyclic voltammograms of 1 mM $K_3[Fe(CN)_6]$ in 0.1 M PBS pH 7.0 at various electrodes: (1) blank at SPCE, (2) bare SPCE, (3) MWNT/SPCE, (4) herring sperm DNA/SPCE, (5) herring sperm DNA-MWNT/SPCE, (6) herring sperm DNA/MWNT/SPCE. Scan rate 50 $mV.s^{-1}$.

Table 2. The CV parameters of 1mM $K_3[Fe(CN)_6]$ in 0.1 M PBS pH 7.0 at individual modified electrodes. ΔE_p – anodic to cathodic peak potential difference, I_a/I_c - anodic to cathodic peak current ratio.

Electrode	ΔE_p, V	I_a/I_c
SPCE	0.63±0.05	0.892
MWNT/ SPCE	0.21±0.01	0.991
Calf thymus DNA-MWNT/SPCE	0.44±0.06	1.003
Calf thymus DNA/MWNT/SPCE	0.45±0.04	0.980
Calf thymus DNA/SPCE	0.71±0.06	1.014
Herring sperm DNA-MWNT/SPCE	0.50±0.01	0.994
Herring sperm DNA/MWNT/SPCE	0.48±0.03	0.974
Herring sperm DNA/SPCE	0.60±0.05	0.822

At the bare SPCE, the CV of $K_3[Fe(CN)_6]$ is characterized by the anodic to cathodic peak potential separation of 0.63±0.05 V (curve 2). When covered by DNA, the peak potential separation was approximately the same (curve 4). The arrangement of the SPCE surface by a MWNT interface significantly improves the electrochemical reversibility of the redox probe (ΔE_p is 0.21±0.01 V) and at

the same time increases its current response (curve 3). The addition of the DNA-layer (curves 5 and 6) leads to an increase of ΔE_p and a decrease in the cathodic current. In all cases, the peak current ratio for anodic to cathodic peaks is in the range from 0.89 to 1.01 that confirmes reversibility of the redox couple. The peak potential separation ΔE_p and anodic to cathodic peak current ratio I_a/I_c obtained for various electrodes are summarized in Table 2.

The data obtained are similar to those reported previously [27]. It should be noted that there is no statistically significant difference in peak potential separation and current values for both types of DNA used and ways of the SPCE modification (the mixed or layer-by-layer coverages). The results allow to use the difference in the current response of the redox probe at DNA/MWNT/SPCE and MWNT/SPCE (i.e. the current difference between the curves 3 and 6) at the potential of 0.0 V as the detection window for DNA damage analysis.

3.3 Detection of a deep DNA damage

Five typical DNA cleavage mixtures have been tested as strong DNA degradating agents. All of them are sources of reactive oxygen species, particularly hydroxyl radicals formed by the Fenton reaction [28]. In general, the reactive oxygen species play an important role in the oxidation of biomolecules such as proteins, amino acids, lipids, and DNA which can lead to cell injury and death [29, 30]. Therefore, the investigation of DNA damage by reactive oxygen species formed *in situ* in the presence of potentially toxic chemicals is of great interest. The evaluation of DNA damage at DNA/MWNT/SPCE was carried out by using the EIS and CV methods in the same experiment. Typical CV and impedance spectra (inset) are shown on Fig. 4.

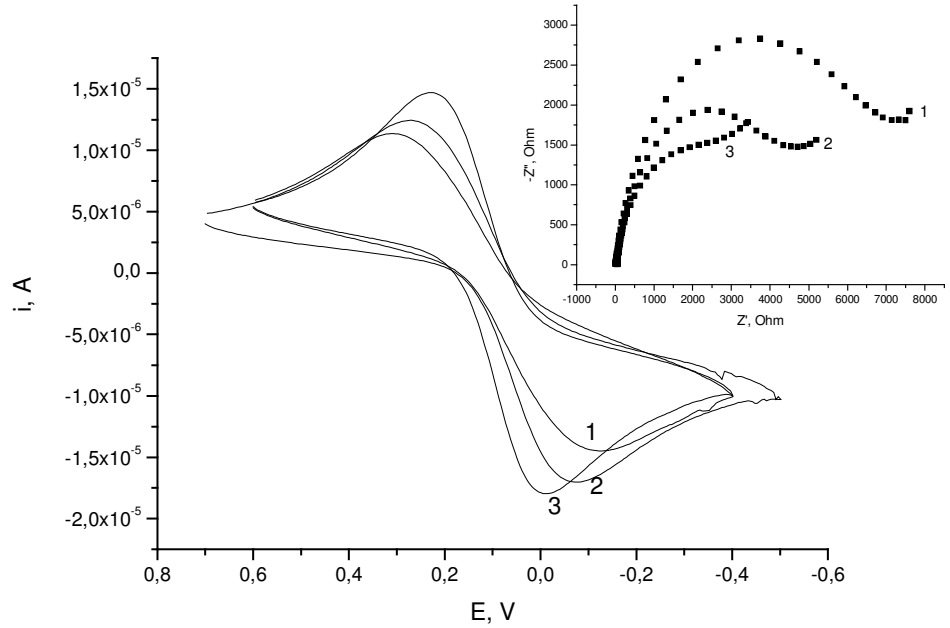

Figure 4. Typical CVs and Niquist plots (inset) for 1mM $K_3[Fe(CN)_6]$ in 0.1 M PBS indicating DNA damage at DNA/MWNT/SPCE in cleavage mixture No. 2 (see Table 3), 5 min. incubation. 1 – DNA/MWNT/SPCE before damage, 2 – DNA/MWNT/SPCE after damage, 3 – MWNT/SPCE.

The deep DNA damage is indicated by the signal of survived DNA (Table 3). This is expressed by the normalized parameters obtained for the DNA-based biosensor according to formulaes 1 and 2 given in Experimental.

All cleavage mixtures have exhibited a degradating effect towards DNA. As one can see, the EIS data agree well with the CV results.. The biosensors proposed have shown high sensitivity for the cleavage agents and can be used for the evaluation of deep DNA oxidative damage.

3.4 Evaluation of the protecticve effect of antioxidants towards DNA oxidative damage

The DNA/MWNT/SPCE biosensor was applied to the detection of antioxidant effect of rutin and teas on calf thymus DNA during the damage process in the cleavage mixture containing $1.0x10^{-6}$ M Fe^{2+}, $2.5x10^{-4}$ M H_2O_2 and $1.0x10^{-6}$ M ascorbic acid.

Table 3. Evaluation of a deep DNA damage at DNA/MWNT/SPCE using the EIS and CV measurements obtained in the same experiment. Conditions: 1 mM $K_3[Fe(CN)_6]$ in 0.1 M PBS after 5 min incubation of the biosensor in cleavage mixtures at ambient temperature. $\Delta R_{ct(rel)}$ – normalized change of the charge transfer resistance before and after incubation in a cleavage mixture, ΔI_{rel} – normalized change of the cathodic current signals before and after incubation in a cleavage mixture.

No	Cleavage mixture	Normalized signals of survived calf thymus DNA		Relative portion of survived herring sperm DNA	
		$\Delta R_{ct(rel)}$	ΔI_{rel}	$\Delta R_{ct(rel)}$	ΔI_{rel}
1	2.5×10^{-8} M Cu^{2+} 2.5×10^{-4} M H_2O_2	0.251	0.257	0.216	0.187
2	2.5×10^{-8} M Cu^{2+} 2.5×10^{-4} M H_2O_2 1.0×10^{-6} M Ascorbic acid	0.561	0.549	0.366	0.401
3	1.0×10^{-6} M Fe^{2+} 2.5×10^{-4} M H_2O_2	0.287	0.274	0.181	0.201
4	1.0×10^{-6} M Fe^{2+} 2.5×10^{-4} M H_2O_2 1.0×10^{-6} M Ascorbic acid	0.214	0.201	0.192	0.203
5	1.0×10^{-6} M Fe^{2+} 2.5×10^{-4} M H_2O_2 4.0×10^{-6} M EDTA	0.212	0.198	0.255	0.263

Well-known polyphenolic antioxidant rutin (quercetin-3-O-beta-rutinoside) was choosen as model standard substrate with strong antioxidative properties [31]. Changes in the electron transfer resistance and CV response of the redox probe after the sensor exposure to the cleavage mixture with various concentrations of rutin have been found (Fig. 5). As expected, with increased concentration of rutin the portion of survived DNA increases, thus the protetive efect of rutin towards surface confined DNA layer was confirmed.

An effect of the commercial tea extracts at the protection of DNA towards deep DNA degradation was also tested. Table 4 represents the results obtained for the tea extracts mixed with the cleavage agents (1:1 volume ratio).

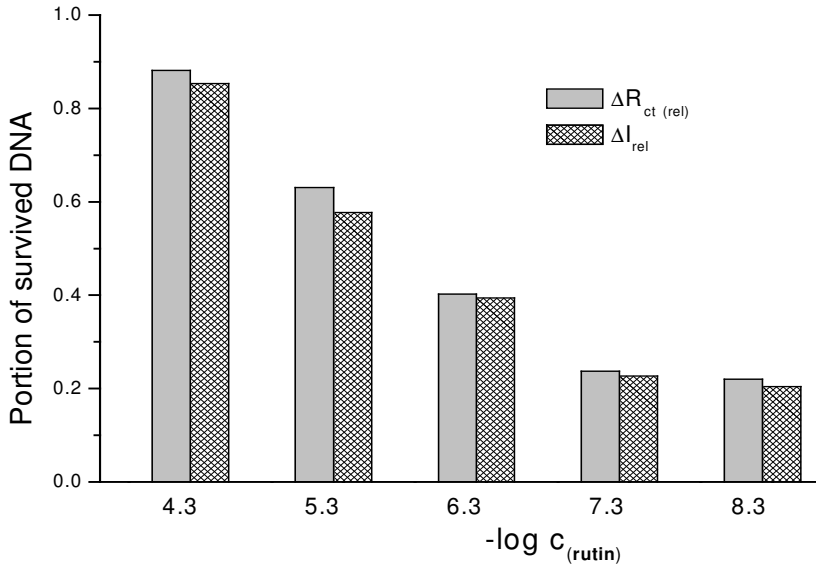

Figure 5. Antioxidantive effect of rutin on DNA damage evaluated using EIS and CV methods. Conditions: 5 min incubation of calf thymus DNA/MWNT/SPCE in the mixture of 1.0×10^{-6} M Fe^{2+}, 2.5×10^{-4} M H_2O_2 and 1.0×10^{-6} M ascorbic acid with rutin in PBS, pH 7.0 at ambient temperature under stirring.

Table 4. Evaluation of antioxidative properties of various tea extracts towards the DNA oxidative degradation using EIS and CV with 1mM $K_3[Fe(CN)_6]$ in 0.1 M PBS. Calf thymus DNA/MWNT/SPCE and the mixture of 1.0×10^{-6} M Fe^{2+}, 2.5×10^{-4} M H_2O_2 and 1.0×10^{-6} M ascorbic acid with the tea extract in 1:1 volume ratio were used. $\Delta R_{ct(rel)}$ – relative change in charge transfer resistance before and after incubation in cleavage mixture with tea extract, ΔI_{rel} – relative change in cathodic current signals before and after incubation in cleavage mixture with tea extract.

Tea	Normalized signals of survived DNA			
	$\Delta R_{ct(rel)}$ at 70 ^{0}C	ΔI_{rel} at 70 ^{0}C	$\Delta R_{ct(rel)}$ at 98 ^{0}C	ΔI_{rel} at 98 ^{0}C
Earl grey	0.587	0.678	0.675	0.658
Black	0.506	0.409	0.838	0.870
Green	0.642	0.567	0.834	0.843
Ginkgo	0.229	0.230	0.507	0.457

There are significant differences between the effects of individual extracts, mainly between that containing ginko and other types of teas. It could be concluded that, in general, they follow known antioxidant protective effect of tea extracts according to the temperature of water used for the tea extract preparation [32, 33].

During the experiments it was also confirmed that the prepared dsDNA/MWNT/SPCE sensor exhibits also good storage stability in dry conditions and ambient temperature.

4. CONCLUSIONS

The application of a nanostructured interface formed by the MWNT/SPCE electrode modification has ensured a good detection window for the impedimetric and voltammetric evaluation of the presence of dsDNA layer. This is based on a significant increase in the charge transfer resistance detected by the impedimetric method as well as a significant decrease of the voltammetric current of the negatively charged redox probe like $[Fe(CN_6)]^{3-}$ at the DNA modified electrode. The proposed biosensors represent mass-produced screen-printed non-toxic disposable chemical toxicity sensors which are cheap and simple prepared. It was demonstrated here that the proposed impedimeric and voltammeric procedures can be effectively used as for the evaluation of both the deep DNA degradation and the DNA protection towards oxidative damage by antioxidats. Commercially available equipments and software allow combine and correlate advantageously the EIS and CV measurements in one experiment. The time of analysis is significantly shorter than that for the procedures using DNA redox indicators which have to be accumulated within the DNA layer before the measurements and removed at a biosensor renewal step. Hence, the results obtained here are promising for a simple and effective evaluation of antioxidants in industrial labs and also for fast tests of DNA damage by biologically active compounds.

ACKNOWLEDGEMENT

This work was supported by the Research and Development Assistance Agency (Contract No. APVT 20-015904) and the Applied Research Project AV/4/0103/06 of the Ministry of Education of the Slovak Republic.

References

1. E. Palecek, in *Electrochemistry of nucleic acids and proteins, Towards electrochemical sensors for genomics and proteomics*, ed. by E. Palecek, F. Scheller and J. Wang, Elsevier, 2005
2. R. Ovádeková and J. Labuda, *Current Topics in Electrochemistry*, 11, (2006) 21
3. B. Pejcic and R. De Marco, *Electrochim. Acta*, 51 (2006) 6217
4. J. Wang, *Analytical Electrochemistry*, 2nd ed., Wiley/VCH, New York, 2000
5. A.J. Bard, L.R. Faulkner, *Electrochemical methods: Fundamentals and Applications*, 2nd ed., John Wiley & Sons, New York, 2001
6. A.W. Bott, *Cur. Separat.*, 19 (2001) 71
7. L. Alfonta, A. Bardea, O. Khersonsky, E. Katz and I. Willner, *Biosens. Bioelectron.*, 16 (2001) 675
8. D.D. Macdonald, *Electrochim. Acta*, 51 (2006) 1376
9. E. Katz and I. Willner, *Electroanalysis*, 15 (2003) 913

10. O.M.S. Filipe and C.M.A. Brett, *Electroanalysis*,16 (2004) 994
11. H. Zhao and H. Ju, *Electroanalysis*, 16 (2004) 1642
12. F. Patolsky, A. Lichtenstein and I. Willner, *J. Am. Chem. Soc*., 123 (2001) 5194
13. Y.T. Long, C.Z. Li, H.B. Kraatz and J.S. Lee, *Biophys. J*., 84 (2003) 3218
14. F. Lucarelli, G. Marrazza and M. Mascini, *Biosens. Bioelectron*., 20 (2005) 2001
15. F. Lucarelli, G. Marrazza and A.P.F. Turner, M. Mascini, *Biosens. Bioelectron*., 19 (2004) 515
16. A.C. Barton, S.D. Collyer, F. Davis, D.D. Gomall, K.A. Law, E.C.D. Lawrence, D.W. Mills, S. Myler, J.A. Pritchard and M. Thompson, S.P.J. Higson, *Biosens. Bioelectron*., 19 (2004) 328
17. J. Galandova, G. Ziyatdinova and J. Labuda, *Anal. Sci*., submited.
18. A. Ferancová, M. Adamovski, P. Grundler, J. Zima, J. Barek, J. Mattusch, R. Wennrich and J. Labuda, *Bioelectrochem.*, 71 (2007) 33
19. J. Labuda, M. Fojta, F. Jelen, E. Paleček, in *Encyclopedia of Sensors, Vol. 3* (*Electrochemical Sensors with DNA Recognition Layer)*, American Scientific Publishers, CA, USA, 2006
20. A. Lasia, in *Modern Aspects of Electrochemistry*, ed. By B.E. Conway, J. Bockris, and R.E. White, Kluwer Academic/Plenum Publishers, New York, 1999
21. L. Yang and Y. Li, *Biosens. Bioelectron*., 20 (2005) 1407
22. H. Peng, C. Soeller, N.A. Vigar, V. Caprio and J. Travas-Sejdic, *Biosens. Bioelectron*., 22 (2007) 1868
23. H. Huang, Z. Liu and X. Yang, *Anal. Biochem*., 356 (2006) 208
24. Y. Fu, R. Yuan, L. Xu, Y. Chai, Y. Liu, D. Tang and Y. Zhang, J. *Biochem. Biophys. Methods*, 62 (2005) 163
25. M. Wang, L. Wang, G, Wang, X, Ji, Y. Bai, T. Li, S. Gong and J. Li, *Biosens. Bioelectron*., 19 (2004) 575
26. A. Li, F. Yang, Y. Ma and X. Yang, *Biosens. Bioelectron*., 22 (2007) 1716
27. R. Ovadeková, S. Jantová, S. Letašiová, I. Štepánek and J. Labuda, *Anal. Bioanal. Chem*., 386 (2006) 2055
28. K.H. Gabriel and S. Xianglin, *Mut. Research*, 533 (2003) 183
29. J.M. McCord, *Am. J. Med*., 108 (2000) 652
30. I. Fridovich, *Ann. N. Y. Acad. Sci*., 893 (1999) 13-18
31. R. Russo, R. Acquaviva, A. Campisi, V. Sorrenti, C.Di Giacomo, G. Virgata, M.L. Barcellona and A. Vanella, *Cell Biol. and Toxicol*., 16 (2000) 91
32. A. Ferancová, L. Heilerová, E. Korgová. S. Šilhár, I. Štepánek and J. Labuda, *Eur. Food Res. Technol.*, 219 (2004) 416
33. R. Zhou, Y. Zhou, D. Chen, S. Li and A. Haug, *Toxicol. Lett*, 115 (2000) 23