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Electrochemical DNA Biosensor for Detection of Aqueous Toxicants

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Abstract: A simple procedure for the voltammetric detection of toxicants in aqueous samples based on their interaction with DNA at the electrode surface is reported. A disposable electrochemical DNA biosensor fabricated as a graphite-based screen-printed electrode modified by a surface layer of the salmon sperm double stranded (ds) DNA was used as a working electrode in combination with a graphite counter electrode and a silver pseudo–reference electrode. The interaction between DNA and toxicant was monitored by the change in the guanine signal using square wave voltammetric. The changes to the signal of guanine after exposure to the toxicants showed conformational change in DNA molecules. Benzene, naphthalene and antrachene derivatives were used as toxicant model compounds in this study. The effect of these toxicants on the surface-modified DNA was found to be linearly related to their concentration in solution. The comparison of the results with a toxicity assay based on the rate of NAD reduction has confirmed the applicability of the DNA biosensors as screening method for detection of the toxicants in aqueous samples. *Copyright © 2008 IFSA.*

Keywords: Electrochemical, Scree-printed electrode, DNA biosensor, Toxicants, Genotoxic compounds

1. Introduction

In order to develop monitoring strategies and methodologies for the characterisation of specific toxicants or pollutants (i.e. genotoxic compounds), there has been an increase in the use of nucleic acid

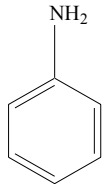
as a tool in the recognition and monitoring of many compounds of analytical interest [1, 2, 3, 4]. Nucleic acid layers combined with electrochemical transducers produce a new type of affinity biosensor for small weigth molecules. Electrochemical DNA biosensor provide a rapid and inexpensive method for the determination of DNA damage caused by chemical, drug and radiactive agents [2, 4]. The sensing principle is based on the molecular interaction between the surface linked DNA and target analyte, which in turn can be used to develop devices for a rapid screening of these compounds. Here, the signal transducer must determine the change that has occurred due to the binding molecules or to hybridization at the recognition layer, converting this into an electronic signal that then can be relayed to the end user. Comparing the pre and post electrochemical signals of a DNA-analyte interaction provides good evidence of the interaction mechanism to elucidated. Moreover, this interaction could be used for the quantification of these analyte [5, 6, 7].

The role of the nucleic acid recognition layer is to detect the changes that occurred in the DNA structure during interaction with DNA-binding molecules, or to selectively detect a spesific sequence of DNA [3, 4, 8]. There have been intensive studies of the applications of modern volatmmetric methods in nucleic acid research and DNA analysis. This is due to the fact that electrochemical DNA biosensor offer several advantages like simple immobilisation of the DNA layer (i.e. adsorption driven by a positive electrode potential), faster, low-cost measurements, simplicity, and the use of a miniaturised and portable instrument as well as hold enourmous potential application in environmental monitoring [2, 3, 5]. For instance, a DNA biosensor can be used for the testing of water, food, soil and plant samples for the presence of interest analytes (pollutants, carcinogens, toxicans, drugs, etc) with binding affinities for the structure of DNA. This binding scheme of small molecules to DNA has been described via the variation of electrochemical signal of guanine [5-7]. Toxic molecules that interact with DNA could also be detected by monitoring the change in the guanine oxidation signal in relation to electrochemical DNA biosensors [9, 10, 11, 12].

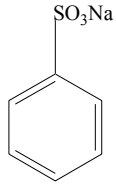
The toxicant model compounds that were used in this study are aniline (AN) and sodium benzenesulfonate (BS) as benzene derivatives, 2-naphtylamine (NA) and sodium 2-naphtalenesulfonate (NS) as naphthalene derivatives, and sodium antraquinone 2-sulfonate (QS), 1-aminoantraquinone (AQ) and 2-antramine (AT) as antrachene derivatives. The molecular structures of compounds used in this study are presented in Fig.1. These toxic compounds are well documented as carcinogen and their use in many industrial as solvent and/or precursor to more-complex chemicals. Aniline for example, it is the simplest and one of the most important aromatic amines, being used in the manufacture of polyurethane. Aniline is toxic by inhalation of the vapor, absorption through the skin or swallowing. It causes headache, drowsiness and mental confusion. Prolonged exposure to the vapor or slight skin exposure over a period of time affects the nervous system and the blood, causing tiredness, loss of appetite, headache, and dizziness [13]. These toxic compounds were used as a model in order to investigate genotoxic effect of the ring number of molecule and the type of group (i.e. amino and sulfonate) at the same molecular concentration on the surface-confined DNA. The genotoxic effect can be revealed from the interaction of DNA with the two benzenes, two naphthalenes and three anthracene derivatives used in this study.

In order to study such genotoxic effect of the model compounds, a disposable biosensor based on the immobilisation of double-stranded DNA on the surface of graphite based screen-printed electrodes (SPE) and the use of the square-wave voltametric method in order to measure guanine oxidation. Disposable or single-use sensor has several advantages, such as avoidance of contamination among samples, constant sensitivity and reproducibility and ease of use [5-7]. The disposable DNA biosensor was used to evaluate the presence of toxicant DNA-binding compounds by measuring changes of electrochemical signal of guanine in salmon sperm DNA extract. Some preliminary experiments showing clear electrochemical effect due to presence of toxicant compounds, which can be extrapolate and evaluate from potentiallly genotoxic compounds present in the samples. Then the application of

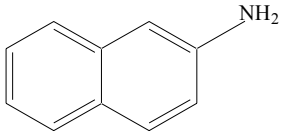
such DNA biosensor was evaluated by analysing the real water samples along with comparison with other toxicity assay.



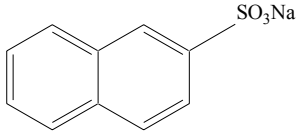
Aniline (AN)



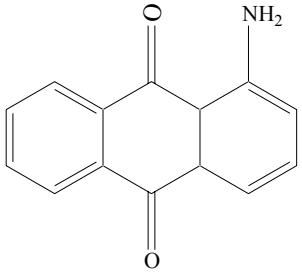
Sodium benzenesulfonate (BS)



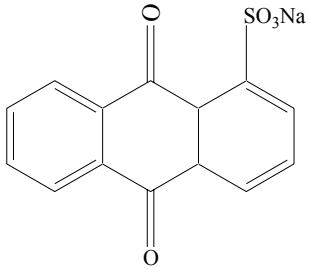
2-naphtylamine (NA)



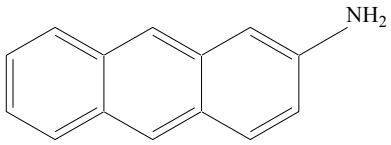
Sodium 2-naphtalenesulfonate (NS)



1-aminoantraquinone (AQ)



Sodium anthraquinone 2-sulfonate (QS)



2-anthramine (AT)

Fig. 1. Structure of compounds used as toxicant model.

2. Experimental

2.1. Materials

Double stranded salmon sperm DNA (dsDNA) was purchased from Fluka. The stock solution of 1000 mg/L dsDNA was prepared with TE solution (10 mmol/L Tris HCl, 1 mmol/L EDTA, pH 8) and kept it frozen. More dilute solutions were prepared with 0.50 mol/L acetate buffer (pH 4.8). Inorganic salts for buffers preparation (sodium acetate, potassium chloride, sodium chloride, acetic acid, sodium and dehydrogen phosphate) and ethanol were obtained from Merck. The toxicant model compounds aniline, sodium benzenesulfonate, 2-naphtylamine, sodium 2-naphtalenesulfonate, sodium antraquinone 2-sulfonate, 1-aminoantraquinone and 2-antramine were purchased from Sigma. All aqueous solutions were prepared using sterilised and double distillate water.

2.2. Instrumentation

Electrochemical measurements were performed with a PalmSens Electrochemical Portable Apparatus (Palm Instrument BV, Houten, The Netherlands), which is controlled by a Pocket PC (Compaq iPAQ). The PalmSens Instrument is used for sensors or cells with two or three electrodes and the dynamic range allows applications as micro as well as macroelectrodes. The Pocket PC software (a PalmScan 1.3 Software package) provides easy control of PalmSens.

A disposable biosensor was based on the immobilized double-stranded DNA on the surface of carbon based Screen-Printed Electrode (SPE) (gift from Prof. M. Mascini Univ. Florence, Italy). The electrochemical cell consisted of a graphite working electrode of 3 mm, a graphite counter electrode and a silver pseudo electrode. These devices are produced in foiled of twenty electrodes each. Because of the mass-production and the low-cost, these devices can be used as disposables. Here, each electrode was used disposable for one measurement only. The detail on the SPE was reported in literature [14].

2.3. DNA Biosensor Procedure

The preparation and the following measurement of the interaction at the DNA-modified SPE in room temperature include four steps as slightly modification procedure from [14]:
(1) Electrode activation: a surface conditioning step is necessary to oxidize the graphite impurities and to obtain a more hydrophilic surface to favour DNA immobilization (+1.6 V vs. Ag SP pseudo-reference electrode for 2 min and +1.8 V for 1 min; electrodes were in 5 ml of 0.5 M acetate buffer containing 10 mM NaCl, pH 4.8, under stirred conditions).
(2) DNA immobilisation: in this step DNA is electrochemically accumulated and adsorbed onto electrode surface by applying a positive potential able to attract negative charged groups of DNA (activated SPE is dipped in a solution of 50 mg/L salmon sperm ds-DNA in 0.5 acetate buffer with 10 mM NaCl, applying a potential of 0.5 V versus Ag-SPE for 5 minutes, under stirred conditions).
(3) Blank and sample interaction: in this step, the response of the guanine signal before (reference signal) or after interaction is evaluated (the DNA-modified SPE is dipped for 2 minutes in a solution containing the analyte dissolved in a buffer solution similar to the blank solution).
(4) Measurement: a square wave voltammetric (SWV) scan is carried out to evaluate the oxidation of quinine residues on the electrode surface (the height of the guanine peak at +0.95 V vs Ag-SPE was measured in 0.5 M acetate buffer, containing 10 mM NaCl, using the following parameters: scan from +0.6 V to +1.2 V, E_{step} = 15 mV, $E_{amplitude}$ = 40 mV, Frequency = 200 Hz).

The four steps DNA biosensor procedure can be summarised as given in Fig. 2. The guanine oxidation peak was used as the transduction signal in this detection system of DNA interaction agents. As a result interaction of ds-DNA with a toxicant agent a decrease of guanine peak (measured by SWV) was detected. DNA interaction was estimated by the percentage change in the guanine peak height (Signal, *S%*), which was taken as the ratio of the guanine peak height after interaction with a sample (S_s), to the guanine peak height in buffer solution or blank (S_b) as given in equation (1).

$$S\% = (S_s/S_b) \times 100\% \quad (1)$$

Potential genotoxic compounds present in water sample can be evaluated simply by changes of the electrochemical signal of guanine using eqn. (1) as the percentage decrease (*S%*), which is the ratio of the guanine peak height after the interaction with the analyte and the guanine peak height after the interaction with blank solution.

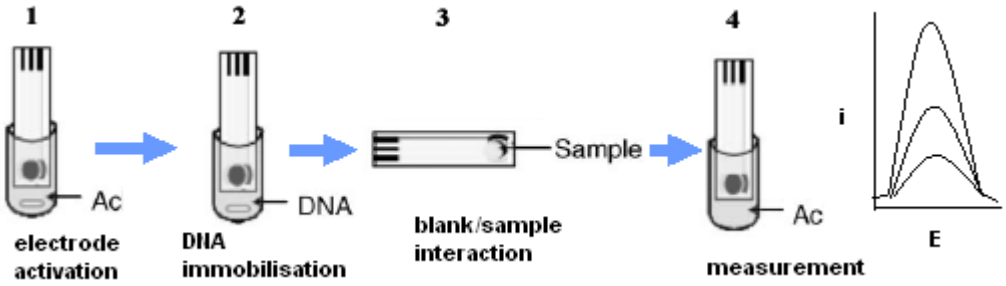


Fig. 2. Schematic drawing of electrochemical DNA biosensor procedure, which consisted in four steps: (1) electrode activation, (2) DNA immobilization on electrode surface, (3) blank or sample interaction, (4) measurement, Ac acetate buffer.

2.4. Sub-Mitocondrial Particle Assay

The sub-mitochondrial particles (SMP) electron transfer inhibition assay is an enzyme in-vitro screening test based on the effects of toxic compounds on the NADH oxidation by SMP. The electron transfer (ET) test allows normal forward electron flow along a proportion of the oxidative pathway, and monitors spectrophotometrically the decrease in the light absorption of the NADH at 340 nm. The absorbency can be related to the rate of enzymatic conversion NADH and to detect retardation of the reaction caused by toxic substances [15]. Here, SMP derived from beef heart were purchased from MitoScan Corp. (Madison, USA). In order to increase sensitivity of SMP, a modified spectrophotometric method measured the rate of NAD reduction in a reverse electron transfer (RET) test at 340 nm was employed also known as SMP reverse reaction [16, 17]. The effluent samples were tested at 70% v/v and the results were compared with a negative control (buffer/blank) solution.

2.5. Water Sample Description

The river water sample from river water of Bedadung (Jember) and the waste water sample from effluent of paddies field in Jember area. River water samples and waste water samples were pre-concentrated by passing 1 l of water sample through an Isolute^{TM} column solid-phase extraction. The extracted organic compounds were eluted using 0.1 mL ethyl acetate. The samples obtained were dried, and then 5 ml of a 2% NaCl solution was added in order to adjust to the neutral range. The final sample solutions were ready to be tested by DNA biosensor and SMP assay procedure. Samples composition was described in Table 1. Each batch comprises river water and wastewater samples. Sample preservation was accomplished by storing bottles at 4^{o}C immediately after sampling and during the transportation.

Table 1. Composition of the seven batches of analyzed samples.

	A	B	C
BATCH 1	AN	R1	S1
BATCH 2	BS	R2	S2
BATCH 3	NA	R3	S3
BATCH 4	NS	R4	S4
BATCH 5	QS	R5	S5
BATCH 6	AQ	R6	S6
BATCH 7	AT	R7	S7

AN: water solution containing aniline 100 μmol/L;
BS: water solution containing sodium benzenesulfonate 100 μmol/L,
NA: water solution containing 2-naphtylamine 50 μmol/L
NS: water solution containing sodium 2-naphtalenesulfonate 100 μmol/L,
QS: water solution containing sodium antraquinone 2-sulfonate 10 μmol/L,
AQ: water solution containing 1-aminoantraquinone 5 μmol/L,
AT: water solution containing 2-antramine 1.0 μmol/L, R1-R7 = river water samples from river side 1 to side 7, S1-S7 = waste water from effluent of paddies field 1 to 7.

3. Results and Discussion

3.1. Optimization of Experimental Parameters

Preliminary studies were performed to identify general assay conditions which affected the electrochemical signal of the guanine oxidation peak, like ionic strength, pH, buffer composition and double-stranded DNA concentration. Table 2 reports the optimization results of experimental parameters. The modified electrode performance was tested in a buffer solution containing methanol up to 10% (v/v) and we did not observe any variation of the electrochemical signal of guanine peak. Methanol is useful for dissolving some organic compounds.

Table 2. The optimisation of experimental parameters.

Parameter	Optimization range	Selected value
Buffer composition	phosphate, citrate, acetate	acetate
pH	4.0-8.0	4.8
Ionic strength	0.1 – 1.0 M	0.5 M
dsDNA concentration	10-80 (mg/l)	50 mg/l

The guanine peak was sharp in acetate buffer; the baseline at this value was lower than other buffer. The guanine peak height obtained as function of pH, shows that the height increase linearly with pH value up to 4.8 and then tend to constant. While for ionic strength (in this case acetate buffer was used) 0.5 M shows to be an optimum value. The guanine peak height obtained as function of double-stranded salmon sperm concentration. The height increases linearly with concentration up to 20 mg l^{-1} and then levels off. These values were generally used for further measurements.

3.2. DNA Biosensor Response

The effects of two benzene, two naphthalene and tree anthracene derivatives were investigated with the dsDNA-modified SPE biosensors using guanine signal. The SWV oxidation signals of guanine obtained with the dsDNA-modified SPE biosensors before and after exposure to 1-aminoantraquinone (5 μM) and 2-antramine (5 μM) standard solution is given in Fig. 3. The decrease in the guanine signals as indicated damage in the oxidation of guanine [1,18]. Different concentrations for each toxicant compound were investigated and the inhibition of the guanine oxidation peak (S %) increased with increasing in toxicants concentration (Figs. 4-6).

The results in Figs. 4-6 demonstrated that the toxicant compounds have different genotoxic effect. In general, the effect was higher with increased in ring number of the molecule (order of increasing ring number and genotoxic effect, Fig. 6 > Fig. 5 > Fig. 4). In this case 2-anthramine (with 3 rings) (Fig. 6) has the highest inhibition of the guanine peak (S%) compared to the rest. This is due to the fact that

naphthalene and antracene derivatives investigated are DNA intercalating agents [7,19], while the benzene derivatives are only a weak DNA interactive [19], and not DNA intercalating agents. The type of the group also had different genotoxic effect. In this case, the amino group was more toxic than a sulfonate one at the same molecular concentration as given in Figs. 4 and 5.

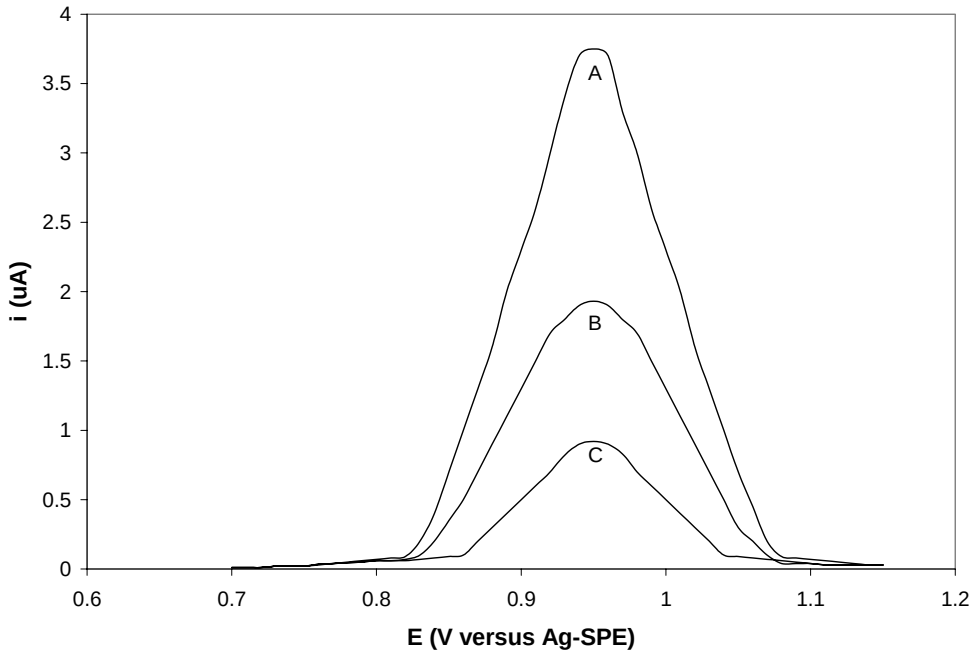


Fig. 3. Square wave voltammogram of a SPE containing 20 mg l^{-1} dsDNA (a) and 3 µl of a solution containing 1-aminoantraquinone (5 µM) and 2-antramine (5 µM). Supporting electrolyte 0.5 M acetate buffer pH 4.8.

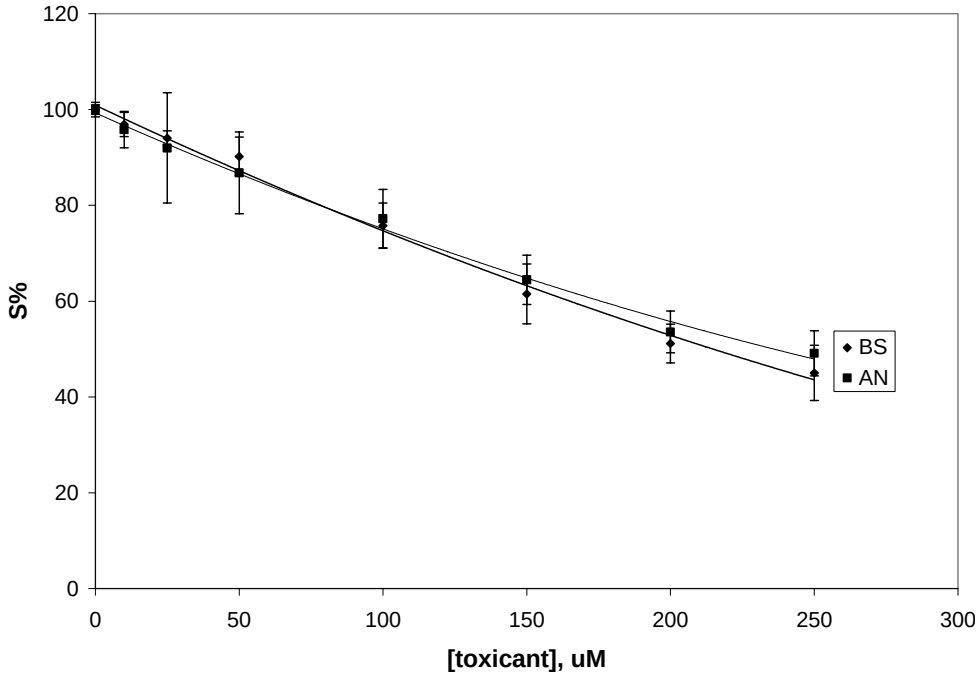


Fig. 4. S% obtained with increasing concentration of aniline (AN) and sodium benzenesulfonate (BS) in 0.5 M acetate buffer (pH 4.8) and interaction time 2 minutes.

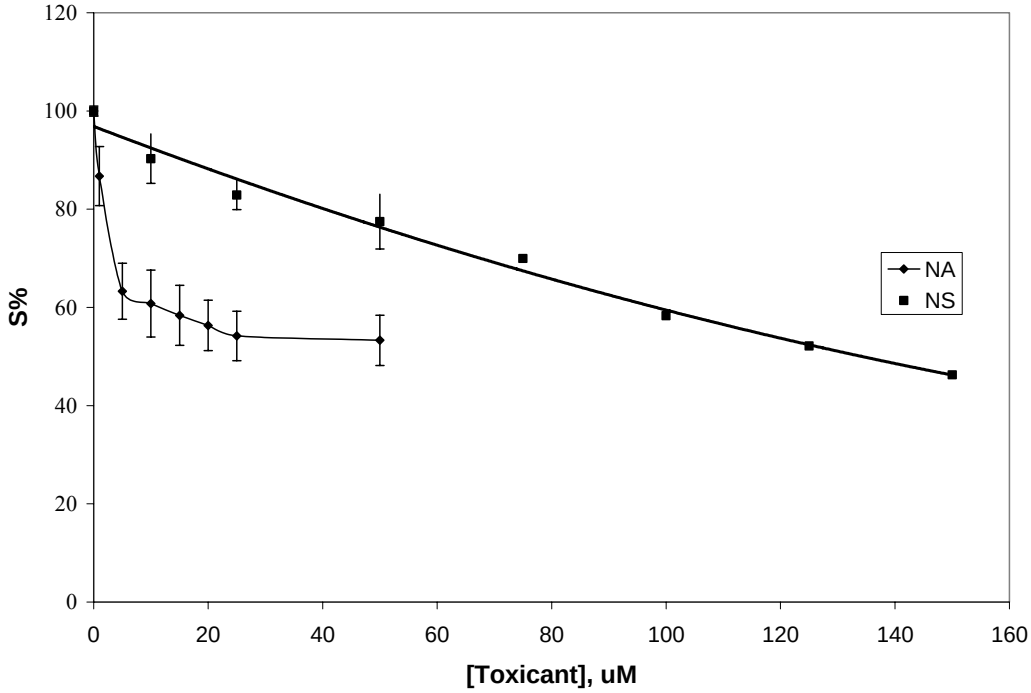


Fig. 5. S% obtained with increasing concentration of 2-naphtylamine (NA) and sodium 2-naphtalenesulfonate (NS) in 0.5 M acetate buffer (pH 4.8) and interaction time 2 minutes.

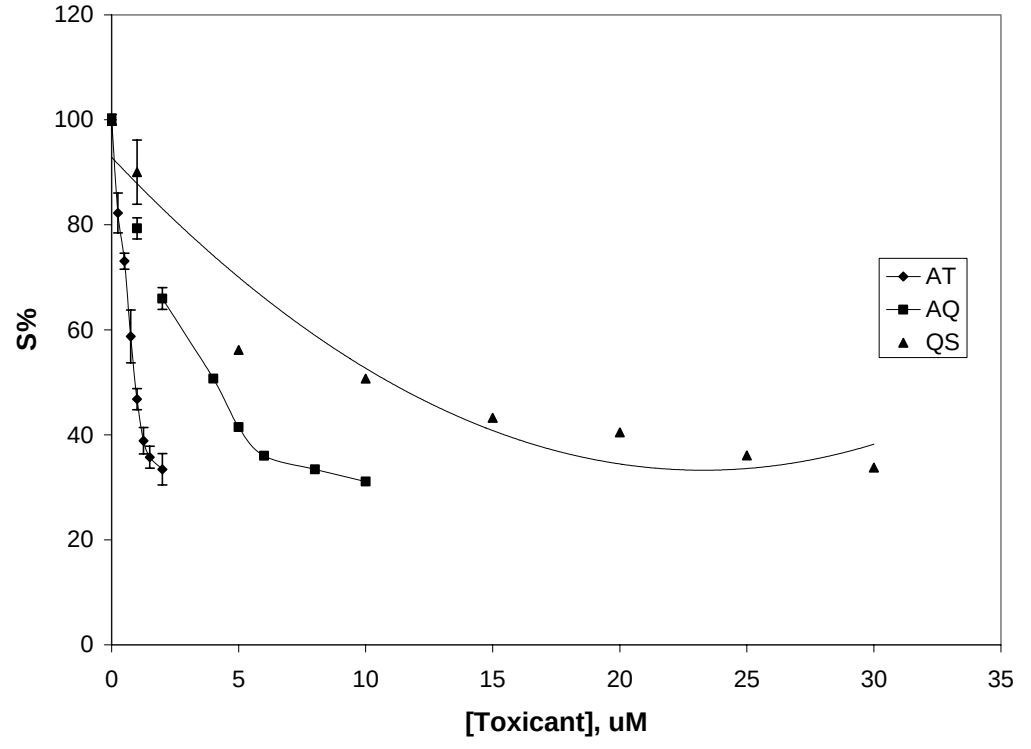


Fig. 6. S% obtained with increasing concentration of 1-aminoantraquinone (AQ), sodium anthraquinone 2-sulfonate (QS) and 2-anthramine (AT) in 0.5 M acetate buffer (pH 4.8) and interaction time 2 minutes.

Figs. 4 and 5 revealed that naphthalene-sulfonate derivatives with two and three aromatic rings and aromatic amines with two and tree rings gave a positive result with the DNA biosensor in a concentration range of micro and submicro-molar. The EC_{50} value (50% toxicity) was 4.0 μM for 1-aminoantraquinone and 1.0 μM for 2-anthramine.

3.3. Analysis of Water Samples

Applicability to the analysis of water samples is also illustrated in Table 3. A decrease of the guanine peak height could be detected with some water samples that have been spiked with toxic compounds and in river water samples as well as waste water samples. By using signal response of the biosensor as given in eqn. 1 to measure toxicity of water sample tested, the toxicity criteria [20] can be categorised as follows:

$$S > 85\% = \text{non toxic}; 50\% < S < 85\% = \text{moderately toxic} ; S < 50\% = \text{toxic} \quad (2)$$

The results of the test of analyzed water samples are presented in Tables 3 and 4. These results indicate that the DNA biosensor constitute a valid platform for detecting toxicity. This is due to the fact that the DNA biosensor was able to discriminate between the toxic and non-toxic samples, indicated a low and high presence of molecules with binding affinity for the DNA. The comparison study suggests a reasonably good level of agreement with SMP assay. It also shows that the DNA biosensor has a higher sensitivity than SMP assay in this case. Furthermore, the DNA biosensor represents a significant improvement for toxicity measurements and environmental monitoring, where this biosensor providing simple, sensitive, rapid and low-cost to perform toxicity screening task in the field.

Table 3. Results of analyzed samples using DNA biosensor and SMP assay.

	A **DNA sensor (S%) SMP(A*)**	**B** **DNA sensor (S%) SMP(A*)**	**C** **DNA sensor (S%) SMP(A*)**			
BATCH 1	77.23 ± 6.11	0.93± 0.08	65.25 ± 5.69	0.85 ± 0.10	73.08 ± 1.53	0.86 ± 0.08
BATCH 2	75.76 ± 4.73	0.88± 0.06	58.73 ± 5.03	0.73 ± 0.08	56.15 ± 6.69	0.71 ± 0.04
BATCH 3	53.29 ± 5.13	0.68± 0.04	56.15 ± 7.11	0.69 ± 0.06	46.39 ± 2.00	0.53 ± 0.06
BATCH 4	58.29 ± 3.61	0.73± 0.05	46.79 ± 2.03	0.56 ± 0.08	41.64 ± 2.00	0.47 ± 0.08
BATCH 5	50.71 ± 5.13	0.65± 0.06	55.81 ± 5.15	0.68 ± 0.06	40.46 ± 8.14	0.43 ± 0.05
BATCH 6	41.44 ± 2.10	0.45± 0.04	38.86 ± 2.08	0.42 ± 0.07	50.78 ± 5.03	0.66 ± 0.09
BATCH 7	46.79 ± 2.13	0.54±0.07	43.26 ± 6.11	0.51 ± 0.04	51.16 ± 4.04	0.68 ± 0.08

*Absorbance

Table 4. Results of simplified assessment of toxicity.

	A DNA sensor	SMP	B DNA sensor	SMP	C DNA sensor	SMP
BATCH 1	MT	NT	MT	NT	MT	NT
BATCH 2	MT	NT	MT	MT	MT	MT
BATCH 3	MT	MT	MT	MT	T	MT
BATCH 4	MT	MT	T	MT	T	T
BATCH 5	T	MT	MT	MT	T	T
BATCH 6	T	T	T	T	MT	MT
BATCH 7	T	MT	T	MT	MT	MT

Note: NT = non toxic; MT = moderate toxic and T = Toxic. Using equal criteria with DNA biosensor SMP can also be classed as A > 0.85 = NT; 0.50 < A < 0.85 = MT; A < 0.50 = T. Since the maximum and minimum absorbance in this case 1.00 to 0.00.

4. Conclusion

In the present work, electrochemical DNA biosensors are demonstrated for the detection of toxicant in aqueous samples. The results show that the DNA biosensor could be a very good test, since it can give sensitive, rapid and easy information to evaluate the presence of genotoxic compounds with the affinity for nucleic acids. This is also indicated by good agreement with SMP assay for the results of the real water samples analyzed. The genosensors may represent an easy and rapid way of analysis of toxic or polluted area, particularly for field applications, being one of the most competitive tools in term of time and cost of analysis. Furthermore, such DNA biosensor could be useful as early warning device for environmental monitoring.

Acknowledgement

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Guide for Contributors

Aims and Scope

Sensors & Transducers Journal (ISSN 1726-5479) provides an advanced forum for the science and technology of physical, chemical sensors and biosensors. It publishes state-of-the-art reviews, regular research and application specific papers, short notes, letters to Editor and sensors related books reviews as well as academic, practical and commercial information of interest to its readership. Because it is an open access, peer review international journal, papers rapidly published in *Sensors & Transducers Journal* will receive a very high publicity. The journal is published monthly as twelve issues per annual by International Frequency Association (IFSA). In additional, some special sponsored and conference issues published annually.

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- Sensor instrumentation;
- Virtual instruments;
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