Available online at www.sciencedirect.com
SCIENCE DIRECT
Talanta 69 (2006) 365–369
Talanta
www.elsevier.com/locate/talanta

A comparison study between a disposable electrochemical DNA biosensor and a *Vibrio fischeri*-based luminescent sensor for the detection of toxicants in water samples

Anca Monica Tencaliec [a], Serena Laschi [b], Vasile Magearu [a], Marco Mascini [b,*]

[a] *University of Bucharest, Faculty of Chemistry, Department of Analytical Chemistry, Blvd. Regina Elisabeta 4-12, 703461 Bucharest, Romania*
[b] *Università degli studi di Firenze, Dipartimento di Chimica, Via della Lastruccia 3, 50019 Sesto Fiorentino, Firenze, Italy*

Received 21 February 2005; received in revised form 26 May 2005; accepted 29 September 2005
Available online 9 November 2005

Abstract

In the present study, a comparison between a disposable electrochemical DNA biosensor and a *Vibrio fischeri*-based luminescent sensor for the detection of toxicants in water samples was made.

In order to realize this study, a disposable electrochemical DNA biosensor has been reported. The DNA biosensor is assembled by immobilizing double stranded *Calf Thymus* DNA onto the surface of a disposable carbon screen-printed electrode. The oxidation signal of the guanine base, obtained by a square wave voltammetric scan, is used as analytical signal to detect the DNA damage; the presence of low molecular weight compounds with affinity for nucleic acids is measured by their effect on the guanine oxidation peak.

Wastewater samples provided during First European Interlaboratory Exercise on water toxicity in the course of the project SWIFT-WFD were analyzed, and biosensor results were compared with a currently used toxicity test ToxAlert®100 based on the bioluminescence inhibition of *Vibrio fischeri*. This test have been used because is rapid, easy handling and cost effectively responses for the toxicity assessment in real water samples.

The results showed a promising correlation between two tests used for the detection of toxic compounds in water samples.

Keywords: Electrochemical DNA biosensor; Toxicity; Screen-printed electrode; Toxalert®100

1. Introduction

The amount of chemicals released into the environment has grown enormously over recent decades. These chemicals may undergo biochemical or chemical transformations leading to new compounds of unknown toxicity. Additionally, some of them may be introduced into the trophic chain and can be bioaccumulated in organisms. These facts have created serious concerns regarding their adverse effects on the ecosystem and public health [1]. In this sense, for example, for a rapid wastewater toxicity assessment, several toxicity procedures are currently used. Numerous biological techniques have been developed as toxic detection systems [2–6].

Toxicity tests using biological responses to measure effects provide valuable information about the significance of chemical contamination. The response of an organism to contaminated effluents provide an integrated measure of effects that reflects the combined action of all material presents in the effluent composition and also take in consideration other factors that affects the whole toxicity (such as synergistic or antagonistic effects) [7]. Most of these biological toxicity tests are expensive, require large sample volumes and usually take more than 24 h [8,9].

In recent years, there has been a huge increase in the use of nucleic acids as a tool in the recognition and monitoring of many toxic compounds of analytical interest. Nucleic acids layers in association with electrochemical or optical transducers produce a new kind of affinity biosensors for small molecular weight molecules, which can be investigated by chronopotentiometric or voltammetric analysis.

In order to develop devices for a rapid screening of these compounds, molecular interaction between the DNA immobilized and the target pollutants or drugs were studied [10,11]. Many small molecules show a high affinity for DNA and they can interact with nucleic acids; these interactions could have either

* Corresponding author. Tel.: + 39 055 457 3283; fax: +39 055 457 3384.
E-mail address: marco.mascini@unifi.it (M. Mascini).

0039-9140/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.talanta.2005.09.042

Table 1
Composition of the six batches of analysed samples

	A	B	C	D	E	F
BATCH 1	3,5 DCP	Phenol	Zn-sulphate	Influent	Effluent	Fortified
BATCH 2	3,5 DCP	Phenol	Zn-sulphate	Influent	Effluent	Fortified
BATCH 3	Phenol	Influent	Fortified	Zn-sulphate	Effluent	3,5 DCP
BATCH 4	Effluent	Fortified	Zn-sulphate	3,5 DCP	Influent	Phenol
BATCH 5	Influent	Fortified	Effluent	Zn-sulphate	Phenol	3,5 DCP
BATCH 6	Fortified	Effluent	Influent	Phenol	3,5 DCP	Zn-sulphate

3,5 DCP: water solution containing 3,5-Dichlorophenol 50 mg/L; phenol: water solution containing phenol 60 mg/L; Zn-sulphate: water solution containing Zn-sulphate 50 mg/L; influent: row influent of a WWTP (WasteWater Treatment Plant); effluent: final effluent of a WWTP; fortified: first settlement of a WWTP fortified with phenol and Zn-sulphate.

favorable or adverse effects in life science related to the replication and transcription of DNA in vivo, mutation of gene, action mechanism of some synthetic chemical nucleases and molecular analysis [12]. The binding of small molecules to DNA and generally DNA damage has been described through the variation of electrochemical signal of guanine [13].

In literature, electrochemical DNA biosensors were already proposed as screening tools for environmental toxicants [14–20]; thus, using electrochemical DNA biosensors interaction of several toxic compounds with DNA have been also studied [21–28].

In this paper, we have reported the results obtained by using a disposable electrochemical DNA biosensor for the detection of toxicants in water samples, which were also analyzed by the commercial toxicity test Toxalert®100, which is based on the bioluminescence inhibition of the bacterium *Vibrio fischeri*. The *V. fischeri* bioluminescence test is a standard toxicity test widely recognized in many legislation [29]. This test is directly linked to the vitality (the metabolic status) of the bacterial cell. A toxic substance cause changes to the cellular state, damage to the cell wall, cell membrane, the electron transport chain, enzymes, cytoplasmatic constituents; these changes are rapidly reflected in a decrease in the bioluminescence signal that can be measured with a photomultiplier in a luminometer. More than 20 aquatic toxicity tests are reported in the 20th edition of the Standard Methods for the Examination of Water and WasteWater [30]. However, the Toxalert® 100 assay was chosen here since bacterial bioluminescence assay are rapid, reproducible and cost effective tests.

Results obtained for the analyzed samples with the two methods were reported and compared here.

2. Material and methods

2.1. Apparatus and reagents

Electrochemical measurements were performed with a PalmSens Electrochemical Portable Apparatus (Palm Instrument BV, Houten, The Netherlands), which is controlled by a Pocket PC. 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 provides easy control of PalmSens.

The screen-printed electrochemical cells are produced at the University of Florence. They consist of a three-electrodes cell composed by a circular graphite working electrode (diameter of 3 mm), a pseudo reference electrode and a graphite counter electrode. The procedure and the reagents to make disposable screen printed electrodes were reported in literature [31]. These planar electrochemical cells can be used as “drop and sensors” and only 10 μL of sample solution is required to perform the measurements.

The luminometer Toxalert®100 and all the reagents to perform cytotoxicity tests were kindly provided from Merck (Darmstadt, Germany). Bacteria *Vibrio Fisheri* as well reconstituent reagents were provided from Merck (Darmstadt, Germany).

Double stranded calf thymus DNA was purchased from Sigma (Milan, Italy); sodium acetate, potassium chloride, sodium chloride, acetic acid, ethanol, sodium dihydrogen phosphate were provided from Merck (Darmstadt, Germany).

2.2. Samples description

Water samples analyzed were provided during First European Interlaboratory Exercise on water toxicity by bioluminescence tests organized by the European Union funded project “Screening methods for water data InFormaTion in support implementation of the Water Framework Directive” (SWIFT-WFD). During the Interlaboratory exercise, six batches of six samples each one were distributed to all the participants.

Samples composition was described in Table 1: each batch comprises row influent of a WWTP (WasteWater Treatment Plant), final effluent of a WWTP and fortified samples. Samples spiked with toxicants as well as 3,5 DCP, phenol and Zn-sulphate are also present: 3,5 DCP (3,5-Dichlorophenol) is an organic substance commonly used as toxicity standard with high toxic level and low dispersion values [32]. Phenol is an acute toxic organic substance; it is a common used toxicity standard due to their stability at low dispersion degree [33]. Zn-sulphate is a heavy metal solution, his toxicity being highly influenced by matrix effect, conditions and concentration [7].

All the samples were analyzed using electrochemical DNA biosensor and commercial device Toxalert®100. Each sample was tested three times with both methods by diluting it as 45.45%, 11.36% and 2.84% in water adjusted to NaCl 2%. The procedure followed for the two methods are reported in next two sections.

2.3. DNA biosensor

Before the DNA immobilization, the electrode surface of the screen-printed sensor was pretreated by applying a potential of +1.6 V for 2 min and a potential of +1.8 V for 1 min, following the procedure previously reported in literature [34].

The biosensor was then assembled by immobilizing double stranded calf thymus DNA at fixed potential (+0.5 V versus Ag screen printed pseudo-reference electrode, for 300 s) onto the screen-printed electrode surface. During the immobilization step, the strip was immersed in acetate buffer solution containing 50 ppm of double stranded calf thymus DNA. Then a washing step was performed by immersion of the biosensor in a clean acetate buffer solution for 30 s, at open circuit condition. The interaction step was performed just by placing 10 μL of the sample on the electrode surface of the DNA biosensor. After 2 min the biosensor was washed, immersed in acetate buffer and a square wave voltammetric scan was carried out to evaluate the oxidation of guanine peak on the electrode surface [34]. The area of the guanine peak (around +1 V versus Ag screen printed pseudo-reference electrode) was measured. Potentially toxic compounds present in water were evaluated by changes of the electrochemical signal of guanine.

The DNA modification was estimated by means of the percentage of the response decrease (S%), which is related to the ratio of the guanine peak area after the interaction with the analyte (GPA_s), and the guanine peak area after the interaction with buffer solution (GPA_b), in accordance with the following equation:

$$S\% = \left[1 - \left(\frac{GPA_s}{GPA_b}\right)\right] \times 100.$$

This equation was already reported in literature by [11], but in our case it was changed in the mathematical sign to be homogeneous with Toxalert® results. The results of the test for one sample can be obtained within 10 min.

According to the Toxalert®100 supplier's notes, only inhibition values over 20% are attributed to a toxic compound [35].

The supporting electrolyte for the voltammetric experiments and for any step in the biosensor set up was acetate buffer 0.25 M pH 4.7, KCl 10 mM.

Square wave voltammetric parameters were: frequency = 200 Hz, step potential = 15 mV, amplitude = 40 mV, potential range +0.2 / +1.2 V versus Ag, pseudo-reference electrode.

2.4. Toxalert®100 procedure

In all the experiments the osmolality of all standard and sample solutions were adjusted to 2% NaCl for optimal reagent performance. To express the toxicity we have used the percentage of inhibition (I%), determined by comparing the response given by a saline control solution to that corresponding to the sample as a function of incubation time. For all the experiments we used an incubation time of 5 min. Therefore the bioluminescence inhibition is determined by:

$$I\% = \left[\frac{(I_{oc} - I_f)}{I_{oc}}\right] \times 100$$

where I_{oc} is the corrected value of luminescence intensity of the control test suspension in relative luminescence unit (RLU) and I_f is the luminescence intensity of the test sample after the contact time of 5 min in RLU [35].

3. Results and discussion

The results obtained with the DNA biosensor and the Toxalert®100 for the six batches of analyzed samples are reported in Table 2. The reproducibility obtained with the DNA biosensor for three repetition was always of %R.S.D. = 10%, whereas a %R.S.D. less that 8% for each sample was obtained for Toxalert®100 (data not reported in the table).

As it can be seen, all samples containing 3,5 DCP and the influent waters were indicated as not detectable (n.d.) using the DNA biosensor. These samples were found electroactive in the potential range of the guanine peak of DNA; then, they could not be analyzed using electrochemical DNA biosensor. Nevertheless, as reported in the table, these samples had a very strong effect on the bioluminescence of *V. fischeri*.

For the effluent samples we observed that these samples did not cause any significant decrease of guanine oxidation peak of DNA: also the inhibition of the bacteria bioluminescence was not important. In conclusion, the effluent samples contained compounds, which were not toxic to both DNA biosensor and bacteria cell.

Analyzing the samples containing phenol, it can be seen the effect of this compound on the DNA biosensor response; it means a significant decrease of the guanine residues signal inducing DNA damage. This compound had also an effect on bacteria cells, inhibiting the bacteria bioluminescence. So we can conclude that phenol is a toxic substance, which affect the response both of DNA biosensor and Toxalert®100 device.

Looking at the samples containing Zn-sulphate, they didn't present any effect on both tests used. So this compound is not toxic for DNA or bacterial cell.

Finally, samples fortified with both phenol and Zn-sulphate showed a moderate toxicity. The results obtained for these samples presented the same trend in both cases for DNA biosensor and Toxalert®100.

Fig. 1 shows the correlation between the data obtained with the two methods for the sample batch 1. The method of least squares mean was used to statistically evaluate this correlation for a number of data $n = 12$. The "ideal" correlation is defined by a line of regression with a slope of 1 and an intercept of 0 [36,37]. Statistical tests on the data at the 95% significance level were performed; the analysis generated a line with the following equation and correlation coefficient: $(0.68 \pm 0.06)x + (7.47 \pm 1.87)$, $r = 0.961$. Thus, a trend between the two methods was found, but a non-linear correlation is present. The 95% confidence bands show also that the accuracy could be better. Similar considerations can be made for the other five sample batches.

Table 2
Results obtained with DNA biosensor and Toxalert® 100 for all the batches analysed

	Dilution (%)	A		B		C		D		E		F	
		DNA biosensor (*S*%)	Toxalert® 100 (*I*%)	DNA biosensor (*S*%)	Toxalert® 100 (*I*%)	DNA biosensor (*S*%)	Toxalert® 100 (*I*%)	DNA biosensor (*S*%)	Toxalert® 100 (*I*%)	DNA biosensor (*S*%)	Toxalert® 100 (*I*%)	DNA biosensor (*S*%)	Toxalert® 100 (*I*%)
Batch 1	45.45	n.d.	99	60	70	7	7	n.d.	99	21	21	43	56
	11.36	n.d	59	33	42	7	8	n.d.	52	15	1	23	15
	2.84	n.d.	9	14	16	15	6	n.d.	14	11	0	8	4
Batch 2	45.45	n.d.	99	56	64	10	4	n.d.	99	11	6	22	16
	11.36	n.d.	63	13	35	5	0	n.d.	43	6	0	4	3
	2.84	n.d.	16	5	13	0	0	n.d.	6	4	1	0	0
Batch 3	45.45	62	64	n.d.	99	35	45	5	2	15	6	n.d.	99
	11.36	24	31	n.d.	37	20	11	3	1	10	0	n.d.	62
	2.84	4	8	n.d.	0	6	0	0	0	5	0	n.d.	10
Batch 4	45.45	18	4	37	34	34	45	n.d.	99	n.d	99	69	74
	11.36	10	8	12	11	21	15	n.d	44	n.d.	47	48	43
	2.84	6	7	0	2	7	1	n.d.	10	n.d.	12	20	16
Batch 5	45.45	n.d.	99	32	38	21	4	47	53	62	74	n.d.	99
	11.36	n.d	43	16	11	14	2	23	14	41	43	n.d.	63
	2.84	n.d.	3	0	1	0	1	10	1	15	15	n.d.	13
Batch 6	45.45	36	49	13	1	n.d.	93	55	76	n.d.	99	37	44
	11.36	23	17	5	5	n.d.	40	27	45	n.d.	70	13	14
	2.84	9	7	0	5	n.d.	10	10	14	n.d.	18	0	2

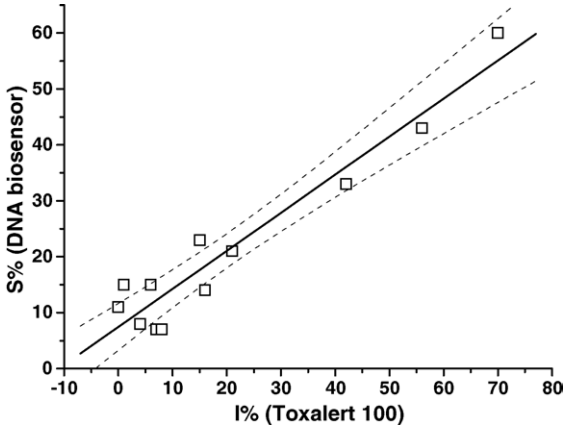

Fig. 1. Correlation between DNA biosensor and Toxalert®100 results obtained for the batch 1.

Nevertheless, analyzing the results obtained for the DNA biosensor and the Toxalert®100, a difference between the two methods was expected; the DNA biosensor response indicates mainly the interaction of DNA with some chemicals, which decrease the guanine oxidation capability. The Toxalert®100 response is more complex, since the metabolic activity of the bacterial cell is involved. Nevertheless the results on samples analysis showed a promising correlation between the two tests.

4. Conclusions

The results show that there is a promising correlation between the results obtained using electrochemical DNA biosensor and commercial device Toxalert®100 on the water samples. The DNA biosensor could be a very good test since can give rapid and easy information to evaluate the presence of small compounds with the affinity for nucleic acids.

Electrochemical DNA biosensors offer other several advantages like an easy immobilization of the DNA layer, fast measurement, portable instrumentation suitable for measurements in situ, in field screening analysis of toxic compounds and cost effective, moreover the disposable single use biosensor avoids contamination among samples and allows constant sensitivity and reproducibility.

References

[1] M.P. Marco, D. Barcelo, Meas. Sci. Technol. 7 (1996) 1547.
[2] D.I. Mount, L. Anderson-Carnahan, Methods for Aquatic Identification Evaluations, EPA/600/3-88/034, US Environmental Protection Agency, Duluth, 1988.
[3] H.C. Sarakinos, N. Bermingham, P.A. White, J.B. Rasmussen, Environ. Toxicol. Chem. 19 (2000) 63.
[4] Q. Zhang, J.C. Crittenden, J.R. Mihelcic, Environ. Sci. Technol. 35 (2001) 1282.
[5] L. Freitas dos Santos, L. Defrenne, A. Krebs-Brown, Anal. Chim. Acta 456 (2002) 41.
[6] A. Gomot de Valfleury, A. Bispo, Environ. Sci. Technol. 34 (2000) 1865.
[7] M. Farré, F. Arranz, J. Ribo, D. Barcelo, Talanta 62 (2004) 549.
[8] Chi-Ying Hsieh, Meng-Hsiun Tsai, David K. Ryan, Oscar C. Pancorbo, Sci. Total Environ. 320 (2004) 37.
[9] Patrick van Beelen, Chemosphere 53 (2003) 795.
[10] J. Wang, G. Rivas, X. Cai, E. Palecek, P. Nielsen, H. Shiraishi, N. Dontha, D. Luo, C. Parrado, M. Chicharro, P.A.M. Farias, F.S. Valera, D.H. Grant, M. Ozsoz, M.N. Flair, Anal. Chim. Acta 347 (1997) 1.
[11] F. Lucarelli, A. Kicela, I. Palchetti, G. Marrazza, M. Mascini, Bioelectrochemistry 58 (2002) 113.
[12] D.W. Pang, H.D. Abruna, Anal. Chem. 70 (1998) 3162.
[13] J. Wang, G. Rivas, D. Luo, X. Cai, F.S. Valera, N. Dontha, Anal. Chem. 68 (1996) 4365.
[14] G. Chiti, G. Marrazza, M. Mascini, Anal. Chim. Acta 427 (2001) 155.
[15] E. Palecek, Talanta 56 (2002) 809.
[16] J. Wang, G. Rivas, X.H. Cai, E. Palecek, Anal. Chim. Acta 347 (1997) 1.
[17] A.M. Oliveira Brett, S.H.P. Serrano, M.A. La Scalea, Kluwer Academic Publishing, Dordrecht, 1998, p. 78.
[18] S.S. Babkina, N.A. Ulakhovich, Y.I. Zyavkina, Zavod. Lab. 66 (2000) 147.
[19] S.S. Babkina, E.P. Medyantseva, H.C. Budnikov, M.P. Tyshlek, Anal. Chem. 68 (1996) 3827.
[20] G. Marrazza, I. Chianella, M. Mascini, Biosens. Bioelectron. 14 (1999) 43.
[21] A.M. Oliveira Brett, S.H.P. Serrano, J.A.P. Piedade, in: G. Handcock (Eds.), Chem. Kinetics, 37 (1999) 91.
[22] E. Palecek, M. Fojta, M. Tomschick, J. Wang, Biosens. Bioelectron. 13 (1998) 621.
[23] M. Mascini, I. Palchetti, G. Marrazza, Fresen. J. Anal. Chem. 369 (2001) 15.
[24] G.M. Blackburn, M.J. Gair, Nucleic Acids in Chemistry and Biology, Oxford University Press, Oxford, 1996.
[25] A.M. Oliveira Brett, L. Antonio da Silva, H. Fujii, S. Mataka, T. Thiemann, J. Electroanal. Chem. 549 (2003) 91.
[26] H. Karadeniz, B. Gulmez, F. Sahinci, A. Erdem, G.I. Kaya, N. Unver, B. Kivcak, M. Ozsoz, J. Pharmaceut. Biomed. 33 (2003) 295.
[27] E. Palecek, M. Fojta, Anal. Chem. 73 (2001) 67A.
[28] G. Marrazza, I. Chianella, M. Mascini, Anal. Chim. Acta 387 (1999) 297.
[29] Italian Legislation Decree no. 152 of 11/05/1999.
[30] Standard Methods for the Examination of Water and WasteWater, in: L.S. Clescrei, A.E. Greenberg, A.D. Eaton (Eds.), Publish by the American Public Health association, 20th ed., The American Water Works Association and the Water Environment Federation, Washington, DC, 1998.
[31] A. Cagnini, I. Palchetti, I. Lionti, M. Mascini, A.P.F. Turner, Sens. Actuators B 24 (1995) 85.
[32] Antje Brack, Jürgen Strube, Peter Stolz, Heinz Decker, Biochim. Biophys. Acta (BBA) General Subjects 1621 (2003) 253.
[33] M. Farré, D. Barceló, Trends Anal. Chem. 22 (2003) 299.
[34] Mauro Ravera, Sara Baracco, Claudio Cassino, Donato Colangelo, Graziana Bagni, Gianni Sava, Domenico Osella, J. Inorg. Biochem. 98 (2004) 984.
[35] Toxalert®100 Operating Manual, Merck, 2000.
[36] R. Kellner, J.M. Mermet, M. Otto, H.M. Widmer, Analytical Chemistry, Wiley/VCH Verlag GmbH, Weinheim, 1998, pp. 726–727.
[37] Ilaria Palchetti, Serena Laschi, Marco Mascini, Anal. Chim. Acta 530 (2005) 61.