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DNA sensor for *o*-dianisidine

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Abstract

o-Dianisidine (3,3′-dimethoxybenzidine) is applied in the production of some dyes and also used in analytical tests. However, this compound is anticipated to be a human carcinogen. An analytical strategy utilizing square wave voltammetry for the determination of *o*-dianisidine is presented. An electrochemical system was consisted of three electrodes: carbon paste working electrode, platinum wire counter electrode and silver—silver chloride (Ag/AgCl) reference electrode. However, square wave voltammograms of direct measurements of *o*-dianisidine were found to be hardly reproducible, exhibiting few peaks due to some labile short-lived intermediates with the only exception of a quite stable peak at +0.7 V vs. Ag/AgCl.

Quantitative determination of *o*-dianisidine gave satisfactory results only when the carbon paste working electrode was replaced by deoxyribonucleic acids (DNA) electrode obtained by immobilization of double-stranded (ds) DNA on carbon electrode. Square wave voltammogram of DNA showed two peaks attributed to adenine and guanine and the latter was used as analytical signal. After interaction with *o*-dianisidine, guanine oxidation peak was reduced to the extent related to the concentration of the analyte. Initial reduction of guanine peak took place already at the concentration of *o*-dianisidine equal to 0.4 μM; high concentrations (above 100 μM) of the analyte quenched completely a guanine response.

The presented electrochemical system enables a specific detection of *o*-dianisidine by the presence of an oxidation peak at +0.7 V and its quantitative determination by measuring a reduction of guanine peak by means of a DNA sensor.

Keywords: *o*-Dianisidine; Analytical strategy; DNA sensor; Square wave voltammetry; Electrochemistry

1. Introduction

Rapid detection and quantitative determination of compounds hazardous for a human health occurrent as environmental pollutants is of great importance. Amine-substituted aromatic derivatives belong to substances extremely harmful and unfortunately present in the environment. Some of these compounds are really dangerous for all living organisms. Such substances require exceptional treatment independently on if they are met on a large scale because of their application in industry or if they occur only occasionally, e.g., in the laboratory use. This special treatment means, at first, a need for sensitive screening tool for the detection of these harmful substances and, secondly, the easy way of their quantitative determination.

Certainly, the analytical method of the determination of toxic compounds should be not only reliable but simple, not requiring highly qualified staff, sensitive and applicable in a wide range of the analyte concentration. Furthermore, it would be of great utility if the method simultaneously supplies the information concerning a toxicity of the compound.

o-Dianisidine (3,3′-dimetoxybenzidine, $C_{14}H_{16}N_2O_2$; see Fig. 1 for the structure) is amine-substituted aromatic compound (benzidine derivative). This class of substances is known as extremely harmful environmental pollutants, mainly water pollutants. *o*-Dianisidine is included in the EPA list of dangerous pollutants (EPA code UO91). On the base of studies on experimental rats having malignant and benign tumors at multiple tissue sites after exposure to *o*-dianisidine, this compound is anticipated to be a human carcinogen [1,2]. *o*-Dianisidine is used as a chemical intermediate for the production of dyes and pigments that are used for leather, paper, plastics, rubber and textiles. It is also applied as a test substance. *o*-Dianisidine is sensitive to heat, air and prolonged exposure to light. It is very slightly soluble in water (0.01 g/100 cm^3), good soluble in ethanol, DMSO and many other organic solvents [3]. A

Abbreviation: E_p, Peak potential (V).

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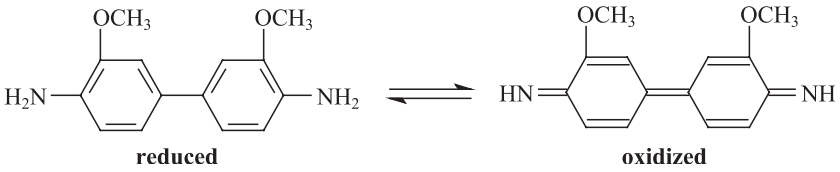

Fig. 1. The structure of *o*-dianisidine (reduced and oxidized forms).

usual water soluble form of *o*-dianisidine is its dihydrochloride derivative.

Because of shattering properties of *o*-dianisidine, there is a need for simple and sensitive methods of its determination. Actually, detection and quantitative determination of *o*-dianisidine is based on chromatographic analysis. These methods are time and labour consuming, require highly qualified operating personnel and instruments, which are usually very expensive. Therefore, simple, rapid and inexpensive analytical procedures are required and these expectations can be accomplished by biosensors. In the last few years, tremendous development of biosensors has been observed, thousands of publications and textbooks edited; however, only few projects were really commercialized and put on the market [4]. It means that, still, a lot of problems have to be solved. Some of them are concerned with a poor operational stability, not reliable selectivity or low reproducibility of measurements. Elaborated biosensors still possess many drawbacks preventing them from massive practical applications.

Recently, biosensors with deoxyribonucleic acids as recognition elements have rapidly been developed [5–11]. Among several types of transducers used in biosensors, the most common are electrochemical devices [11]. Immobilization of a single-stranded (ss) deoxyribonucleic acids (DNA) chain or an oligonucleotide of specific sequence on the electrode creates a biosensor that detect the DNA hybridization. Another type of device is a biosensor with immobilized double-stranded (ds) calf thymus DNA detecting small molecules interacting with DNA. This kind of biosensor is supposed to be used for detecting harmful substances present, e.g., in the environment but also in food [12–14]. Wang et al. [14] proposed to use a biosensor for detecting aromatic amines present in the environment, e.g., in water. Furthermore, other numerous reports have described sensors with immobilized calf thymus DNA for detection molecules interacting with nucleic acids [5,11,12]. Almost all of them are designed for total determination of so-called toxicity or genotoxicity pretending that change of electrochemical signal of DNA immobilized on the sensor means a detection of molecules that could be dangerous for human [5]. And all of them aimed to detect total toxicity but not detection and determination of any particular substance. Electrochemical techniques usually applied for detection of this kind of interaction are chronopotentiometry and differential pulse voltammetry [5,14]. Only recently, Lucarelli et al. [15] reported an application of a square wave voltammetry for the determination of toxic compounds in water.

The aim of this work was to develop a specific analytical tool destined for the detection, identification and the quantitative determination of one particular toxic substance—*o*-dianisidine. This is an electroactive compound and it is supposed to be determined directly by some electrochemical methods. Furthermore, the usefulness of electrochemical DNA sensor for the determination of *o*-dianisidine was examined. For all electrochemical analyses, a square wave voltammetry technique was used as a detection method.

2. Experimental

o-Dianisidine and *o*-dianisidine dihydrochloride were purchased from Sigma, USA. Deoxyribonucleic acid from calf thymus, graphite powder, mineral oil were also from Sigma. All other chemicals were from Merck, Germany and Polskie Odczynniki Chemiczne, Poland.

Electrochemical measurements were performed with a potentiostat μAutolab with GPES, version 4.8 software (Eco Chemie, Utrecht, The Netherlands). The experimental conditions for electrochemical analysis were: the three electrode system consisted of a carbon paste working electrode, Ag/AgCl reference electrode and a platinum wire counter electrode. The carbon paste was prepared by mixing graphite powder with mineral oil with the ratio 70:30. The resulting paste was packed into Teflon tube of 0.1 cm internal diameter. Electrical connection was supplied with a copper wire. The surface of the working electrode was always renewed before use by polishing to a smoothed finish on a weighing paper [16].

Direct electrochemical measurements of *o*-dianisidine were done by immersing three electrodes in an electrochemical cell containing 2.5 cm^3 sample solution (*o*-dianisidine in the concentrations from 0.4 to 500 μM in 0.2 M acetate buffer, pH 4.7, with add 0.01 M KCl) and performing of square wave voltammetry analysis.

Experimental conditions for square wave voltammetry were (unless otherwise stated): scan range from 0.5 to 1.75 V, frequency of 200 Hz, amplitude of 0.04 V and step potential of 0.015 V.

DNA sensor was obtained by immobilizing calf thymus DNA on the carbon paste electrode. The measurement procedure with the use of a DNA sensor consisted of four steps: electrode pretreatment, DNA immobilization, interaction with the sample and detection of the analyte (*o*-dianisidine) by a square wave voltammetry. Electrode

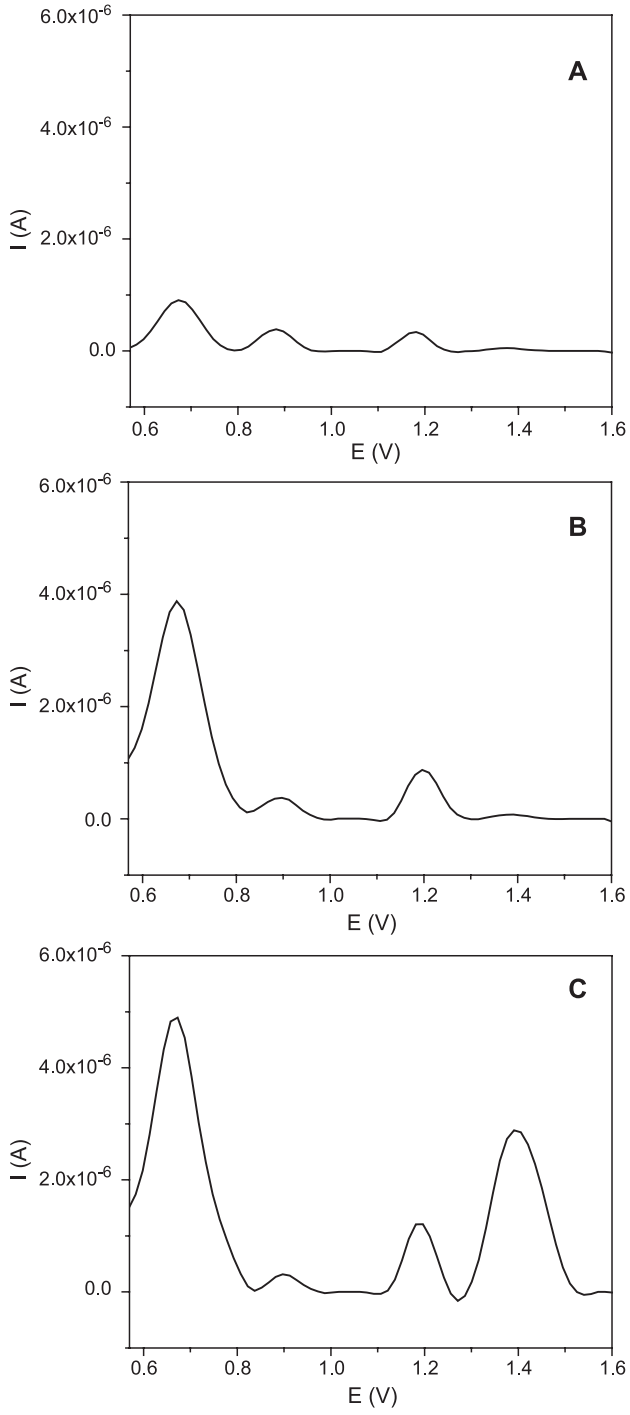

Fig. 2. Square wave voltammograms of *o*-dianisidine in acetate buffer, pH 4.7, after base line correction. The *o*-dianisidine concentration: (A) 5 μM; (B) 40 μM; (C) 100 μM.

pretreatment was performed by an immersion of a smoothed carbon paste working electrode for 2 min in a stirred acetate buffer solution (polarization at +1.7 V). For immobilization, the electrode was immersed for 2 min in DNA solution (calf thymus DNA in acetate buffer, 20 mg/l^{-1}) and polarizated at +0.5 V. In the next step, the DNA electrode was transferred to a sample solution for 1 min, then the electrode was immersed and washed in an acetate buffer solution where finally a square wave voltammetric scan was recorded. Upon interaction of *o*-dianisidine with DNA immobilized on the electrode, a guanine peak on the SW voltammogram was decreased. The percentage of this peak reduction (% R) was evaluated by calculation the ratio of the guanine peak height after interaction with *o*-dianisidine and before this interaction (i.e., after immersion of a DNA electrode in a buffer solution) [15].

For the presentation of all experimental voltammograms origin, version 6.0 (Microcal Software) was used.

All potentials are referred to Ag/AgCl electrode.

All measurements were done in room temperature.

3. Results and discussion

3.1. Direct electrochemistry of o-dianisidine

o-Dianisidine is able to be oxidized eletrochemically (Fig. 1), and this attribute was used in the analytical procedure. Several electrochemical detection methods including cyclic voltammetry and square wave voltammetry have been applied and results obtained with the latter technique are shown here. *o*-Dianisidine in 0.2 M acetate buffer solution (pH 4.7) presents rather complex electrochemistry as shown in the SW voltammograms on Fig. 2. The response exhibits three to four peaks depending on the *o*-dianisidine concentration, their positions shift slightly with the concentration although the average values were: $E_{p1} = 0.7$ V, $E_{p2} = 0.9$ V, $E_{p3} = 1.2$ V, $E_{p4} = 1.4$ V.

An increase in the concentration of *o*-dianisidine reflected in the change of voltammograms; however, it seemed there was no direct relationship between the area or the height of peaks and the concentration. For example, the peak at +0.9 V diminished when the *o*-dianisidine

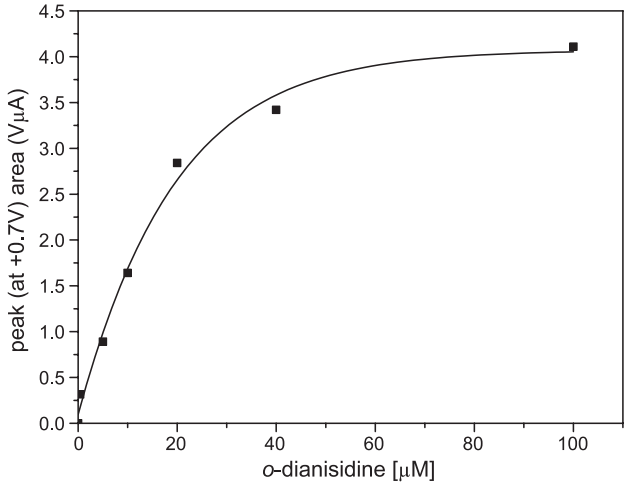

Fig. 3. The relationship between the *o*-dianisidine concentration and the area of the peak at 0.7 V of SW voltammograms.

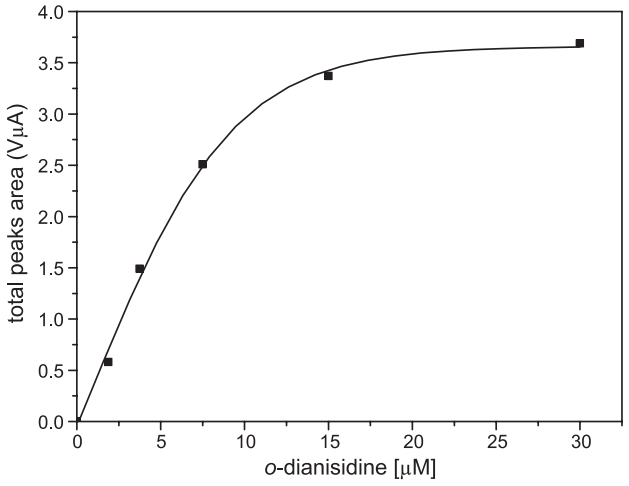

Fig. 4. The relationship between the *o*-dianisidine concentration and the total area of all peaks at SW voltammograms.

content increased from 0.4 to 100 μM. The peak at ca. +1.4 V is visible only at high concentrations (100 μM). Electrochemical response *o*-dianisidine indicates not only one but more oxidation centers in the molecule of the compound and also evidences probably some unstable intermediates present in a solution only at some analyte concentrations [17]. Some of the electrochemical waves come probably from electroactive labile intermediates resulting from an interaction of *o*-dianisidine with a solvent. This phenomenon does not help in quantitative determination of *o*-dianisidine. However, a sufficient relationship has been found between the area (or the height) of a peak at +0.7 V and *o*-dianisidine concentration (Fig. 3). The graph contains a linear range between 0.4 and 20 μM. Likewise, a good relationship has been found between *o*-dianisidine concentration and a total area of all peaks of voltammograms (Fig. 4), although linear range here looks shorter (4–8 μM). Unfortunately, a reproducibility of repetitive experiments ($n = 6–8$) of electrochemical measurements of *o*-dianisidine concentration was not satisfactory. Relative standard deviation (RSD) values (Table 1) of the area of the peak at 0.7 V were usually above 10%. The lowest values were found with the higher concentrations (above 15 μM). These data rather exclude application of square wave voltammetry method for the quantitative determination of *o*-dianisidine; however, complex SW voltammograms enable a simple identification of this compound.

Table 1
Relative standard deviations of *o*-dianisidine direct measurements by SWV

[*o*-Dianisidine]/μM	RSD (%)
0.18	25
3.75	8
7.50	45
30.00	10

3.2. Quantitative determination of o-dianisidine using DNA sensor

It was already reported [12,14] that amine-substituted aromatic compound interact with deoxyribonucleic acids, and this feature was used in this work for the detection of *o*-dianisidine by DNA modified electrodes. It was found and also presented here (Fig. 5) that this interaction resulted in a decrease of guanine electrochemical response. This lowering of the DNA signal was attributed to changes in the

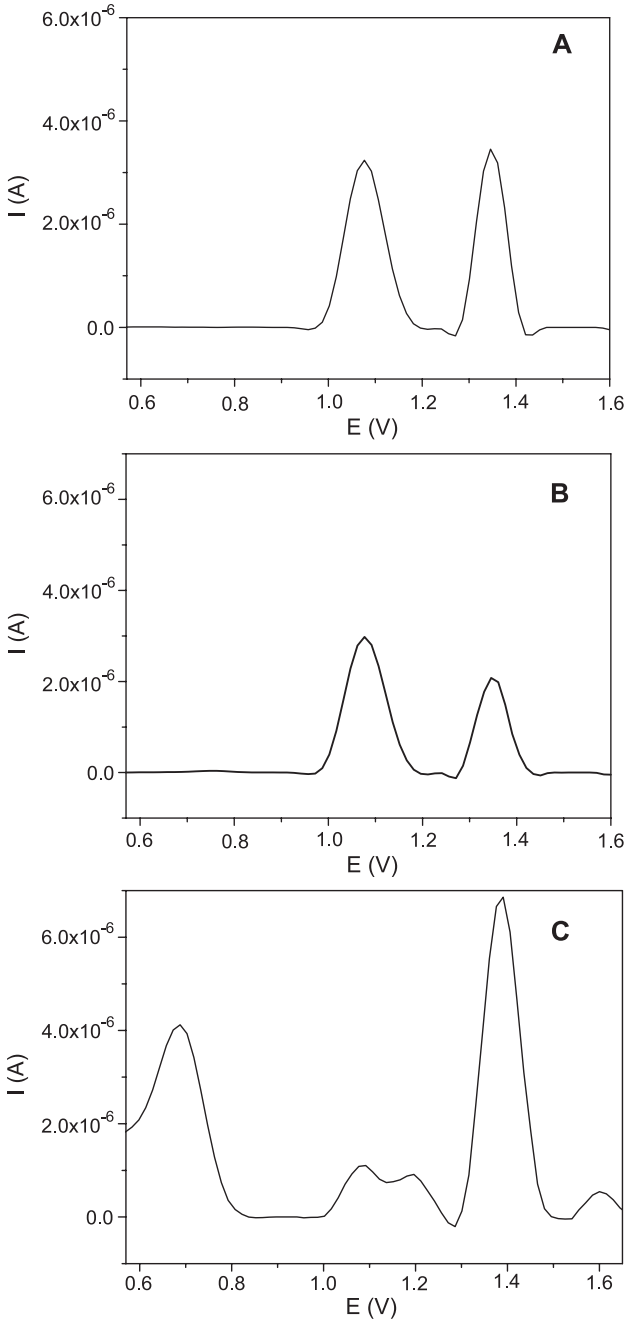

Fig. 5. Square wave voltammograms (after base line correction) of DNA carbon paste electrode in acetate buffer, pH 4.7 (A) and *o*-dianisidine—0.4 μM (B) and 20 μM (C)—after interaction with DNA electrode.

surface accessibility of the guanine moiety upon binding of the aromatic amines to the DNA duplex [14]. However, it was also reported by Marrazza et al. [12] that guanine peak increased after interaction with some intercalating compounds like daunomycin, cisplatin and bisphenol. This phenomenon was explained that intercalation of some compounds between the base pairs of dsDNA resulted in an easy oxidation of guanine [12]. However, hydrazine, another intercalator, evokes a decrease of a guanine response [12]. It is difficult to accept any general explanation for differentiated changes of a guanine peak after an interaction of some molecules with a DNA electrode and, in consequence, to foresee a result of an interaction of unknown compound with a DNA electrode.

To study electrochemically the interaction between *o*-dianisidine and DNA, a carbon paste electrode with immobilized DNA was prepared and electrochemical measurements were performed using square wave voltammetry as a detection method.

Square wave voltammogram obtained with the use of DNA sensor immersed in the acetate buffer solution is presented on Fig. 5A. Two peaks are generated from DNA immobilized on the carbon paste electrode, one coming from guanine (at +1.1 V) and second one from adenine (at +1.3 V). Both peaks have changed after interaction of the DNA electrode with *o*-dianisidine (Fig. 5B and C). However, adenine peak fluctuated irregularly when the concentration of *o*-dianisidine varied. On the contrary, a guanine peak diminished proportionally when the concentration of *o*-dianisidine in the sample increased in the range from 0.4 to 100 μM and completely disappeared at the concentration of 500 μM. In repetitive measurements, results obtained with *o*-dianisidine samples of different concentration in the range 0.4–40 μM showed good satisfactory reproducibility (Table 2). The RSD values of *o*-dianisidine measurements by DNA sensor did not exceed 7%, whereas the highest values occurred at concentration above the linearity of calibration graph (above 20 μM). Furthermore, the RSD values are mainly influenced by results of blank sample, i.e., reproducibility of DNA electrode. Improvement in the preparation of working electrode would be surely resulting in lower RSD values.

Table 2
Relative standard deviations of *o*-dianisidine measurements with the use of DNA sensor

[*o*-Dianisidine]/μM	RSD (%)
0[a]	6
0.4	1
3.7	4
5	3
7.5	6
40	7

[a] RSD of repetitive measurements of guanine peak height in the voltammograms with the use of DNA electrode in a blank sample, i.e., acetate buffer not containing *o*-dianisidine.

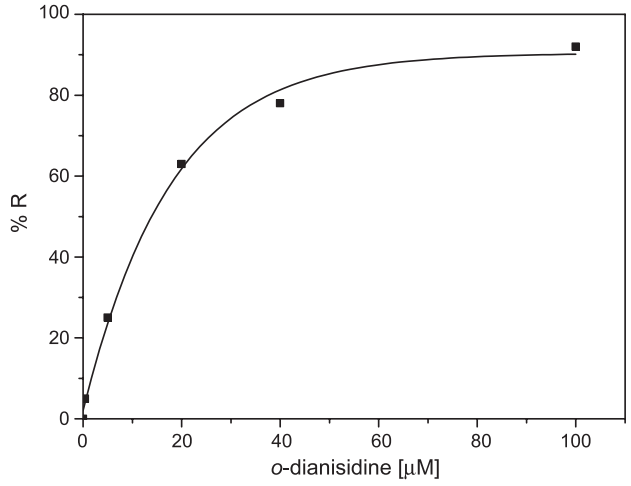

Fig. 6. Calibration curve for *o*-dianisidine reflecting relationship between guanine peaks height reduction (% R) after interaction of *o*-dianisidine with DNA sensor and the *o*-dianisidine concentration.

On the ground of reduction percentage of guanine peak height (% R), a calibration curve was crossed out and this graph could be used for quantitative determination of *o*-dianisidine in the range from 0.4 to 100 μM (Fig. 6), whereas the linear range attained only the value 20 μM. Above 100 μM, a guanine peak is not visible, and this would only indicate the presence of a toxic substance but precise quantitative determination is not possible. Anyway, the calibration graph covers relatively wide range of *o*-dianisidine concentration, and this is an extremely significant and useful feature of the method.

4. Conclusions

The analytical procedure targeting the detection of any health hazard substance should not be only a simple way of confirming the presence of a toxic compound in the investigated sample but should also embrace the method of its identification and quantitative determination. The extent of threat and a direction of hazardous effect is surely dependent on the nature and amount of a toxic substance.

o-Dianisidine is an electroactive amine-substituted compound and can be determined by electrochemical methods. It was evidenced that *o*-dianisidine interacts with DNA that reflects in a guanine peak reduction and confirms its toxic and possibly carcinogenic activity.

Direct electrochemical determination of *o*-dianisidine by square wave voltammetry resulted in large errors (high relative standard deviations). However, quite complex SW voltammograms of *o*-dianisidine can be used for its qualitative identification.

Quantitative determination is possible with DNA sensor. The obtained results show a sufficient relationship between *o*-dianisidine concentration in a wide range between 0.4 to 100 μM and guanine peak reduction in SW voltammograms.

Relative standard deviation values of such measurements did not exceed 7%.

In summary, it was presented a simple tool for detection and quantitative determination of *o*-dianisidine. The proposed analytical procedure consists of two steps: the first step involving direct square wave voltammetry of a sample containing *o*-dianisidine enabling its identification, and the second step resulting in quantitative determination with the use of DNA sensor. This method could be also useful in the control of foods, water and some other environmental samples for the presence of harmful substances, i.e., small molecules interacting with DNA. In such a case, the procedure starts with a second step-based on using DNA sensor and positive results of these attempts give raise to perform direct square wave voltammetry of the sample to identify a harmful molecule. There are some electroactive substances (aromatic amines) that can interfere with *o*-dianisidine. However, SW voltammetry gives a great chance to identify it even in a complex sample. This has to be confirmed by testing of many real samples. Such work is in progress and will be published at a later date.

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