Selective and sensitive electrochemical device for direct VB_2 determination in real products

Agata Kowalczyk, Monika Sadowska, Beata Krasnodebska-Ostrega, Anna M. Nowicka

www.elsevier.com/locate/talanta

PII: S0039-9140(16)30837-2
DOI: http://dx.doi.org/10.1016/j.talanta.2016.10.087
Reference: TAL17007

To appear in: *Talanta*

Received date: 18 August 2016
Revised date: 20 October 2016
Accepted date: 23 October 2016

Cite this article as: Agata Kowalczyk, Monika Sadowska, Beata Krasnodebska Ostrega and Anna M. Nowicka, Selective and sensitive electrochemical devic for direct VB_2 determination in real products, *Talanta* http://dx.doi.org/10.1016/j.talanta.2016.10.087

Selective and sensitive electrochemical device for direct VB_2 determination in real products

Agata Kowalczyk*, Monika Sadowska, Beata Krasnodebska-Ostrega, Anna M. Nowicka

Faculty of Chemistry, University of Warsaw, ul. Pasteura 1, PL-02-093 Warsaw, Poland.

***Corresponding Author.** Tel: +48 22 55 26 361. akowalczyk@chem.uw.edu.pl A

ABSTRACT

: The developed by us electrochemical device for vitamin B_2 (VB_2; riboflavin) determination, without preconcentration step, in real products exhibits high sensitivity, selectivity, stability and low detection limit compared to those described in the literature. The determination procedure was based on the monitoring of the reduction current signal of VB_2 bound with dsDNA anchored to the electrode surface through intermediary - carboxyphenyl layer. The application of such intermediary layer formed during electroreduction of appropriate diazonium salt at CV peak potential guarantees high efficiency of hybridization process and thus fully available places for VB_2 interaction. Moreover, such intermediary layer provides good electrical contact, what is very important in the case of electrochemical sensors. The analytical range of work of the proposed VB_2 sensor was between 0.08 ÷ 1 μM (30 ÷ 377 $\mu g \cdot L^{-1}$) of riboflavin concentration. The obtained detection (LOD) and quantification limits (LOQ) were 24 ± 2 and 55 ± 5 $\mu g \cdot L^{-1}$, respectively. The proposed VB_2 detection method was used for determination of riboflavin content in commercially available dietary supplements and yolk of hen egg samples. The accuracy of the obtained data was proved using comparison with an independent method (HPLC FLD).

KEYWORDS: Direct VB_2 determination, Square wave voltammetry, DNA biosensor, Dietary supplements, Intermethod comparison.

1. Introduction

Riboflavin (6,7-dimethyl-9-(D-1-ribityl)-isoalloxazine) is an extremely important compound for the proper functioning of the human organism. Vitamin B_2 participates in redox reactions in animal organisms as a hydrogen carrier in the processes of carbohydrate, protein and fat metabolism, and hemoglobin synthesis [1]. It is also known to be the central component of flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN), which are important cofactors of dehydrogenase *in vivo*. Numerous diseases are related to the deficiency of riboflavin [2,3]. Human and animals organisms have limited ability to store vitamin B_2 and its excess will be excreted in the urine. Hence, vitamin B_2 should be taken and supplemented regularly to avoid shortage. For this reason, a constant supply of vitamin B_2 is required for the optimal maintenance of large number of metabolic processes requiring the flavin coenzymes [3,4,5]. Due to this fact, the control of the content of vitamin B_2 in supplements and food is very important.

Various analytical methods for determination of riboflavin including high-performance liquid chromatography, spectroscopy or fluorescence have been used [6-10]. However, these analytical techniques are relatively time-consuming, require expensive and complicated instruments, or suffer from poor sensitivity and selectivity. The electrochemical techniques are simple, rapid, cheap, selective, and highly sensitive. Different types of electrochemical procedures of the determination of riboflavin have been developed [11-15].

Nucleic acids offer a powerful tool in the recognition and monitoring of many important compounds such as drugs [16-18]. The investigation of DNA interactions with small molecules is a fundamental issue in life science [19-21]. Since riboflavin interacts with double-stranded DNA in groove-binding way and the electrostatic effect [22], the investigations based on DNA interactions are an attractive approach for riboflavin detection. Additionally, DNA and riboflavin are electroactive, so the electrochemical detection can be based on redox signals of both riboflavin and DNA. It is known that the current signal of the molecules bound with DNA strongly depends on its packing density in dsDNA structure and the efficiency of electron transfer through DNA over long distance [23,24]. Due to this fact action of the most of the described in the literature electrochemical DNA biosensors for VB_2 detection are based on the DNA voltammetric signal.

In clinical and pharmaceutical analysis proving of the results' correctness is very important. The availability of two independent methods (measurements based on different phenomena) is a necessity in analytical chemistry. The recovery study and intermethod comparison are useful tools in data validation. Among all various available methods for VB_2 determination [25,26,27] the DNA-biosensors seems to be interesting approach for this purpose. Therefore we proposed an additional, independent methodology for direct VB_2 determination, based on DNA biosensor an alternative to the methods commercially used. DNA biosensors for riboflavin detection, which were so far described in the literature, are based either on the electrochemical signal of DNA (oxidation signals of guanine and adenine) [13,28] or the electrochemical signal of the mediator but not on the VB_2 current signal [29]. In this paper we present a highly selective, rapid and sensitive electrochemical device for successful determination of riboflavin in commercially available dietary supplements and yolk of hen's egg. The detection was based on the direct voltammetry of riboflavin bound with dsDNA. To our best knowledge such studies have not been carried out yet. The assembly

of the DNA strands via chemical bonding with a carboxyphenyl layer (electrode modifier; formed during electroreduction of appropriate diazonium salt) guaranteed efficient electron transfer through DNA over long distance and in consequence low detection limit. Moreover such electrode modifier provided uniform distribution of DNA chains in the sensing layer with optimal dense packing, thereby the amount of VB_2 molecules in biosensor was sufficient to generate electrochemical signal at low level.

2. Experimental section

2.1. Chemicals

All chemicals were of the highest purity available: CH_3COOH, NaCl, NaOH, $MgCl_2$, $CaCl_2$ (POCH, Poland), $CH_3COONa \cdot 3H_2O$ (Chempur, Poland), MeOH (HPLC grade, Fisher Scientific, UK), HEPES (2-[4-(2-hydroxyethyl)piperazin-1-yl]ethanesulfonic acid), 4-aminobenzoic acid (H_2N-C_6H_4COOH), sodium nitrite ($NaNO_2$), *N*-hydroxysuccinimide (NHS), *N*-(3-dimethylaminopropyl)-*N′*-ethylcarbodiimide hydrochloride (EDC), riboflavin (VB_2), vitamin E (VE), vitamin C (VC), glucose (GL) were used as received (Sigma Aldrich). All oligonucleotides were purchased from MWG-Operon (Eurofins). The following oligonucleotide sequences were used:

amino probe DNA (5'⇒3'): H_2N-C6-GAGCGATGCCGAACTGCTCG

complementary DNA (5'⇒3'): CGAGCAGTTCGGCATCGCTC (designed from the strain of *Pseudomonas aeruginosa* Pilin Genes).

The single DNA solutions (probe and complementary strands; aqueous solutions of 400 $\mu g \cdot mL^{-1}$) were at first heated for 10 minutes at the melting temperature (specified by the manufacturer). Next the solution was immediately cooled down in the ice bath and diluted to the appropriate concentration by the immobilization buffer (0.02 M HEPES, pH 7.40) in the case of probe DNA, and hybridization buffer (0.02 M HEPES buffer with addition of 1 M

NaCl and 1 mM EDTA, pH 7.40) in the case of complementary DNA. All electrochemical measurements were performed in degassed with pure argon 0.1 M acetate buffer containing 0.02 M NaCl, pH 4.80.

2.2. Electrochemical measurements

The square wave voltammetry (SWV) was performed using an Autolab PGSTAT 30 potentiostat. The electrochemical measurements were carried out in a three-electrode system, consisting of a glassy carbon disc electrode (GC; 3 mm in diameter, BAS, UK) used as the working electrode, a KCl saturated Ag/AgCl electrode served as the reference electrode and a platinum wire was used as the auxiliary electrode. The surface of the working electrode was polished with 1 μm Al_2O_3 powder on a wet pad. After each polishing, to completely remove alumina from the electrode surface, the electrode was rinsed with a direct stream of ultrapure water (Hydrolab, conductivity of ~ 0.060 $\mu S \cdot cm^{-1}$) and dried with argon.

2.3. UV-vis measurements

UV-vis spectra of the solution containing VB_2 in concentration 10 μM and with addition of dsDNA in appropriate concentration (0, 10, 20, 30, 40 and 50 μM) were obtained in the range of 200 ÷ 600 nm. A Perkin-Elmer (Lambda-25) spectrometer was used. Measurements were performed three times for each of three independently prepared solutions.

2.4. Fabrication of device sensing platform

The key element of the proposed electrochemical device sensing platform was dsDNA fragments, more precisely the efficiency of the VB_2 and dsDNA interactions. Therefore the density and the distribution of the dsDNA fragments on the electrode surface are very important. We have proven that the intermediary layer formed by electroreduction of an appropriate diazonium salt provides stable and appropriately packed of DNA strands in the sensing platform [30].

After the cleaning step the surface of a glassy carbon electrode was modified with the carboxyphenyl layer obtained according to the procedure described in our previous paper [31]. The presence of phenyl rings on the electrode surface provided good conductivity of the film which is especially important in the case of electrochemical detection, so the transport of electrons through double bonds was not limited. It should be stressed here that the intermediary layer ought to be homogenous, thin, well conducting, tight, durable, resistant to cracking and it should not influence the biological properties of the immobilized molecules. In the next step the carboxyl groups of the intermediary layer were activated in an aqueous solution of EDC/NHS mixture (40 and 10 mM, respectively) for 1 h [32]. After the activation, the electrode was rinsed with pure, deionized water and incubated overnight in probe H_2N-ssDNA solution in concentration 4 μM, and finally rinsed with pure water to remove physically adsorbed DNA strands. After the probe DNA immobilization step the hybridization process with complementary DNA, in concentration 4 μM, took place for (1 h). The amount of dsDNA fragments anchored to carboxyphenyl layer was determined according to the procedure described by Steel et al. [33]. In those experiments the chronocoulograms were recorded in 0.02 M PBS buffer with addition of 50 μM $Ru(NH_3)_6^{3+}$. The determined, from the Steel formula, dsDNA surface coverage was $(2.49\pm0.8)\cdot10^{12}$ molecule$\cdot$cm^{-2}.

Shortly afterwards the electrode was immersed in the riboflavin solution (1 μM in 0.1 M acetate buffer, pH 4.80, with 0.02 M NaCl) for 2 h. The optimal incubation time of immersing of the DNA biosensor in the solution of electrochemical tag (VB_2) was based on the data presented in Fig. 1S in Supplementary Materials. This Figure shows a plot of reduction peak current height of VB_2 versus incubation time of the biosensor in the riboflavin solution. In the experiments 1 μM VB_2 solution was used to assure an appropriate excess of electrochemical tag. In such case the time of saturation of the dsDNA layer by VB_2 would be reasonably short and particularly any nonspecific binding would be limited. Finally, the

electrode was rinsed with the buffer solution to remove the unbound VB_2, and characterized using SWV. The preparation of electrochemical VB_2 DNA-biosensor including several steps is illustrated in Scheme 1.

2.5. Sample preparation for determination of vitamin B_2 in dietary supplements

For determination of vitamin B_2 in tested dietary supplements, an amount of 6.50 mg of the supplement was weighed and dissolved in 40 mL of acetate buffer solution. The solution was intensively mixed in ThermoMixer C (Eppendorf) for 15 minutes. The DNA biosensor was immersed in such prepared solution (250 μL) for 2 hours and then the SWV measurement was carried out in degassed 0.1 M acetate buffer with addition of 0.02 M NaCl, pH 4.80.

2.6. Extraction of riboflavin from yolk of hen egg

The egg was hard-boiled (6 min in boiling water bath) and the yolk was separated. To 1.0 g of yolk 20.0 mL 0.02 $mol \cdot L^{-1}$ acetic acid was added and shaken for 15 min at room temperature. After extraction the sample was filtered through 0.45 μm PTFE syringe filter. In order to ensure that the extract contains vitamin B_2 the chromatographic analysis (HPLC) with fluorescence detection (FLD; Agilent 1200, Agilent, USA) with a quaternary pump and a manual injector (20 μL injection loop) was carried out. The obtained sample was injected onto the column (Zorbax Eclipse XDB-C18; 4.6 × 250 mm) and separated at ambient temperature. The mobile phase was consisted of 65 % methanol and 35 % 0.10 $mol \cdot L^{-1}$ acetate buffer (pH 5.20), and the flow rate was 1.5 $mL \cdot min^{-1}$. The measurements were carried out with an excitation wavelength of 450 nm and an emission wavelength of 530 nm. The identification of riboflavin was based on the retention time and the quantification analysis was based on the standard calibration curve.

3. Results and discussion

3.1. Voltammetric characteristic of VB_2 biosensor

The constructed biosensor was tested for the riboflavin concentration in the range from 0.06 to 1.40 μM. The representative SW voltammograms are presented in inset in Fig. 1. With the increase of concentration of riboflavin interacting with DNA biosensor an increase of its reduction peak was observed. However, for concentrations of VB_2 higher than 1 μM no changes in the intensity of the reduction signal of riboflavin bound with DNA layer were observed. Such stabilization of the current signal was due to the saturation of the biosensor by VB_2 molecules, thus further increase of riboflavin concentration had no effect on the amount of the VB_2 molecules bound to DNA. On the other hand, for concentrations of VB_2 lower than 0.08 μM the recorded signal did not differ statistically from the background. Probably, in this case the amount of riboflavin bound with the DNA biosensor was not sufficient to generate the analytical signal.

To be sure that the observed reduction signal of VB_2 corresponds to the riboflavin bound only with dsDNA (GC/-C_6H_4COOH/dsDNA), after each step of modification a new electrode was immersed in 1 μM riboflavin solution for 2 hours, and then the SW voltammogram was recorded pure buffer solution. Typical recorded voltammograms are shown in Fig. 2S in Supplementary Materials. The modification of the electrode surface by subsequent biosensor layers leads to a change of the riboflavin reduction peak position, compared to the signals obtained at the bare electrode (inset in Fig. 2S in Supplementary Materials). The signal at -0.32 V comes from riboflavin bound only with the DNA layer of the biosensor. Since riboflavin interacts with DNA mainly in groove-binding way the drop in the peak current after the interaction with ssDNA is understandable [22]. Although the way of VB_2 binding to DNA is random, the DNA damage, revealed as strand cleavage did not take place. Most of the strand cleavage occurs at guanine residues [34]. The interaction of riboflavin with DNA was proceeded in the presence of oxygen, so the VB_2 molecules bound

to DNA was in oxidized form. The introduction of riboflavin in oxidized form to DNA duplex led to the oxidation of guanine residues. Among all DNA bases guanine is the most easily oxidized to guanine radical cation, $G^{\bullet+}$ [35]. In such case the electron transport through DNA strands takes place in multistep hopping mechanism with all guanines as carriers of the positive charge [36,37]. The G-hopping mechanism holds only if the $(A:T)_n$ bridge between the guanines is small ($n < 4$). At longer $(A:T)_n$ sequences adenines also become charge carriers [37]. Charge transport in DNA via hopping occurs over very long distances, 5-30 nm without significant attenuation [38]. The application of carboxyphenyl layer did not affect the charge transport.

3.2. Analytical parameters

The sensitivity of the developed VB_2 biosensor was estimated as the slope of the calibration curve (reduction current of VB_2 versus its concentration, Fig. 1). In the range of VB_2 concentration (0.08 ÷ 1 μM) an almost perfect linear response was observed: I_{VB_2} (in nA) = (-45.9 ± 0.9)C_{VB_2} (in μM) + (-2.1 ± 0.5), r = 0.9992 (the slope and the intercept are accompanied by their standard deviations; each point taken for the regression was the mean of five independent measurements). In the whole range of VB_2 concentration the reproducibility was good; the relative standard deviation was approx. 4.30 % (n = 5). The electrode-to-electrode reproducibility was also examined between five different electrodes in the acetate buffer, and the relative standard deviation was calculated to be 9.50 %.

The detection (LOD) and quantification limits (LOQ) were 0.06 ± 0.005 and 0.15 ± 0.01 μM (24 ± 2 and 55 ± 5 $\mu g \cdot L^{-1}$) respectively. The value of LOD was calculated as a mean value increased by 3-times the standard deviation of VB_2 concentration in the blank sample ($\bar{x} \pm 3\sigma_x$; n = 5). The value of LOQ was calculated as a mean value increased by 10-times the standard deviation of VB_2 concentration in the blank sample ($\bar{x} \pm 10\sigma_x$; n = 5). Also the LOD

and LOQ were estimated from the equation of the best-fit line of the calibration curve (Fig. 1) drawn as the intensity of the reduction signal of VB_2 bound with DNA layer, in the range of VB_2 concentration 0.08 ÷ 1 μM. Both methods led to the same values of LOD and LOQ. The proposed DNA biosensor has a better detection limit of VB_2 compared with the other voltammetric sensors described in the literature, especially towards DNA-biosensor (see Table 1). In our case, in contrast to all available electrochemical biosensors for riboflavin detection, the biosensor signal was generated by riboflavin bound with dsDNA not by marker / redox probe. The application of proposed by us DNA biosensor with carboxyphenyl intermediary layer decreased the detection limit 15 times compared to the DNA-biosensor described in the literature [13], which showed that it could be used for the sensitive, rapid, simple and cost effective detection and determination of riboflavin via dsDNA interactions. However, there is a significant difference between LOD of VB_2 obtained using the described sensor, and reached by using differential pulse adsorptive stripping voltammetry (DPAdSV) at pretreated pencil graphite electrode (PPGE) but without DNA layer [13]. The detection limit estimated by the authors was $0.76 \cdot 10^{-4}$ mg·L^{-1}, which proved that determination of riboflavin by DPAdSV at PPGE was more sensitive than other electrochemical methods reported in the literature (including ours). It should be stressed that the surface area of interaction of VB_2 with activated pencil graphite electrode is much bigger than the size of DNA groove area (place of interaction of riboflavin with DNA). It means that the number of VB_2 molecules (redox probe) was also different. In the case of the sensor described by Wang et al. [15] ssDNA fragments (A_{32}) guaranteed stable anchoring of nanocomposite (grapheme-rMoS_2) to gold surface, so the riboflavin interacted only with the composite. Despite of that the detection limit was very low we should take into account that the preparation time of the sensor is very long, more than 3 days.

High stability is a very important parameter defining the biosensor's functionality. The stability of the used biosensor was examined by measuring the changes in the value of reduction current of riboflavin in acetate buffer as a function of time elapsed since the biosensor was constructed until its use (interaction with riboflavin). After the construction the DNA biosensor was kept under the cover at room temperature for 3 months. The proposed DNA biosensor was characterized by a good stability for the first month since its construction. The differences in the intensities of the reduction current signals of riboflavin bound with DNA layer were smaller than 8 %. For the longer storage time (>1 month) the current decreases by 20 % compared to the initial value (biosensor used just after its construction).

The range of selectivity of the proposed DNA-biosensor for VB_2 determination was checked in the presence of potential interfering substances commonly found in pharmaceutical and biological samples such as: glucose; vitamins: E, C, and ions: Ca^{2+} and Mg^{2+}. The selectivity experiments were carried out at saturation (1 μM) and unsaturation (0.5 μM) VB_2 concentrations. This was designed to check whether the interfering substances (i) react with DNA biosensor, (ii) form more stable complexes with DNA than riboflavin (displace VB_2 from the complex DNA-VB_2). After preparation the DNA biosensor (GC/-C_6H_4COOH/dsDNA) was immersed in acetate buffer (0.1 M, pH 4.80, with 0.02 M NaCl) containing riboflavin at appropriate concentration and 500-fold excess of interfering substances for 2 hours, and then the SW voltammograms were recorded in pure degassed acetate buffer. Typical SWV curves obtained after the interaction of constructed biosensor with VB_2 in the presence of different interferents are presented in Fig. 2. The current signals recorded in the presence of interferents varied in the range of 2 ÷ 9 % versus the signal obtained for pure riboflavin. It meant that none of tested interferents blocked the interaction sites of DNA with vitamin B_2. The influence of the interfering substances on the reduction current signal of riboflavin ($I_{VB_2+interferent}$ / I_{VB_2}) is presented in Fig. 3. The obtained results

clearly showed that the proposed procedure was selective and can be successfully used for VB_2 determination in real samples. The fact that the interferent did not displace VB_2 from the complex with double-strand DNA was a result of high stability of the complex. In order to prove the high stability of the VB_2-DNA complex the UV-vis measurements were carried out (Fig. 3S in Supplementary Materials). Based upon the variation in absorbance, the binding constant (K) of the VB_2 with dsDNA was determined from the intercept-to-slope ratio of the dependence A_0 / (A – A_0) versus 1/C_{DNA}, presented in Fig. 4S in Supplementary Materials, constructed according to the Benesi-Hildebrand formula [39]. The calculated K value was equaled $(2.2 \pm 0.5)\cdot 10^5$ M^{-1}. Practically identical K value was obtained from McGhee and von Hippel equation [40].

3.3. Analytical application of VB_2 DNA-biosensor in natural samples

Vitamin B_2 can be supplied to the body through ingestion of foods rich in vitamin B_2 or dietary supplements, and the necessity of monitoring VB_2 content in such products creates the analytical market for methods of riboflavin determination in natural matrices. The functionality of the developed method was tested by determination of riboflavin in two dietary supplements and in an animal tissue naturally rich in VB_2 – yolk of hen egg. The SWV responses are shown in Fig. 5S in Supplementary Materials. For all samples the reduction current signals of riboflavin were observed at the same position as the isolated VB_2 (Sigma Aldrich). The results of quantitative determination of riboflavin in supplements and yolk are presented in Table 2. To prove the correctness of the data obtained using the proposed biosensor the statistical evaluation based on the significance two-tailed t-test of mean values was applied. For supplements the obtained results were compared with the value given by the manufacturer – the reference value. The comparison of parameters t_{crit} (critical value – tabulated data) and t_{exp} (experimental value) revealed that there were no significant differences between these two values (two-tailed, P = 95 %, n-1 = 4, t_{crit} = 2.776, t_{exp} = 0.149

÷ 0.559), t_{exp} was lower than t_{crit}. In the case of yolk, to estimate the differences between the results obtained by DNA-biosensor and HPLC FLD the intermethod comparison (two independent analytical methods) was performed, see inset in Fig. 3S in Supplementary Materials. Both sets (the two-tailed, P = 95 %, n-2 = 8, t_{crit} = 2.306, t_{exp} = 2.303) of results did not differ significantly, t_{exp} was lower than t_{crit}. Therefore it could be concluded that the determination of VB_2 in natural samples by the proposed DNA-biosensor lead to correct results.

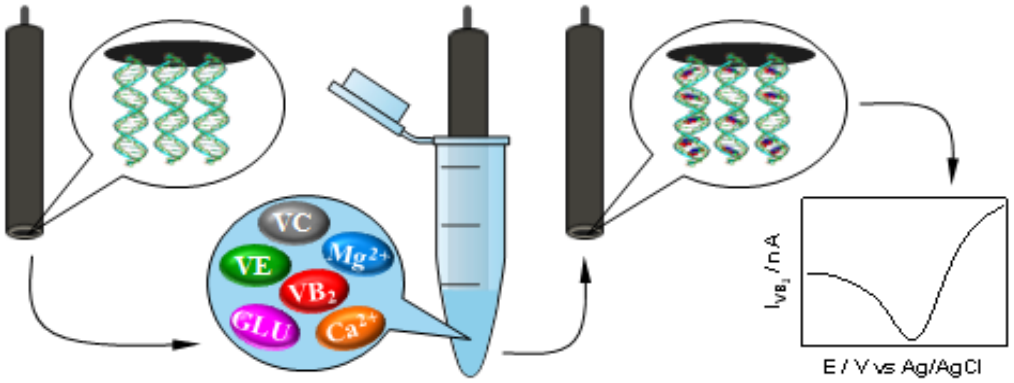
4. Conclusions

Herein, for the first time, we demonstrate a simple, rapid and direct electrochemical method of determination of riboflavin in pharmaceuticals and food samples by application of DNA biosensor. Proposed approach was based on directly recorded reduction current of riboflavin preferentially bound to the DNA double helix. The excellent analytical performance of DNA biosensor may be attributed to the carboxyphenyl intermediary layer that guaranteed high packing density of VB_2 in DNA duplex and the efficiency of electron transfer through DNA chains. These features allowed to observe a well-defined reduction current signal of riboflavin bound to the DNA biosensor.

Presented in this work DNA biosensor for direct electrochemical riboflavin determination is very simple in construction, not limited to DNA sequence, does not require special treatment of DNA or advanced mathematical treatment of the data. Furthermore, this DNA biosensor was characterized by a good stability for the first month since its construction. The differences in the intensity of the reduction current signals of riboflavin bound with DNA layer were lower than 8 %.

The DNA biosensor described in this paper allowed to selectively detect riboflavin at low limit of detection (LOD = 24 ± 2 $\mu g \cdot L^{-1}$). The most popular analytical techniques offer

similar or lower detection limits (1 μg·L^{-1}) [41,42], however these methods lack sufficient selectivity and require complicated procedures and expensive instruments. Presented here simple method of analysis, based on DNA biosensor, has been successfully used to determine riboflavin in dietary supplements and yolk of hen's egg. The data were in statistical compatibility with the results obtained using alternative (the classic one) method, which proved that the proposed DNA biosensor is feasible to be used in natural samples.



GRAPHICAL ABSTRACT

Table 1. Comparison of the efficiency of reported electrochemical methods employing carbon electrodes in riboflavin detection.

ELECTRODE MODIFICATION	ANALYTICAL SIGNAL	DETECTION LIMIT [mg·L^{-1}]	REFERENCE
	without DNA		
Screen-printed carbon electrode	DPV signal of VB_2	0.90 (in the absence of Cu^{2+}) 0.005 (in the presence of Cu^{2+})	[14]
Plain carbon paste electrode (PCPE)	SWV signal of VB_2	0.08	[43]
Pretreated pencil graphite electrode (PPGE)	DPAdSV	0.000076	[13]
	with DNA		
PGE/dsDNA	DPV signal of DNA	0.34	[13]

Au/A_{32}/graphene-rMoS_2	DPV	0.0075	[15]
GC/-C_6H_4COOH/dsDNA	SWV signal of VB_2	0.024	this work

SWV: square wave voltammetry; DPAdSV: differential pulse adsorptive stripping voltammetry; DPV: differential pulse voltammetry; PGE: pencil graphite electrode

Table 2. Results of determination of riboflavin in dietary supplements and yolk of hen's egg (n = 5).

SAMPLE	FOUND [mg]	DECLARED [mg]
Yolk*	6.1 ± 0.5	5.5 ± 0.3**
Supplement 1	1.4 ± 0.3	1.4
Supplement 2	1.4 ± 0.2	1.4

* amount of VB_2 [μg] per 1 g of yolk;

** the value found in the extract using HPLC FLD

Conflict of interest

The authors declare that they have no conflict of interest.

Acknowledgment

This work was supported by a National Science Center of Poland Grant No. 2014/15/D/ST4/02989. We thank student Karolina Twardowska for assistance in voltammetric experiments.

REFERENCES

[1] L.S. Anisimova, E.V. Mikheeva, V.F. Slipchenko, Voltammetric determination of riboflavin in vitaminized supplements and feeds, J. Anal. Chem. 56 (2001) 658-662.

[2] W. Friedrich, Vitamin B_2: Riboflavin and its bioactive variants, in: Vitamins. Walter de Gruyter, Berlin, 1988, pp. 405-463.

[3] H.J. Powers, Riboflavin (vitamin B-2) and health, Am. J. Clin. Nutr. 77 (2003) 1352-1360.

[4] S. Escott-Stump, Nutrition and Diagnosis-Related Care, 6th ed., Lippincott Williams & Wilkins, Philadelphia, 2008.

[5] F.A. Sarubin, C. Thomson, The Health Professional's Guide to Popular Dietary Supplements, third ed., American Dietetic Association, Chicago, USA, 2007.

[6] T.R.I. Cataldi, D. Nardiello, V. Carrara, R. Ciriello, G.E.D. Benedetto, Assessment of riboflavin and flavin content in common food samples by capillary electrophoresis with laser-induced fluorescence detection, Food Chem. 82 (2003) 309-314.

[7] R. Engel, E. Stefanovits-Bányai, L. Abrankó, LC simultaneous determination of the free forms of B group vitamins and vitamin C in various fortified food products, Chromatographia 71 (2010) 1069-1074.

[8] J. Ghasemi, B. Abbasi, A. Niazi, E. Nadaf, A. Mordai, Simultaneous spectrophotometric multicomponent determination of folic acid, thiamine, riboflavin, and pyridoxal by using double divisor–ratio spectra derivative–zero crossing method, Anal. Lett. 37 (2004) 2609-2623.

[9] A.E. Karatapanis, Y.C. Fiamegos, C.D. Stalikas, HILIC separation and quantitation of water-soluble vitamins using diol column, J. Sep. Sci. 32 (2009) 909-917.

[10] A. Kokaislová, P. Matějka, Surface-enhanced vibrational spectroscopy of B vitamins: what is the effect of SERS-active metals used?, Anal. Bioanal. Chem. 403 (2012) 985-993.

[11] J. Bai, J.C. Ndamanisha, L. Liu, L. Yang, L. Guo, Voltammetric detection of riboflavin based on ordered mesoporous carbon modified electrode, J. Solid State Electrochem. 14, (2010) 2251-2256.

[12] B. Baś, M. Jakubowska, Ł. Górski, Application of renewable silver amalgam annular band electrode to voltammetric determination of vitamins C, B_1 and B_2, Talanta 84 (2011) 1032-1037.

[13] A.A. Ensafi, E. Heydari-Bafrooei, M. Amini, DNA-functionalized biosensor for riboflavin based electrochemical interaction on pretreated pencil graphite electrode, Biosens. Bioelectron. 31 (2012) 376-381.

[14] R.O. Kadara, A.G. Fogg, B.G.D. Haggett, B.J. Birch, Enhancement by copper(II) of the voltammetric signal of vitamin B_2 applied to its determination in breakfast cereals, J. Agric. Food Chem. 57 (2009) 804-806.

[15] Y. Wang, Q. Zhuang, Y. Ni, Fabrication of riboflavin electrochemical sensor based on homoadenine single-stranded DNA/molybdenum disulfide–graphene nanocomposite modified gold electrode, J. Electroanal. Chem. 736 (2015) 47-54.

[16] B. Dogan-Topal, B. Uslu, S.A. Ozkan, Voltammetric studies on the HIV-1 inhibitory drug Efavirenz: The interaction between dsDNA and drug using electrochemical DNA biosensor and adsorptive stripping voltammetric determination on disposable pencil graphite electrode, Biosens. Bioelectron. 24 (2009) 2358-2364.

[17] M. Mascini, I. Palchetti, G. Marrazza, DNA electrochemical biosensors, Fresenius J. Anal. Chem. (Anal. Bioanal. Chem.) 369 (2001) 15-22.

[18] L. Strasak, J. Dvorak, S. Hason, V. Vetterl, Electrochemical impedance spectroscopy of polynucleotide adsorption, Bioelectrochemistry 56 (2002) 37-42.

[19] P.K. Brahman, R.A. Dar, S. Tiwari, K.S. Pitre, Electrochemical behavior of gatifloxacin at multi-walled carbon nanotube paste electrode and its interaction with DNA, Rev. Anal. Chem. 31 (2012) 83-92.

[20] B. Dogan-Topal, S.A. Ozkan, A novel sensitive electrochemical DNA biosensor for assaying of anticancer drug leuprolide and its adsorptive stripping voltammetric determination, Talanta 83 (2011) 780-788.

[21] A.-E. Radi, Application of electrochemical methods for analysis of fluoroquinolones antibacterial agents and fluoroquinolones-DNA interactions, Open Chem. Biomed. Methods J. 3 (2010) 27-36.

[22] Y.M. Hu, X.M. Wang, D. Fei, F.Q. Dong, L.S. Ding, Study on the interaction between Riboflavin and herring sperm DNA by fluorescence spectrometry, Acta Chimica Sinica 66 (2008) 1245-1251.

[23] E.L.S. Wong, P. Erohkin, J.J. Gooding, A comparison of cationic and anionic intercalators for the electrochemical transduction of DNA hybridization via long range electron transfer, Electrochem. Commun. 6 (2004) 648-654.

[24] E.L.S. Wong, J.J. Gooding, Charge transfer through DNA: A selective electrochemical DNA biosensor, Anal. Chem. 78 (2006) 2138-2144.

[25] G. Liu, Y.M. Wang, D.M. Sun, Simultaneous determination of vitamins B2, B6 and C using silver-doped poly(L-arginine)-modified glassy carbon electrode, J. Anal. Chem. 71 (1) (2016) 102-109.

[26] J. Chen, B.Q. Li, Y.Q. Cui, E. Yu, H.L. Zhai, A fast and effective method of quantitative analysis of VB1, VB2 and VB6 in B-vitamins complex tablets based on three-dimensional fluorescence spectra, J. Food Composit. Anal. 41 (2015) 122-128.

[27] T. Nie, J.-K. Xu, L.-M. Lu, K.-X. Zhang, L. Bai, Y.-P. Wen, Electroactive species-doped poly(3,4-ethylenedioxythiophene) films: Enhanced sensitivity for electrochemical simultaneous determination of vitamins B_2, B_6 and C, Biosens. Bioelectron. 50 (2013) 244–250.

[28] C. Sumathi, P. Muthukumaran, S. Radhakrishnan, G. Ravi, J. Wilson, Riboflavin detection by α-Fe_2O_3/MWCNT/AuNPs-based composite and a study of the interaction of riboflavin with DNA, RSC Adv. 5 (2015) 17888-17896.

[29] J. Li, Y. Zhang, T. Yang, H. Zhang, Y. Yang, P. Xiao, DNA biosensor by self-assembly of carbon nanotubes and DNA to detect riboflavin, Mater. Sci. Eng.: C 29 (2009) 2360-2364

[30] A. Kowalczyk, M. Fau, A.M. Nowicka, Z. Stojek, Formation of intermediate layers for immobilization of biomacromolecules by self-assembling and reduction of phenyldiazonium salts. A comparative study, Electroanalysis 24 (2012) 2053-2060.

[31] A.M. Nowicka, M. Fau, A. Kowalczyk, M. Strawski, Z. Stojek, Electrografting of carboxyphenyl thin layer onto gold for DNA and enzyme immobilization, Electrochim. Acta 126 (2014) 11-18.

[32] J. Liu, J.J. Gooding, M.N. Paddon-Row, Unusually rapid heterogeneous electron transfer through a saturated bridge 18 bonds in length, Chem. Commun. 5 (2005) 631-633.

[33] A. Steel, T.M. Herne, M.J. Tarlov, Electrochemical quantitation of DNA immobilized on gold, Anal. Chem. 70 (1998) 4670-4677.

[34] G.B. Schuster, Long-Range Charge Transfer in DNA: Transient Structural Distortions Control the Distance Dependence, Acc. Chem. Res. 33 (2000) 253-260.

[35] S. Steenken, S.V. Jovanovic, How easily oxidizable is DNA? One-electron reduction potentials of adenosine and guanosine radicals in aqueous solution, J. Am. Chem. Soc. 119 (1997) 617-618.

[36] P. Daublain, A.K. Thazhathveetil, Q. Wang, A. Trifnov, T. Fiebig, F.D. Lewis, Dynamics of photochemical electron injection and efficiency of electron transport in DNA, J. Am. Chem. Soc. 131 (2009) 16790-16797.

[37] B. Giese, Long-distance electron transfer through DNA, Annu. Rev. Biochem. 71 (2002) 51-70.

[38] M. Bixon, B. Giese, S. Wessely, T. Langenbacher, M.E. Michel-Beyerle, J. Jortner, Long-range charge hopping in DNA, PNAS 96 (1999) 11713-11716.

[39] H.A. Benesi, J.H. Hildebrand, A spectrophotometric investigation of the interaction of iodine with aromatic hydrocarbons, J. Am. Chem. Soc. 71 (1949) 2703-2707.

[40] J.D. McGhee, P.H. von Hippel, Theoretical aspects of DNA-protein interactions: Co-operative and non-co-operative binding of large ligands to a one-dimensional homogeneous lattice, J. Mol. Biol. 86 (1974) 469-489.

[41] P. Vinas, N. Balsalobre, C. Lopez-Erroz, M. Hernandez-Cordoba, Liquid chromatographic analysis of riboflavin vitamers in foods using fluorescence detection, J. Agric. Food Chem. 52 (2004) 1789-1794.

[42] Ch. Zhang, H. Qi, Highly sensitive determination of riboflavin based on the enhanced electrogenerated chemiluminescence of lucigenin at a platinum electrode in a neutral aqueous solutions, Anal. Sci. 18 (2002) 819-822.

[43] R.M. Kotkar, P.B. Desai, A.K. Srivastava, Behavior of riboflavin on plain carbon paste and aza macrocycles based chemically modified electrodes, Sens. Actuat. B 124 (2007) 90-98.

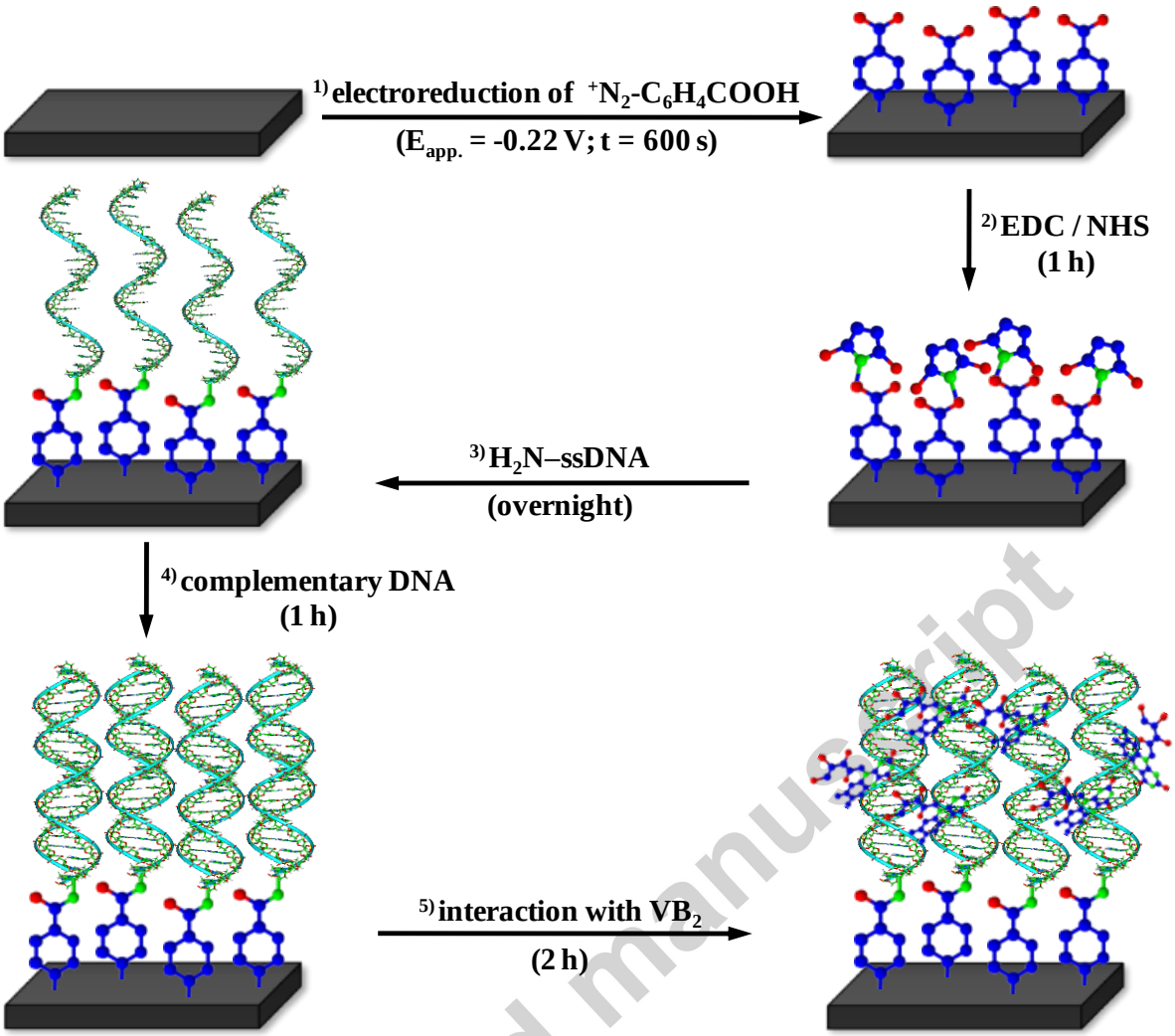
Figure and scheme legends

Scheme 1. Scheme of the entire procedure of preparation of VB_2 DNA-biosensor.

Fig. 1 Calibration plots of changes in reduction current of VB_2. Inset: Background subtracted SW voltammograms obtained in degassed 0.1 M acetate buffer containing 0.02 M NaCl; pH 4.8 at modified glassy carbon disc electrode (GC/-C_6H_4COOH/dsDNA) after interaction with VB_2 at different concentrations: 0.08; 0.2.; 0.4; 0.6; 0.8 and 1 μM (solid lines); background (dashed line). Experimental conditions: SWV parameters: step potential = 2.5 mV, frequency = 8 Hz; amplitude = 25 mV; glassy carbon disc electrode (ϕ = 3 mm).

Fig. 2 Background subtracted SW voltammograms obtained in 0.1 M degassed acetate buffer containing 0.02 M NaCl; pH 4.8 at modified glassy carbon disc electrode (GC/-C_6H_4COOH/dsDNA) after interaction with VB_2 in the presence of appropriate interfering substances. Experimental conditions: concentration of VB_2: 0.5 μM; concentration of interfering species 0.25 mM. SWV conditions as in Fig. 1. VE: vitamin E, VC: vitamin C, GL: glucose. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Fig. 3 Effect of the presence of interfering substances on the reduction current of riboflavin. Experimental conditions: concentration of VB_2: 0.5 μM and 1.0 μM. Other conditions as in Fig. 2.



Scheme 1

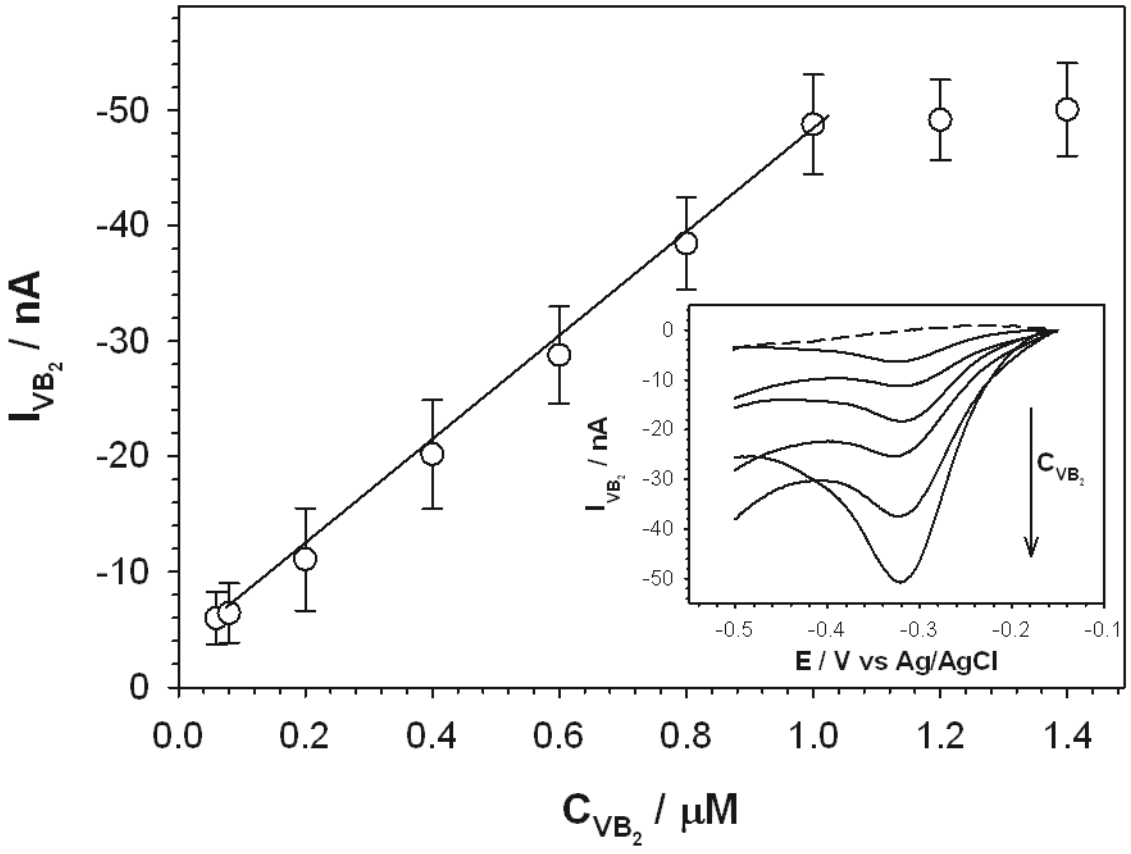


Fig. 1

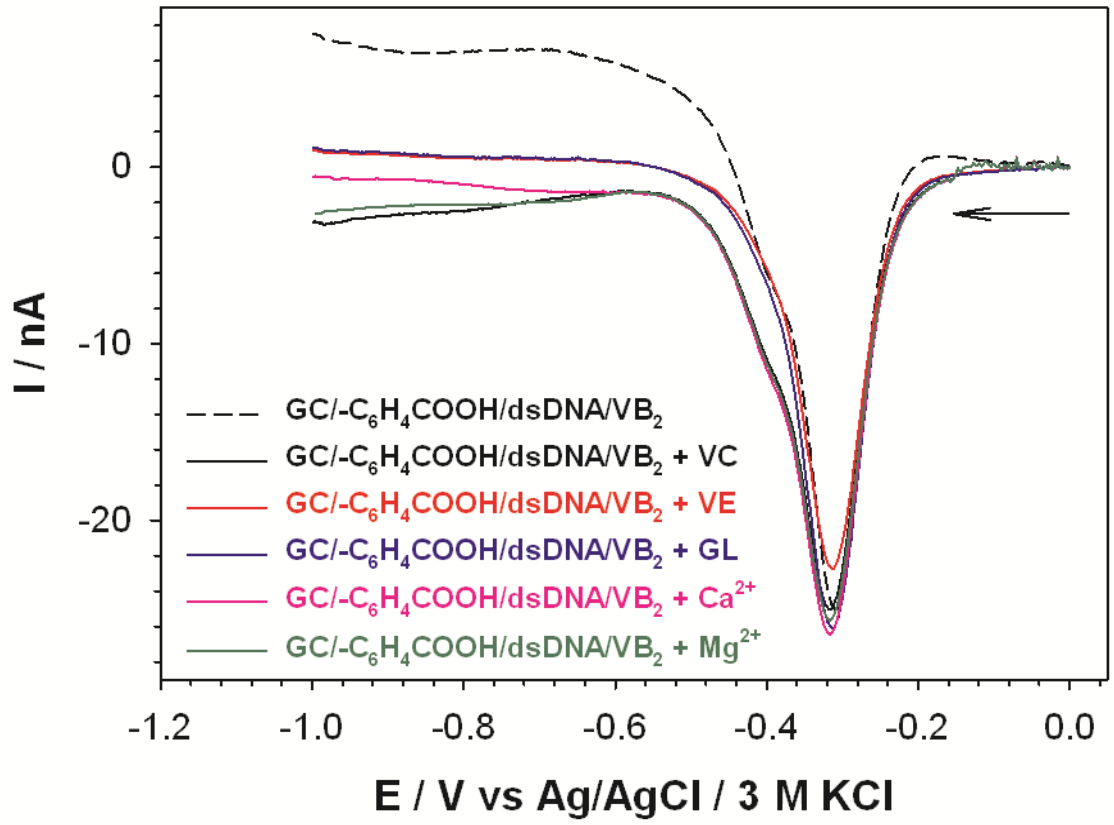


Fig. 2

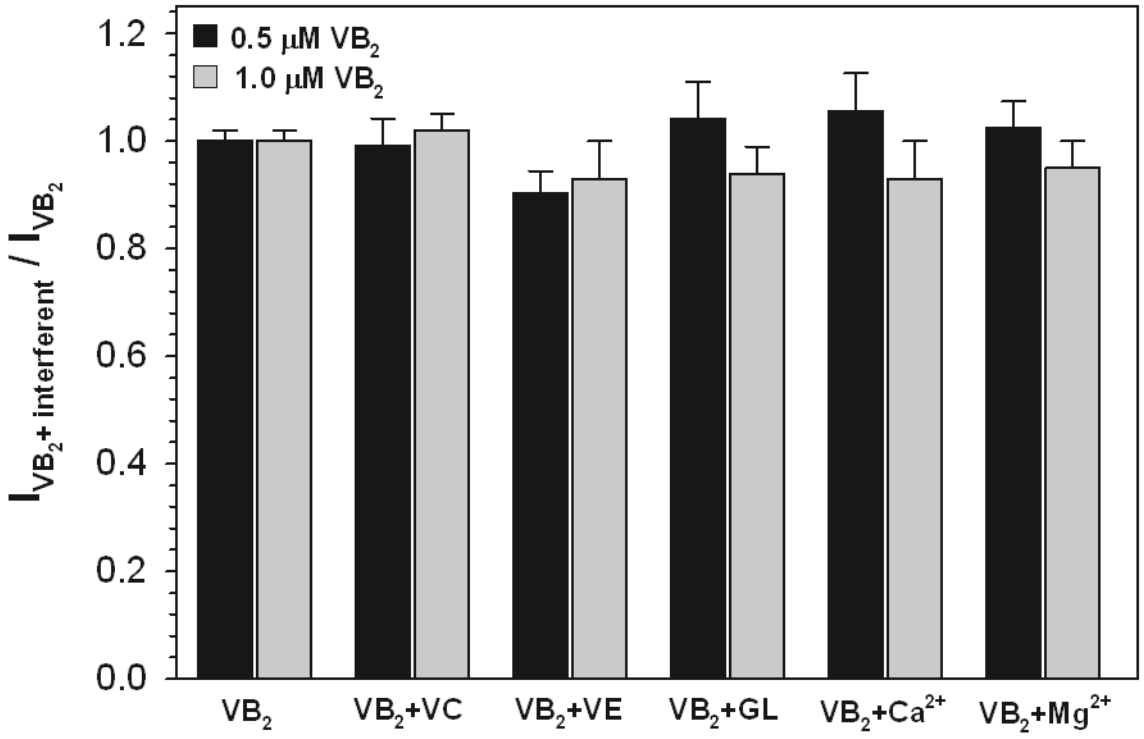


Fig. 3

Highlights

- Direct determination of VB_2 in natural samples using DNA biosensor.
- Voltammetric detection based on reduction signal of VB_2.
- Low LOD and LOQ (0.063 and 0.15 μM of VB_2) achieved.
- Applicability proved by intermethod comparison with HPLC FLD.