Journal of the Serbian Chemical Society
JSCS@tmf.bg.ac.rs • www.shd.org.rs/JSCS

J. Serb. Chem. Soc. 74 (12) 1467–1476 (2009)
JSCS–3933

UDC 663.12:547.963.32+543.552
Original scientific paper

Sensitive voltammetric detection of yeast RNA based on its interaction with Victoria Blue B

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(Received 7 January, revised 25 May 2009)

Abstract: Voltammetric studies of the interaction of yeast RNA (y-RNA) with Victoria Blue B (VBB) are described in this paper. Furthermore, a linear sweep voltammetric method for the detection of y-RNA was established. The reaction conditions, such as acidity and amount of buffer solution, the concentration of VBB, the reaction time and temperature, *etc*., were carefully investigated by second order derivative linear sweep voltammetry. Under the optimal conditions, the reduction peak current of VBB at –0.75 V decreased greatly after the addition of y-RNA to the solution without any shift of the reduction peak potential. Based on the decrease of the peak current, a new quantitative method for the determination of y-RNA was developed. The effects of co-existing substances on the determination were carefully investigated and three synthetic samples were determined with satisfactory results. The stoichiometry of the VBB–y-RNA complex was calculated by linear sweep voltammetry and the interaction mechanism is discussed.

Keywords: interaction; linear sweep voltammetry; Victoria Blue B; yeast RNA.

INTRODUCTION

Nucleic acids (NAs) are very important for their specific functions in life science. The determination of the content of NAs is very useful in mutation detection and clinical diagnostics. Hitherto, many methods have been proposed for the determination of NAs, including UV–Vis spectrophotometry,[1–3] fluorescence,[4] the light-scattering technique,[5,6] *etc*. However, spectrophotometric methods are limited by their low sensitivity, while fluorometric methods often suffer from inherent interference from proteins and other compounds present in biological samples. Recently, the light-scattering technique was extensively studied and applied to the determination of deoxyribonucleic acid (DNA).[7,8] Compared with

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doi: 10.2298/JSC0912467Z

these analytical methods, electrochemical methods have some advantages, such as cheaper and smaller devices, a wider linear range and a lower detection limit. Electrochemical methods have been widely used to study the interaction of NAs in solution with molecules such as metal chelates,[9] dyes[10] and drugs.[11] Most of these studies were, however, focused on investigations with DNA because of its importance in relation to replication and transcription, mutation of genes, action mechanisms of some DNA-related diseases and DNA-targeted drugs, specific sequence gene detection, *etc*. Otherwise, to the best of our knowledge, reports concerning interactions with ribonucleic acid (RNA) are seldom. RNA also plays important roles in the process of transcription and some gene information is concerned with RNA. Proteins can also take advantage of conformational polymerphism in the RNA backbone. Thus, it is also important to study the electrochemical behavior of RNA. Palecek investigated the voltammetric behavior of RNA on hanging mercury working electrodes using cyclic voltammetry[12] and differential pulse voltammetry.[13] The results indicated that, in a weakly alkaline electrolyte, RNA produced a cathodic peak at –1.36 V (*vs.* SCE). The interaction of some metal chelates, such as rhodium(III) phenanthroline,[14,15] ruthenium(II) polypyridine,[16,17] lead(II),[18] *etc*., with RNA have been reported for recognition or hydrolysis reactions. Sun *et al*. investigated the interaction of pyronine B with RNA by an electrochemical method and further applied it to the quantitative detection of RNA.[19] Zhang *et al.* studied the interaction of a ciprofloxacin–copper complex with RNA by linear sweep voltammetry and established a new approach for RNA determination.[20] Jia *et al*. developed a method for detecting RNA by the resonance light scattering quenching technique.[21]

In this work, the electrochemical behavior of Victoria Blue B (VBB) in the absence and presence of yeast RNA (y-RNA) was examined. VBB is a cationic dye, the structure of which is shown in Fig. 1. It is a commonly used as a cheaper price indicator. In pH 3.5 Britton–Robinson (B–R) buffer solution, VBB has a sensitive linear sweep voltammetric reduction peak at a potential of –0.75 V (*vs.* SCE) and the addition of y-RNA into a VBB solution resulted in changes of the reduction peak current, which could be further used for the detection of y-RNA.

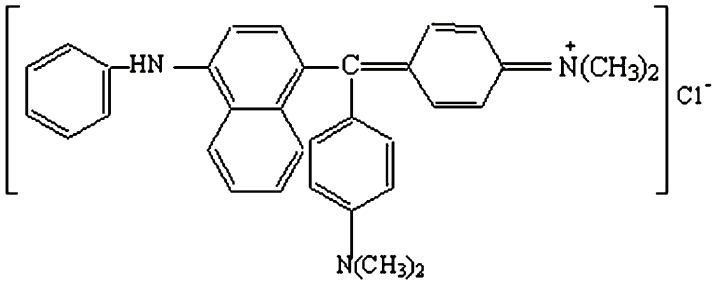

Fig. 1. The molecular structure of Victoria Blue B.

The optimal conditions for the interaction were selected. Under the optimal conditions, the binding number and the binding constant were calculated from the electrochemical data.

EXPERIMENTAL

Apparatus

All the electrochemical experiments were performed using a JP model 303 polarographic analyzer (Chengdu Apparatus Factory, China) with the traditional three-electrode system using a dropping mercury electrode (DME) as the working electrode, a platinum wire counter electrode and a saturated calomel reference electrode (SCE). All the potentials given in this paper are related to the SCE. A Cary 50 probe UV–Vis spectrophotometer (Varian Company, Australia) was used to record the UV–Vis absorption spectra. A pHS-25 acidimeter (Shanghai Leici Instrument Factory, China) was used for measuring the pH of the solutions. All the experiments were performed at 25±2 °C, except when otherwise stated.

Reagents

Stock solutions of yeast RNA (y-RNA, Tianjin Damao Chemical Reagent Company, China) and fish sperm DNA (fs-DNA, Beijing Jingke Biochemical Reagent Company, China) (1.0 g L^{-1}) were prepared by dissolving them in doubly distilled water. The 1.0×10^{-3} mol L^{-1} solution of Victoria Blue B (VBB, Tianjin Kermel Chemical Reagent Company, China) was obtained by dissolving 0.0506 g VBB into 100 mL water. A Britton–Robinson (B–R) buffer solution (0.20 mol L^{-1}) was used to control the acidity of the interaction system. All other reagents were of analytical reagent grade and doubly distilled water was used throughout this study.

Procedure

Solutions of VBB (0.40 mL, 1.0×10^{-3} mol L^{-1}), pH 3.5 B–R buffer (2.5 mL) and an appropriate amount of y-RNA (or samples) were mixed in a 10 mL volumetric flask, diluted to the mark and mixed thoroughly. After reacting at room temperature for 15 min, the second order derivative linear sweep voltammetric curve was recorded in the potential range from –0.3 V to –1.0 V. The peak current of VBB reduction at a potential of –0.75 V (*vs.* SCE) was recorded as the blank response (Ip_0'') and the peak current of the VBB–y-RNA mixture was recorded as Ip''. The difference of the peak current ($\Delta Ip'' = Ip_0'' - Ip''$) was used for quantitative analysis.

RESULTS AND DISCUSSION

Absorption spectra

The UV–Vis absorption spectra of VBB in the absence and presence of different amounts of y-RNA are shown in Fig. 2. In pH 3.5 B–R buffer solution and in the scanning range from 350 to 800 nm, VBB had an absorption peak maximum at 612 nm (curve 1) and y-RNA had no absorption (curve 4). When y-RNA was mixed with VBB, the absorbance of VBB at 612 nm decreased (curves 2 and 3), with an isobestic point appearing at 646 nm. The more the y-RNA was added, the greater was the absorbance decrease, which indicates that a binding reaction between VBB and y-RNA had occurred in the mixture solution and a new biosupramolecular complex was formed under these experimental conditions.

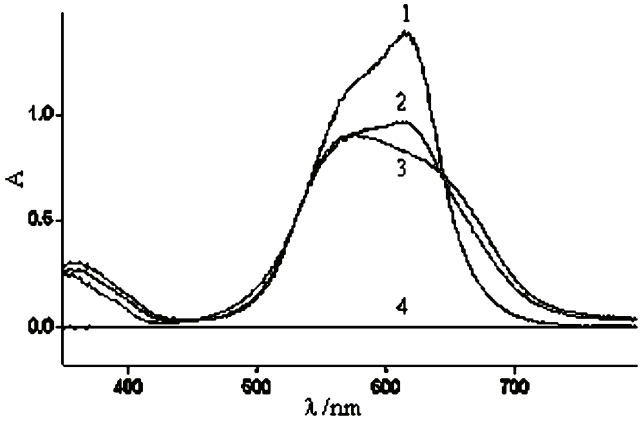

Fig. 2. UV–Vis Absorption spectra of the interaction of VBB with y-RNA. Reaction conditions: 1) pH 3.5 B–R buffer + 4.0×10^{-5} mol L^{-1} VBB; 2) and 3) pH 3.5 B–R buffer + 10.0 and 50.0 mg L^{-1} y-RNA, respectively; 4) pH 3.5 B–R buffer + 20.0 mg L^{-1} y-RNA.

Linear sweep voltammograms

Second order derivative linear sweep voltammetry can give a peak shape curve with high sensitivity; hence it was employed in this study. The second order derivative linear sweep voltammograms of VBB with different amounts of y-RNA are shown in Fig. 3. It can be seen that the B–R buffer did not have any electrochemical response (curve 1) and VBB had a sensitive second order derivative linear sweep voltammetric reduction peak at –0.75 V (*vs.* SCE) (curve 2), which was due to the electrochemical reduction of VBB on the mercury electrode, while y-RNA showed no electrochemical response in this potential range. After the addition of y-RNA into the VBB solution, the reduction peak current at the potential of –0.75 V decreased gradually with increasing y-RNA concentration (curves 3 and 4). The phenomena indicated that an interaction occurred in the mixture solution, which resulted in a decrease of free concentration of VBB

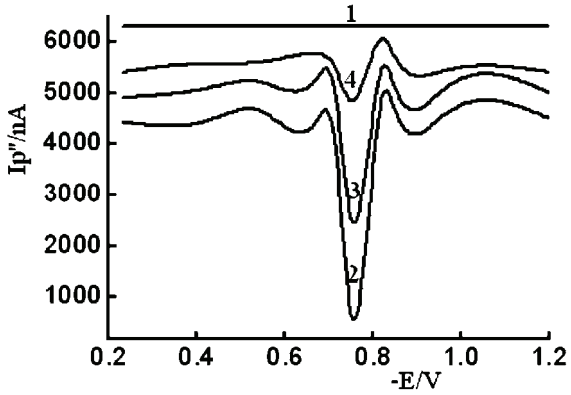

Fig. 3. Second order derivative linear sweep voltammograms of the interaction of VBB with y-RNA. Reaction conditions: 1) pH 3.5 B–R buffer; 2) pH 3.5 B–R buffer + 4.0×10^{-5} mol L^{-1} VBB; 3) and 4) pH 3.5 B–R buffer + 4.0×10^{-5} mol L^{-1} VBB + + 10.0 and 20.0 mg L^{-1} y-RNA, respectively.

in the solution and a decrease of the reduction peak current. Since the isoelectric point (p*I*) of y-RNA is in the range of 2.0 to 2.8 and the value of the pK_a of VBB is 8.25, in the selected pH 3.5 buffer solution, the phosphate in the backbone of y-RNA was highly negatively charged, while the VBB molecules were positively charged. Thus a strong electrostatic attraction reaction between VBB and y-RNA occurred in the solution to form a supramolecular complex. Based on the decrease in the peak current, a new voltammetric method for the quantification of NAs was further established.

Optimization of experimental conditions

The influence of pH on the difference of peak currents was examined in the pH range from 1.5 to 6.0 and the results are shown in Fig. 4, from which it can be seen that the Δ*I*p value reached its maximum at pH 3.5, hence this pH value was employed in the following experiments. Additionally, the experiments indicated that the response to the VBB–y-RNA reaction was larger in B–R buffer solution than in other buffers, such as NH_3–NH_4Cl, HOAc–NaOAc, *etc*. Hence, a B–R buffer solution of pH 3.5 was selected as being optimal. The effect of the concentration of the B–R buffer solution on the peak current difference was also studied in the range from 0.010 to 0.20 mol L^{-1} and the results showed that the Δ*I*p″ value reached a maximum when the concentration of the B–R buffer solution was 0.05 mol L^{-1}.

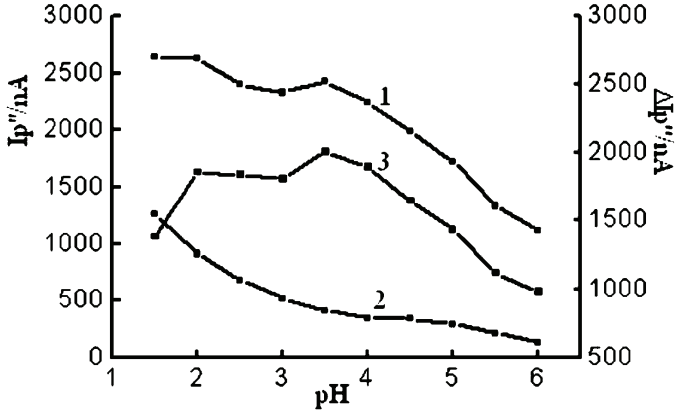

Fig. 4. The influence of buffer pH on the peak current (1 and 2) and the difference of peak currents (3). Reaction conditions: *c*(VBB) = 4.0×10^{-5} mol L^{-1}; 1) *c*(y-RNA) = 0; 2) *c*(y-RNA)= 20.0 mg L^{-1}; 3) Δ*I*p″ = *I*p1″ – *I*p2″.

The influence of the VBB concentration on the difference in the reduction peak current was measured using 20.0 mg L^{-1} y-RNA. As shown in Fig. 5, the Δ*I*p″ value increased with increasing VBB concentration and then decreased gra-

dually. The maximal value of $\Delta Ip''$ was obtained at a concentration of VBB of 4.0×10^{-5} mol L^{-1}; hence a 4.0×10^{-5} mol L^{-1} concentration of VBB was selected for use. Since the y-RNA concentration was fixed at 20.0 mg L^{-1}, when the VBB concentration was smaller than 4.0×10^{-5} mol L^{-1}, the interaction of VBB with y-RNA did not reach equilibrium, hence the value of $\Delta Ip''$ value increased gradually. When the VBB concentration was more than 4.0×10^{-5} mol L^{-1}, the reaction reached to the equilibrium and all the y-RNA was bound to VBB; hence any further increase of the VBB concentration in the reaction solution increased the concentration of free VBB in the reaction solution and then the $\Delta Ip''$ value decreased gradually.

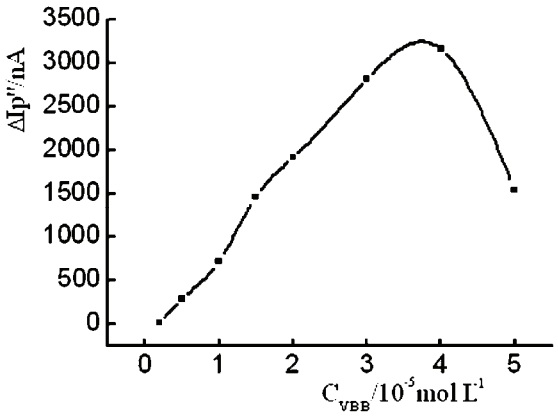

Fig. 5. The influence of the VBB concentration on the difference of peak currents ($\Delta Ip''$). Reaction conditions: 20.0 mg L^{-1} y-RNA and different concentrations of VBB in pH 3.5 B–R buffer solution.

The binding reaction occurred rapidly after y-RNA was mixed with VBB. The $\Delta Ip''$ value reached its maximum within 15 min and remained constant for at least 2 h. Therefore, this system gave ample time to measure the reduction current of a large number of real samples. In the reaction temperature range from 10 to 40 °C, no great differences were observed for the determination. When reaction temperature was more than 40 °C, y-RNA may be denatured. Hence, a temperature of 25 °C was used throughout in the following procedure.

The instrumental conditions of the polarographic analyzer, such as the scan rate and the dropping mercury standing time (lifetime of the mercury drop) were also selected. With increasing scan rate, the peak current increased, which is in accordance with the Ilkovic equation. The maximal $\Delta Ip''$ value was obtained at a scan rate as 900 mV s^{-1}, hence this scan rate was selected. The reduction peak current also increased with increasing standing time of the dropping mercury. However, when the dropping mercury standing time was more than 13 s, the mercury drop fell down naturally. Hence, a 12-second standing time of the dropping mercury was selected.

Generally speaking, biosamples are often diluted with NaCl solution to keep the bioactivity and biomicroenvironment of the target. Hence, the influence of ionic strength was also investigated by the addition of 1.0 mol L^{-1} NaCl to the mixture. As shown in Fig. 6, the peak current decreased greatly with increasing ionic strength, which was due to a decrease of the electrostatic force between the VBB anion and y-RNA. With increasing ionic strength, the shielding effect of the charges on the y-RNA was unbeneficial to the formation of the VBB–y-RNA complex.

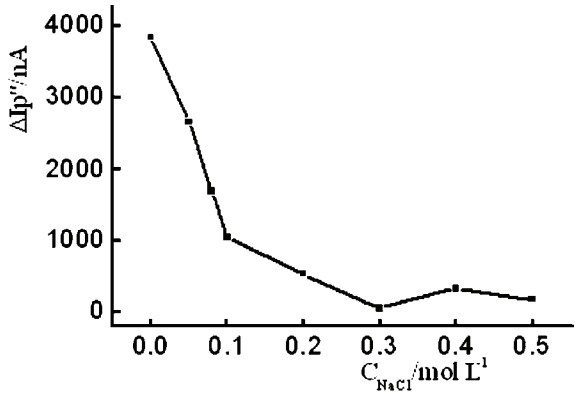

Fig. 6. The influence of ionic strength on the difference of peak currents ($\Delta Ip''$). Reaction conditions: 4.0×10^{-5} mol L^{-1} VBB + 20.0 mg L^{-1} y-RNA in pH 3.5 B–R buffer solution.

Interferences

The interferences of some co-existing substances, such as amino acids, metal ions, glucose, *etc*., on the determination of y-RNA were studied and the experimental results are shown in Table I. As can be seen, most of the investigated substances could be tolerated at higher concentrations without interference.

TABLE I. Tolerance to co-existing substances on the determination of 20.0 mg L^{-1} y-RNA in pH 3.5 B–R buffer solution with a VBB concentration of 4.0×10^{-5} mol L^{-1}

Coexisting substance	Concentration mg L^{-1}	Relative error %	Coexisting substance	Concentration μmol L^{-1}	Relative error %
L-Serine	0.5	4.99	Cu^{2+}	0.5	–4.98
L-Tyrosine	0.5	–3.17	Mn^{2+}	0.5	–0.29
L-Valine	0.5	2.08	Ca^{2+}	0.5	–0.06
L-Arginine	0.5	–2.28	Sn^{2+}	0.5	0.61
L-Leucine	0.5	1.25	Zn^{2+}	0.5	–3.19
L-Glutamine	0.5	–1.42	Mg^{2+}	0.5	–2.72
Glycine	0.5	–3.19	Co^{2+}	0.5	–2.36
Citric acid	0.5	2.92	Urea	0.5 mg L^{-1}	1.01
6-Amino caproic	0.5	12.02	Glucose	0.5 mg L^{-1}	–2.46

Calibration curves

Under the optimal conditions, calibration curves for the determination of NAs were constructed. As shown in Table II, the differences of the reduction peak current in the absence and presence of the two examined NAs were proportional to the concentration of the NA with a good linear relationship. The detection limit was calculated according to the equation of $LOD = KS_0/S$, where K is a constant related to the confidence level. According to the suggestion of the IUPAC, the value of K is 3 at the 99 % confidence level. S_0 is the standard deviation of ten blank-solution measurements (no added y-RNA) and S is the slope of the calibration graph. The relative standard deviation (*RSD*) for 11 parallel determinations of 20.0 mg L^{-1} y-RNA was 1.98 %.

TABLE II. Analytical parameters for the determination of different nucleic acids in pH 3.5 B–R buffer solution with a VBB concentration of 4.0×10^{-5} mol L^{-1}

NAs	Linear range mg L^{-1}	Standard regression equation	Detection limits (3σ), mg L^{-1}	Regression coefficient (γ)
y-RNA	6.0–20.0	$\Delta Ip'' = 190.08c - 889.72$	1.34	0.993
fs-DNA	6.0–16.0	$\Delta Ip'' = 287.68c - 1509.00$	0.57	0.991

Sample determinations

Artificial y-RNA samples containing metal ions and amino acids, *etc*., were determined and the results are listed in Table III. It can be seen that y-RNA in the artificial samples could be determined with satisfactory results and the recoveries were in the range of 99.67–100.80 %, which indicates that this method is practical and reliable.

TABLE III. Results of the determination of y-RNA in synthetic samples (n = 5) in pH 3.5 B–R buffer solution with a VBB concentration of 4.0×10^{-5} mol L^{-1}

Sample	Coexisting substance[a]	Added mg L^{-1}	Found mg L^{-1}	*RSD* %	Recovery %
1	Glycine, citric acid, Zn^{2+}, Mn^{2+}	10.00	10.08	1.67	100.80
2	L-Arginine, urea, Ca^{2+}, Mg^{2+}	15.00	14.97	0.89	99.67
3	L-Valine, L-glutamine, Cu^{2+}, Co^{2+}	20.00	20.08	0.76	100.40

[a]Concentration of coexisting substances: 0.50 μmol L^{-1}

Stoichiometry of the VBB–y-RNA complex

In the selected pH 3.5 buffer solution, the VBB molecules were positively charged, while deprotonation of the phosphate groups resulted in negative charges on the y-RNA chains. Hence, the interaction of VBB with y-RNA was caused by electrostatic attraction. The stoichiometry of the VBB–y-RNA complex was calculated from the voltammetric data. According to a proposed method,[22,23] it was assumed that only a single complex of y-RNA–mVBB was formed when

VBB interacted with y-RNA. The binding number (m) and the equilibrium constant (β_s) of the binding reaction can be deduced as follows:

$$\text{y-RNA} + m\text{VBB} \leftrightarrow \text{y-RNA}–m\text{VBB} \quad (1)$$

The equilibrium constant is deduced as follows:

$$\beta_s = \frac{[\text{y-RNA}–m\text{VBB}]}{[\text{y-RNA}][\text{VBB}]^m} \quad (2)$$

as:

$$\Delta I_{max} = kc_{\text{y-RNA}} \quad (3)$$

$$\Delta I = k[\text{y-RNA}–m\text{VBB}] \quad (4)$$

$$[\text{y-RNA}] + [\text{y-RNA}–m\text{VBB}] = c_{\text{y-RNA}} \quad (5)$$

Therefore:

$$\Delta I_{max} - \Delta I = k(c_{\text{y-RNA}} - [\text{y-RNA}–m\text{VBB}]) = k[\text{y-RNA}] \quad (6)$$

Introducing Eqs. (2), (4) and (6) gives:

$$\log [\Delta I/(\Delta I_{max} - \Delta I)] = \log \beta_s + m \log [\text{VBB}] \quad (7)$$

where ΔI is the difference between the peak current of the sample and blank, ΔI_{max} corresponds the maximum value of difference of peak currents, $c_{\text{y-RNA}}$, [y-RNA–mVBB] and [y-RNA] correspond to the total, bound and free concentrations of y-RNA in the solution, respectively.

From Eq. (7), the relationship of log ($\Delta I/(\Delta I_{max} - \Delta I)$) with log [VBB] was calculated and a linear regression equation was obtained as:

$$\log (\Delta I/(\Delta I_{max} - \Delta I)) = 2.48\log [\text{VBB}] + 11.97 \quad (n = 6, \gamma = 0.992)$$

From the intercept and the slope, the values $m = 2.5$ and $\beta_s = 9.33\times10^{11}$ were deduced, which indicated that a stable 2:5 complex of 2y-RNA–5VBB was formed under the selected conditions.

CONCLUSIONS

The linear sweep voltammetric method was shown to be a useful method for bioanalysis with the advantages of a low detection limit, wide dynamic range and instrumental simplicity with moderate costs. Since the electrode reaction occurred at the electrode/solution interface, it can be applied to small amounts of samples. Based on the decrease of the reduction peak current of VBB after the addition of y-RNA under the selected conditions, a new voltammetric method for the determination of y-RNA was developed. The method is sensitive, reproducible and not affected by commonly co-existing substances. The stoichiometry of the VBB–y-RNA complex was calculated from the voltammetric data.

Acknowledgements. This work received support from the National Natural Science Foundation of China (20405008) and the Doctoral Foundation of QUST (0022125).

ИЗВОД

ОСЕТЉИВА ВОЛТАМЕТРИЈСКА ДЕТЕКЦИЈА РНК КВАСЦА БАЗИРАНА НА ИНТЕРАКЦИЈИ СА ВИКТОРИЈАПЛАВИМ Б

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У раду је описана волтаметријска анализа интеракције РНК квасца (РНКк) са викторијаплавим Б као и метода линеарне промене потенцијала за детекцију РНКк. Реакциони услови, као што су киселост, количина пуфера, концентрација викторијаплавог Б, реакционо време и температура, испитивани су диферцијалном линеарном променом потенцијала другог реда. Под оптималним условима, струјни врх редукције викторијаплавог Б на –0,75 V смањује се нагло по додатку РНКк у раствор, без промене потенцијала струјног врха. Метода за одређивање РНКк је базирана на смањењу струјног врха. Испитан је ефекат утицаја споредних компоненти на одређивање РНКк и три синтетичка узорка су успешно анализирана. Стрехиометријски састав комплекса викторијаплаво Б–РНКк је израчунат на основу волтаметријских података, а механизам интеракција дискутован је у раду.

(Примљено 7. јануара, ревидирано 25. маја 2009)

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