Scientific paper

Determination of Yeast RNA Using Safranine T with Linear Sweep Voltammetry

Wei Sun*, Na Zhao, Kui Jiao

College of Chemistry and Molecular Engineering, Qingdao University of Science and Technology, Qingdao 266042, P. R.China

** Corresponding author: Tel: +86-532-4022681, Fax: +86-532-4023927, E-mail: sunwei@qust.edu.cn*

Received: 01-02-2007

Abstract

A new linear sweep voltammetric method was developed for the determination of trace amount of yeast RNA (yRNA) by using safranine T (ST) as an electrochemical probe. In a pH 3.5 Britton-Robinson (B-R) buffer solution, ST had a sensitive linear sweep voltammetric reduction peak at –0.39 V (vs. SCE) and the addition of yRNA can decrease the reduction peak current of ST without the shift of the peak potential. The decrease of the peak current was proportional to the concentration of yRNA and further used for yRNA determination. The optimum conditions for the determination of yRNA were investigated. Under the optimal conditions a linear relationship of the peak current with the concentration of yRNA was got in the range of 8.0–25.0 mg L^{-1} with the detection limit as 0.84 mg L^{-1}. The method was simple, practical and free of the interferences from coexisting substances. Three synthetic samples were analyzed with satisfactory results. The binding constant (β_s) and the binding number (m) of yRNA with ST was calculated by voltammetric method with the results as $\beta_s = 1.05 \times 10^{11}$ and $m = 2.5$.

Keywords: Yeast RNA, safranine T, linear sweep voltammetry, interaction

1. Introduction

The determination of nucleic acids (NAs) is a basic prerequisite in analytical biochemistry related to life science. Many analytical methods were reported, such as UV–Vis spectrophotometry,[1–2] fluorimetry,[3–4] chemiluminescence,[5–6] light scattering technique[7–8] and electrochemistry.[9–10] Among them, the most commonly used technique is the spectrophotometric technique, which is simple and easy to perform with inexpensive instrumentation. But the disadvantages of spectrophotometry are its low sensitivity, while fluorometric methods often suffer from inherent interferences from proteins and other compounds in biological samples.

Electrochemical techniques have some advantages such as wider linear range and lower detection limit. Since the electrochemical reactions occur at the electrode/solution interface, the electrochemical response is related to the surface concentration of the detected species and is thus especially suitable for small sample concentrations. Different electrochemical methods had been applied to the study on electrochemical behaviors of NAs. Paleček first discovered the direct electrochemical activity of NAs on the mercury electrode.[11] Patricia *et al* had reviewed the development of electrochemistry of NAs on different types of solid electrodes.[12] Electrochemical studies on the interaction of NAs with different kinds of small molecules such as metal complexes,[13–14] drugs[15–16] and dyes[17–18] had been extensively studied. Most of the references are focused on the investigation of the electrochemical behavior of DNA, which is important in relation to replication and transcription, mutation of genes, action mechanisms of some DNA-related diseases and DNA-targeted drugs, electrochemical DNA biosensor and so on. But there are rare reports on electrochemical studies of the ribonucleic acid (RNA). In life sciences, RNA is also important in the process of transcription and some of the gene information is transferred by RNA. So the structure and nature of RNA is also of great research interest and it is necessary to investigate and establish a sensitive method for RNA detection. Barton *et al* used the coordination complex tris (4,7-diphenyl- 1,10-phenanthroline) rhodium (III) as a unique probe of guanine-uracil (G-U) mismatches and as a model in identifying G-U mismatches within double-helical regions of folded RNAs.[19] Cater *et al* investigated the binding specificity for the intercalating $Ru(tpy)(dppz)O^{2+}$

complex (tpy = 2,2´,2″-terpyridin, dppz = dipyridophenazine) for duplex DNA, HIV-1 TAR DNA, RNA and t-RNA^{Phe}.[20] Ji *et al* studied the interaction mechanism of the $[Ru(phen)_2(pMIP)]^{2+}$ (phen = 1,10-phenanthroline, pMIP = 2-(4-methylphenyl) imidazo[4, 5-f][1, 10]phenanthroline) with yeast tRNA using a spectroscopic method and found that this method was suitable.[21] Lindell *et al* used the divalent ion, lead (II), as a structural probe to describe a simple and reliable method for RNA structure determination in vivo.[22] Sun *et al* used pyronine B (PB) as an electrochemical probe to investigate the quantitation of yRNA.[23]

In this paper, the electrochemical behaviors of safranine T (ST) before and after the addition of yeast RNA (yRNA) in buffer solution were discussed. ST was a cationic dye with its structure shown in Figure 1. In the selected pH 3.5 Britton-Robinson (B-R) buffer solution, ST had a sensitive linear sweep voltammetric reductive peak at –0.39 V (vs. SCE). The interaction of yRNA with ST resulted in the decrease of the reduction peak current, which can be further used to establish a method for yRNA detection. Compared with the report using PB for yRNA detection[23], the reductive peak potential was 0.51 V lower than that of PB, which was appeared at –0.90 V (vs. SCE). The result indicated that the reduction of ST was easily taken place and not interfered by the reduction of oxygen dissolved in the solution. Under the optimal conditions, the binding number and the binding constant were calculated by the voltammetric data.

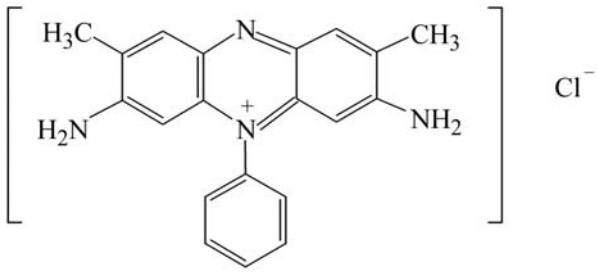

Figure 1. The molecular structure of safranine T

2. Experimental

2. 1. Apparatus

All the electrochemical experiments were performed on a JP model 303 polarographic analyzer (Chengdu Apparatus Factory, China) with the traditional three-electrode system composing of a dropping mercury electrode (DME) as working electrode, a platinum wire as counter electrode and a saturated calomel as reference electrode (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 carried out at 25 °C ± 2 °C except otherwise stated.

2. 2. Reagents

A 1.0 g L^{-1} stock solution of yeast RNA (yRNA, Tianjin Damao Chemical Reagent Company, China) were prepared by dissolving it in doubly distilled water, stored at 4 °C and used without further purification. The concentration of yRNA was determined according to the absorbance at 260 nm after establishing the absorbance ratio A_{260}/A_{280} of RNA in the range of 1.9~2.0, which indicated that yRNA was free of protein.[24] The molarities of yRNA were calculated by using ε_{RNA} = 7800 L mol^{-1} cm^{-1}.[25] The 1.0×10^{-3} mol L^{-1} safranine T (ST, Shanghai Guoyao Chemical Reagent Station, China) solution was prepared by dissolving 0.03650 g ST in 100 mL water. 0.2 mol L^{-1} Britton-Robinson (B-R) buffer solution was used to control the pH of the interaction system. All other reagents were of analytical regent grade and doubly distilled water was used throughout the experiments.

2. 3. Procedure

1.0 mL of 1.0×10^{-3} mol L^{-1} solution of ST, 3.0 mL of pH 3.5 B-R buffer solution and an appropriate quantity of yRNA solution were added in 10 mL calibrated tube in sequence, diluted to the mark and stirred thoroughly. After reaction at room temperature for 20 min, the second order derivative linear sweep voltammetric curve was recorded in the potential range from 0 V to –0.7 V. The reduction peak current of ST at –0.39 V (vs. SCE) was recorded as the blank (Ip_0'') and the difference of peak current ($\Delta Ip'' = Ip_0'' - Ip''$) was used for yRNA determination.

3. Results and Discussion

3. 1. UV–Vis Absorption Spectra

Figure 2 showed the UV–Vis absorption spectra of ST in the absence and presence of yRNA at the pH 3.5 B-R buffer solution and in the interval 350–800 nm. ST had a maximum absorption peak at 515 nm (curve 1) and yRNA showed no absorption (curve 4). When yRNA was mixed with ST, the absorbance of ST at 515 nm decreased without a shift of the maximum and no new absorption peak appeared (curve 2, 3). The more yRNA added, the greater was the decrease in absorbance. According to Long´s results,[26] the hypochromic effect is characteristic of electrostatic interactions between ST and yRNA in a mixed solution.

3. 2. Linear Sweep Voltammogram

The typical second order derivative linear sweep voltammograms of ST and its mixture with different

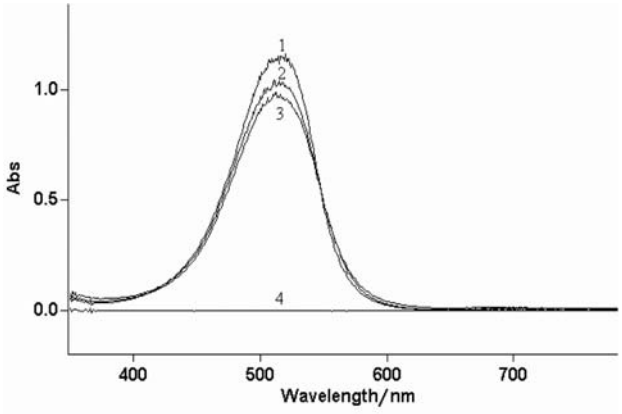

Figure 2. Absorption spectra of ST-yRNA reaction system. 1. pH 3.5 B-R buffer + 5.0×10^{-5} mol L^{-1} ST; 2→3. 1 + 50.0, 100.0 mg L^{-1} yRNA; 4. pH 3.5 B-R buffer + 20.0 mg L^{-1} yRNA

amounts of yRNA are shown in Figure 3. It can be seen that B-R buffer shows no voltammetric response (curve 1) and ST exhibits a sensitive linear sweep voltammetric reductive peak at –0.39 V (vs. SCE) (curve 2). After the addition of yRNA into ST solution, a decrease of reductive peak current appeared without a shift in peak potential (curves 3, 4), which indicates that yRNA can interact with ST in the solution to form a supramolecular complex.

The electrochemical behavior of ST in the absence and presence of yRNA were investigated. The relationship of $I\mathrm{p}''$ and scan rate was examined in the absence and presence of yRNA and the plots are shown in Figure 4. It can be seen that regardless of whether yRNA was present or not, the reductive peak current obtained from the ST (curve 1) and ST-yRNA (curve 2) reaction solution showed linear dependence on the square root of scan rate ($v^{1/2}$), which indicated that in the absence and presence of yRNA, the electrode process was controlled by diffusion mass transport of the electroactive species to the surface of mercury electrode. The diffusion coefficient was calculated according to the Delahay equation:

$$I_\mathrm{p}'' = 3.01 \times 10^5 \, n^{3/2} \alpha^{1/2} D_0^{1/2} A \, C_0^{*} \, v^{1/2} \qquad (1)$$

where D_0 is the apparent diffusion coefficient, A is the surface area of one mercury drop (which can be calculated from the mass of 100 drops), α is the electron transfer coefficient, C_0^{*} is the concentration of ST and v is the scan rate. From the slope of the linear of I_p'' versus $v^{1/2}$, the diffusion coefficient was calculated as 1.63×10^{-5} cm^2/s for free ST and 2.71×10^{-6} cm^2/s for the ST-yRNA complex. Thus the apparent diffusion coefficients were decreased after the interaction of yRNA with ST and a decrease of the diffusion coefficient resulted in a decrease of the reductive peak current. Based on the decrease of the peak current, a new linear sweep voltammetric method for yRNA was established.

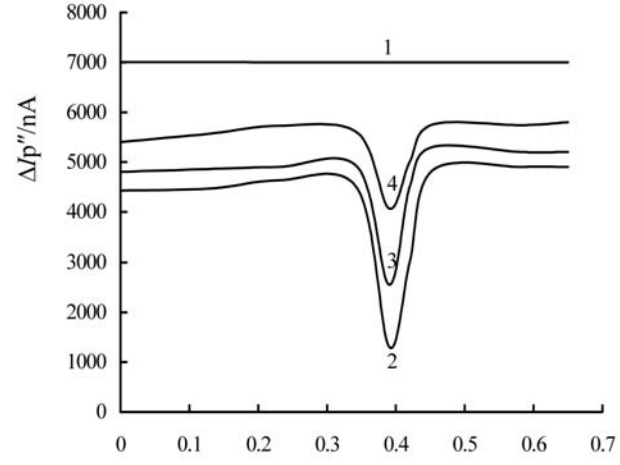

Figure 3. Second order derivative linear sweep voltammograms of ST interaction with yRNA 1.pH 3.5 B-R buffer; 2. 1 + 1.0×10^{-4} mol L^{-1} ST; 3→4. 2 + 15.0, 25.0 mg L^{-1} yRNA

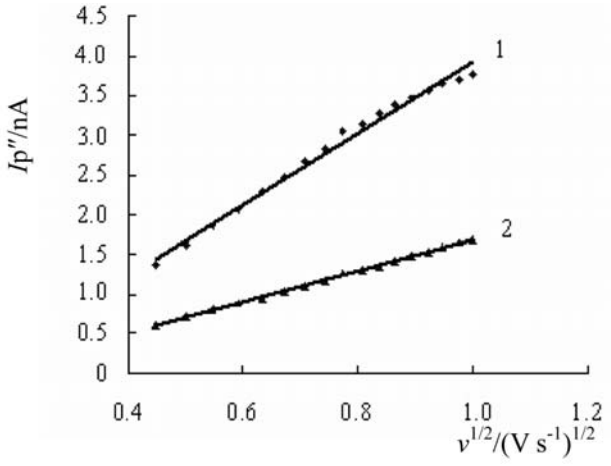

Figure 4. Dependence of the peak current on the square root of scan rate 1. pH 3.5 B-R buffer+1.0×10^{-4} mol L^{-1} ST; 2. 1+30.0 mg L^{-1} yRNA

3. 3. Optimization of Reaction Conditions

3. 3. 1. Effect of the pH

The medium pH had a great effect on the reductive peak current of ST and its mixture with yRNA. As shown in Figure 5, the value of $\Delta I\mathrm{p}''$ ($I\mathrm{p}_0''$– $I\mathrm{p}''$) reached its maximum at pH 3.5. Therefore pH 3.5 buffer solution was selected for further investigations. The volume of 0.2 mol L^{-1} B-R buffer solution was also investigated and 3.0 mL of B-R was chosen in a final 10 mL total volume solution.

3. 3. 2. Effect of the Amount of ST

The difference of peak current increased with an increasing ST concentration. As shown in Figure 6, when yRNA concentration was fixed at 30.0 mg L^{-1}, the differen-

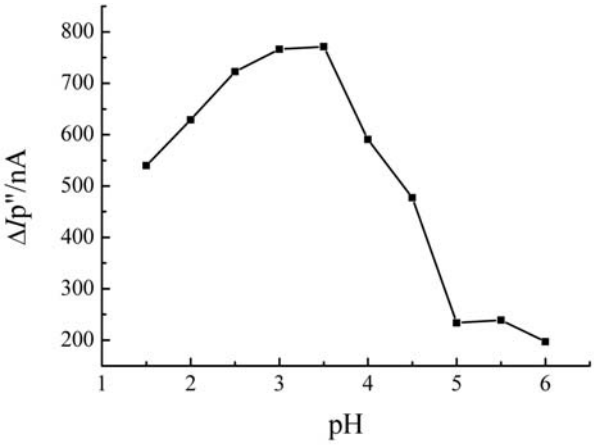

Figure 5. Influence of pH on binding interactions in 1.0×10^{-4} mol L^{-1} ST and 30.0 mg L^{-1} yRNA in B-R buffers of different pH

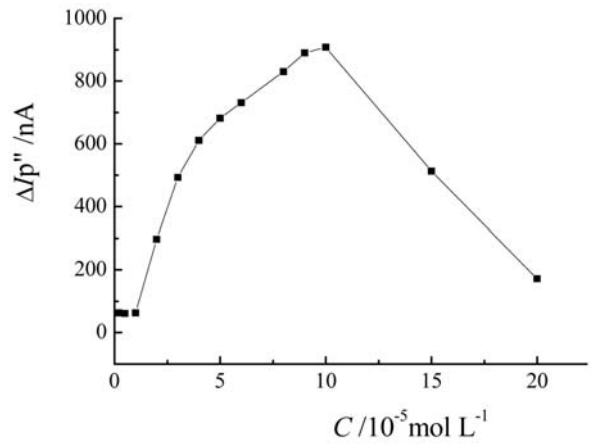

Figure 6. Influence of ST concentration on binding interactions in 30.0 mg L^{-1} yRNA and different concentration of ST in pH 3.5 B-R buffer

ce of peak current reached its maximum when the concentration of ST was up to 1.0×10^{-4} mol L^{-1}, so 1.0×10^{-4} mol L^{-1} ST was selected for further studies.

3. 3. 3. Effect of the Reaction time

The binding reaction of ST with yRNA occurs quickly at room temperature, the electrochemical response reaches a maximum after 20 min reaction and remains constant for about 2 h, which is enough for routine measurements.

3. 3. 4. Effect of Addition Sequences

Different sequences of addition of various reagents had little influences on the reduction peak current, so the order of addition of ST, buffer and yRNA was used for further study.

3. 3. 5. Effect of Coexisting Substances

Under the optimum conditions, a study of the effect of various foreign substances such as metal ions, amino acids and similar on the determination of yRNA was carried out. The experimental results are given in Table 1 and it can be seen that a variation of the relative error is within ±5% for the established level of yRNA. Most coexisting substances have no influence in determination.

3. 3. 6. Optimal of Instrumental Conditions

The instrumental conditions including the scan rate and dropping mercury standing time were studied under the optimal reaction conditions. According to Ilkovič equation, the increase of scan rate and standing time can increase the value of peak current and thus increase the detection sensitivity. Within the range provided by the instrument, the scan rate and the dropping mercury standing time were selected as 1000 mV s^{-1} and 24 s, respectively.

3. 4. Linear Range and Detection Limit

Under the optimal experimental conditions, we obtained a linear relationship between the decrease of the reductive peak current and the yRNA concentration in the range from 8.0 to 25.0 mg L^{-1}. The obtained linear regression equation was $\Delta Ip''$ (nA) = 95.11C (mg L^{-1}) – 637.72 (γ = 0.992, n = 7). The detection limit was calculated to be

Table 1. Effect of coexisting substances on the determination of 30.0 mg L^{-1} yRNA

Coexisting substances	Concentration (mg L^{-1})	Relative error (%)	Coexisting substances	Concentration C(μ mol L^{-1})	Relative error (%)
L–Serine	0.5	2.62	Cu^{2+}	0.5	2.88
L–Tyrosine	0.5	–5.30	Mn^{2+}	0.5	0.77
L–Valine	0.5	–4.09	Ca^{2+}	0.5	–0.67
L–Arginine	0.5	–2.28	Sn^{2+}	0.5	–1.80
L–Leucine	0.5	–1.95	Zn^{2+}	0.5	1.59
L–Glutamine	0.5	–2.09	Mg^{2+}	0.5	0.31
Glycine	0.5	–4.58	Co^{2+}	0.5	3.19
Glucose	0.5	–2.09	Urea	0.5 (mg L^{-1})	0.62

Table 2. Determination of yRNA in synthetic samples (n = 5)

Samples	Coexisting substances	Added (mg L^{-1})	Found (mg L^{-1})	RSD (%)	Recovery (%)
1	L–Arginine, Glucose, Zn^{2+}, Ca^{2+}	10.00	10.06	2.01	100.60
2	L–Leucine, L–Tyrosine, Sn^{2+}, Mg^{2+}	15.00	15.14	4.11	100.93
3	L–Serine, Urea, Co^{2+}, Cu^{2+}	20.00	20.25	2.11	101.25

*Coexisting substances: L–Arginine, L–Leucine, L–Tyrosine, L–Serine, Urea, Glucose: 0.5 mg L^{-1}; Zn^{2+}, Ca^{2+}, Sn^{2+}, Mg^{2+}, Co^{2+}, Cu^{2+}: 0.5 μmol L^{-1}

0.84 mg/L with the definition of IUPAC with the K value as 3 [27] and the relative standard deviation for 10 parallel detections of 25.0 mg L^{-1} yRNA was 0.37%.

3. 5. Analysis of Synthetic Samples

Three synthetic samples containing different amounts of amino acids, metal ions and yRNA were determined according to the general procedure and the results are shown in Table 2. The recovery was in the range of 100.60% ~ 101.25% and the proposed method can be easily performed with high accuracy.

3. 6. Stoichiometry of the ST-yRNA Complex

The stoichiometry of the ST-yRNA complex was calculated using voltammetric data. According to the method used by Li,[28] it was assumed that ST interacting with yRNA only formed a single complex yRNA-*m*ST. The binding number (m) and the equilibrium constant (β_s) of the binding reaction could be deduced as following:

$$\text{yRNA} + m\ \text{ST} \leftrightarrow \text{yRNA-}m\ \text{ST} \quad (2)$$

The equilibrium constant was deduced as follows:

$$\beta_S = \frac{[\text{yRNA-}m\text{ST}]}{[\text{yRNA}][\text{ST}]^m} \quad (3)$$

Because of:

$$\Delta I_{max} = kC_{yRNA} \quad (4)$$

$$\Delta I = k[\text{yRNA-}m\text{ST}] \quad (5)$$

$$[\text{yRNA}] + [\text{yRNA -}m\text{ST}] = C_{yRNA} \quad (6)$$

Therefore:

$$\Delta I_{max} - \Delta I = k(C_{yRNA} - [\text{yRNA -}m\text{ST}]) = k[\text{yRNA}] \quad (7)$$

Introducing equations (2), (4) and (6) gave:

$$\log[\Delta I/(\Delta I_{max} - \Delta I)] = \log\beta_s + m\log[\text{ST}] \quad (8)$$

where ΔI is the difference between the peak current of sample and blanks, ΔI_{max} corresponds the maximum value of difference of peak current, C_{yRNA}, [yRNA – *m*ST] and [yRNA] correspond to the total, bound and free concentration of yRNA in the solution, respectively.

From the equation (8), the relationship of $\log[\Delta I/(\Delta I_{max}-\Delta I)]$ with log[ST] was calculated and plotted with a linear regression equation as $\log[\Delta I/(\Delta I_{max}-\Delta I)] = 2.43\log[\text{ST}] + 11.02$ ($n = 8$, $\gamma = 0.990$). From the intercept and the slope $m = 2.5$ and $\beta s = 1.05 \times 10^{11}$ were deduced, which indicated that a stable 2:5 complex of 2yRNA –5ST was formed in the selected conditions.

4. Conclusion

This paper presents a new electrochemical method for the determination of yeast RNA (yRNA) using safranine T (ST) as voltammetric probe in low concentrations. ST and yRNA form a 5 : 2 supramolecular complex, which results in a decrease of the diffusion coefficient compared with that of free ST, and the decrease of the reductive peak current. The method is highly sensitive, reproducible and practicable. It was further applied to determination of yRNA in synthetic samples with satisfactory results.

5. Acknowledgements

This work has received support from the National Natural Science Foundation of China (20405008, 20635020).

6. References

1. S. C. Weatherly, I. V. Yang, P. A. Armistead, H. H. Thorp, *J. Phys. Chem. B.* **2003**, *107*, 372–378.
2. C. Wilms, J. W. Noah, D. Zhong, P. Wollenzien, *RNA* **1997**, *3*, 602–612.
3. Q. E. Cao, Y. K. Zhao, Y. Y. Xu, C. Z. Li, Z. D. Hu, Q. H. Xu, *Aanl. Biochem.* **2000**, *277*, 214–220.
4. W. Chen, N. J. Turro, D. A.Tomalia, *Langmuir* **2000**, *16*, 15–19.

5. V. Pavlov, Y. Xiao, R. Gill, A. Dishon, M. Kotler, I. Willner, *Anal. Chem.* **2004**, *76*, 2152–2156.
6. M. Verhaegen, T. K. Christopoulos, *Anal. Chem.* **1998**, *70*, 4120–4125.
7. F. Gao, L. Zhang, G. R. Bian, L. Wang, *Spectrochim. Acta A* **2004**, *60*, 2505–2509.
8. Y. F. Li, C. Z. Huang, M. Li, *Anal. Chim. Acta* **2002**, *452*, 285–294.
9. W. Sun, J. Y. You, X. Hu, K. Jiao, *Anal. Sci.* **2006**, *22*, 691–696.
10. E. L. S. Wong, J. J. Gooding, *Anal. Chem.* **2003**, *75*, 3845–3852.
11. E. Paleček, *Nature* **1960**, *188*, 656–657.
12. S. A. Patricia, L. C. M. Jesus, J. M. O. Arturo, T. B. Paulino, *Anal. Bioanal. Chem.* **2004**, *378*, 104–118.
13. M. T. Carter, A. J. Bard, *J. Am. Chem. Soc.* **1987**, *109*, 7528–7530.
14. K. Jiao, Q. X. Wang, W. Sun , F. F. Jian, *J. Inorg. Biochem.* **2005**, *99*, 1369–1375.
15. A. Radi, M. A. ElRies, S. Kandil, *Anal. Chim. Acta* **2003**, *495*, 61–67.
16. D. T. Breslin, G. B. Schuster, *J. Am. Chem. Soc.* **1996**, *118*, 2311–2319.
17. S. F. Wang, T. Z. Peng, C. F. Yang, *Electroanalysis* **2002**, *14*, 1648–1653.
18. W. Sun, J. Y. You, X. Hu, K. Jiao, *Anal. Lett.* **2006**, *39*, 33–45.
19. C. S. Chwo, J. K. Barton, *Biochemistry* **1992**, *31*, 5423–5429.
20. P. J. Cater, C. C. Cheng, H. H. Thorp, *J. Am. Chem. Soc.* **1998**, *120*, 632–642.
21. H. Xu, H. Deng, H. Y. Hu, J. Z. Liu, H. Chao, J. Liu, L. N. Ji, *Chem. J. Chin. Univ.* **2003**, *24*, 25–27.
22. M. Lindell, P. Romby, E. G. Wagner, *RNA* **2002**, *8*, 534–541.
23. W. Sun, J. Y. You, Q. X. Wang, K. Jiao, *Chem. Anal.* **2006**, *51*, 477–488.
24. H. Y. Zhang, H. Q. Wan Eds.; *Biochemistry*; Chemical Industry Press: Beijing, **2001**; p. 201.
25. J. A. Pachter, C. H. Huang, V. H. Duvernay, A. W. Prestayko, S. T. Crooke, *Biochemistry* **1982**, *21*, 1541–1547.
26. E. C. Long, J. K. Barton, *Acc. Chem. Res.* **1990**, *23*, 271–273.
27. IUPAC, Analytical Chemistry Division, *Spectrochim. Acta* **1978**, *33B*, 242–245.
28. N. Q. Li, J. Min, *Chin. J. Anal. Chem.* **1989**, *17*, 346–348.

Povzetek

Razvili smo voltametrično metodo z linearnim preletom za določitev RNA kvasovk (yRNA) v sledovih z uporabo safranina T (ST). Pri pH 3,5, kar smo dosegli z uporabo Britton-Robinsonovega pufra, je izkazal ST redukcijski vrh pri –0,39 V napram SCE, pri čemer dodatek yRNA lahko zmanjša tok pri vrhu brez premika potenciala. Zmanjšanje je bilo sorazmerno koncentraciji, kar smo uporabili za razvoj metode za kvantitativno določitev yRNA. Pri optimalnih pogojih je bil linearen odziv v območju 8,0–25,0 mg L^{-1} z mejo zaznavnosti 0,84 mg L^{-1}. Metoda je enostavna, pri čemer spojine v analizni matrici ne motijo določitev. Določili smo konstanto vezave ($\beta_s = 1{,}05 \cdot 10^{11}$) in vezavno število $m = 2{,}5$.