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## Investigation of Electrochemical Properties of Xanthine, Adenine and Thymine on a Glassy Carbon Electrode by Voltammetry

D.A. Vishenkova <sup>a,\*</sup>, E.I. Korotkova <sup>a</sup>, E.V. Dorozhko <sup>a</sup>, K.V. Derina <sup>a</sup>

<sup>a</sup> National Research Tomsk Polytechnic University, Lenin avenue, 30, Tomsk, 634050, Russia

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### Abstract

In this paper for investigation of electrochemical properties of nitrogenous bases by voltammetry xanthine (Xa), adenine (A) and thymine (T) with constant-current potential sweep with differentiation were used. The electrochemical behavior of Xa, A and T on the surface of a glassy carbon electrode were investigated. The conditions of registration of their joint detecting in the solution were defined. It is demonstrated that the oxidation peak currents of Xa, A and T increased linearly with their concentration in the range of  $4.0 \cdot 10^{-8} - 1 \cdot 10^{-4}$  mol/dm<sup>3</sup> for Xa,  $3.0 \cdot 10^{-7} - 1.0 \cdot 10^{-4}$  mol/dm<sup>3</sup> for A, and  $1.0 \cdot 10^{-5} - 1.1 \cdot 10^{-3}$  mol/dm<sup>3</sup> for T with correlation coefficients of 0.996, 0.996 and 0.999, respectively.

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**Keywords:** voltammetry, glassy carbon electrode, xanthine, adenine, thymine

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### 1. Introduction

The nitrogenous bases known as «purines» and «pyrimidines» are two of the essential building blocks of deoxyribonucleic (DNA) and ribonucleic acid (RNA)<sup>1</sup>. The derivatives of purine are adenine, guanine, xanthine, hypoxanthine and the derivatives of pyrimidine are thymine, cytosine and uracil. The purine and pyrimidine bases in cells are linked to carbohydrate and in this form are termed, nucleosides which are found in the cell primarily in

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\* Corresponding author. Tel.: +79631942232; fax 8(3822)56-38-32.  
E-mail address: vishenkova\_darya@mail.ru

their phosphorylated form – nucleotides<sup>2</sup>.

Nucleotides have a variety of roles in cellular metabolism. They are the energy currency in metabolic transactions, the essential chemical links in the response of cells to hormones and other extracellular stimuli, and the structural components of an array of enzyme cofactors and metabolic intermediates. The structure of every protein, and ultimately of every biomolecule and cellular component, is a product of information programmed into the nucleotide sequence of cell's nucleic acids. The ability to store and transmit genetic information from one generation to the next is a fundamental condition for life.

Investigations of the redox behavior of biologically occurring compounds by means of electrochemical techniques have the potential for providing valuable insights into biological redox reactions of such biomolecules<sup>3</sup>. Electroactivity of nucleic acids was discovered fifty years ago<sup>4</sup>, and afterwards in this field a lot of intensive efforts have been made to apply modern electrochemical methods in research of nucleic acids, purines, pyrimidines, their analysis and determination<sup>5-9</sup>.

This work is devoted to the investigation of electrochemical properties of xanthine, adenine and thymine on a glassy carbon electrode by a voltammetric method for the purpose of their further determination in various objects.

## 2. Materials and methods

### 2.1. Electrochemical apparatus

All voltammetric measurements for investigation of electrochemical properties of nitrogenous bases were performed on the TA-2 analyzer («Tomanalyt», Tomsk) with a three-electrode system. The indicator electrode was a glassy carbon electrode (GCE) with a working surface area of 1.507 cm<sup>2</sup> («Tomanalyt», Tomsk). For the experimental error decrease, the indicator GCE was on preliminary polarization in the field of potentials from -2.0 V to 2.0 V by cyclic voltammetry with a scan rate of 60 mV/sec for 10 cycles in the phosphate buffer solution of an equimolar mixture of KH<sub>2</sub>PO<sub>4</sub> and Na<sub>2</sub>PO<sub>4</sub> with pH=6.86. A silver chloride electrode (Ag/AgCl/KCl, 1M) as a reference one and platinum wire as a supporting electrode were used. All experiments were carried out at a room temperature.

### 2.2. Chemicals

Adenine and thymine reagents were purchased from «Chemical Line» Co. (Russia, Saint Petersburg), xanthine (Xa) came from Sigma-Aldrich Co. (St. Louis, the USA). The thymine (T) solution was prepared by dissolving 0.0126±0.0002 g of its sample with the classification “pure for analysis” in 10 ml of bidistilled water. Based on the fact that adenine (A) is highly soluble in water, especially hot, and with water temperature decreases, its solubility falls<sup>10</sup>, so adenine solution was prepared by dissolving 0.0135±0.0002 g of its sample in 10 ml of bidistilled water under constant heating. Due to the fact that xanthine is poorly soluble in water, ethanol and ether but is highly soluble in acid and alkaline solutions, xanthine solution was prepared by dissolving 0.0152 ± 0.0002 g of its sample in 10 ml of 0.05 M NaOH solution. The prepared solutions were stored at 4 °C when not in use.

The investigation of electrochemical properties of nitrogenous bases was carried out with the use of background solutions having different values of pH. For the preparation of aqueous background solutions with the specified value of pH a set of standard titles was used, working standards of the third category:

- pH 1.65 - potassium tetraoxalate, 0.05M KH<sub>3</sub>C<sub>4</sub>O<sub>8</sub>·2H<sub>2</sub>O;
- pH 4.01 – potassium hydrogen phthalate, 0.05M KC<sub>8</sub>H<sub>5</sub>O<sub>4</sub>;
- pH 6.86 – potassium phosphate monobasic 0.025M KH<sub>2</sub>PO<sub>4</sub>, sodium phosphate dibasic 0.025 M Na<sub>2</sub>HPO<sub>4</sub>;
- pH 9.18 – sodium tetraborate, 0.01 M Na<sub>2</sub>B<sub>4</sub>O<sub>7</sub>·10H<sub>2</sub>O;
- pH 12 – sodium hydroxide NaOH.

All background solutions were prepared by dissolving contents of the vial or accurately weighed sample in bidistilled water into the flask with volume of 1000.0 cm<sup>3</sup>.

Other chemicals were of analytical reagent grade and were used without further purification.

### 3. Results and discussion

Electrochemical properties of Xa, A and T were investigated by cyclic and anodic voltammetry with constant-current potential sweep with differentiation on a glassy carbon electrode.

#### 3.1. Cyclic voltammetry of Xa, A and T.

Typical cyclic voltammograms of  $2.0 \cdot 10^{-5}$  mol/dm<sup>3</sup> of Xa, A and T on GCE were recorded and shown in Fig. 1.

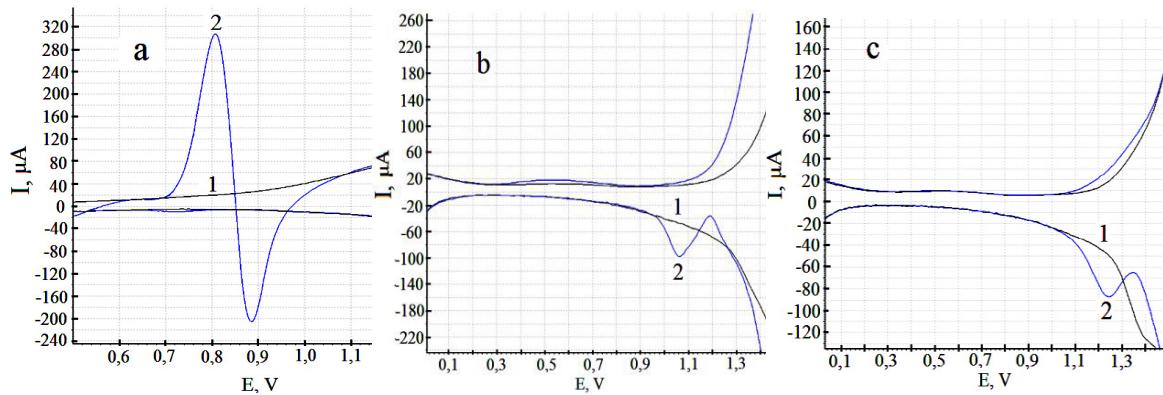


Fig. 1. Cyclic voltammograms of Xa (a), A (b) and T (c) on GCE: 1 - background curve; 2 - Xa (a), A (b) and T (c) with  $C = 2.0 \cdot 10^{-5}$  mol/dm<sup>3</sup>. pH= 6.86, W = 40 mV/sec.

The electrooxidation peaks potentials of Xa appeared at + 0.9 V, A - at + 1.1 V and T - at + 1.3 V on GCE without any reduction peaks, that indicated the fact that those were totally irreversible electrode processes under selected conditions. All further studies on the electrochemical properties of nitrogenous bases presented in this work were carried out by anodic voltammetry in the potential range from 0.2 V to 1.8 V.

#### 3.2. Influence of pH buffer

The pH of supporting electrolyte is an important parameter that affects redox behavior of different compounds. The effect of pH on oxidation response of nitrogenous bases was investigated by anodic voltammetry in the pH range from 1.65 to 12 (Fig. 2).

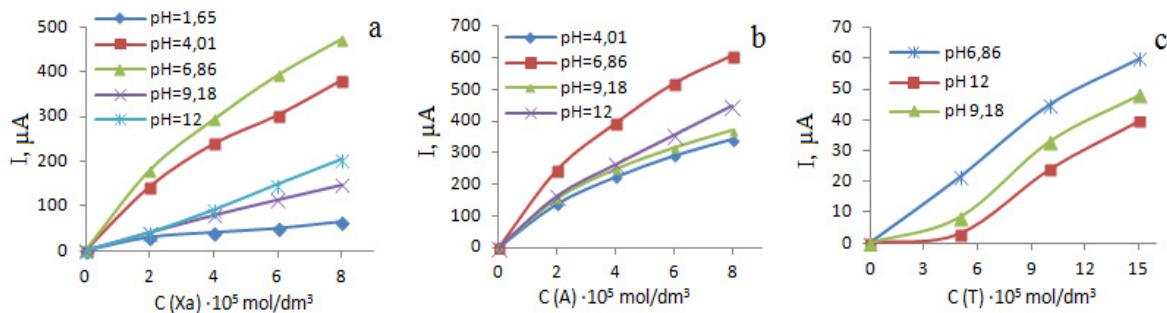


Fig. 2. Dependence of current of Xa (a), A (b) and T (c) electrooxidation from their concentration on GCE at different values of pH

Figure 2 shows that the maximum value of currents as for Xa, A and T is observed at pH=6.86, whereas in

alkaline ( $\text{pH}=9.18, 12$ ) and acidic ( $\text{pH}=1.65$ ) media Xa has a smaller value of current, and the signal from A at  $\text{pH}=1.65$  and T at  $\text{pH}=1.65 - 4.01$  was not found at all.

During the research it was observed that peak potentials of Xa, A and T shifted to less positive potential with the increase in pH (Table. 1)

Table 1. Potentials of electrooxidation of Xa, A and T, depending on pH of the supporting electrolyte

pH of the supporting electrolyte	$E_{\text{Xa}}, \text{V}$	$E_{\text{A}}, \text{V}$	$E_{\text{T}}, \text{V}$
1.65	1.147	-	-
4.01	1.104	1.421	-
6.86	0.909	1.201	1.439
9.18	0.780	1.072	1.275
12	0.723	0.966	0.99

On the basis of the data presented in Figure 2 and Table 1 it is possible to assume that for determination of Xa, A and T on GCE the more appropriate pH value of the supporting electrolyte is  $\text{pH} = 6.86$ .

### 3.3. Effect of scan rate

The influence of scan rate on the oxidation peak currents of nitrogenous bases was investigated in the range from 10 to 300 mV/sec at  $\text{pH}=6.86$ . Studying the influence of the scan rate (W) on electrooxidation peaks of Xa, A and T showed that for Xa and A with gradually increasing W the intensity signal increases, whereas for T, the value of current reaches the maximum at  $W = 40 \text{ mV/sec}$  and at the subsequent increase of W current falls. So the peak currents of  $2.0 \cdot 10^{-5} \text{ mol}/\text{dm}^3$  of Xa and A in electrochemical cell shows a linear increase with square root of square wave frequency, suggesting that the electrode reactions for the oxidation of Xa and A are diffusion controlled processes. By reference to the obtained data it is possible to assume that for joint determination of Xa, A and T  $W = 40 \text{ mV/sec}$  is the most optimal.

### 3.4. Calibration curve

It is well known that the peak current depends on the concentration of analytes. Hence, the anodic voltammograms were recorded for varying concentrations of Xa, A and T in the phosphate buffer solution with  $\text{pH}=6.86$ . It was observed that with increase in concentration of Xa, A and T peak currents were found to increase linearly (Fig. 3).

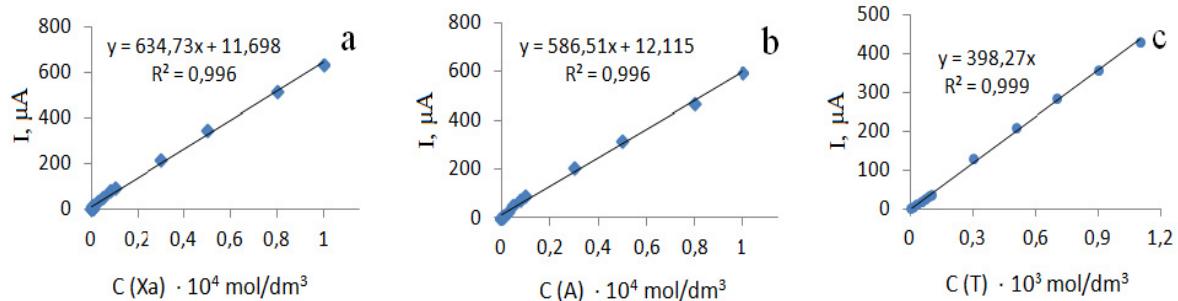


Fig. 3. Dependence curves of currents of Xa (a), A (b) and T (c) oxidation on their concentration in the electrochemical cell

Under optimal conditions the increase of oxidation peak currents was proportional to concentration of Xa in the range from  $4.0 \cdot 10^{-8} \text{ mol}/\text{dm}^3$  to  $1.0 \cdot 10^{-4} \text{ mol}/\text{dm}^3$  ( $R^2=0.996$ ), of A from  $3.0 \cdot 10^{-7} \text{ mol}/\text{dm}^3$  to  $1.0 \cdot 10^{-4} \text{ mol}/\text{dm}^3$  ( $R^2=0.996$ ), and of T from  $1.0 \cdot 10^{-5}$  to  $1.1 \cdot 10^{-3} \text{ mol}/\text{dm}^3$  ( $R^2=0.999$ ).

### 3.5. Simultaneous determination of Xa, A and T

Simultaneous determination of nitrogenous bases (Xa, A and T) was studied by anodic voltammetry with constant-current potential sweep ( $W = 60 \text{ mV/sec}$ ) with differentiation in the potential range from 0.0 V to 2.0 V in the phosphate buffer solution with pH = 6.86. It was observed that with increase in concentrations of Xa, A, and T oxidation peak currents were found to increase linearly. The oxidation peak currents versus concentration dependence showed a good linearity for Xa, A in concentration range of  $2.0 \cdot 10^{-5} - 8.0 \cdot 10^{-5} \text{ mol}/\text{dm}^3$  and for T of  $4.0 \cdot 10^{-5} - 1.2 \cdot 10^{-4} \text{ mol}/\text{dm}^3$  as depicted in Figure 4.

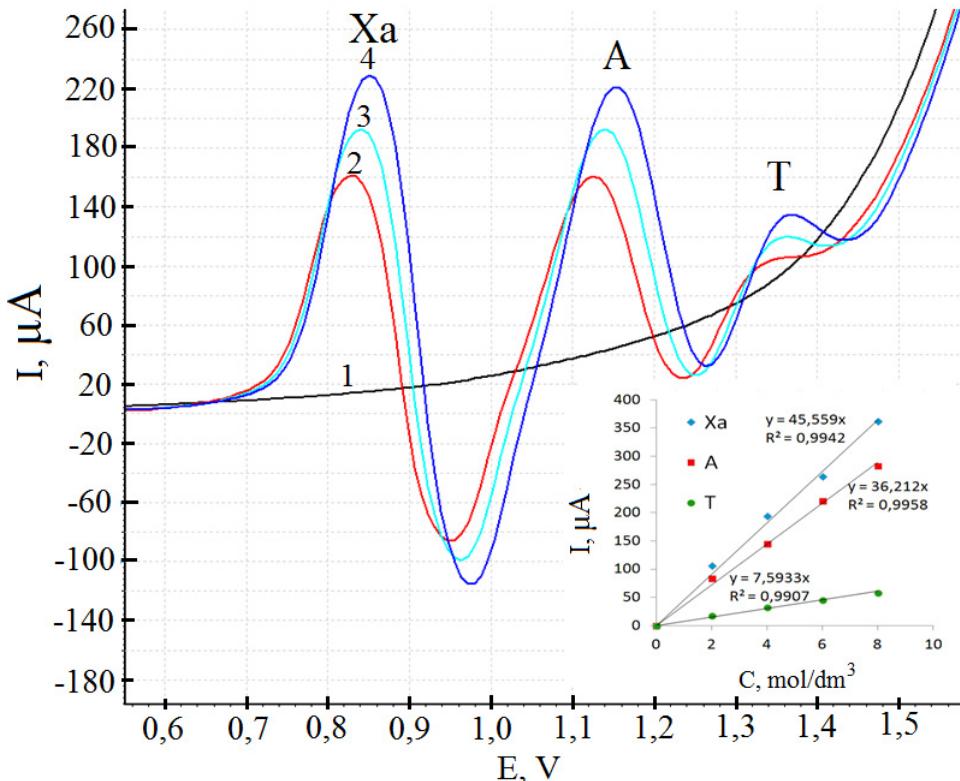


Fig. 4. Voltammograms for simultaneous determination of Xa, A and T on GCE.

1-background curve; 2 - Xa  $2.0 \cdot 10^{-5} \text{ mol}/\text{dm}^3$ , A  $2.0 \cdot 10^{-5} \text{ mol}/\text{dm}^3$ , T  $4.0 \cdot 10^{-5} \text{ mol}/\text{dm}^3$ ; 3- Xa  $4.0 \cdot 10^{-5} \text{ mol}/\text{dm}^3$ , A  $4.0 \cdot 10^{-5} \text{ mol}/\text{dm}^3$ , T  $8.0 \cdot 10^{-5} \text{ mol}/\text{dm}^3$ ; 4- Xa  $6.0 \cdot 10^{-5} \text{ mol}/\text{dm}^3$ , A  $6.0 \cdot 10^{-5} \text{ mol}/\text{dm}^3$ , T  $1.2 \cdot 10^{-4} \text{ mol}/\text{dm}^3$ ; W =  $40 \text{ mV/sec}$ , pH = 6.86.

Thus, it can be concluded that the proposed method can be successfully applied for simultaneous determination of Xa, A and T in the mixture with the ratio of 1:1:2.

### 4. Conclusions

Electrochemical oxidation of Xa, A and T on GCE was investigated. The influence of pH of the supporting electrolyte and scan rate on the electrooxidation currents of nitrogenous bases was identified by voltammetry with constant-current potential sweep with differentiation. According to the research the background solution with pH equal to 6.86 at the scan rate of 40 mV/sec was chosen as an optimal value for the joint determination of Xa, A and T. Under optimal conditions the increase of oxidation peak currents was proportional to concentration of Xa in the range from  $4.0 \cdot 10^{-8} \text{ mol}/\text{dm}^3$  to  $1.0 \cdot 10^{-4} \text{ mol}/\text{dm}^3$  ( $R^2=0.996$ ), of A from  $3.0 \cdot 10^{-7} \text{ mol}/\text{dm}^3$  to  $1.0 \cdot 10^{-4} \text{ mol}/\text{dm}^3$  ( $R^2=0.996$ ), and of T from  $1.0 \cdot 10^{-5} \text{ mol}/\text{dm}^3$  to  $1.1 \cdot 10^{-3} \text{ mol}/\text{dm}^3$  ( $R^2=0.999$ ). The proposed method was successfully applied to determine Xa, A and T simultaneously.

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