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ANALYSIS OF SUBSTANCES

Voltammetric Determination of Carmoisine in Soft Drinks

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Abstract—A voltammetric study of the electrochemical behavior of red food azo dye Carmoisine on a glassy carbon electrode (GCE) is performed. The influence of various factors on the cathode signal of the dye is shown: pH and values of accumulation time and potential sweep rate. The operating conditions of Carmoisine identification in model media are selected: pH = 1.65, $E_{acc} = -0.1$ V, $t_{acc} = 10$ s, $W = 100$ mV/s. A linear dependence of the electrolytic reduction current of the dye on its concentration at –0.15 V is observed within 0.05–0.5 mg/L; detection limit of Carmoisine is 0.02 mg/L. A comparative determination of Carmoisine in soft drinks is performed by voltammetric and spectrophotometric methods.

Keywords: azo dyes, Carmoisine, voltammetry, spectrophotometry, soft drinks

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Dyes are an important part of our everyday life. Natural sources of vegetable or animal origin were initially used for the obtainment of dyes, but natural dyes were replaced by synthetic ones as organic synthesis was developing.

Azo dyes are the largest class of synthetic organic dyes [1]. They are obtained by azo coupling reaction between aromatic diazo compounds and phenols, aromatic amines, or their derivatives [2].

Carmoisine (azorubine, E122) is one of the azo dyes; it is a synthetic red dye obtained from coal tar pitch. It is widely used in the food industry to color various foodstuffs and drinks (Fig. 1). In Russia, the Carmoisine content is strictly regulated. It should not exceed 50 μg/kg in soft drinks and 500 μg/kg in sauces and seasonings [3].

The World Health Organization together with the Food and Agriculture Organization of the United Nations set the maximum permissible daily rate of Carmoisine consumption at 4 mg per kilogram of body weight [4].

The consequences of excessive consumption of azo dyes, including Carmoisine, can vary: allergic reactions, anemia, reticulocytosis, hematuria, diseases of the kidneys and liver, and even fatal outcome. Azo dyes are also considered carcinogenic because their metabolism is followed by the formation of aromatic amines [5]. However, azo dyes are still used nowadays in the food industry because of their low cost and stability against light, heating, and pH change [6].

Owing to this fact, interest in the problems of quality control of foodstuffs has been increasing recently. Electrochemical methods are widely used along with such widespread methods of determination of synthetic dyes as chromatography [7], spectrophotometry [8], and capillary electrophoresis [9]. Their unchallengeable advantages are low cost, high sensitivity with wide ranges of determined concentrations of both inorganic and organic substances, rapidity of analysis, and the possibility to identify several components simultaneously [10, 11].

In modern studies, identification of azo dyes in foodstuffs is related to the application of toxic mercury electrodes [12, 13] or it is complicated by the modification of various types of electrodes. For example, identification of Carmoisine is performed with glassy carbon electrode (GCE) modified by bismuth [14, 15].

The goal of the present study was to develop a technique of voltammetric identification of Carmoisine is soft drinks using available modified GCE which does not require a long preparation for analysis. Spectrophotometry was used as a comparative method.

A TA-2 voltammetric analyzer (OOO NPP Tom'analit, Tomsk, Russia) was used in the work. The three-electrode cell was composed of indicator GCE and silver/silver chloride electrodes used as auxiliary and comparison electrodes immersed in electrolyte solution.

The process solution of Carmoisine (C = 10.0 mg/L) was prepared from a standard sample of dye which contained no less than 85% of the dye substance (ZAO Vekton, St. Petersburg, Russia). The following buffer solutions were used as stock electrolytes: tetraoxalate, pH = 1.65 ($KH_3(C_2O_4)_2 \cdot 2H_2O$); phosphate, pH = 6.86 (KH_2PO_4, Na_2HPO_4); and tetrabo-

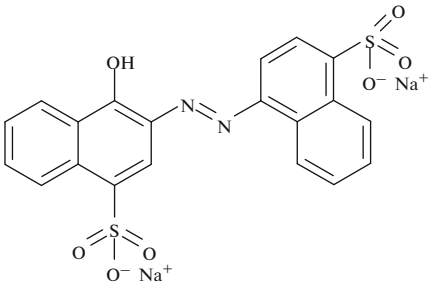

Fig. 1. Carmoisine structural formula.

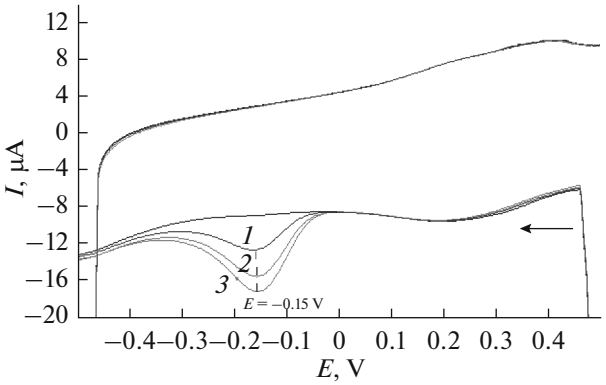

Fig. 2. Cyclic voltammograms of reduction of various concentrations of Carmoisine on GCE (pH = 1.65, W = 100 mV/s): (*1*) 0.2, (*2*) 0.4, (*3*) 0.6 mg/L.

rate, pH = 9.18 ($Na_2B_4O_7 \cdot 10H_2O$). A 150M laboratory pH meter (Russia) was used to control pH.

Voltamograms of cathode reduction of the dye on GCE were registered in the constant current mode with differentiation at potential sweep rate W = 100 mV/s; the operating range of potentials was varied from 0.5 to –0.5 V; the time of accumulation of substance on the electrode was 10 s; the accumulation potential was 0.1 V; the relaxation time was 20 s. Nitrogen was fed through the solution under pressure for 10 min before every experiment to exclude the interfering influence of oxygen.

The following soft drinks containing Carmoisine were chosen as test objects: Priyatnyi den' (vitamin mix, OOO PO Zapsibkola, Novosibirsk, Russia), Korolevskii pingvin (cherry, OOO Ob''edinennaya vodnaya kompaniya, Stavropol krai, Russia) and Shampusenok (cherry, watermelon, OOO PO Zapsibkola, Novosibirsk, Russia).

In performing the experiment, 10 mL of buffer solution was placed in the cell, and the voltammogram of stock electrolyte was registered. A certain volume of studied solution was introduced using a dosing unit after proving the absence of contaminants in the stock solution and demonstrating the reproducibility of the stock curve. The value of the current of electrolytic reduction of Carmoisine was registered. The additives of studied samples differed depending on the degree of intensity of coloring and were 0.1, 0.2, and 0.5 mL for Korolevskii pingvin, Priyatnyi den', and Shampusenok, respectively. The content of dye in the sample under study was determined according to the calibration curve.

Spectrophotometric identification of Carmoisine was performed using an Agilent Technology Cary 60 UV-Vis spectrophotometer in the following conditions: integration time was 1 s; spectral channel capacity was 2 nm; scanning pitch was 0.1 s. Standard Carmoisine solutions with concentrations of 2.0–20.0 mg/L were used to plot a calibration curve. Korolevskii pingvin and Priyatnyi den' drinks were diluted with distilled water 2.5 times at identification of dye in analyzed samples.

Preliminary investigation of the electrochemical behavior of the dye on GCE in model solutions, the regularities of processes taking place on the electrode, and the influence of various factors (pH, time and potential of accumulation, sweep rate) on the electrochemical signal is required to develop a technique of Carmoisine determination in real objects.

Cyclic voltammograms were registered in the mode of constant current linear potential sweep to investigate the electrochemical behavior of Carmoisine on GCE. A single peak within the cathode region is observed on the voltammogram in the region of potentials from 0.5 to –0.5 V. This peak is related to the reduction of the dye molecule. No signal was observed within the anode region (Fig. 2).

The dependences of current (I) on $W^{1/2}$ and of potential on $\log(W^{1/2})$ at potential sweep rates of 20–200 mV/s were constructed (Fig. 3) for evaluating the mechanism of electrolytic reduction of the dye. Figure 3a shows that the dependence of I on $W^{1/2}$ exhibits a linear character, which is typical of reversible and irreversible processes. Moreover, the linear dependence of the potential of the peak of electrolytic reduction of Carmoisine on $\log(W^{1/2})$ characterized by shift of the potential toward the negative region at the increase in the sweep rate shows that electrode process is irreversible (Fig. 3b).

Thus, electrolytic reduction of Carmoisine on GCE probably exhibits an irreversible character. One can suppose on the basis of the structural formula of the dye that azo-group –N=N– exhibits electrochemical activity. The supposed mechanism of Carmoisine reduction on GCE in acidic aqueous media including step-by-step reduction to hydrazo compound then to aromatic amines is in good accordance with published data [17]:

$$R{-}N{=}N{-}R_1 + 2\bar{e} + 2H^+ \rightleftarrows R{-}NH{-}NH{-}R_1, \quad (1)$$

$$R{-}NH{-}NH{-}R_1 + 2\bar{e} + 2H^+ \rightarrow R{-}NH_2 + R_1{-}NH_2. \quad (2)$$

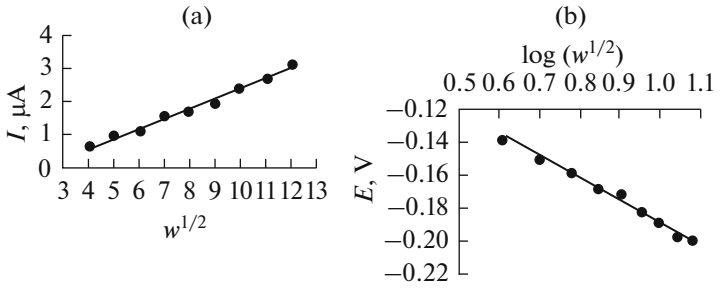

Fig. 3. Dependence of electrolytic reduction current of Carmoisine on GCE on $W^{1/2}$ (a); dependence of electrolytic reduction potential of Carmoisine on $\log(W^{1/2})$ (b). C_{Carm} = 0.4 mg/L.

Selection of Operating Conditions of Determination of Carmoisine

The acidity of the medium is one of the important factors affecting the value of electrode potential. Voltammograms registered in the differential mode at various pH values show that the value of the current of the Carmoisine peak decreases as pH is increased. Moreover, a significant shift of electrolytic reduction potential of the dye from –0.15 V at pH = 1.65 to –0.56 V at pH 9.18 is observed, indicating the participation protons in the electrochemical reaction (Fig. 4).

Tetraoxalate buffer solution (pH 1.65) was used in further investigations because the highest current of electrolytic reduction of the dye is observed in a strong acidic medium.

The potential range between –2 and 2 V was studied to select the potential of accumulation. The dependence of the value of current of electrolytic reduction of Carmoisine on accumulation potential is given in Fig. 5a: the current of the peak exhibits insignificant changes within E_{acc} equal to –2.0 to 0.2 V; however, a drastic decrease in electrolytic reduction current is observed at more positive values of potential. The accumulation potential of –0.1 V was selected owing to this fact.

The region from 0 to 30 s was studied to estimate the accumulation time to obtain a more pronounced signal. The current of electrolytic reduction of the dye increases for 10 s; a further increase in accumulation time does not affect significantly the value of the analytical signal (Fig. 5b). Thus, the accumulation time of 10 s was selected.

The influence of the potential sweep rate on the signal of Carmoisine electrolytic reduction was studied at 10–200 mV/s in tetraoxalate buffer solution (pH 1.65) (Fig. 6).

The current of electrolytic reduction of the dye increases as the potential sweep rate is raised. The sweep rate of 100 mV/s was selected to reduce the time of analysis and to increase the sensitivity of determination.

Thus, the following operating conditions of Carmoisine determination in model solutions were selected: GCE, differential mode of constant current voltammetry, pH = 1.65, W = 100 mV/s, E_{acc} = –0.1 V, t_{acc} = 10 s.

Series of standard solutions of Carmoisine characterized by different concentrations were prepared to plot a calibration curve. The value of the current of electrolytic reduction of the dye was measured under similar conditions. The calibration dependence is described by the equation I (μA) = 17.31C (mg/L) + 0.4925, r = 0.9988; its linear character is preserved within the concentration range of 0.05–0.5 mg/L. The detection limit calculated according to the 3S criterion is 0.02 mg/L [18]. Relative standard deviation S_r does

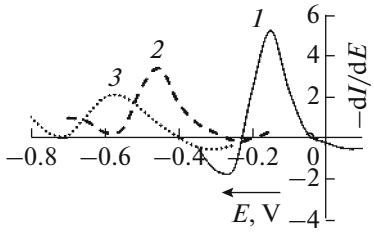

Fig. 4. Differential voltammograms of electrolytic reduction of Carmoisine on GCE at various pH values (C_{Carm} = 0.4 mg/L, W = 100 mV/s): (*1*) 1.65 (E = 0.15 V); (*2*) 6.86 (E = –0.45 V); (*3*) 9.18 (E = –0.56 V).

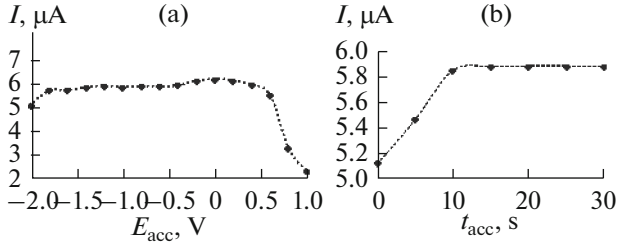

Fig. 5. Dependence of electrolytic reduction current of Carmoisine on accumulation potential (a) and accumulation time (b) (C_{Carm} = 0.4 mg/L, W = 100 mV/s).

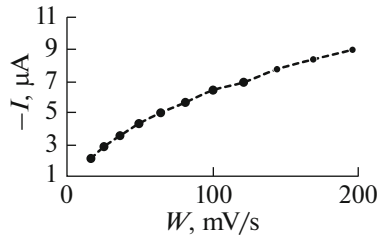

Fig. 6. Dependence of electrolytic reduction current of Carmoisine on potential sweep rate (C_{Carm} = 0.4 mg/L).

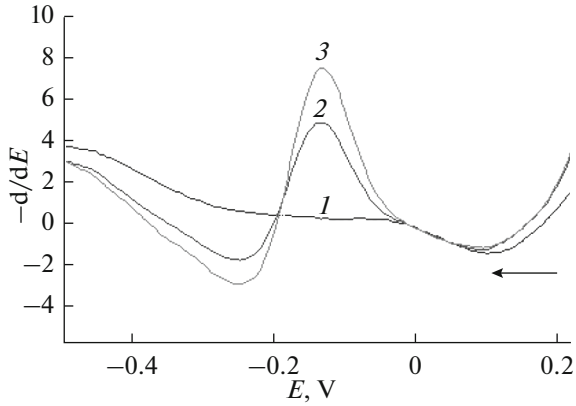

Fig. 7. Differential voltammograms of Carmoisine electrolytic reduction: (*1*) background curve; (*2*) 0.1 mL of Korolevskii pingvin drink; (*3*) addition of 0.2 mL of standard solution of Carmoisine (C = 0.01 mg/L; pH = 1.65; W = 100 mV/s).

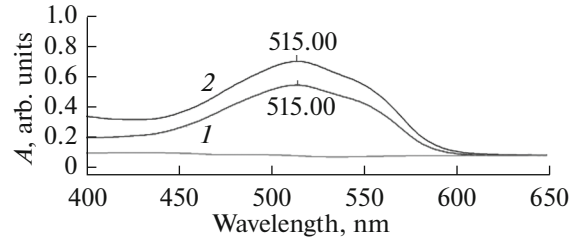

Fig. 8. Absorption spectra of standard solution of Carmoisine (C = 10 mg/L) (*1*); Korolevskii pingvin drink diluted 2.5 times (*2*).

not exceed 8% within the whole range of studied concentrations.

Carmoisine was determined in soft drinks according to the proposed technique. The cathode voltammogram of reduction of Carmoisine in Korolevskii pingvin soft drink is given in Fig. 7. The addition of a standard solution of Carmoisine of known concentration leads to the increase in the current of the peak of electrolytic reduction of the dye at –0.15 V; i.e., the cathode signal of studied sample of drink is due to reduction of the dye. The concentration of Carmoisine in the samples under study was determined according to the calibration curve.

Spectrophotometry was used as a comparative method: the optical density of the solution was measured at the characteristic wavelength. The spectra of standard solutions of Carmoisine and drinks under study were registered at wavelengths of 400–650 nm (Fig. 8) to determine the wavelength corresponding to the maximum of light absorption. The absorption maxima of the standard and studied solutions correspond to 515.0 nm, matching published data [19].

Series of standard solutions of Carmoisine characterized by different concentrations were prepared to plot a calibration curve; the absorption intensity of solution was measured at the wavelength of 515 nm. The calibration dependence is described by the equation $A = 0.04565C + 0.06457$ ($R = 0.99970$); its linear character is preserved within the concentration range of 2.0–20.0 mg/L.

The results of Carmoisine determination using two methods are given in Table 1.

Comparison of the parameters of linearity and relative standard deviations shows that the results of voltammetric and spectrophotometric determination satisfactory match each other.

In all three samples, the dye content does not exceed the norm (under the condition of moderate consumption) owing to the fact that according to SanPiN the maximum acceptable concentration of Carmoisine in soft drinks is 50 mg/L.

Thus, performed investigations show that process of electrolytic reduction of Carmoisine azo dye on GCE involves the action of protons. The higher the pH value, the lower the electrochemical signal of the dye. A mechanism of electrolytic reduction of Carmoisine in an acidic medium on GCE which includes two step-by-step stages of reduction of the dye into aromatic amines is proposed on the basis of cyclic voltammetry data and linear dependences of current and potential on $W^{1/2}$ and $\log(W^{1/2})$, respectively. Operating conditions of determination of the dye in soft drinks using the voltammetry method are proposed on basis of the results of analysis of model solutions. A

Table 1. Results of Carmoisine determination (mg/L) in soft drinks by voltammetric and spectrophotometric methods (n = 6, p = 0.95, t_{tab} = 2.57)

Drink	Voltammetry	S_r	Spectrophotometry	S_r	t_{exp}
Korolevskii pingvin (cherry)	36.43 ± 2.19	0.06	36.08 ± 2.06	0.05	1.65
Priyatnyi den' (vitamin mix)	12.18 ± 1.01	0.08	13.6 ± 1.11	0.08	2.08
Shampusenok (cherry, watermelon)	4.09 ± 0.23	0.05	4.37 ± 0.14	0.03	1.87

comparative determination of Carmoisine in soft drinks by the voltammetric and spectrophotometric method was performed. The results of dye determination using the two methods match each other.

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