Carbon-paste electrode modified by β-cyclodextrin as sensor for voltammetric determination of Tartrazine and Carmoisine from one drop

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Abstract
For food quality control methods, low cost, speed, and simplicity are essential. Electrochemical methods can satisfy all of these requirements. In this paper, we propose a fast and simple voltammetric method using a carbon-paste electrode modified with β-cyclodestrin for the determination of two common food azo dyes: Tartrazine and Carmoisine. To reduce the amount of sample required for analysis, in this work, we explored the prospect of another methodology similar to adsorption stripping voltammetry. The redox behavior of dyes, the influence of pH and scan rate on oxidation currents were investigated. Based on the results the scheme of oxidation of azo dyes was proposed. The use of the proposed approach in combination with the developed sensor makes it possible to determine Tartrazine and Carmoisine within their concentrations of 314–5024 ng/mL and 167–5340 ng/mL with calculation LOD 101 ng/mL and 60 ng/mL respectively. The proposed sensor was tested during analysis of model solutions and soft drinks and showed good results with high reproducibility.

Keywords Voltammetry · Carbon-paste electrode · Adsorption · Modification · Food azo dyes · β-Cyclodextrin

Introduction

Tartrazine and Carmoisine are classed as food coloring sulfazo dyes and are widely used in the food and pharmaceutical industries to provide pleasant and long-lasting color for products [1]. The WHO sets the maximum permitted daily dose for Tartrazine and Carmoisine at 10 and 4 mg/kg, respectively [2, 3]. According to studies conducted by the WHO and EFSA [2–5], Tartrazine and Carmoisine do not exhibit any carcinogenic and mutagenic effects like other classes of azo dyes, such as textile benzidine dyes (direct blue 1, pigment yellow 12, etc.) or non-sulfur fat-soluble (Sudan I-III) [6]. However, recent studies on rats have shown their toxicity [7–11]. Thus, Tartrazine and Carmoisine, when administered orally for prolonged periods, can increased oxidative stress in brain cells [7], structural changes in the liver and kidneys [8–10] and decreased of thinking ability and memory [11]. Thus, the food azo dyes Tartrazine and Carmoisine, when consumed for a long time, may have a negative impact on human health.

Voltammetric methods of analysis are noted for their simplicity, sensitivity low cost and mobility [12–16]. For the analysis of food azo dyes, in particular Tartrazine and Carmoisine, were developed sensors, which include modifiers of different nature. For example, have been described sensors that include modifiers such as carbon nanotubes [17–19], gold nanoparticles [20, 21], surfactants [22–24], conductive polymers [25, 26], metal oxides [27–30], and ionic liquids [31, 32].

β-Cyclodextrin (β-CD) is a cyclic oligosaccharide consisting of 7 glucopyranose residues [33]. Its property to form supramolecular guest–host complexes is widely used in various fields of science and pharmacy [33]. On the basis of β-CD were developed chemical sensors [34], new types of sorbents [35], and stationary phases for the separation of enantiomer pairs in chromatography [33]. β-CD also found application in the development of voltammetric sensors [36–39]. A number of researchers have shown that β-CD-based sorbents can be effective for the purification water from various azo dyes [40–44]. Thus, β-CD may be a...
promising modifier for developing voltammetric sensors for the determination of azo dyes.

Adsorption stripping voltammetry is a powerful technique for increasing detection sensitivity [45]. When using this method, the analyzed compounds can accumulate on the electrode surface both when a potential is applied to the working electrode or not, and when the solution is stirred or not. At the end of the adsorption process, the analyst can choose one of two ways: carry out the electrolysis in the same solution in which the accumulation takes place, or to transfer the electrode to another solution (for example, in a pure buffer solution with a different composition or pH).

In this work, a methodology similar to adsorption stripping voltammetry was used. To reduce the volume of the analyzed sample, it was proposed to carry out the adsorption process from the solution pipetted directly onto the electrode surface. This can be represented as follows (Fig. S1).

Using a dispenser, 10 μL of the analyzed sample solution is pipetted onto the electrode surface. After the accumulation step, the analyzed solution is washing off with water, and the electrode is thoroughly wash, and then transfer to a suitable buffer solution and the determination is provide.

This work is devoted to study the possibility of determining Tartrazine and Carmoisine using the carbon-paste electrode (CPE) modified with β-CD (CPE/β-CD) with methodology as described above.

**Experimental**

**Reagents and apparatus**

Tartrazine, Carmoisine, β-CD, and silicon oil were provided by Merck (Germany). C1 colloidal graphite powder with a particle size of ≤ 15 μm (Ukraine) was used to prepare the carbon-paste electrode. Double distilled water (DE-10C, MICROmed, Ukraine) was used to prepare standard dye solutions and buffer systems.

For recording cyclic and square-wave voltammetry Ecotest VA potentiostat (LLC “Econix Expert”, Russia) paired with auxiliary platinum electrode, silver chloride reference electrode and working CPE/β-CD electrode was used. To control the pH value an I-500 ionometer (Aquilon LLC, Russia), complete with glass electrode ESK-10601/7 was used.

**Preparing CPE/β-CD**

The unmodified CPE was prepared by homogenization in a ceramic mortar of carbon powder with silicone oil at a ratio of 2.3:1 (by weight). To prepare the modified electrode, different contents of β-CD (from 2.5 to 10 wt%) were added to the carbon powder so that the dry matter to binder ratio of 2.3:1 was maintained. A polytetrafluoroethylene tube with an inner diameter of 3 mm was used as the electrode body. A copper wire was used to contact the carbon paste with the potentiostat output. The electrode surface was refreshed before each measurement by pushing through a small portion of the paste, cutting off and polishing on a piece of weighing paper.

**Studying the redox behavior of dyes and establishing the optimal conditions for its determination**

To study the redox behavior of dyes, cyclic voltammetry was used. To do this, the working electrode was placed in a solution of dyes (or in a pure buffer solution after preliminary accumulation of dyes on the electrode surface) and the potential sweep was immediately carried out according to the following values: 0 to 1200 mV, 1200 to − 200 mV and − 200 to 1200 mV with scan rate 50 mV/s. To optimize the determination conditions, square-wave voltammetry was used. To do this, dyes were accumulated on the working electrode and, after washing the surface, the electrode was placed in a clean buffer solution and the potential was immediately swept from 300 to 1200 mV at a scan rate of 50 mV/s. To obtain a stable signal after each surface renewal, the electrode was placed in a Britton–Robinson buffer solution with pH 2 and the potential was swept from 0 to 1000 to 0 mV for 3 cycles at scan rate 100 mV/s.

**Determination of azo dyes in soft drinks**

The soft drink sample was degassed in an ultrasound bath for 15 min. In cases of suspended matter, the drink was filtered through a hydrophilic PTFE filter (0.22 μm) after degassing. After that, 5 mL of aliquot of the filtrate was pipetted into 10 mL volumetric flask and topped up to the mark with Britton–Robinson buffer solution with pH 2 and stirred thoroughly. Then, 10 μL of the analyzed sample was pipetting onto the sensor surface and incubated for 5 min, after which the electrode was carefully washed with double distilled water. Measuring was performed in square-wave mode at a scan rate of 50 mV/s from 300 to 1200 mV. The amount of azo dye was determined by the standard addition method.

**Results and discussion**

**Setting the optimal sensor composition**

To establish the optimal content of the modifier, the effect of the β-CD content in CPE on the oxidation current of the dyes was studied. As can be seen (Fig. 1), the oxidation current of Tartrazine and Carmoisine first increases
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slightly with increasing of the modifier content. At a modi-
fier content of 10 wt%, the signal reaches the maximum
time. Increasing the mass fraction of the modifier to
15 wt% made the sensor become very dense for opera-
tion, so 10 wt% was chosen as the optimal value. It should
be noted that the oxidation current on the CPE/β-CD for
Tartrazine and Carmoisine increased 5.8 and 2.7 times,
respectively, compared to the unmodified CPE.

Electrochemical behavior of Tartrazine
and Carmoisine onto the CPE/β-CD sensor

To study the electrochemical behavior of the dyes on the
proposed sensor, a number of cyclic voltammograms were
recorded at different pH values. As can be seen (Fig. 2a,
b) in the cyclic voltammograms of Tartrazine (at pH 7)
and Carmoisine (at pH 2) there is only one oxidation peak
at 961 mV and 930 mV, respectively. The absence of a
corresponding reduction peak on the cyclic voltammogram indicates an irreversible oxidation process of these dyes on the proposed sensor [46]. Other researchers have noted the same behavior of the dyes on sensors with different modifiers [18, 21–23, 47].

In the case of Tartrazine, with pH values between 2 and 10, no new redox pairs were detected on the cyclic voltammogram of the following cycles. In the case of Carmoisine, after the oxidation process and change of the sweep direction, a new redox pair was detected in the cyclic voltammogram, which has oxidation and reduction potentials of 306 and 341 mV, respectively. At pH 4, another redox pair was also detected (Fig. 2b-inset). The formation of new redox pairs can be caused by the oxidative degradation of Carmoisine, in particular, the azo group included in the structure of the molecule. The difference between the oxidation potentials of Tartrazine and Carmoisine at pH 2 is 220 mV, which make it possible simultaneous determination on the proposed sensor.

**pH effect**

The pH of the buffer solution can have a strong influence on the condition of food dyes in solution [48–52] and, consequently, on their sorption preconcentration [53]. Figure 3 shows the dependence of the oxidation peak current of azo dyes on different pH values of the solution during its accumulation onto sensor. As the pH value of dyes accumulation on the sensor surface increases, the oxidation current decreases for both Tartrazine and Carmoisine (at pH 6 and above the Tartrazine peak is absent). This dependence indicates a significant decrease in dye sorption by the modifier at pH > 2. A similar effect of pH was observed for the sorption of azo dyes by β-CD-based sorbents [40, 43]. When considering the possible mechanism of sorption of azo dyes on the surface of the proposed electrode, it is necessary to take into account not only the possibility of the formation of supramolecular complexes with β-CD [54–56], but also their interaction with the surface of the carbon included in the electrode [57]. As is known, the surface of carbon always contains various hydrophilic groups such as hydroxyl, carbonyl, and carboxyl, which additionally can interact with analyte molecules on the electrode surface [58]. Also, π–π interactions play an important role in the sorption of azo dyes on the surface of CPE [57]. Considering all this, we can assume that the mechanism of sorption of azo dyes on the surface of the sorbent will include several combinations of forces, such as supramolecular, electrostatic, and π–π interactions. With an increase in pH, the degree of ionization of dyes increases, which can lead to a greater polarity of the compound and an increase in the electrostatic repulsion of dye molecules from the electrode surface, which is observed as a decrease in the oxidation current. In our further studies, the adsorption step was performed at buffer solution with pH 2.

While studying the effect of pure buffer solution pH on the oxidation peaks of the azo dyes, it was found (Fig. 3) that the optimal value for Tartrazine and Carmoisine are pH 7 and pH 3, respectively.

To determine the ratio of transferred protons to electrons in the oxidation process, the dependences of the oxidation potentials of the azo dyes on the pH of the solution were analyzed. The following equation was obtained: \( E_p = -36.40 \) pH + 1222.30 \( (R^2 = 0.9863) \) for Tartrazine and \( E_p = -59.90 \) pH + 1064.60 \( (R^2 = 0.9969) \) for Carmoisine. The slopes

![Fig. 3](image-url)
correspond to the proton/electron ratio as 1:2 and 1:1 for Tartrazine and Carmoisine respectively [12].

**Scan rate effect**

To establish the current nature of dye oxidations we studied the dependence of the natural logarithm of the peak current on the natural logarithm of the potential sweep rate ($\ln(I_p) = f(\ln(\nu))$) (Fig. S2a). These dependencies can be described by the following linear equations: $\ln(I_{pa}(TAR)) = 0.79\ln(\nu) + 0.17 \quad (R^2 = 0.992)$ and $\ln(I_{pa}(CAN)) = 0.81\ln(\nu) - 0.18 \quad (R^2 = 0.993)$. The slopes of these straight lines indicate a mix between the adsorption and diffusion nature of the current for both dyes [46].

The dependence of the oxidation peak potential of the decimal logarithm of the scan rate ($E_p = f(\log(\nu))$) was also studied (Fig. S2b). Due to the adsorptive nature of the current, the Laviron model and equations for the irreversible oxidation process (1) were used [59]. The linear dependencies that are described by the following equations (Fig. 4b) were obtained.

$$E_p = (2.3RT/((1 - a)nF)) \log(\nu) + \text{const.} \quad (1)$$

Using the slope of these lines and assuming that the charge transfer coefficient for irreversible systems is close to 0.5, the number of electrons that take part in the oxidation of Tartrazine ($n = 1.97 \approx 2$) and Carmoisine ($n = 0.86 \approx 1$) was calculated. Based on the data obtained, the following dye oxidation scheme can be assumed (Scheme 1).

**Optimization of colorants determination parameters on CPE/β-CD sensor**

For quantitative determination of azo dyes on the proposed sensor, square-wave voltammetry was used. The potential amplitude ($\Delta E$), oscillation frequency ($f$), accumulation time (tads), and potential sweep rate ($\nu$) were chosen as optimized parameters (Fig. S3).

According to obtained data the resultant oxidation current of azo dyes increases with increasing potential amplitude and frequency (Fig. S3a, b). However, at very large values of the amplitude and frequency, significant distortions of the resulting oxidation peak form are observed. On this basis, 35 mV and 50 mV for Tartrazine and Carmoisine as the optimal potential amplitude values were chosen, respectively. A value of 15 Hz was chosen as the optimal frequency.

By studying the sorption time, the resulting current reached a plateau after 5 min of accumulation for both Tartrazine and Carmoisine (Fig. S3c). A scan rate of 50 mV/s was chosen as the optimum for both dyes.

The selected optimal parameters for the determination of azo dyes on the CPE/β-CD sensor are presented in Table S1. The possibility of reusage of the sensor for the determination of dyes was also studied (Table S2). According to the obtained data, the proposed sensor can detect azo dyes without updating the surface up to five times with a relative standard deviation not greater than 5%. In subsequent measurements, a decrease in the signal value was observed, which may indicate a partial blocking of the surface by the oxidation products of the dyes.

For investigation of the stability proposed sensor, the model solution of Tartrazine and Carmoisine was analyzed.
according to proposed procedure for days (Table S3). As can see from the obtained data the stability of the proposed sensor was good, and the oxidation current of azo dyes practically did not change which indicates good sensor stability.

**Determination of Tartrazine and Carmoisine on CPE/\(\beta\)-CD in soft drinks**

To determine the metrological characteristics, a calibration curve was plotted for the determination of Tartrazine and Carmoisine on the proposed sensor (Fig. 4). The obtained calibration curves are linear in the concentration range of 5340–167 ng/mL and 5024–314 ng/mL for Tartrazine and Carmoisine, respectively. As recommended [60], six parallel measurements of Tartrazine and Carmoisine solution with concentrations of 167 and 314 ng/mL, respectively, was performed to assess reproducibility. Using a 3\(\sigma\) and 10\(\sigma\) approach and data of reproducibility at the lower calibration point the limit of detection (LOD) and limit of quantification (LOQ) were found to be for Tartrazine (LOD = 60 ng/mL, LOQ = 338 ng/mL) and Carmoisine (LOD = 101 ng/mL; LOQ = 338 ng/mL). Model solutions and soft drink samples, which contain no dyes, were used to validate the proposed sensor. The results of azo dyes determination are presented in Table 1.

**Conclusions**

The proposed carbon-paste electrode modified with \(\beta\)-cyclodextrin proved to be a promising sensor at the determination of Tartrazine and Carmoisine in sweet drinks. The results showed that Tartrazine and Carmoisine had an irreversible oxidation peak on the prepared sensor. The effects of pH and scan rate were studied and an oxidation scheme for the azo dyes were proposed based on the obtained data. The square-wave conditions for the determination of azo dyes were optimized, and the method demonstrated its suitability.

**Table 1** Results of Tartrazine and Carmoisine determination in model solutions and soft drink (\(n = 3, P = 0.95\))

| Analyte     | Sample       | Spiked, µg/mL | Found, µg/mL | Recovery, % | RSD, % |
|-------------|--------------|---------------|--------------|-------------|--------|
| Tartrazine  | Model solution | 5             | 4.9 ± 0.58   | 98          | 4.8    |
|             |              | 2.5           | 2.4 ± 0.35   | 96          | 5.9    |
|             |              | 0.5           | 0.49 ± 0.09  | 97          | 7.1    |
|             | Soft drink   | 5             | 4.6 ± 0.66   | 92          | 5.8    |
|             |              | 2.5           | 2.2 ± 0.42   | 88          | 7.6    |
|             |              | 0.5           | 0.43 ± 0.10  | 85          | 8.9    |
| Carmoisine  | Model solution | 5             | 4.8 ± 0.68   | 95          | 5.8    |
|             |              | 2.5           | 2.4 ± 0.43   | 95          | 7.3    |
|             |              | 0.5           | 0.48 ± 0.10  | 96          | 8.6    |
|             | Soft drink   | 5             | 4.7 ± 0.84   | 94          | 7.2    |
|             |              | 2.5           | 2.3 ± 0.49   | 92          | 8.6    |
|             |              | 0.5           | 0.44 ± 0.11  | 89          | 10.5   |

Scheme 1 Proposed mechanism of Carmoisine and Tartrazine oxidation

![Scheme 1 Proposed mechanism of Carmoisine and Tartrazine oxidation](image-url)
for the soft drinks analysis. The proposing changed methodology of adsorption stripping voltammetry promising solution for reducing the sample amount and increasing sensitivity and selectivity by selective sorption of desired analytes.

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Data availability The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Informed consent On behalf of other authors, informed consent was obtained from all individual participants included in the study.

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