

Electrochemical interference study for determination of carminic acid in beverages

(Elektrochemická interferenční studie pro stanovení kyseliny karmínové v nápojích)

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Abstract

The electrochemical behaviour of carminic acid in the environment of model lemonade, imitating the real composition of common soft drinks, was investigated at four types of commercially available screen-printed electrodes using cyclic voltammetry. The carminic acid displayed quasi-reversible waves in cyclic voltammograms which show the shift of the redox peak potentials to more negative values with increasing the pH. This phenomenon is caused by easier deprotonation of hydroxyl groups in an alkaline environment and their subsequent anodic oxidation to form the corresponding quinone counterpart with the participation of two electrons and two protons, which is typical for hydroxyanthraquinones. This study should lead in the development of a completely new screening electrochemical assay for field monitoring of total sum of carminic acid and carmine, commonly referred to as food additive E120.

Key words: Carminic acid, Cyclic voltammetry, Interferences, Soft drinks.

Introduction

Carminic acid and carminic lake (E120 food additive) are widely used as food colourant of animal origin (*Dactylopius coccus*), which is able to cause allergic reactions in some sensitive individuals, hyperactivity in children, anaphylaxis, asthma, urticaria, and hay fever [1]. According to the update from 46th Session of the Codex Alimentarius Commission (2023), the E120 content must not exceed 20 mg per 100 mL for liquid foods (soft drink, alcoholic beverage, fruit juice, jam, jelly, and marmalade) and 5–50 mg per 100 g for solid foods (soft candy, raw meat, cheese, salami, sausages, fish, and canned meat) [2]. As a rule, raw foods supposed for heat treatment can usually contain a higher amount of E120 food additive. Despite the large number of already developed analytical methods for monitoring of E120 content in food a simple [3], reliable, and cheap screening analytical method, that could be used directly in the field monitoring without sample preparation, has not been introduced yet.

Experimental part

Model lemonades were composited from numerous ingredients (Table 1), which were purchased from Merck KGaA (Darmstadt, Germany). A deionized water with resistivity of 18.3 MΩ cm obtained from Milli-Q[®] deionization unit from Merck Millipore (Burlington, USA) was used for preparation these lemonades of pHs varying from 2–6.

Table 1. Composition of a model lemonade.

Total volume	500 mL
Sugars	27.5 g sucrose, 2.76 g fructose, 2.75 g glucose
Salts	0.0757 g sodium benzoate, 0.071 g sodium citrate, 0.255 g, sodium chloride, 0.255 potassium chloride, 0.205 g potassium monophosphate monobasic, 0.069 g aluminium chloride, 0.104 g magnesium chloride, and 0.069 g calcium chloride
Organic acids	1.5 g citric acid, 0.1 g carminic acid (20 mg per 100 mL), and 0.850 g ascorbic acid
Other ingredients	0.055 g caffeine, 0.015 g pyridoxine, and 5% (v/v) ethanol

Note: The model lemonade was saturated with CO₂ using a carbonated sparking water maker.

For assessment of aluminum contamination [4], the microwave mineralization of randomly selected beverages (Red-Bull energy drink, Coca-Cola soft drink, and Pilsner Urquell beer) was realised as follows: 2 mL of sample, 5 mL of 65% HNO₃, and 1 mL of 30% H₂O₂ were added into TFM™ - PTFE vessels DAK100. Microwave digestions were performed in a closed microwave oven system Speedwave XPERT from Berghof Products + Instruments GmbH (Eningen, Germany) with the power output of dual magnetrons 2×1000 W and the optical sensors for contactless real-time recording of the sample temperature and pressure in each vessel. The resulting mineralized samples were transferred to 10 mL volumetric flasks, which were subsequently filled by redistilled water up to mark. For itself ICP-MS analysis, the obtained stock sample solutions had to be minimally twice diluted. For direct ICP-MS analysis, the original (non-mineralized) samples were diluted ten times with redistilled water. ICP-MS measurements were performed by using the Agilent 7900 quadrupole mass spectrometer (Q-ICP-MS) from Agilent Technologies, Inc. (Santa Clara, CA, USA) equipped with an octopole-based collision cell for interference removal using kinetic energy discrimination (KED).

The electrochemical investigation was carried out at commercially available screen-printed electrodes (SPE), namely 250AT, C550, and C110 from Metrohm DropSens (Oviedo, Spain) and BDDE from The Slovak Diamond Group (Bratislava, Slovakia), all with working electrode surface diameter of 3 mm. A repetitive cyclic voltammetry of 500 μmol L⁻¹ carminic acid in 0.1 mol L⁻¹ citrate buffers, differing in pH values, in potential range from -0.3 to +1.0 V at scan rate of 50 mV s⁻¹ and potential step of 5 mV was used as suitable electrochemical technique. Each SPE was connected to an EmStat USB potentiostat through a sensor connector operated via PStTrace 4.8 software, all from PalmSens BV (Houten, Netherlands).

Results and discussion

In general, the development of any analytical method must include a detailed interference study in order to be variably applied with respect to the complex matrix of analyzed samples. Substances with similar electrochemical properties, subject to oxidation or reduction at the same peak potential, represent one of the most common interferences in the voltammetric analysis. Other substances can include those that react with the target analyte, passivate the electrode surface, affect the conductivity of the working medium and pH. For food samples, these interferences can be naturally occurring substances, including additives, or potential contaminants.

Due to presence of organic (citric, ascorbic, and sorbic) and inorganic (carbonic and phosphoric) acids, the pH of soft drinks can usually range from 2.5 to 6. If the electrochemical behaviour of carminic acid is investigated in different 0.1 mol L⁻¹ citrate buffers with pH values from 2 to 7, a typical Nernst dependence of the oxidation peak potential (E_p^a) on pH will be obtained, described by the equation $E_p^a(\text{V}) = -0.0583\text{pH} + 0.709$ and $R^2 = 0.9838$. As evident from Fig. 1, the carminic acid displays quasi-reversible waves in cyclic voltammograms, when the value of peak separation (ΔE_p) decreases with lower pHs. This phenomenon corresponds to the electrode reaction described in Fig. 2. Despite the presence of aluminum salts, contaminants originating from cans (Tab. 1), carminic acid provides a relatively constant anodic peak current response (I_p^a) at pH 3–5.

Some soft drinks, especially energy drinks, usually contain high amount accompanying ingredients (ascorbic acid, pyridoxine, and caffeine) having also electroactive properties, and which could be simultaneously determined together with carminic acid, as predictable from sufficient difference in their values of anodic peak potentials (E_p^a) in Tab. 2.

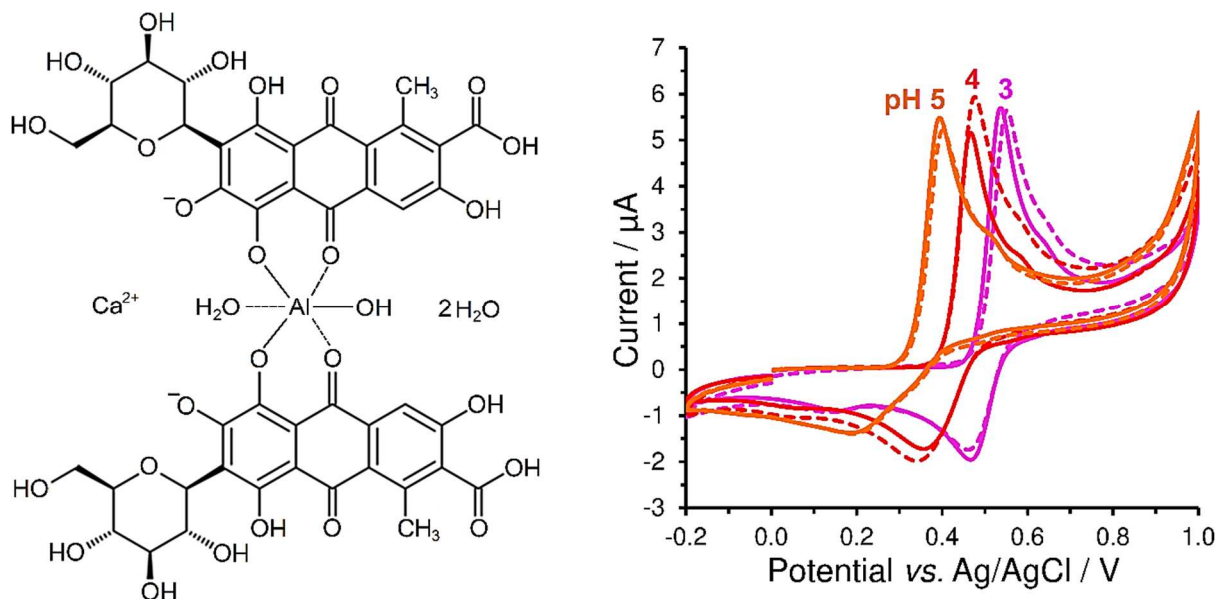


Fig. 1. Molecular structure of curcumin and cyclic voltammograms of 20 mg curcumin added in 100 mL in model lemonades (solid) with 1 mg aluminium chloride (dotted lines) recorded on SPCE at scan rate of 50 mV s^{-1} .

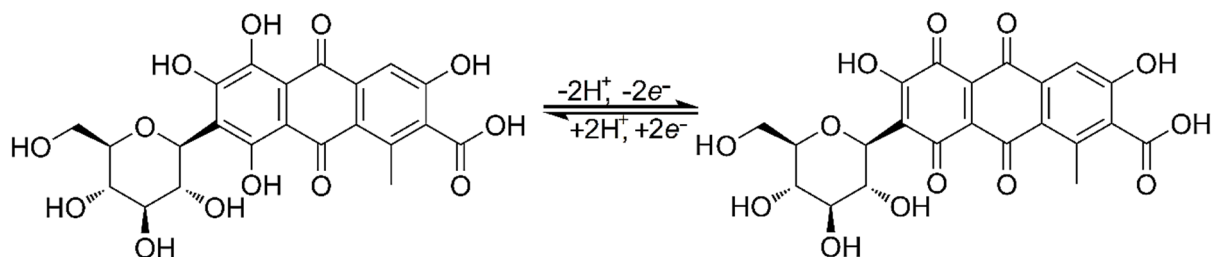


Fig. 2. The quasi-reversible electrode reaction of curcumin [3].

Table 2. Determined aluminum contents with^a and without^b the use of mineralization.

Sample	Aluminum content ^a [µg per 100 mL]	Aluminium content ^b [µg per 100 mL]
Pilsner Urquell	8.9 ± 0.6	9.0 ± 0.6
Pilsner Urquell	35.9 ± 1.3	35.5 ± 1.3
Pilsner Urquell	10.2 ± 0.4	10.2 ± 0.4
Red Bull	60.3 ± 4.3	62.4 ± 4.5
Red Bull	70.5 ± 2.1	73.6 ± 2.2
Red Bull	60.8 ± 1.8	63.6 ± 1.9
Coa-Cola	14.9 ± 0.4	15.7 ± 0.4
Coa-Cola	26.9 ± 1.2	28.2 ± 1.2
Coa-Cola	10.9 ± 0.5	11.6 ± 0.5

Note: Determined aluminium contents given as confidence intervals $\bar{x} \pm 2SD$, where \bar{x} is the arithmetic mean and $2SD$ twice the standard deviation for 10 repetitions of each analysis at $\alpha = 0.05$ (95% probability).

This assumption was confirmed by analysis of a model mixture containing all four potentially interesting analytes, whether with regard to nutritional contents or compliance with legislative standards. In order to sufficiently distinguish the oxidation peaks of individual analytes, especially curcumin from ascorbic acid, it was necessary to modify the electrode surface with thin layer of Nafion[®] copolymer, as shown in Fig. 3.

Table 3. Anodic potentials of ascorbic acid and accompanying ingredients in energy drinks.

pH (citrate buffer)	E_p^a / V (Ascorbic acid)	E_p^a / V (ascorbic acid)	E_p^a / V (Pyridoxine)	E_p^a / V (Caffeine)
2	0.329	0.604	1.168	1.410
3	0.250	0.533	1.040	1.384
4	0.168	0.473	0.932	1.382
5	0.112	0.402	0.856	1.379
6	0.083	0.347	0.796	1.362
7	0.027	0.322	0.743	1.468

Note: Data obtained from differential pulse voltammetry of $200 \mu\text{mol L}^{-1}$ investigated substance at 110 at potential step of 5 mV, potential amplitude of 50 mV, and scan rate of 50 mV s^{-1} .

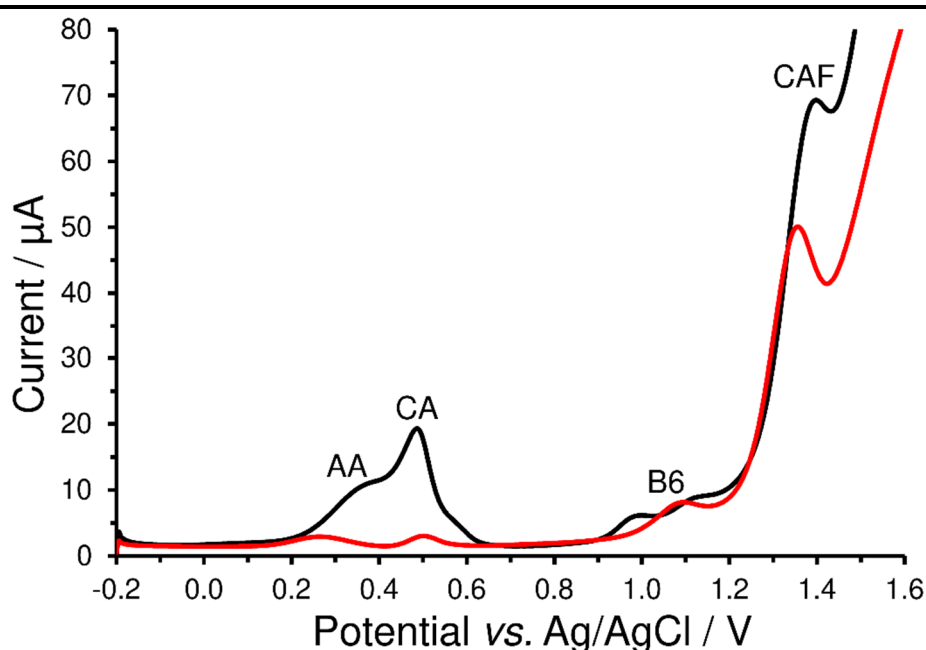


Fig. 3. Differential pulse voltammograms of model lemonade containing 0.2 mg ascorbic acid (AA), 0.2 mg ascorbic acid (CA), 15 μg pyridoxine (B6), and 0.32 mg per mL caffeine (CAF) obtained at bare (black) and glassy carbon electrode modified with Nafion® (red line) in 0.1 mol L^{-1} citrate buffer (pH 4) at potential amplitude of 50 mV and scan rate of 50 mV s^{-1} .

Conclusions

The detailed electrochemical interference study shows that it is possible to develop a highly selective screening assay for field monitoring of food additive E120 content with other ingredients in various beverages without any sample pretreatment.

Acknowledgements

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References

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