A NEW SCREENING ELECTROCHEMICAL ASSAY FOR FIELD MONITORING OF FOOD ADDITIVE E120

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Abstract: For field monitoring of food additive E120 concentration levels in foods, a simple, reliable, and inexpensive screening electrochemical method has been developed that based on direct drop casting of 50 μL sample onto the surface of a commercially available screen-printed voltammetric sensor and subsequent anodic oxidation of the carminic acid present using square-wave voltammetry. Before each screening analysis, it is necessary to determine the pH of the target sample at least with indicator paper due to the statistically significant influence of pH on the current yields of the respective electrode reaction. The peak area response obtained could be calibrated within a linear range 1–50 mg per 100 mL^{-1} carminic acid characterised with coefficient of determination >0.9930, a sensitivity of 2.181, 3.225, and 8.910 $\mu AVmg^{-1}L$ at pH 4, 3 and 2, respectively, and a detection limit of 2 mg L^{-1} carminic acid. Time-consuming optimization with a detailed interference study using a model lemonade, composited from a variety of possible ingredients and eventual contaminants, had to be included, which led to a significant simplification of the entire analytical procedure. Practical applicability of the developed screening electrochemical assay in the food safety control was successfully verified in analysis of candies, soft drinks, and alcoholic beverages. The determined E120 contents were in a good accordance with those obtained by the reference colorimetric method.
Keywords: carminic acid, disposable sensors, electrochemical assay, food colorants, field analysis

INTRODUCTION

As of autumn 2018, you will no longer find the term "cochineal" under the E120 designation, only carmine or carminic acid. Since carmine is insoluble in water, carminic acid is most often present in soft drinks and candies, where it acts as a food colourant. Both anthraquinone derivatives are obtained from grinded bugs to powder, namely from red nopal beetle (*Dactylopius coccus*). Anthraquinones, including carminic acid, are toxic by ingestion and may cause vomiting, diarrhea, kidney and liver damage, and allergic skin reactions (hives), as mentioned in National Library of Medicine, National Center for Biotechnology Information. For this reason, the E120 content is strictly regulated by law. 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). 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 (Alizadeh et al., 2022), a simple, reliable, and cheap screening analytical method, that could be used directly in the field monitoring without sample preparation, has not been introduced yet. In this contribution, a completely new electrochemical assay is therefore proposed, which is based on anodic oxidation of carminic acid in one sample drop casted onto a commercially available screen-printed carbon electrode (SCPE) using square-wave voltammetry (SWV). The necessary validation has to be included, in terms of sample matrix interference, accuracy, precision, and sensor's service life. These analytical parameters are critically evaluated and discussed below.

MATERIAL AND METODOLOGY

Ethanol, carminic acid, glucose, sucrose, fructose, sodium benzoate, citric acid, sodium citrate, sodium chloride, potassium monophosphate monobasic, anhydrous calcium chloride, magnesium chloride hexahydrate, caffeine, ascorbic acid, pyridoxine, and aluminium nitrate, all of purity higher than 95% (*w/w*), were purchased from Merck KGaA (Darmstadt, Germany). Ultrapure water characterised with resistivity lower than 18.3 MΩ cm was obtained from a Milli-Q® deionization unit from Merck Millipore (Burlington, USA).

The electrochemical cell assembly, comprised of a glassy carbon electrode (GCE) of disk diameter 3 mm (type 6.09395.014) from Metrohm Česká republika, s.r.o. (Prague, Czech Republic) as working electrode, a reference silver/silver chloride electrode with a salt bridge containing 3 mol L^{-1} KCl, and platinum rod as an auxiliary electrode, was connected to potentiostat/galvanostat AUTOLAB model PGSTAT101 operated via software NOVA 1.11.0 version, all from abovementioned Metrohm company. This system was employed in investigation of carminic acid electrochemical behaviour in an aqueous environment imitating the composition of common soft drinks. For this purpose, a repetitive cyclic voltammetry of 500 µmol L^{-1} carminic acid in 0.1 mol L^{-1} citrate buffers, differing in pH values, was performed in potential range from –0.3 to +1.0 V at scan rate of 50 mV s^{-1} a potential step of 5 mV.

The itself voltammetric analysis with SPCE (type DRP-C110) from Metrohm DropSens (Oviedo, Spain) were carried out always in 50 µL drop of each real sample and model lemonade using square-wave voltammetry (SWV) at a potential begin of 0 V, potential end of +0.8 V, potential step (E_{step}) of 0.005 V, amplitude potential (E_{ampl}) of 0.025 V, equilibrium time (t_{eq}) of 5 s, and frequency (f) of 10 Hz. A calibration curve method (1–50 mg per 100 mL) was used to determine E120 content in randomly selected food products. As demonstrated in Figure 1, SPE used was connected to an EmStat USB potentiostat trough a sensor connector operated via PSTrace 4.8 software, all from PalmSens BV (Houten, Netherlands).

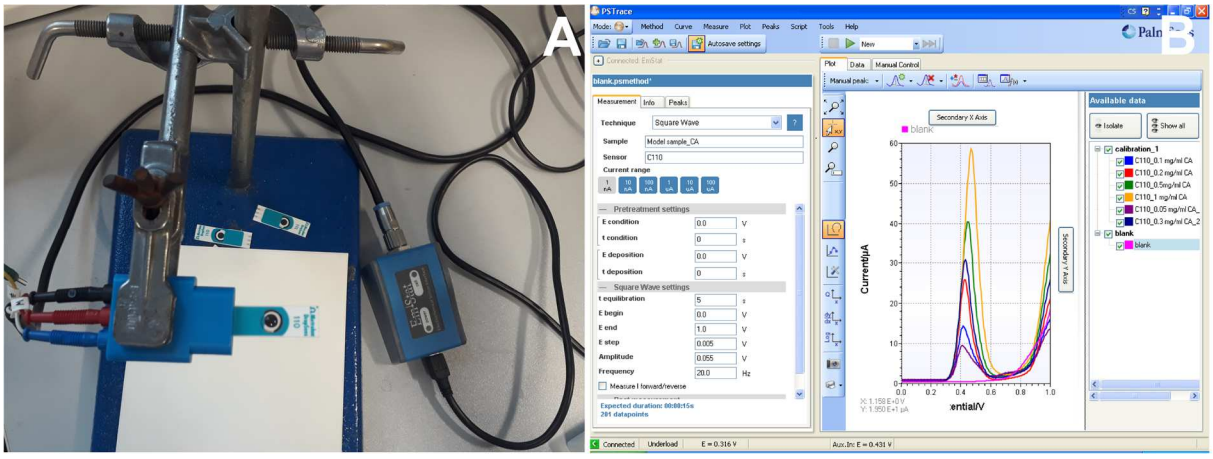

Figure 1 SPCE (type DRP-C110) with 50 µL soft drink dropped connected to EmStat USB potentiostat (A) and PSTrace 4.8 software with voltammetric curves obtained during calibration measurements (B).

The model lemonade with a volume of 500 mL was composited from 27.5 g sucrose, 2.76 g fructose, 2.75 g glucose, 0.0757 g sodium benzoate, 1.5 g citric acid, 0.071 g sodium citrate, 0.255 g sodium chloride, 0.255 potassium chloride, 0.205 g potassium monophosphate monobasic, 0.055 g caffeine, 0.015 g pyridoxine, and 0.1 g carminic acid. Within detailed

interference study, this soft drink was enriched with other ingredients, such as 0.069 g aluminium, 0.104 g magnesium, and 0.069 g calcium salt, 10 mL pure ethanol, and 0.850 g ascorbic acid. Furthermore, the model lemonade was saturated with CO_2 using a carbonated sparking water maker.

First, the selected candy for analysis was weighed, then transferred to a 10 mL volumetric flask, filled with 0.1 mol L^{-1} citric buffer (pH 2) up to mark, and sonicated until whole candy was dissolved. Without subsequent filtration, 50 µL of the resulting sample solution was drop-casted onto SPCE and analysed using SWV.

A rapid colorimetric assay, being recommended as the reference methods for determination of E120 in soft drinks (Marshall, et al., 1974), was used. The corresponding measurements employing visible spectrophotometry (VIS) were performed in 1 cm quartz cuvette (Fisher Scientific, Pardubice, Czech Republic) using a spectrophotometer UV2450 (Shimadzu, Kyoto, Japan) operated via the UV-Probe software. A method of calibration curve (1–20 mg carminic acid per 100 mL) was used for determination of E120 content in real samples, when the maximum adsorption band appears at a wavelength of 492 nm.

All real samples were purchased from Czech stores. Analysis of model lemonade, soft drinks, and an alcoholic beverage was always five times repeated (n = 5) and the final results were calculated and presented as confidence intervals $\bar{x} \pm st_{1-\alpha}/\sqrt{n}$ where $\bar{x}$ is the arithmetic mean, s the standard deviation, and $t_{1-\alpha}$ the critical value of Student's t-distribution for ten (4 degrees of freedom) determinations (2.776) at a significance level α of 0.05 (95% probability). Finally, y-intercept significance of the calibration curve constructed from the average of five replicate measurements (n = 5) at the SPCE was determined by means of data analysis *via* the Microsoft Excel 2016 version.

RESULTS AND DISCUSSION

In aqueous and aqueous-ethanol mixtures, 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, as evident from Figure 2.

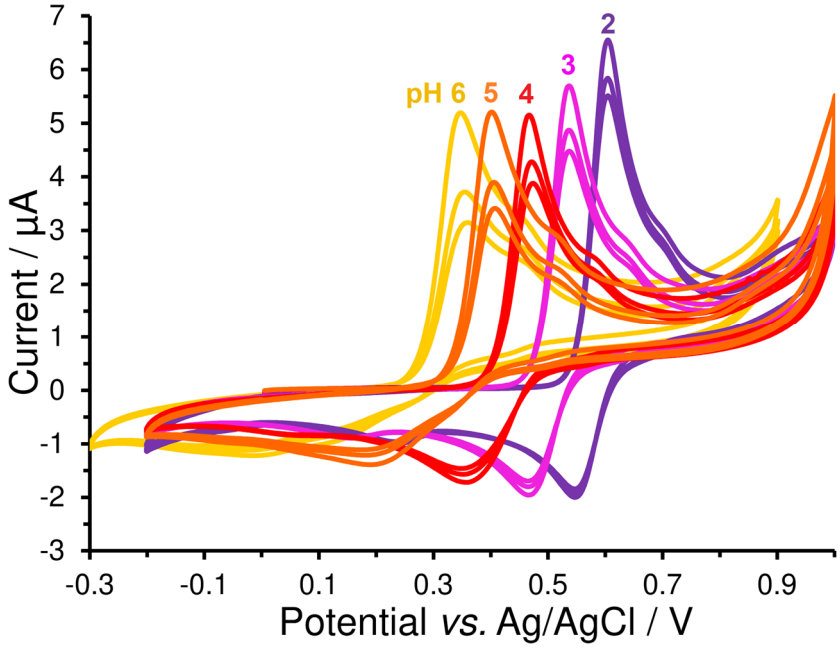

Figure 2 Repetitive cyclic voltammograms (3 cycles) of 500 µmol L^{-1} carminic acid recorded on GCE in different 0.1 mol L^{-1} citrate buffers at scan rate of 50 mV s^{-1}.

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 (Figure 3), which is typical for naphthazarin (5,8-dihydroxy-1,4-naphthoquinone) and similar hydroxyanthraquinones (Yao et al., 2017; Brousse et al., 2018). The corresponding peak current responses also significantly depended on the chosen pH of the working medium, while the highest current yields of electrode reactions can be achieved in a strongly acidic environment. Unfortunately, this fact must be kept in mind during real analysis.

Hence, the pH of analysed soft drinks was determined to vary from 2.8 to 4, which reflects their composition, especially the presence of citric acid, ascorbic acid, phosphoric acid, and sodium benzoate in the mixture. To correctly assign the cutoff values of current response for 20 mg E120 per 100 mL at different pHs (Table 1), it will be necessary to approximately estimate the pH of the analyzed soft drinks and alcoholic beverages at least using pH indicator paper. For solid samples, such as sausages and candies, the pH is given by the used 0.1 mol L^{-1} citrate buffer into which E120 will be released during sample pretreatment.

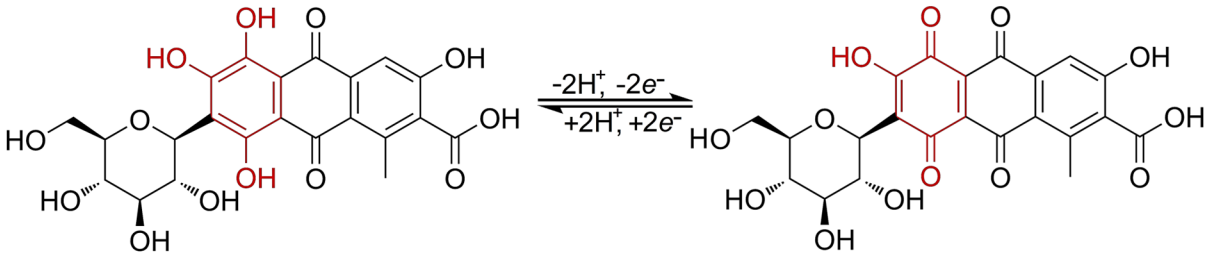

Figure 3 The reversible electrochemical reaction of carminic acid (Alizadeh et al., 2022).

Within optimisation of the electrochemical detection, SWV parameters obtained were deliberately not chosen, in order to guarantee the widest possible linear range (1–50 mg per 100 mL^{-1}) that would cover the commonly occurring E120 content of foods (Figure 4). Therefore, potential range from 0 to +0.8 V, E_{step} = 5 mV, E_{ampl} = 25 mV, t_{eq} = 5 s, and f = 10 Hz were considered as an acceptable compromise. The peak area response (A_p) obtained could be calibrated within a linear range 1–50 mg per 100 mL^{-1} carminic acid characterised with coefficients of determination higher than 0.9930, a sensitivity of 2.181, 3.225, and 8.910 μAVmg^{-1}L at pH 4, 3 and 2, respectively, statistically insignificant y-intercepts, and a detection limit of 2 mg L^{-1} (4×10^{-6} mol L^{-1}) carminic acid. Due to passivation of the working electrode surface, manifested in the gradual decrease of the signal during repeated analysis, SPCE seemed to be used only for disposable application. Fortunately, it has been shown that it is possible to renovate the surface using repetitive cyclic voltammetry (15 cycles) in 0.5 mol L^{-1} H_2SO_4 at potential range from 0 to +1 V and scan rate of 50 mV s^{-1}.

Table 1 Cutoff values of peak current responses for 20 mg per 100 mL E120 food additive at different pH.

pH	2	3	4	5
A_p (μAV)	1.533 ± 0.050	0.865 ± 0.036	0.536 ± 0.021	0.539 ± 0.018
Notes: Values given as confidence intervals $\bar{x} \pm st_{1-\alpha}/\sqrt{n}$, where $\bar{x}$ is the arithmetic mean, s the standard deviation, and $t_{1-\alpha}$ the critical value (2.776) of Student's t-distribution for 5 repetitions (4 degrees of freedom) of each analysis at α = 0.05.				

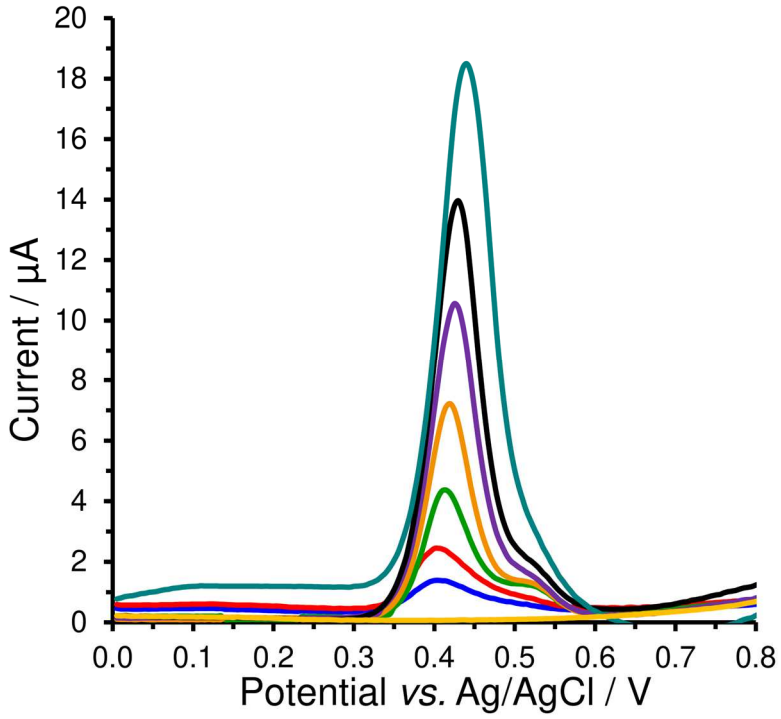

Figure 4 Square-wave voltammograms of 0, 1, 3, 5, 10, 20, 30, and 50 mg carminic acid per 100 mL model lemonade recorded on SPCE (type DRP-C110) at E_{step} = 5 mV, E_{ampl} = 25 mV, t_{eq} = 5 s, and f = 10 Hz.

The precision of the developed screening electrochemical assay was determined using repetitive analyses of the model lemonade containing maximally allowed carminic acid content (20 mg per 100 mL) to be 3.5 % relative standard deviation. As for accuracy, it was verified in the framework of the analysis of the model lemonade, soft drinks, and candies. As shown in Table 2, the developed electrochemical test is able to provide statistically comparable results with the reference colorimetric method, which demonstrates its potential use in the food safety control.

Table 2 Comparison of the developed electrochemical assay with colorimetric reference method in analysis of several foodstuffs.

Soft drinks	SWV (mg per 100 mL)	VIS (mg per 100 mL)	Allowed content (mg per 100 mL)
Model lemonade	21.55 ± 0.89	21.03 ± 0.06	20
Aloe Vera watermelon	9.29 ± 0.21	9.12 ± 0.05	20
Tiger watermelon	4.82 ± 0.23	5.04 ± 0.13	20
Tiger strawberry	2.24 ± 0.19	2.50 ± 0.08	20
Relax strawberry	2.77 ± 0.12	2.69 ± 0.05	20
Candies	SWV (mg per 100 g)	VIS (mg per 100 g)	Allowed content (mg per 100 g)
MandM	2.765 ± 0.08	2.717 ± 0.05	30
Tutti frutti	3.701 ± 0.17	3.912 ± 0.06	30

Notes: Values given as confidence intervals $\bar{x} \pm st_{1-\alpha}/\sqrt{n}$, where $\bar{x}$ is the arithmetic mean, s the standard deviation, and $t_{1-\alpha}$ the critical value (2.776) of Student's t-distribution for 5 repetitions (4 degrees of freedom) of each analysis at $\alpha = 0.05$.

CONCLUSION

In this post, it has been shown that square-wave voltammetry in combination with commercially available screen-printed sensor offers an effective electroanalytical tool for reliable determination of E120 food additive in soft drinks, alcoholic beverages, and candies. The sensor together with a vial of 0.5 mol L^{-1} sulfuric acid for sensor renovation, a vial with pure 0.1 mol L^{-1} citrate buffer (pH 2), pH indicator papers, and list of peak current responses for 20 mg carminic acid per 100 mL at different pH, could be served as portable screening kit for field monitoring in the food safety control.

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