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Determination of the food dye carmine in milk and candy products by differential pulse polarography

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ABSTRACT

As a basis for the development of a sensitive analytical method for the determination of carmine food dye, a study of the differential pulse polarographic reduction of carminic acid (CA) on a dropping mercury electrode was performed. For the analytical differential pulse polarographic method running at pH 2.0 Britton–Robinson (B–R) buffer solution (peak at −489 mV), the relationship between the peak current and CA concentration was linear in the range of 1 μM to 90 μM with a detection limit of 0.16 μM. The proposed electrochemical procedure was successfully applied to the determination of carmine food dye in spiked commercially available strawberry flavored milk. The method was extended to the determination of CA in candy and results were in agreement with that obtained by a spectrophotometric comparison method. A cyclic voltammogram of CA in 2.0 B–R buffer electrolyte was obtained on the dropping mercury electrode at pH 2.0 during potential scans from 0.00 mV to 1000 mV versus Ag/AgCl. From repetitive cyclic voltammograms, one cathodic peak at −500 mV and three anodic peaks on the reverse scan between approximately −340 mV and −460 mV were recorded. The influences of some other commonly found inorganic and organic salts on the determination of CA were also examined. The sufficiently good recoveries and low standard deviations for the data reflect the high accuracy and precision of the proposed differential pulse polarographic method.

1. Introduction

Carminic acid (CA) is obtained from aqueous, aqueous alcoholic, or alcoholic extracts from cochineal, which consists of the dried bodies of the female *Dactylopius coccus* Costa insect. CA (7-α-D-glycopyranosyl-9, 10-dihydro-3,5,6,8-tetrahydroxy-1-methyl-9,10-dioxo-2 anthracenecarboxylic acid), the principal component of the food dye cochineal, is an anionic, anthraquinone glycoside widely used as a coloring dye in foodstuffs [1], drugs, and cosmetics [2]. Its identification code as a food additive is E-120. Its structure is shown in Scheme 1. The molecular structure consists of an anthraquinone chromophore, a sugar residue, and a carboxyl group. Thus, CA has good solubility in water [3].

CA belongs to types of antitumor and antibiotic anthracycline derivatives. They are believed to develop their cytotoxic

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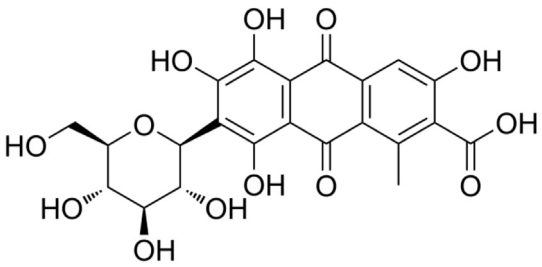

Scheme 1 – Structure of carminic acid (CA).

effect by penetrating into the tumor cell nucleus and interacting there with DNA [4,5].

Increased hyperactivity has been reported in a few cases. Carmines and possibly CA in food and beverages may provoke allergic reactions in some individuals [6]. CA has been associated with IgE-mediated anaphylaxis, urticaria, and angioedema [7–9]. This increased demand for carminic red highlights the importance of better understanding its chemical behavior and developing trustworthy, simple analytical methods to quantify the amount of colorant in cochineal for quality control purposes.

Several analytical methods have been reported for the determination of CA using fluorometric [10], spectrophotometric [11–13], and chromatographic [14–17] methods. However, the control range on the absorbance is very small, whereas the determined accuracy acquired is high. Furthermore, the methods based on chromatography need expensive instrumentation, extra pure solvents, and a high degree of operator training. This makes the analytical determination of CA time consuming [18].

In a study, a fluorometric method for the determination of CA is developed. Under optimized conditions, the enhanced intensities of fluorescence are quantitatively in proportion to the concentrations of CA in the range of 0.01231–12.31 μg/mL. The detection limit is 10.92 ng/mL [10]. A simple analytical method based on the second-order calibration of the pH gradient spectrophotometric data was developed for assay of CA in human plasma and orange juice over the concentration range of 1.5–14.0 μM [12]. Gonzalez et al [16] developed a procedure for the extraction and determination of color pigments in cochineals (*Dactylopius coccus* Costa). The procedure was based on the solvent extraction of pigments in insect samples using methanol/water (65:35, v/v) as extractant. A two-level factorial design was used to optimize the solvent extraction parameters: temperature, time, methanol concentration in the extractant mixture, and the number of extractions. The results suggest that the number of extractions is statistically the most significant factor. The separation and determination of the pigments was carried out by high-performance liquid chromatography (HPLC) with UV-visible detection [16]. In most of the spectrophotometric methods for the determination of tungsten, procedures such as extraction, separation, and enrichment methods are needed. These methods are time consuming in addition to the danger of pollution. The limit of detection (LOD) values are also not as low as the LOD values obtained with electroanalytical methods.

Electrochemical methods, for example, polarography, voltammetry [19], and square wave voltammetry, are used to determine either organic or inorganic electroactive species. The limitations of electroanalytical procedures and their advantages compared with HPLC and gas chromatography (GC), such as speed, sensitivity, and speciation, are discussed elsewhere by Zuman [20].

To date, we only found two available papers for electrochemical determination of CA. The adsorption behavior of carmine (E-120) on the hanging mercury drop electrode has been examined using square wave adsorptive stripping voltammetry in pH 3 acetate buffer [21]. Under optimal conditions, a detection limit of 1.43×10^{-9} mol/L and a linear calibration graph in the range of 5×10^{-8} M to 1.25×10^{-7} M were obtained. The proposed electrochemical procedure was applied to the determination of carmine food dye in spiked commercially available ice cream and soft drinks. In a study, the determination of CA in cochineal extracts by the differential pulse polarographic method has been used [22]. The detection limit was found to be 0.55 μg/mL.

The purpose of the present study was to develop a new, rapid, simple, selective, and inexpensive polarographic method at a dropping mercury electrode (DME) for the direct determination of CA in real samples without any time-consuming extraction or evaporation steps prior to CA assay. The sufficiently good recoveries, UV-spectrophotometric method comparison results, and low relative standard deviations reflect the high accuracy and precision of the proposed polarographic method. The influences of some interfering species will also be investigated. In addition, electrochemical behaviors of CA are investigated with cyclic voltammetry (CV).

2. Methods

2.1. *Apparatus*

A BAS model electrochemical analyzer (Bioanalytical Systems, Epsilon Basic Plus Potentiostat/Galvanostat, West Lafayette, IN, USA) was used for differential pulse polarography (DPP) and CV measurements. A three-electrode system was used, consisting of a platinum counter electrode, an Ag/AgCl (3 M NaCl) reference electrode, and a DME as a working electrode. pH values were measured with a WTW pH/ION 735 (WTW Instruments, Weilheim, Germany) pH meter. Absorption spectra and absorbances were recorded using a PerkinElmer LAMBDA 25 double beam UV/Visible Spectrophotometer, California, USA.

2.2. *Reagents*

The reagents and solutions in the present study were all of reagent grade.

CA, analytical grade, was purchased from Sigma (St Louis, MO, USA). Stock solution was prepared in ethanol–water (50:50, v/v) at a concentration of 0.1 M and stored in a refrigerator. Working standard solutions were prepared by dilution of stock solution with water. All chemicals (electrolyte, solvents, and other reagents) used were of analytical reagent

grade (pro-analysis). Triply distilled water was used in the preparation of all solutions. Solutions of 10^{-3} M and more dilute ones were prepared prior to every use in order to avoid aging.

The mercury used in the DME was obtained from Merck (Darmstadt, Germany). Used mercury was cleaned by successively passing it through dilute HNO_3 (3.0 M) and water columns in the form of fine droplets by using a platinum sieve. The collected mercury was dried between sheets of filter paper. Prior to use, a DP polarogram of this mercury was recorded in order to confirm the absence of impurities.

Britton–Robinson (B–R) buffer solution was prepared in such a way that 2.3 mL of glacial acetic acid, 2.7 mL of phosphoric acid (85%), and 2.47 g of boric acid were dissolved by dilution with water to 1.0 L; 50 mL portions of this solution were taken, and the pH was adjusted by the addition of an appropriate amount of 2.0 M NaOH to the desired value.

2.3. Analytical procedure

In total, 10 mL of supporting electrolyte solution of B–R buffer was placed into the polarographic cell and deoxygenated with high-purity nitrogen (99.999%) for approximately 5 minutes. The background polarograms were obtained by scanning the potential from 0.0 V to approximately −600 mV to −2200 mV (vs. Ag/AgCl) depending on the pH of the solution, and then the polarographic responses of 1×10^{-5} M to 1×10^{-3} M CA at the DME were analyzed in various electrolyte solutions. The analytical curves for CA were obtained by standard addition of CA. The linear concentration range was between 1 μM and 90 μM. The optimum conditions for the analytical determination of CA using DPP were found to be: pH 2.0 B–R buffer electrolyte, peak potential of −489 mV, scan rate of 5 mV/second, pulse amplitude of 50 mV.

2.4. Sample collection

Commercially available candy samples and strawberry flavored milk were purchased from the local market of Nevsehir, Turkey. The samples were stored at 4 °C until processed for extraction of CA.

2.5. Sample preparation

Weighing 12 g of candy samples were diluted with 8 mL pH 2.0 B–R buffer solution, and the resulting solution was homogenized by shaking for 5 minutes. Appropriate volumes of this candy sample were transferred into the polarographic flask and diluted up to the volume with B–R buffer at pH 2.0. The UV-spectrophotometric method was also applied to the candy samples using the above procedure. A filtration step was required prior to dilution for UV-spectrophotometry.

A total of 2.0 mL strawberry flavored milk sample was spiked with 8.0 mL acetonitrile, which removes milk proteins more effectively [23]. After homogenizing the sample for 20 minutes, centrifugation for 20 minutes at 9000 rpm (6758.32*g*) to get rid of milk protein residues, then the supernatant was discarded. After completion of the above procedure for milk, 4.0 mL aliquots were then added to the cell containing 6.0 mL of B–R buffer solution at pH 2. DP polarograms were recorded and CA determination in strawberry flavored milk was performed from the peak current at approximately −526 mV using CA standard additions.

3. Results and discussion

3.1. Effect of pH and selection of a supporting electrolyte

The B–R buffer (0.04 M) was chosen for its wide pH range applicability. For the basic study of the electrochemical behavior of CA, DP polarographic responses were examined over the pH range of 1.0 to 12.0. The polarographic behavior of CA exhibited a single well-defined DP peak over the whole pH range (1.0–12.0) in B–R buffer solution (Table 1). Fig. 1 shows typical DP polarograms of 20 μM CA. The reduction potential of CA at the DME which shifted to more negative values upon increasing pH was found to be pH-dependent. As shown in Table 1, pH versus I_p, a maximum current intensity was obtained at a pH value of 6.0 but there was no linear relationship between the height of this peak and the concentration of CA. In optimization of the pH value, not only was peak current chosen as an important parameter but also peak shape and peak symmetry were chosen as other important parameters. As a result, optimum conditions for analytical determination of CA by DPP were as follows: pH 2 B–R buffers, at a reduction potential of −489 mV, 2 second drop time, 50 mV pulse amplitude.

3.2. Linear range and detection limit

Under the recommended conditions (pH 2.0, B–R buffer, at a reduction potential of −489 mV and −50 mV pulse amplitude, and 1 second drop time), the consecutive additions of CA (Fig. 2) gives a linear relationship between the peak currents and concentrations in the range of 1–90 μM with the linear regression equation given by: $I_p/\mu A = 0.003\ C/\mu M + 0.004$ ($R^2 = 0.994$) ($n = 14$).

The LOD and limit of quantification (LOQ) values were obtained as 0.16 μM and 0.55 μM, respectively. LOD and LOQ values were calculated using the following equations [24,25]: LOD = 3 *Sb/m*, LOQ = 10 *Sb/m*, where *Sb* is the standard deviation of the fortified blank solution containing 10 μM CA

Table 1 – Polarographic behavior of carminic acid Britton–Robinson buffer electrolytes.

Ion	Electrolyte (pH)	*E* peak (mV)	*I* peak (nA)	Peak shape	*C* (μM)
Carminic acid	1	−436	62.10	Broad	20
	2	−489	71.5	Sharp	
	3	−515	80.52	Sharp	
	4	−600	86.67	Broad	
	5	−676	70.60	Broad	
	6	−700	134.4	Broad	
	8	−830	94.2	Broad	
	9	−942	54.00	Broad	
	10	−975	67.7	Broad	
	12	−1106	73.43	Broad	

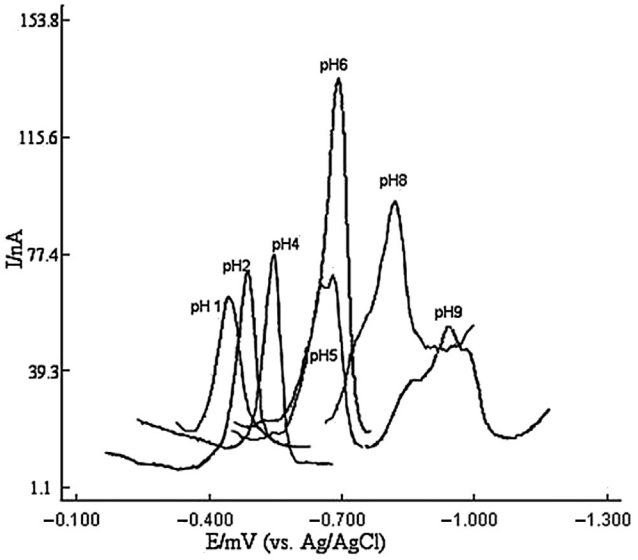

Fig. 1 – Differential pulse polarograms of 20 μM carminic acid in pH 1–12 Britton–Robinson buffer solution.

(10 runs) and *m* is the slope of the calibration curve. The high values of the slope (3×10^{-3} μA/μM) and correlation coefficient ($R^2 = 0.994$) reflected the sensitivity of the proposed method. The reproducibility of the proposed methodology was determined from the 10 different measurements of 10 μM CA and the relative standard deviation was obtained as 3.3%.

3.3. *Cyclic voltammetry*

Electrochemical behavior, diffusion, and adsorption properties of CA were studied using the result of CV. In cyclic voltammetric studies, a single well-defined reduction peak was

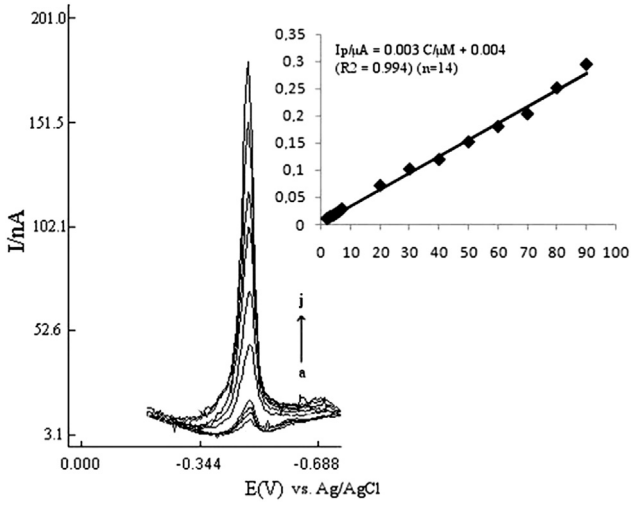

Fig. 2 – Differential pulse polarograms for linear calibration curve in pH 2.0, Britton–Robinson buffer. (a) 2 μM, (b) 3 μM, (c) 4 μM, (d) 5 μM, (e) 10 μM, (f) 20 μM, (g) 30 μM, (h) 40 μM, (i) 50 μM, (j) 60 μM.

observed at a potential of approximately −500 mV (vs. Ag/AgCl) at pH 2.0. There is no peak when a blank B–R solution was scanned at the same conditions, and peak intensity increases linearly with increasing concentration of CA, showing that this reduction peak is due to the reduction of CA molecules. As can be seen in Fig. 3, there are also three anodic peaks at reverse scan. Existence of these anodic peaks depends on the potential scan rate. The scan rate increased the peak potential (*Ep*) and shifted in a negative direction (Fig. 3). This behavior shows the irreversible character of electrode reaction, but as can be seen in Fig. 3 there are also anodic peaks indicating reversibility. In fact, for an ideal reversible electrode reaction, peak potential is not affected by scan rate and ratio of anodic peak current ($I_{p,a}$) to cathodic peak current ($I_{p,c}$) is in agreement [26]. In the present study, peak potential was affected by scan rate and peak current ratio is not in agreement.

The influences of the potential scan rate on cathodic peak current ($I_{p,c}$) and cathodic peak potential ($E_{p,c}$) were investigated for 10 μM CA in the 0.0–1000 mV/second range. In this range, a linear dependency between cathodic peak current, $I_{p,c}$, and scan rate, υ, was found [$I_{p,c}$ (μA) = 0.508 υ(mV/second) + 138.9; $R^2 = 0.994$; Fig. 3A]. The linear relationship between peak current and scan rate confirms an adsorption controlled mechanism [27,28]. In addition, cyclic voltammograms of CA at approximately −530 mV exhibited a pre-peak to the cathodic CV peak, indicating strong adsorption (Fig. 4) [29,30]. Also a plot of the logarithm of the peak current versus the logarithm of the scan rate (mV/second) was studied. This relationship was found to be linear with a slope of 0.622 (Fig. 3B) [31].

Some extra studies were carried out to control the adsorption phenomena according to the literature [29,32]. As a result, it was found that the value of the ratio of cathodic peak current to concentration ($I_{p,c}/C$) decreases with increasing concentration and the value of the ratio of cathodic peak current to multiplication of concentration and square root of scan rate ($I_{p,c}/C\ \upsilon^{1/2}$) increases with increasing scan rate. Results of all these experimental investigations suggest that electroreduction of CA molecules on the hanging mercury drop electrode is mainly controlled by diffusion with some adsorption contribution.

3.4. *Interference studies*

The selectivity of the proposed method for CA was investigated in the presence of some inorganic ions [e.g., Pb(II), Ca(II), Zn(II), Cd(II), Na(I), Mg(II)] and organic species (gallic and tannic acids). The interference studies were performed using the various interfering ions, most of them being electroactive, for example, Cu(II), Fe(III), Pb(II), Zn(II), Cd(II), NO_2^-, SO_3^{2-}, Se(IV), and the others inactive, for example, K(I), Na(I), NH_4^+, Ca(II), Mg(II), NO_3^-, SO_4^{2-}, Cl^-. The degree of interference effects was treated as the recoveries of 10 μM CA in the presence of the 10 times larger concentration of interfering inorganic or organic species. The sufficiently good recoveries could be attributed to the inorganic ions or organic species in which they do not reduce or form complex with the analyte species. Recovery results in the presence of coexisting species are shown in

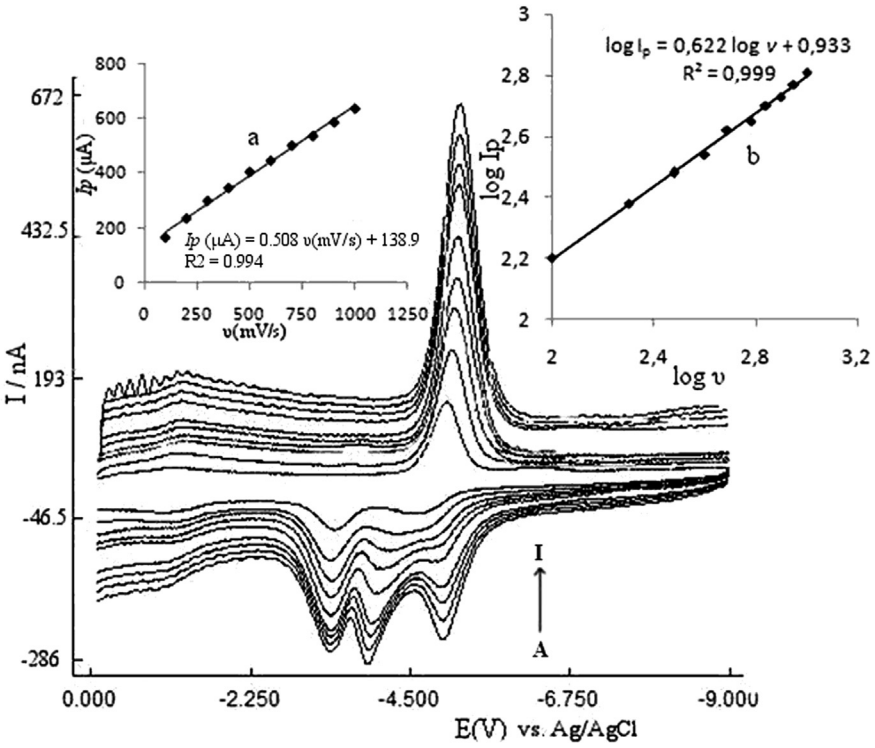

Fig. 3 – Cyclic voltammogram of 10 μM carminic acid: (A) 100 mV/second, (B) 200 mV/second, (C) 300 mV/second, (D) 400 mV/second, (E) 500 mV/second, (F) 600 mV/second, (G) 700 mV/second, (H) 800 mV/second, (I) 900 mV/second.

Table 2. High percentage recovery data also show that the proposed methods are free from interferences.

3.5. *Applications*

To investigate the analytical potential of the proposed method, it was applied for the determination of CA in strawberry flavored milk and candy. For the determination of CA in strawberry flavored milk, a 4.0 mL milk sample was added into a polarographic cell containing 6.0 mL pH = 2 B–R buffer and a polarogram was taken (Fig. 5A). CA was determined by the standard additions method (Table 3). CA was extracted with distilled water from candy samples. And then for the determination of CA in candy samples, a 2.0 mL candy sample was added into a polarographic cell containing 8.0 mL pH 2 B–R buffer and polarograms were taken (Fig. 5B). As can be seen in Fig. 5B, CA was determined by the standard additions method (Table 3).

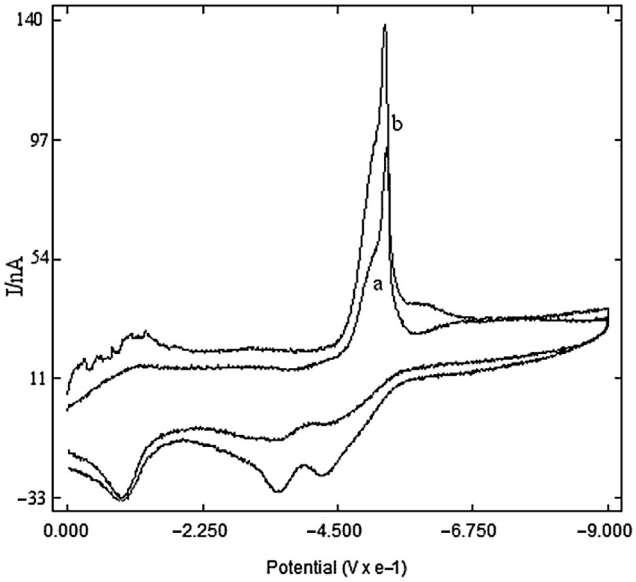

Fig. 4 – Cyclic voltammogram of 10 μM carminic acid. a = 25 mV/second; b = 50 mV/second.

Table 2 – Influence of interfering species (100 μM) on the recovery of 10 μM carminic acid.

Interfering species	Recovery (%)	Interfering species	Recovery (%)
Pb^{2+}	93	Al^{3+}	95
Ni^{2+}	99	K^{+}	87
Cd^{2+}	93.2	Ba^{2+}	102
Fe^{3+}	85.3	Mg^{2+}	88
Zn^{2+}	105	Hg^{2+}	105
Se^{4+}	98	NO_2^-	103
Co^{2+}	84.3	SO_4^{2-}	87
Cu^{2+}	94.3	NO_3^-	100
Mn^{2+}	98	Na^{2+}	100
Ca^{2+}	97.4	SO_3^-	100
Cl^-	85.3	Gallic acid	91.3
		Tannic acid	100

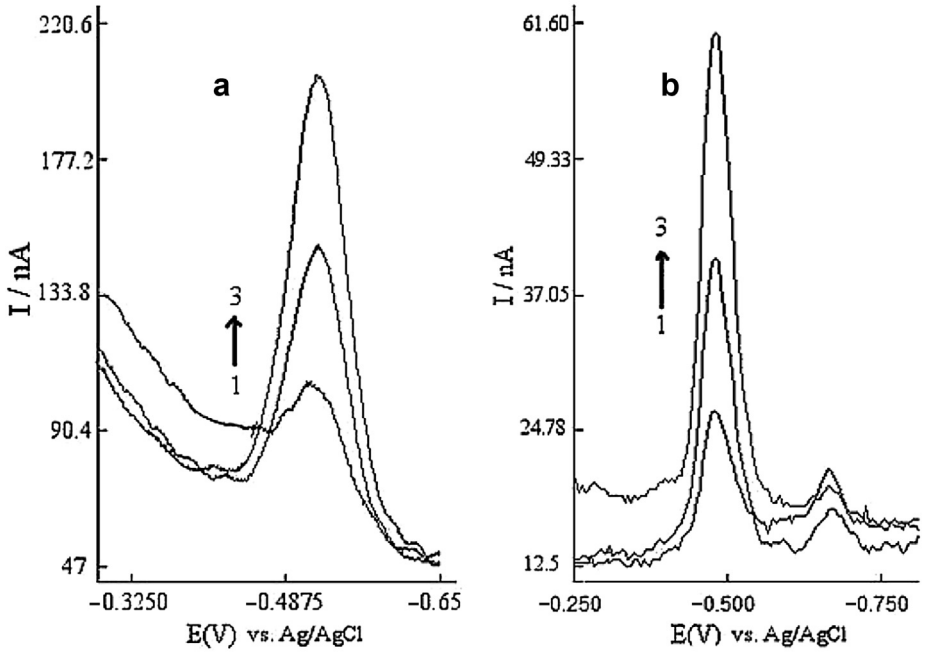

Fig. 5 – (a) Determination of CA in milk sample: (1) 6 mL of B–R buffer (pH = 2) + 4.0 mL milk sample, (2) 1 + 0.1 mL 1×10^{-3} M CA, (3) 2 + 0.1 mL 1×10^{-3} M CA. (b) Determination of CA in candy samples: (1) 8 mL of B–R buffer (pH = 2) + 2.0 mL candy sample, (2) 1 + 0.1 mL 1×10^{-3} M CA, (3) 2 + 0.1 mL 1×10^{-3} M CA. B–R = Britton–Robinson; CA = carminic acid.

A UV-spectrophotometric method was developed to compare the validity of the electroanalytical method. CA absorption spectra in pH 12.00 B–R solution exhibited three well-resolved maxima at 235 nm, 292 nm, and 567 nm. The maxima at 567 nm showed better absorption and had a linear dependence with CA concentration. The linear domain range was 1×10^{-7} M to 2×10^{-6} M for CA with a correlation coefficient of 0.999 and can be expressed by the following regression equation: $A = 1.2 \times 10^{5}$ (C) + 0.055, where A is the absorbance of CA at 567 nm and C molar concentration in pH 12.0 B–R solution. As can be seen in Table 3, the DPP and UV methods showed similar accuracy and precision, which indicate the validity of the proposed method. The DPP method offers high sensitivity, low limit of determination, easy operation, and simple instrumentation.

Table 3 – Application of the DPP and UV-spectrophotometric methods for the determination of CA in strawberry flavored milk and candy samples.

Technique	Sample	*n*	CA found	RSD (%)
DPP	Milk	5	121 ± 4 μg CA/mL milk	2
	Candy	5	28.4 ± 1.5 mg CA/g candy	4
UV	Milk	4	—	—
	Candy	4	27.1 ± 2.5 mg CA/g candy	7

95% confidence interval (CI) is used to indicate the reliability of an estimate.
CA = carminic acid; DPP = differential pulse polarography; RSD = relative standard deviation.

4. Conclusions

The electrochemical behavior of CA was investigated at a DME using CV and DPP. The reduction peak appeared at −489 mV and quantifications were performed on the basis of this peak by successive standard additions. The recommended polarographic method was successfully applied to strawberry flavored milk and candy samples. The sufficiently low standard deviations for the data reflect the high accuracy and precision of the proposed DP polarographic method. This method has some other advantages such as low cost and the possibility of analysis without the need of extraction or pretreatment, as well as the short time required for analysis. The results obtained in the proposed method are very reproducible because with the use of DME the surface of the electrode is always new and the behavior of the electrode is independent of its past history.

Conflicts of interest

All contributing authors declare no conflicts of interest.

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