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Original Article

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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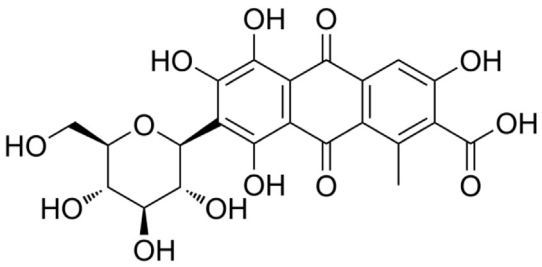

Scheme 1 – Structure of carminic acid (CA).

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