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A simple method for determination of carmine in food samples based on cloud point extraction and spectrophotometric detection

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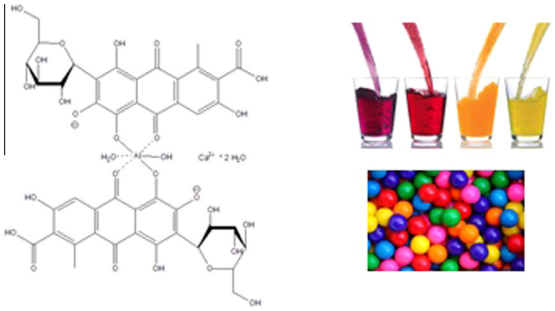
HIGHLIGHTS

- A simple, cost effective and green method was developed for determination of carmine.
- This is the first report which uses the CPE for extraction of carmine in real samples.
- The results demonstrate the method performance to determine the carmine in food samples.

GRAPHICAL ABSTRACT



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ABSTRACT

In this paper, a simple and cost effective method was developed for extraction and pre-concentration of carmine in food samples by using cloud point extraction (CPE) prior to its spectrophotometric determination. Carmine was extracted from aqueous solution using Triton X-100 as extracting solvent. The effects of main parameters such as solution pH, surfactant and salt concentrations, incubation time and temperature were investigated and optimized. Calibration graph was linear in the range of 0.04–5.0 μg mL^{-1} of carmine in the initial solution with regression coefficient of 0.9995. The limit of detection (LOD) and limit of quantification were 0.012 and 0.04 μg mL^{-1}, respectively. Relative standard deviation (RSD) at low concentration level (0.05 μg mL^{-1}) of carmine was 4.8% ($n = 7$). Recovery values in different concentration levels were in the range of 93.7–105.8%. The obtained results demonstrate the proposed method can be applied satisfactory to determine the carmine in food samples.

Introduction

Synthetic colorants are used in different industries such as paper, textile, inks, plastics, cosmetics, drugs, edible drinks and food. Generally, synthetic dyes have complex aromatic structures making them stable and difficult to be biodegraded [1]. The synthetic dyes have also been utilized in foods to make them more attractive and appetizing for centuries [2].

Carmine is a pigment of a bright-red color which obtained from the aluminum salt of carminic acid. The pigment produced from the cochineal which is the female *Dactylopius coccus Costa* insect [3]. Carmine is used as a food dye in many different products including juices, ice cream, yogurt and candy, as well as in drug formulations and cosmetic products such as eye shadow and lipstick [4]. Carmine, one of the synthetic food dyes, is authorized to be used in USA, Canada, Korea and European Union [5]. It is

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worth nothing that the acceptable daily intake (ADI) value of carmine is 5 mg kg^{-1} based on weight [5]. Although the amounts of carmine added to food and drinks are strictly controlled, their use may exceed the authorized levels. Thus monitoring the levels of carmine dye in high consumption products such as beverages is essential.

In terms of rapid methods, the current trend in analytical chemistry is towards user-friendly increasingly miniaturised instruments particularly for quality control applications. The use of sample preparation methods for selective extraction of targets is likely to become commonplace as user-friendly and cost-effective techniques are becoming available. Sample preparation methods with pre-concentration and clean-up operations enable the analysis of several classes of food additives simultaneously with greater specificity and better detection limits [6–8].

The number of studies on this issue over the past few years gives testimony to the importance of this problem and highlights the need for developing fast, accurate and selective techniques for synthetic dye analysis [9]. Up to now several methods for the determination of carmine and carminic acid in food samples have been proposed. These methods are including differential pulse polarography (DPP) [10], stripping voltammetry (SV) [11], high-performance liquid chromatography (HPLC) [12,13] and spectrophotometric method [14]. Some of these methods, e.g. chromatography and polarography are not considered as green analytical methods due to the use of hazardous organic solvents in chromatography and dropping mercury in polarography. On the other hand, HPLC and capillary electrophoresis (CE) methods are construed as more efficient alternative methods. However, they are expensive, time-consuming and produce waste with a high percentage of organic solvents. Despite the high sensitivity of electroanalytical methods, they suffer from low selectivity. The disadvantages of stripping voltammetry (SV) are including longer analysis time than spectroscopic methods, and also interferences which can lead to limitations.

Separation methods based on cloud point extraction (CPE) are practical application of surfactants in analytical chemistry and have become an alternative to solvent extraction. Compared with conventional solvent extraction, CPE uses surfactants and avoids utilizing a large amount of expensive, toxic, and flammable organic solvents. CPE procedure has been widely used for separation, purification and pre-concentration of variety of substances such as organic and inorganic compounds in water, food, drug and biological samples [15–24].

The aim of this study was to develop a simple and sensitive cloud point extraction method for determination of carmine in food samples by using spectrophotometry detection. The method is used for extraction, clean-up and pre-concentration of carmine from aqueous samples using Triton X-100 as extracting solvent. The influences of main parameters on the extraction efficiency of carmine were investigated and optimized. Finally, figures of merit of the proposed method were compared with several reported methods in literature.

Experimental

Reagents and materials

All chemicals used in this work were analytical reagent grade and double-distilled water was used throughout. Carmine and Triton X-100 were purchased from Merck Chemicals Company (Darmstadt, Germany). A solution of nonionic surfactant (40% w/v) Triton X-100 was prepared by dissolving accurately 40 g of Triton X-100 in water and diluting to 100 mL in a volumetric flask. Buffer solution pH 5 was prepared by adding 1.0 mol L^{-1} of sodium hydroxide solution to acetic acid (0.1 mol L^{-1}) and adjusting the pH to 5 using a pH meter. Edible drink and smarties samples were purchased from local supermarkets in Khorramabad (Lorestan, Iran).

Instrumentation

Absorption spectra and absorbance measurements were achieved by a Jenway spectrophotometer (model 6715, UK) using 1 cm glass cells. A Metrohm digital pH meter (model 632, Switzerland) with a combined glass electrode was used to measure pH values. A centrifuge (Behsan, Iran) was used to accelerate the phase separation process. A thermostatic water bath (Memmert, Germany) was used to maintain the temperature in CPE experiments.

Preparation of standard solutions

Stock solution of carmine (1000 μg mL^{-1}) was prepared by dissolving 0.1 g of carmine dye in water and diluting to 100 mL in a volumetric flask. Fresh working standard solutions were obtained by appropriate dilution of the stock solution and were stable during the day.

Preparation of sample solutions

Appropriate amounts of edible drink and smarties samples were dissolved in deionized water. After dissolve in water, sample solutions were filtered using membrane filter (0.45 μm). The filtrated sample solutions were diluted to 5 mL in a volumetric flask. An aliquot of solutions was treated under the recommended procedure for CPE and subsequent determination of carmine.

Analytical procedure

4 mL of the acetate buffer solution (pH 5) containing of carmine (so that its final concentration would be in the range of 0.04–5 μg mL^{-1}) was transferred to a 10 mL centrifuge tube. Then 1 mL of 40% (w/v) of Triton X-100 and 0.75 g of Na_2CO_3 salt were added to this solution. After dissolving the salt, the mixture was then placed in a thermostat bath at 55 °C for 15 min. The phase separation was accelerated by centrifuging the test tube for 5 min at 4000 rpm. The surfactant-rich phase became a viscous and was collected at the upper of the tube. Therefore, the aqueous phase was carefully removed using a syringe with a long needle that passed through the surfactant-rich phase. The surfactant-rich phase was diluted with water up to 1 mL. The absorbance of the solution was measured at 513 nm. A blank solution (without carmine) was also submitted to the same procedure and measured in parallel to the samples.

Results and discussion

The absorption spectrum of carmine shows that maximum absorbance occurs at 513 nm and the presence of surfactant does not have significant effect on maximum wavelength. Therefore, all the absorbance measurements were performed at this wavelength. Carmine is extracted into non-ionic surfactant, Triton X-100. The influences of main parameters in CPE method including pH of the medium, surfactant and salt concentrations, incubation time and temperature were optimized in order to obtain the highest sensitivity and recovery.