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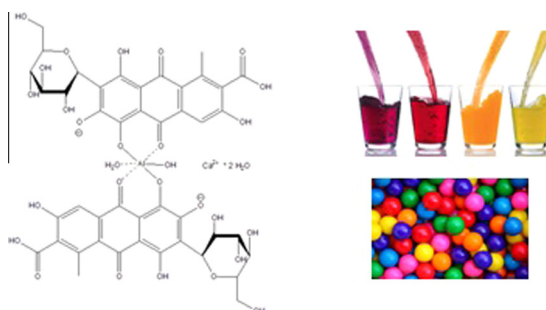
A simple method for determination of carmine in food samples based on cloud point extraction and spectrophotometric detection

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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 $\mu\text{g mL}^{-1}$ 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 $\mu\text{g mL}^{-1}$, respectively. Relative standard deviation (RSD) at low concentration level (0.05 $\mu\text{g mL}^{-1}$) 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.

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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 lip-stick [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 (Mettler, Germany) was used to maintain the temperature in CPE experiments.

Preparation of standard solutions

Stock solution of carmine ($1000 \text{ } \mu\text{g mL}^{-1}$) 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 ($0.45 \text{ } \mu\text{m}$). 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 $0.04\text{--}5 \text{ } \mu\text{g mL}^{-1}$) 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 $55 \text{ } ^\circ\text{C}$ 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.

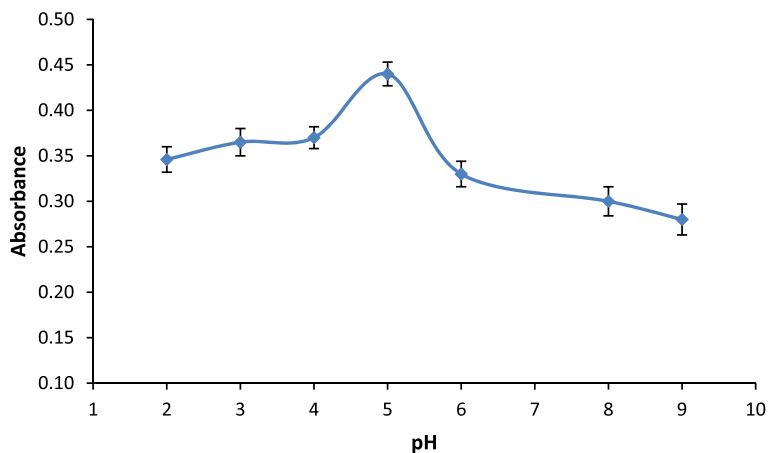


Fig. 1. The effect of pH on the extraction efficiency of carmine. Extraction conditions: Triton X-100 concentration, 8% w/v; incubation temperature, 55 °C; incubation time, 15 min.

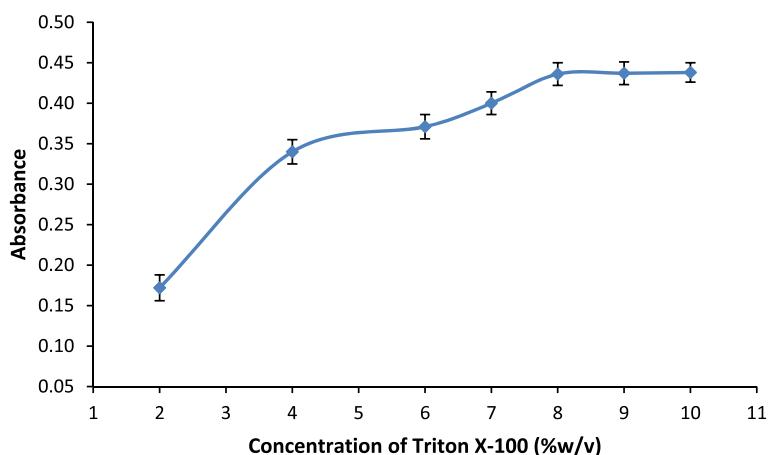


Fig. 2. The influence of Triton X-100 concentration on the extraction efficiency of carmine. Extraction conditions: incubation temperature, 55 °C; incubation time, 15 min; sample pH, 5.

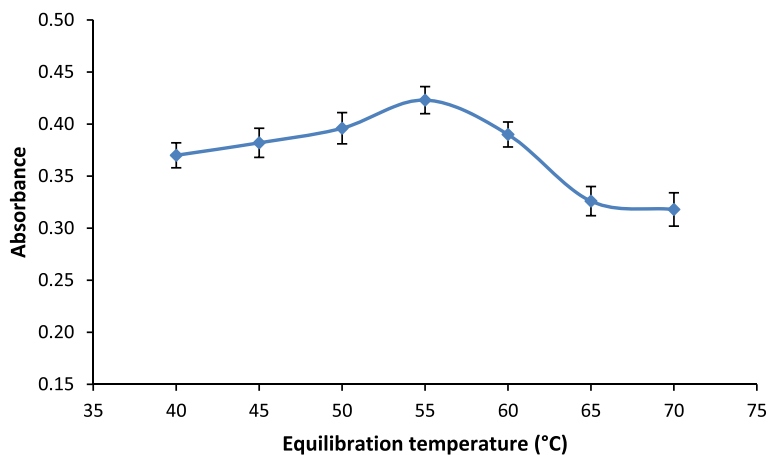


Fig. 3. The effect of temperature on the extraction efficiency of carmine. Extraction conditions: Triton X-100 concentration, 8% w/v; incubation time, 15 min; sample pH, 5.

Effect of pH

The pH is evaluated as a critical factor to increase the partition coefficient of the analyte between aqueous and surfactant-rich phases which lead to increase the extraction efficiency. Therefore

the effect of pH on extraction efficiency of carmine was investigated in the pH range of 2–8. The absorbance of surfactant-rich phase containing carmine was measured at 513 nm. As the results shown in Fig. 1, increase of pH up to 5 lead to an increase in absorbance and then decreased. Decrease in extraction efficiency at

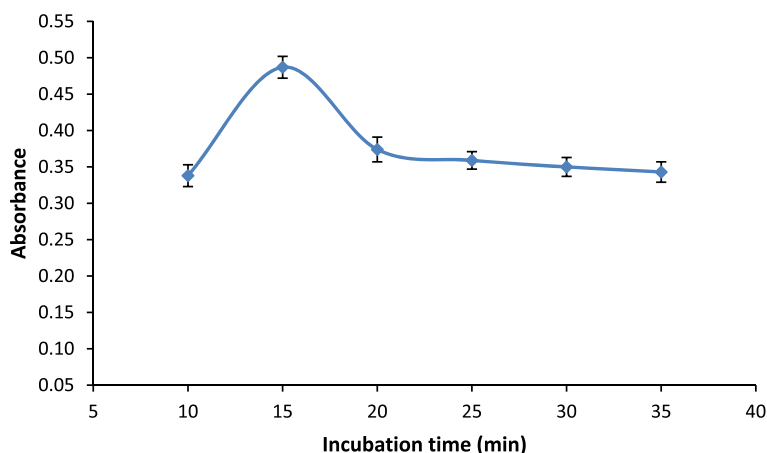


Fig. 4. The effect of incubation time on the extraction efficiency of carmine. Extraction conditions: Triton X-100 concentration, 8% w/v; incubation temperature, 55 °C; sample pH, 5.

Table 1

Tolerance limits of interfering ions.

Foreign ions	Tolerance limits ($\mu\text{g mL}^{-1}$)
Na ⁺ , Cl ⁻	5000
CH ₃ COO ⁻	2000
NH ₄ ⁺	700
K ⁺ , CO ₃ ²⁻ , SO ₄ ²⁻ , H ₂ PO ₄ , I ⁻ , Cr ³⁺ , Ni ²⁺ , Co ²⁺ , Al ³⁺ , Cd ²⁺ , Pb ²⁺ , Mg ²⁺	600
Hg ²⁺	500
Mn ²⁺ , Ag ⁺ , Zn ²⁺	200
Cu ²⁺	50

higher pHs (pH > 5) can be attributed to dissociation of carboxyl group of carmine to carboxylate ion. Therefore, pH 5 was selected as the optimum pH.

Effect of Triton X-100 concentration

Optimization of this factor was carried out in order to use a minimum surfactant concentration with maximum extraction efficiency. The effect of Triton X-100 concentration on the absorbance of carmine dye was studied in the range of 2–10% w/v. The results in Fig. 2 indicate the absorbance of extracted carmine increases up to 8% w/v and then remains constant. In order to obtain the highest pre-concentration 8% w/v was used as the appropriate concentration in this work.

Effect of equilibrium temperature and incubation time

Equilibration temperature and incubation time are two important parameters in cloud point extraction. The effect of

Table 2

Results of accuracy test for carmine in various food matrix.

Sample	Added concentration ($\mu\text{g mL}^{-1}$)	Founded concentration ($\mu\text{g mL}^{-1} \pm \text{SD}^a$)	Recovery (%) (n = 5)
Smarties	0.05	0.05 \pm 0.001	100.0
	1.00	1.04 \pm 0.001	104.0
Peach juice	0.05	0.053 \pm 0.002	106.0
	1.00	1.00 \pm 0.003	100.0
Pomegranate juice	0.05	0.052 \pm 0.002	104.0
	1.00	1.00 \pm 0.002	100.0
Lemon soft drink	0.05	0.050 \pm 0.002	100.0
	1.00	1.00 \pm 0.002	100.0
Grape soft drink	0.05	0.050 \pm 0.003	100.0
	1.00	0.94 \pm 0.003	94.0

^a Standard deviation.

equilibration temperature on the extraction efficiency of carmine was optimized in the range of 40–70 °C. The results in Fig. 3 illustrated the extraction efficiency was increased with an increase in temperature from 40 to 55 °C and then decreased. Hence, 55 °C was selected as the optimum temperature in this study.

In order to obtain acceptable extraction efficiency the incubation time of sample solution was investigated in the range of 10–35 min. Fig. 4 shows an incubation time of 15 min is the best time for maximum extraction.

Effect of salt concentration

In CPE technique addition of salt to sample solution helps to phase separation and increased the mass transfer of analyte from aqueous phase to surfactant-rich phase. Also, presence of small

Table 3

Comparison of analytical parameters of the proposed CPE method with some of the methods reported in literature.

Sample preparation	Matrix	Detection	LOD ($\mu\text{g mL}^{-1}$)	LOQ ($\mu\text{g mL}^{-1}$)	LR ^a ($\mu\text{g mL}^{-1}$)	RSD (%)	Recovery (%)	Refs.
Solvent extraction	Foodstuffs	HPLC ^b	0.4	1.0	1.0–100.0	6.8	94.1	[13]
Direct	Ice cream and soft drinks	SV ^c	0.002	0.05	0.05–0.14	2.2	97.2	[11]
Direct	Candy and milk	DPP ^d	0.18	0.61	1.1–100.5	7.0	95.0	[10]
Cloud point extraction	Foodstuffs	Spectrophotometry	0.012	0.04	0.04–5.0	4.8	100.0	This work

^a Linear range.

^b High-performance liquid chromatography.

^c Stripping voltammetry.

^d Differential pulse polarography.

amounts of inorganic salts leads to decrease in the cloud point temperature. Therefore, it is essential to consider the secondary effects of the electrolyte, i.e. salting-out. Based on these reasons, different salts such as NaCl, Na₂SO₄ and Na₂CO₃ were chosen and their effects on the extraction process were investigated. The results of experiments were revealed that all the salts affected on the process by decreasing the coacervate phase volume but Na₂CO₃ also increased the absorbance of the extracted dye. Thus sodium carbonate chosen as the suitable salt and different concentrations for this salt were tested. The results shown the absorbance was increased up to Na₂CO₃ concentration of 15% w/v and then leveled off. Consequently, 15% w/v of Na₂CO₃ was chosen as the optimum salt concentration.

Analytical performance

Under the optimized conditions, the analytical parameters of the proposed method for carmine determination were calculated. Calibration curve was obtained in the range of 0.04–5.0 µg mL⁻¹. Equation and regression coefficient are $A = 0.245C + 0.06$ and 0.9995, respectively. The limit of detection (LOD) based on $S/N = 3$ was 0.012 µg mL⁻¹ and the relative standard deviation (RSD) in low concentration level (0.05 µg mL⁻¹) of carmine was 4.8% ($n = 7$). The method was applied to the determination of carmine in several food samples such as smarties and soft drinks.

Interferences

In this work, potential interfering ions were examined under the optimized conditions at concentration level of 1.6 µg mL⁻¹ for carmine. Variation over ±5% in the analytical signal that resulted from foreign ions was taken as interference. The results are shown in Table 1. As it is observed from these results most of the ions tested do not have significant effect on the carmine signal.

Applications

The proposed procedure has been applied to determine of carmine in different food matrixes. Since this additive is not used in the food industry of Iran, spike method was used to demonstrate the practical application of the proposed method. The results are given in Table 2. According to these results from Table 2, the spiked concentration of carmine can be quantitatively recovered from the food samples by the proposed procedure. These results demonstrate the applicability of the proposed method for measurement of carmine in smarties and several soft drinks.

The analytical parameters of the proposed method were compared with several reported methods in the literatures (Table 3). The results show the LOD, LOQ and recovery values were improved by using the proposed CPE method. In the other hand, analysis time for this method was shorter than other methods especially chromatographic methods. In this method, no organic solvent was used. The proposed method can be successfully used to extract and quantify carmine in food samples.

Conclusion

In this work, a simple, cost effective and green method was developed for the determination of carmine in food samples by using CPE prior to its spectrophotometric detection. The effects of main parameters such as solution pH, surfactant and salt concentrations, incubation time and temperature were investigated and optimized. The results of this study show the potential of the proposed method for the determination of carmine in food samples. To the best our knowledge, this is the first report which uses

the cloud point extraction for determination of carmine in real samples. The method is simple, selective and sensitive for determination of carmine in food samples and does not require sophisticated instruments such as high-performance liquid chromatography or stripping voltammetry. The results were shown the proposed method can be applied satisfactory to determine the carmine in food samples.

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