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Simultaneous determination of Tropaeolin O and brilliant blue in food samples after cloud point extraction

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Abstract

In this study, a simple and low-cost method was developed for extraction and pre-concentration of brilliant blue(BB) and Tropaeolin O(TO) in food samples using cloud point extraction (CPE) prior to spectrophotometric determination. The effects of main factors such as pH, surfactant and salt concentrations, incubation time and temperature on the cloud point extraction of both dyes were investigated and optimized. Linear range of calibration curves were obtained in the range of 50–4000 ng mL^{-1} for BB and 50–5000 ng mL^{-1} for TO under the optimum conditions. The limit of detection values for BB and TO was 10 and 20ng mL^{-1}, respectively. The relative standard deviation (RSD) values of both dyes for repeated measurements (n=6) were less than 2.2 %. The obtained results demonstrate that the proposed method can be applied satisfactorily to determine these dyes in different food samples.

Keywords: Brilliant blue; Tropaeolin O; Triton X-100; cloud point extraction; spectrophotometric; food samples.

Introduction

Food additive is a general term for compounds which are used in order to sustain or improve the appearance of food or protection of edible products from microbial spoilage [1,2]. Synthetic dyes are added to food additive compounds and have adverse effects on human health. In general, the dyes are used to enhance the attractiveness of the appearance of food products [3,4]. Durability and brightness are two main advantages of synthetic dyes in comparison with natural dyes. But using synthetic dyes can have toxic effects on humans. Thus, in order to control and monitor concentration levels of synthetic dyes in food products, continuous measurement of the amount of these additives is essential [5,6]. BB and TO are used as food dyes in many different products including juices, ice cream, yogurt, jelly and candy [7]. These dyes are the synthetic food additive authorized to be used in very countries. The acceptable

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daily intake (ADI) values of BB and TO based on body weight are 10 and 2.5 mg/kg, respectively [8]. Many methods such as capillary electrophoresis (CE) [9], differential pulse polarography (DPP) [10], high-performance ion chromatography (HPIC) [11], high-performance liquid chromatography (HPLC) [12,13],mass spectrometry (MS) [14], spectrophotometry [15] and spectrofluorimetry [16] were suggested for detrmination of various synthetic dyes in food products. Some of these methods, e.g. polarography and chromatography techniques, due to use of organic solvents in chromatography and mercury in polarography, cannot be classified as environmentally friendly methods. In the other hand, HPLC and CE techniques are interpreted as more impressive alternative methods. These methods are costly, time-consuming and generate waste with a high amount of organic solvents. In spite of high sensitivity of electroanalytical methods, the selectivity of these methods is low. The disadvantages of stripping voltammetry (SV) are including longer analysis time and existence of interferences than spectrophtometric method which can lead to restrictions in real samples analysis.

Cloud point extraction (CPE) is an alternative solvent extraction techinque used to extract different analytes from various matrixes. Compared with traditional solvent extraction methods, CPE uses surfactants as extracting solvent which are non-taxic and compitable with different analytical instruments[17]. CPE method has been widely applied for extration, pre-concentration and clean-up of target analytes in food and drug samples [18-22].

The purpose of this work was to develop a simple and sensitive CPE method for determination of BB and TO in food samples using spectrophotometric detection. Extraction, clean-up and pre-concentration of BB and TO from aqueous samples were performed simultaneously using Triton X-100 as extracting solvent. The effects of main factors on the extraction yield of BB and TO were optimized. Figures of merits 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 for sample preparation. BB, TO and Triton X-100 were purchased from Merck Chemicals Company (Darmstadt, Germany). A solution of nonionic Triton X-100 surfactant (40% w/v) 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. Food samples were purchased from local supermarkets in Khorramabad (Lorestan, Iran).

Apparatus

Absorption 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 keep the temperature in desired values.

Standard and sample preparation

Stock solutions of 1000 µg mL^{-1} of BB and TO were prepared by dissolving 0.1 g of each 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 daily.

Appropriate amounts of food samples were dissolved in deionized water. After dissolve in water, sample solutions were filtered using membrane filter (0.45 µm). 1 mL of the filtrated sample solutions were diluted to 10 mL in a volumetric flask using acetate buffer (pH 5) as the diluent. Finally, 8 mL of these solutions were treated under the optimized CPE procedure for the determination of BB and TO.

Cloud point extraction procedure

8 mL of the acetate buffer solution (pH 5) consisting of BB and TO was transferred to a 15 mL centrifuge tube. Then 1.5 mL of 40% (w/v) of Triton X-100 and 1.5 g of NaCl salt were added to this solution. After the dissolving of salt, up to the 10 mL volume the solution, buffer is added. The mixture was then placed in a thermostat water bath at 50 °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 part of the tube. Hence, the aqueous phase was attentively removed using a syringe with a long needle that passed through the surfactant-rich phase. The surfactant-rich phase was diluted with water. The absorbance of the solution was measured at 630 and 420 nm for BB and TO, respectively. A blank solution (without BB and TO) was also prepared according to the same procedure and measured in parallel to the samples.

Results and discussion

Figure 1 showed the structures of TO and BB. The absorption spectrum of BB and TO indicate that maximum absorbance occurs at 630 and 420 nm, respectively. Also the attendance of surfactant does not have considerable effect on the maximum wavelengths of these dyes. Accordingly, all the absorbance measurements were accomplished at these two wavelengths. The effects of main factors in CPE method such as pH of the medium, surfactant and salt concentration, incubation time and temperature were optimized in order to acquire the maximum sensitivity and recovery.

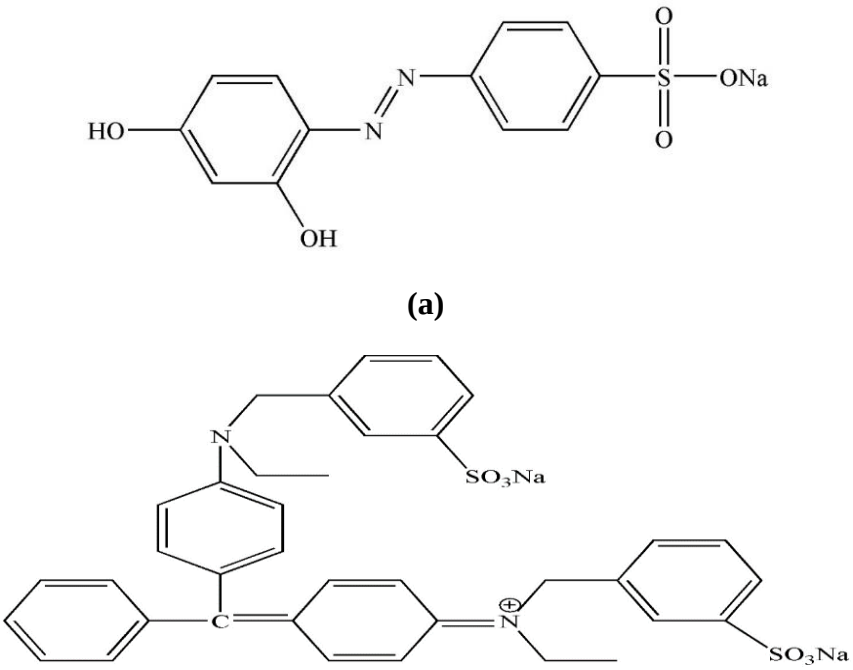

Figure 1. The structures of TO (a) and BB (b)

Effect of pH

The pH is an influential factor in CPE method because it can affect the partition coefficient of the analytes between aqueous and surfactant-rich phases. In CPE method with nonionic surfactants as extracting solvent, the neutral analytes are more extracted into the surfactant phase. Therefore, the effect of pH on extraction efficiency of BB and TO was examined in the pH range of 2–8. The absorbance of surfactant-rich phase containing both dyes was recorded at 630 and 420 nm for BB and TO, respectively. Since both dyes have acidic and basic functional groups, illustration of their behaviors in various pHs can be more difficult. The pKa values of BB are 5.63 and 6.58, which means that the pH can influence on its extraction efficiency. On the other hand, the pKa value for TO is 11.0. However, the decrease in extraction efficiencies at higher pHs (pH>5) is in agreement with pKa values. As it was observed from the results in Figure2, absorbance value of surfactant phase for both dyes at pH 5 is higher than other pHs. Consequently, pH 5 was chosen as the optimum pH for subsequent experiments.

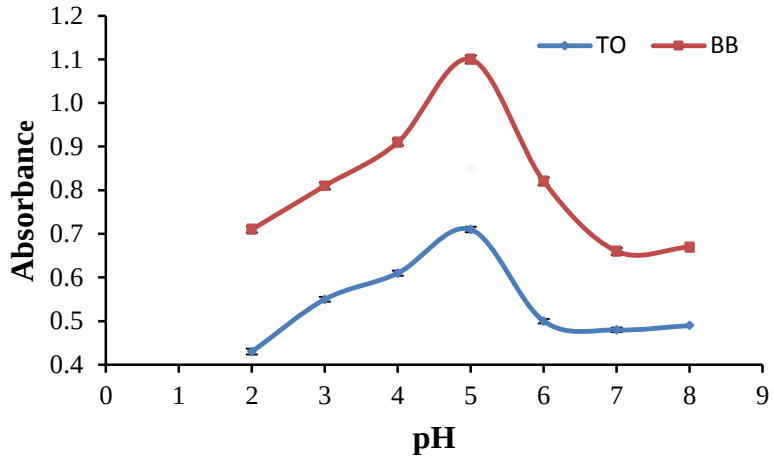

Figure 2. The effect of pH on the extraction efficiency of BB and TO.Extraction conditions: Triton X-100 concentration, 8 %w/v; incubation temperature, 50 °C; incubation time, 15 min; salt concentration, 20 %w/v.

Effect of Triton X-100 concentration

Surfactant concentration can affect the extraction efficiency of analytes and their enrichment factors; hence the concentration of surfactant should be optimized. The effect of Triton X-100 concentration on the absorbance of BB and TO was investigated in the range of 2–12% w/v. The results in Figure3 illustrate that the absorbance of bothextracted dyes increase up to 8% w/v and then reduce. As observed from Figure 3, increasing Triton X-100 concentration, due to increase of extration capacity, leads to increase of extraction efficiencies. Deacreasing of extraction efficiencies after 8 %w/v attributed to increase of surfactant phase which leads to dilute of analytes. In order to obtain the most pre-concentration,8% w/v was chosen as the suitable surfactant concentration in this study.

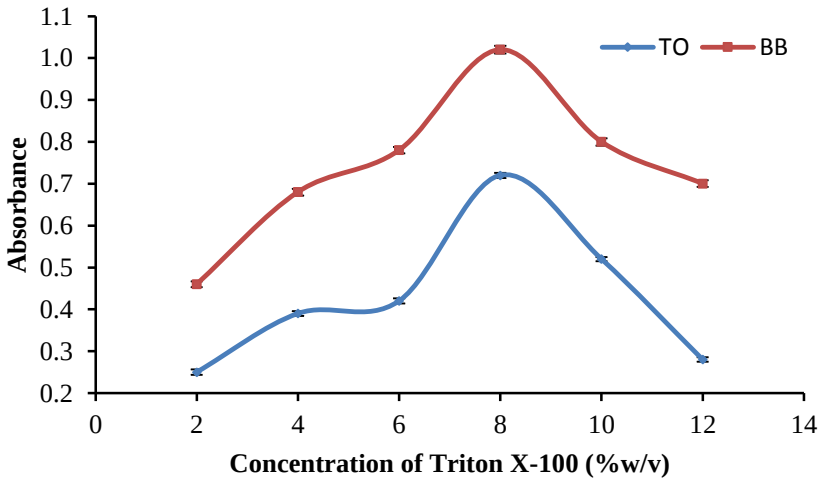

Figure 3. The influence of Triton X-100 concentration on the extraction efficiency of BB and TO. Extraction conditions: incubation temperature, 50 °C; incubation time, 15 min; sample pH, 5; salt concentration, 20 %w/v

Effect of equilibrium temperature and incubation time

Equilibrium temperature and incubation time are two momentous factors in CPE technique. The effect of equilibrium temperature on the extraction efficiency of BB and TO was optimized in the range of 50–75 °C. As it was indicated from the results in Figure4, absorbance value of extraction phase for both dyes at temperature50 °C is higher than other temperatures. Therefore, 50 °C was chosen as the optimum temperature.

In order to acquire acceptable extraction efficiency, the incubation time of sample solution was studied in the range of 5–30 min. Figure5 indicates that incubation time of 15 min is the convenient time for maximum analytes extraction. As observed from Figure 5, increasing incubation time leads to decrease in extraction efficiencies of two analytes. Due to high temperature, this phenomenon can be attributed to degradation of micelle structure and analytes. Therfore, 15 min was chosen as the optimum incubatrion time for further experiments.

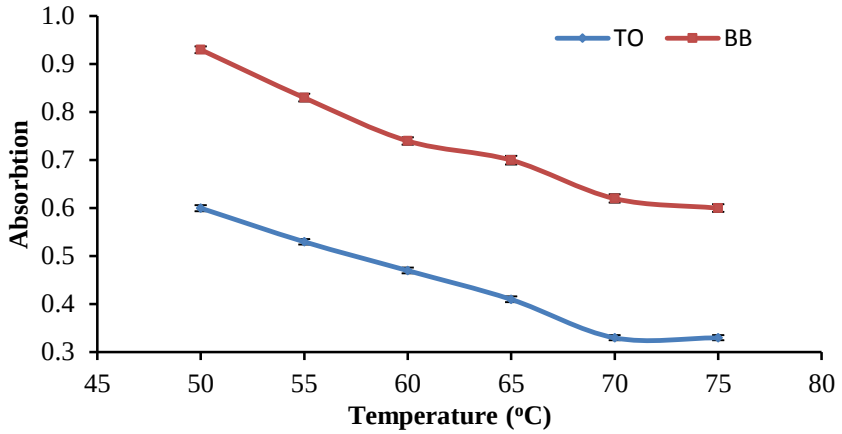

Figure 4. The effect of equilibrium temperature on the extraction efficiency of BB and TO. Extraction conditions: Triton X-100 concentration, 8 %w/v; incubation time, 15 min; sample pH, 5; salt concentration, 20 %w/v.

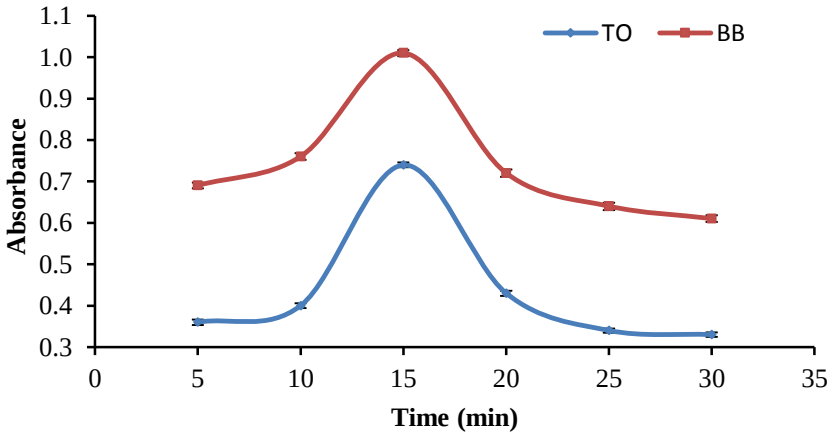

Figure 5. The effect of incubation time on the extraction efficiency of BB and TO. Extraction conditions: Triton X-100 concentration, 8 %w/v; incubation temperature, 50 °C; sample pH, 5; salt concentration, 20 %w/v.

Effect of salt concentration

Addition of salt to the mixture of aqueous sample and surfactant has several advantages including change of density of the aqueous phase and facilitate the phase separation, decrease in the cloud point temperature and increase in analyte transfer from aqueous phase to surfactant phase due to salting-out phenomenon. Therefore, it is essential to investigate the effect of salt type and its concentration on extraction process. For these reasons, various salts such as NaCl, Na_2SO_4 and Na_2CO_3 were selected and their influence on the extraction process was studied. The results of tests indicated that NaCl has the greatest effect on the extraction efficiency of analytes. Thus, NaCl chosen as the suitable salt and different concentrations for this salt were tested. The results (Figure 6) show that dyes absorbance signals were raised up to20% w/v of NaCl and then leveled off. According to the results of Figure 6, 20% w/v of NaCl was selected as the optimum salt concentration.

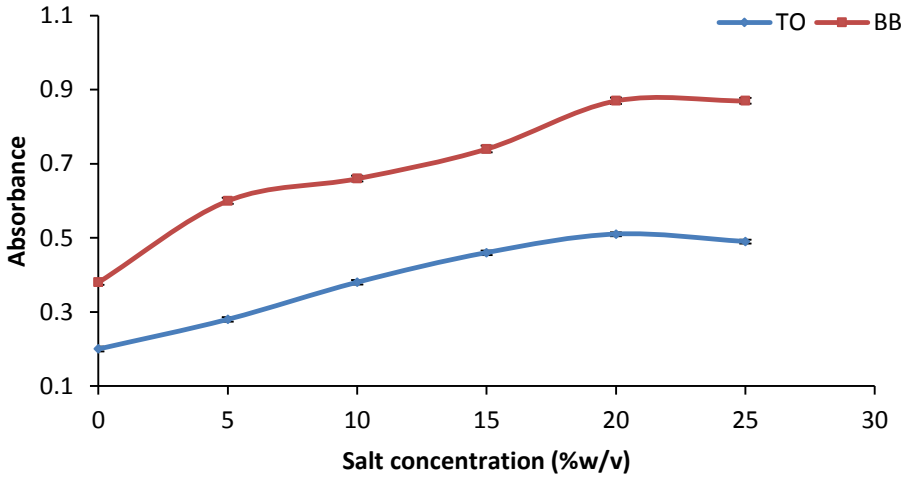

Figure 6. The influence of NaCl concentration on the extraction efficiency of BB and TO. Extraction conditions: Triton X-100 concentration, 8 %w/v; incubation temperature, 50 °C; incubation time, 15 min; sample pH, 5.

Analytical performance

Under the optimized conditions, the correlation between the analytical signal and the dyes concentration was obtained by calibration curves. Linear calibration graphs were obtained in the range of 50–4000 ng mL^{-1} for BB and 50–5000 ng mL^{-1} for TO under the optimum conditions. Equations were A= 0.278C+0.039and A= 0.144C+0.014for BB and TO, respectively. The regression coefficients were 0.9992 and 0.9990 for BB and TO, respectively. Detection limits based on theoretical calculations ($DL=3S_b/m$) were 10 and 20 ng mL^{-1} for BB and TO, respectively. The relative standard deviation (RSD) values at the lowest concentration of linear ranges were 2.17 and 1.45% (n = 6), for BB and TO, respectively.

Interferences

In this paper, the effect of foreign ions on the determination of BB and TO was studied. The tolerance ratio was defined as the ratio of the concentration of interfering ion over the concentration of analyte that caused a relative error of ±5%.The results are shown in Table 1. Standard solutions containing 660 ng mL^{-1} of both dyes and different

concentration of other ions or compounds were prepared and subjected to the CPE procedure. As it is observed from these results, most of the tested ions do not have significant effect on the both deys signals.

Table 1. The effect of several ions on the signals of BB and TO

Foreign ions	Tolerance limit (μg mL^{-1})
Na^+, Cl^-,CH_3COO^-,NH_4^+	5000
K^+ CO_3^{2-}, SO_4^{2-}	1000
Cr^{3+}, Ni^{2+}, Co^{2+}, Al^{3+}, Pb^{2+} Cu^{2+}, Mn^{2+}, Zn^{2+}, Fe^{3+}, Mg^{2+}, I^-, $H_2PO_4^-$	400
Cd^{2+}	200
Ag^+	100

Applications

The proposed method has been applied to determine BB and TO in different food matrixes. The results are given in Table 2. According to these results from Table 3, the spiked concentration of BB and TO can be quantitatively recovered from the food samples by the proposed method. These results demonstrate ability of the CPE procedure to determine both dyes in food samples. The analytical factors of the proposed method were compared with various reported methods in the literature (Table 4). The results show that the LOD, RSD% and recovery values were enhanced by using the proposed CPE method. On the other hand, analysis time for this method was shorter than other methods particularly chromatographic methods. In this method, no organic solvent was used. The recommended method can be successfully applied to extract and quantify BB and TO in food samples.

Table 2. Results of analyzed food samples for BB and TO

Sample (n=5)	TO (ng mL^{-1}) ±SD[a]	BB (ng mL^{-1}) ±SD[a]
Soft beverage	-	-
Soft beer	-	-
Smarties	-	470±0.03
Jelly	-	400±0.09

[a]Standard deviation

Table 3. Results of accuracy test using spike samples with different concentrations of analytes

Sample (n=5)	Tropaeolin O			Brilliant blue		
	Added (ng mL^{-1})	Founded (ng mL^{-1})±SD	Recovery (%)	Added (ng mL^{-1})	Founded (ng mL^{-1})±SD[a]	Recovery (%)
Soft beverage	-	-	-	-	-	-
	660	720±0.02	109	660	660±0.03	100
	1000	1030±0.01	103	1000	996±0.04	99.6
Smarties	-	-	-	-	470±0.03	-
	660	720±0.03	109	660	1110.2±0.02	97
	1000	1000±0.01	100	1000	1460±0.01	99

Table 4. Comparison of analytical parameters of the proposed CPE method with some of the methods reported in literature

Sample preparation	Matrix	Analyte	Detection	Extraction solvent or adsorbent	Analysis Time (min)	LOD (ng mL^{-1})	LR[a] (ng mL^{-1})	RSD (%)	Ref.
Direct	Foodstuffs	BB	SV[c]	-	1	1.53	8.0-80.0	2.20	[24]
SPE[b]	Foodstuffs	BB	Spectrophotometry	β-Cyclodextrin Polymer	40	16.0	50.0-1200	3.40	[25]
CPE	Candy and smarties	BB	Spectrophotometry	Triton X-100	35	16.0	50.0-3500	3.30	[17]
Direct	Soft drinks	BB	Ion-pair HPLC[d]	C_{18}	20	3.0	7.5-300	<5	[23]
CPE	Drinks and smarties	BB	Spectrophotometry	Triton X-100	20	10	50-4000	2.17	This work
CPE	Drinks and smarties	TO	Spectrophotometry	Triton X-100	20	20	50-5000	1.45	This work

[a]Linear range, [b]Solid-phase extraction, [c]Stripping voltammetry, [d]High-performance liquid chromatography.

Conclusion

A simultaneous cloud point extraction method has been introduced for the determination of BB and TO in food samples. Figure of merits the proposed method are comparable to the other previously reported methods for monitoring of both deys in mixtures or alone. Moreover, the proposed method has several advantages including simplicity, environmentally friendly, low-cost, appropriate accuracy and precision. In this method, no organic solvent was used in extraction process. Also, detection was achieved using spectrophotometer and sophisticated instruments such as chromatography or electroanalytical systems were avoided. The obtained results demonstrate that the proposed method can be applied satisfactorily to determine BB and TO in different food samples.

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