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Short communication

Simultaneous cloud point extraction and spectrophotometric determination of carmoisine and brilliant blue FCF in food samples

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ABSTRACT

A novel simultaneous cloud point extraction method for the determination of carmoisine and brilliant blue FCF by spectrophotometry has been developed. The method is based on the cloud point extraction of carmoisine and brilliant blue FCF from aqueous solution using Triton X-100, diluting the extracted surfactant rich phase with water and measuring the absorbance at 522 and 640 nm for carmoisine and brilliant blue FCF, respectively. The effects of different parameters such as pH, concentration of surfactant and temperature on the cloud point extraction of both dyes were investigated and optimum conditions were established. Linear calibration curves were obtained in the range of 0.02–3.50 μg mL^{-1} for carmoisine and 0.05–3.50 μg mL^{-1} for brilliant blue FCF under optimum conditions. Detection limit based on three times the standard deviation of the blank ($3S_b$) was 0.017 and 0.016 μg mL^{-1} ($n = 10$) for carmoisine and brilliant blue FCF, respectively. The relative standard deviation (RSD) for 0.1 μg mL^{-1} was 4.14 and 3.30% ($n = 10$), for carmoisine and brilliant blue FCF, respectively. The method was applied to the simultaneous determination of the dyes in different food samples.

1. Introduction

The development of chemical and instrumental methods for the separation, identification and quantitative analysis of synthetic food colors has become extremely important for the food and beverages industry, academic and governmental institutions to assess the quality and safety of food products [1]. Although the amounts of synthetic colors that are added to food and drinks are strictly controlled, they may exceed the authorized levels. Thus monitoring of the levels of dyes in high consumption products such as beverages becomes of great importance. The number of works published during the last years demonstrates the importance of this problem and the need for developing fast, accurate and selective techniques for synthetic dyes analysis [2]. Various techniques have been introduced for the determination of synthetic dyes in food samples. These include capillary electrophoresis [3] differential pulse polarography [2], high-performance ion chromatography [4], HPLC [5,6] electrospray mass spectrometry [7], spectrophotometry [8] and spectrofluorimetry [9].

Separation methods based on cloud point extraction (CPE) are practical application of surfactants in analytical chemistry and have become as an alternative to solvent extraction. Compared with conventional solvent extraction, CPE uses water and avoids the use of large amount of expensive, toxic, and flammable organic solvents [10]. When the aqueous solutions of nonionic surfactant are heated at a temperature higher than a certain temperature known as cloud point, the solution separates into a surfactant rich phase of small volume, composed almost totally of the surfactant, and a diluted aqueous phase [11]. The phase rich in surfactant micelles is called micellar-rich phase or coacervate phase. This phenomenon has been said to be due to an increase in micellar size and the dehydration of the hydrated outer micellar layers with the increase in temperature. Species that can interact with micellar systems either as such, or after derivatization can readily become concentrated in a small volume of the surfactant-rich phase upon heating [12]. We have recently reported a developed cloud point extraction methodology for the spectrophotometric determination of malachite green in different fish farming water samples [13] and rhodamine B [14].

This paper describes a novel, simple and sensitive simultaneous cloud point extraction method for the determination of carmoisine and brilliant blue FCF by spectrophotometry. The method is based on simultaneous cloud point extraction of carmoisine and brilliant blue FCF from aqueous solution using Triton X-100, diluting the extracted surfactant rich phase with water and measuring the absorbance at 522 and 640 nm for carmoisine and brilliant blue FCF, respectively.

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2. Experimental

2.1. Reagents and materials

All chemicals used in this work were of analytical reagent grade and distilled water was used throughout. Stock solutions of 1000 μg mL^{-1} of carmoisine (BDH, England) or brilliant blue FCF (SERVA, Germany) were prepared by dissolving 0.5 g of each dye in water and diluting to 500 mL in a volumetric flask. Working standard solutions were obtained by appropriate dilution of the stock solution and were stable during the day. A solution of nonionic surfactant (0.5 mol L^{-1}) Triton X-100 was prepared by dissolving accurately 161.7175 g of Triton X-100 (Aldrich, USA) in water and diluting to 500 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. A 1.0 mol L^{-1} of sodium chloride was prepared by dissolving 5.85 g of NaCl (Merck, Germany) in water and diluting to 100 mL in a volumetric flask.

2.2. Apparatus

The absorbance measurements were performed by a GBC Model Cintra 101 (Australia) UV–visible spectrophotometer with 1-cm glass cell. A Metrohm digital pH-meter model 632 (Switzerland) with a combined glass electrode was used for pH adjustments. A Colora thermostat bath (England) was applied to maintain the desired temperature within ±1.0 °C.

2.3. General procedure

Aliquots of carmoisine and brilliant blue FCF (10 μg mL^{-1}), 5 mL of Triton X-100 solution (0.5 mol L^{-1}), 2 mL of NaCl electrolyte (1.0 mol L^{-1}) and 4.5 mL of acetate buffer pH 5.0 were added to a 50 mL volumetric flask and diluted to the mark with water and transferred to a 50 mL glass tube. The glass tube was then incubated in the thermostat bath at 76 °C for 30 min. After the two phase separation was occurred, the test tube was immersed in an ice bath for increasing the viscosity of the surfactant rich phase, which coacervate to the bottom of the vial. The removal of the water-rich phase was performed by a syringe. The surfactant-rich phase was delivered to a 5 mL volumetric flask and diluted to the mark with water. The absorbance of the solution was measured at 522 and 640 nm for carmoisine and brilliant blue FCF, respectively. A blank solution was also run using the entire mentioned components except carmoisine and brilliant blue FCF.

2.4. Sample pre-treatment procedure

Appropriate amounts of raspberry jelly (Tabriz, Iran), fruity candy (Tabriz, Iran), smarties (m&m, USA), smarties (CheePuf, Iran) and ball shaped candy (CheePuf, Iran) samples were dissolved in water, filtered if necessary and diluted to 25 mL in a volumetric flask. An aliquot of the above solutions was treated under the general procedure for cloud point extraction and subsequent determination of carmoisine and brilliant blue FCF.

3. Results and discussion

Carmoisine and brilliant blue FCF (Fig. 1) are present in a variety of food samples to impart a purple color to the samples. The absorption spectra recorded for carmoisine and brilliant blue FCF after cloud point extraction showed that their maximum absorption occur at 522 and 640 nm, respectively and they do not interfere in the determination of each other. Fig. 2 shows the spectra for carmoisine and brilliant blue FCF alone and in the mixture after cloud point extraction.

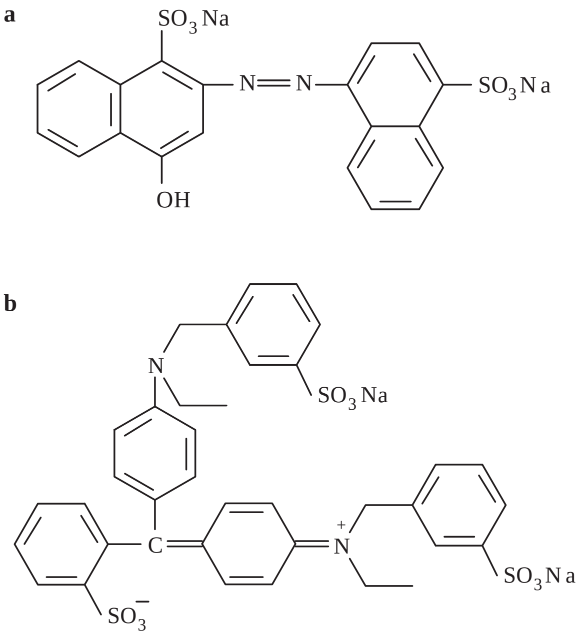

Fig. 1. The structure of (a) carmoisine (b) brilliant blue FCF.

3.1. Effect of pH on the extraction efficiency

The pH is evaluated as a critical parameter for regulating the partitioning of the analyte in the surfactant-rich phase which seems to control the extraction efficiency. Therefore the effect of pH on cloud point extraction of 1.0 μg mL^{-1} of both carmoisine and brilliant blue FCF was investigated. The absorbance of carmoisine and brilliant blue FCF was measured at 522 and 640 nm, respectively after performing cloud point extraction in the pH range of 2–8. The pH of the solution was adjusted to the desire value by the addition of hydrochloric acid and sodium hydroxide and using a pH meter. As the results shown in Fig. 3 indicate pH 5 is suitable for both dyes and was selected as optimum. Thus 4.5 mL of acetate buffer pH 5 was added to the solutions to maintain this pH in further experiments.

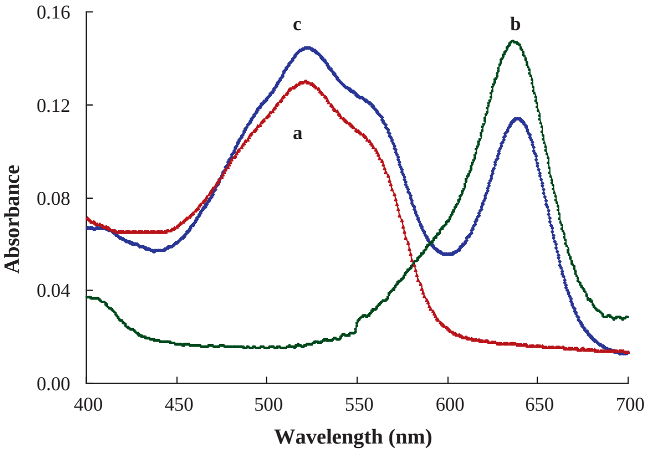

Fig. 2. The absorption spectrum of (a) 1.2 μg mL^{-1} of carmoisine, (b) 1.5 μg mL^{-1} of brilliant blue FCF, and (c) 1.4 μg mL^{-1} of carmoisine and 1.1 μg mL^{-1} of brilliant blue FCF in a mixture after simultaneous cloud point extraction.

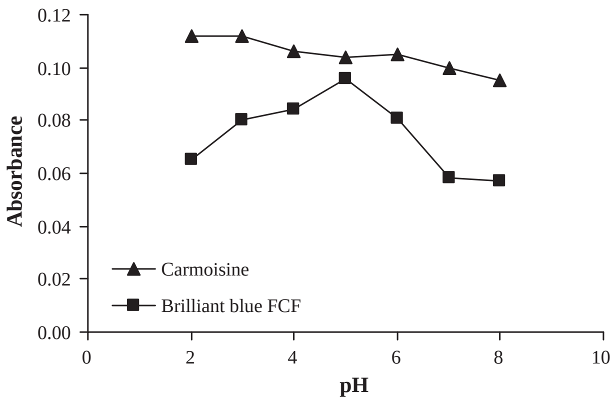

Fig. 3. The effect of pH on the absorbance of 1.0 μg mL^{-1} of carmoisine and brilliant blue FCF after cloud point extraction.

3.2. *Effect of Triton X-100 concentration on extraction efficiency*

Optimization of this parameter was carried out in order to accomplish a minimum desirable surfactant concentration with maximum extraction efficiency. The effect of Triton X-100 concentration on the absorbance of both dyes was studied in the range of 0.01–0.06 mol L^{-1}. The results shown in Fig. 4 indicate that the absorbance and hence the extraction efficiency of both dyes increase up to 0.05 mol L^{-1} and remain constant above that. In order to use less surfactant a Triton X-100 concentration of 0.05 mol L^{-1} was selected for further studies.

3.3. *Effect of salt concentration*

The electrolytes play an important role on the cloud point of nonionic surfactant systems. When small amounts of inorganic salts are added to the system, a decrease in the cloud point temperature is noted. This fact indicates that it is necessary to consider the secondary effect of the electrolytes, i.e. salting-out. Based on these discussion different electrolytes such as NaCl, KCl and $CaCl_2$ were chosen and their effect on the system was investigated. The results of this study revealed that all the salts affected the cloud point extraction by decreasing the coacervate phase volume but NaCl also increased the absorbance of the extracted dyes. Thus the effect of different concentration of NaCl was studied. It was observed that the absorbance was almost constant in the concentration range of 0.04–0.10 mol L^{-1} for carmoisine and 0.02–0.06 mol L^{-1} for brilliant blue FCF. In this way, NaCl concentration of 0.04 mol L^{-1} was used in all further experiments.

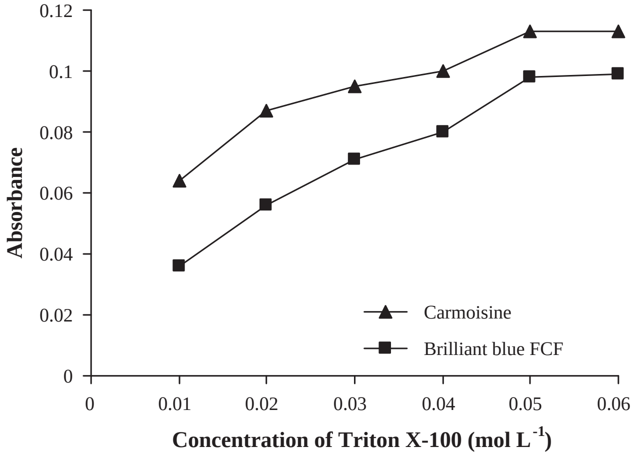

Fig. 4. The influence of Triton X-100 concentration on the absorbance of μg mL^{-1} of carmoisine and brilliant blue FCF after cloud point extraction.

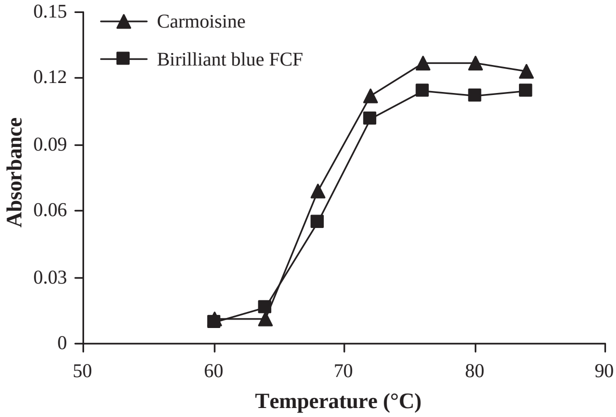

Fig. 5. The effect of temperature on the absorbance of 1.0 μg mL^{-1} of carmoisine and brilliant blue FCF after cloud point extraction.

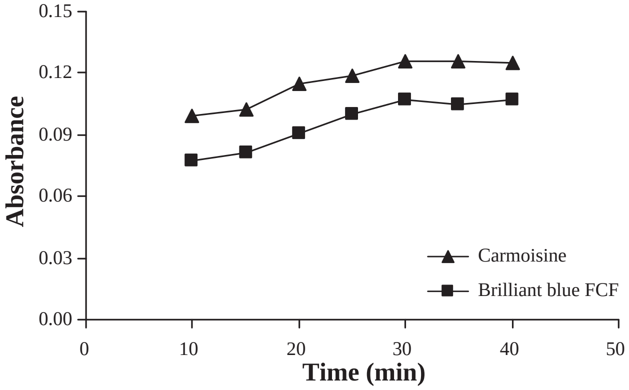

Fig. 6. The effect of time on the absorbance of 1.0 μg mL^{-1} of carmoisine and brilliant blue FCF after cloud point extraction.

3.4. *Effect of the equilibration temperature and incubation time*

In order to achieve efficient preconcentration and easy phase separation, the equilibration temperature and incubation time were optimized. The effect of equilibration temperature in the range of 60–84 °C was studied. The results presented in Fig. 5 indicated that when temperature is raised from 76 to 84 °C, the absorption scales are almost constant. Hence a temperature of 76 °C was adequate to achieve quantitative extraction in this system. In order to achieve satisfactory extraction the incubation time was prolonged in range of 10–40 min (Fig. 6). Accordingly, an incubation time of 30 min was chosen for use in next experiments.

Table 1
The effect of other species on the determination of 1.0 μg mL^{-1} of carmoisine and brilliant blue FCF.

Foreign ions	Tolerance limit (μg mL^{-1})
Ni^{2+}, Cd^{2+}, Ca^{2+}, Mg^{2+}, $NH_4{}^+$, F^-, $PO_4{}^{3-}$, K^+	1000
$NO_3{}^-$, $SO_4{}^{2-}$, $SO_3{}^{2-}$	900
Cu^{2+}, S^{2-}	800
Pb^{2+}, Al^{3+}, Mn^{2+}	400
$CO_3{}^{2-}$, Zn^{2+}, Hg^{2+}, oxalate, citrate, tartarate	100
Fe^{2+}, Co^{2+}	60
Fe^{3+}, Cr^{3+}, Br^-, Ni^{2+},	50
I^-,	5
Amaranth	2

Table 2
Determination of carmoisine and brilliant blue in food sample solutions and recovery tests.

Sample	Carmoisine			Brilliant blue		
	Added ($\mu g\,mL^{-1}$)	Found[a] ($\mu g\,mL^{-1}$)	Recovery (%)	Added ($\mu g\,mL^{-1}$)	Found[a] ($\mu g\,mL^{-1}$)	Recovery (%)
Raspberry jelly	–	3.55 ± 0.03	–	–	0.80 ± 0.02	
	1.5	5.09 ± 0.03	103	0.5	1.31 ± 0.02	102
	3.0	6.61 ± 0.03	102	1.0	1.78 ± 0.03	98
Fruity candy	–	3.04 ± 0.03	–	–	1.29 ± 0.02	
	0.5	3.52 ± 0.03	96	0.5	1.81 ± 0.03	104
	1.0	4.03 ± 0.03	99	1.0	2.27 ± 0.03	98
Smarties m&m	–	2.45 ± 0.02	–	–	0.94 ± 0.02	
	0.5	2.94 ± 0.03	98	0.5	1.46 ± 0.03	104
	1.0	3.41 ± 0.03	96	1.0	1.91 ± 0.03	97
Smarties CheePuf	–	1.82 ± 0.02	–	–	0.95 ± 0.02	
	0.5	2.31 ± 0.02	98	0.5	1.44 ± 0.03	98
	1.0	2.79 ± 0.03	97	1.0	1.94 ± 0.03	99
Ball shaped candy	–	2.57 ± 0.03	–	–	1.37 ± 0.02	
	0.5	3.05 ± 0.03	96	0.5	1.87 ± 0.02	100
	1.0	3.58 ± 0.03	101	1.0	2.35 ± 0.03	98

[a] $\bar{x} \pm ts\sqrt{n}$ at 95% confidence ($n = 5$).

Table 3
Amounts of carmoisine and brilliant blue present in food sample.

Sample	Carmoisine ($\mu g\,g^{-1}$)[a]	Brilliant blue ($\mu g\,g^{-1}$)[a]
Raspberry jelly	148 ± 5	33 ± 1
Fruity candy	176 ± 6	75 ± 1
Smarties m&m	637 ± 8	244 ± 3
Smarties Cheepuf	2271 ± 12	1194 ± 7
Ball shaped candy	1860 ± 10	990 ± 7

[a] $\bar{x} \pm ts\sqrt{n}$ at 95% confidence ($n = 5$).

3.5. Analytical performance of the method

Under the optimized conditions, the relationship between the analytical signal and the dyes concentration was obtained by utilizing different concentrations. Linear calibration curves were obtained in the range of 0.02–3.50 $\mu g\,mL^{-1}$ for carmoisine and 0.05–3.50 $\mu g\,mL^{-1}$ for brilliant blue FCF under optimum conditions. The equations for the lines are $A = 0.099C + 0.008$ and $A = 0.098C + 0.003$ for carmoisine and brilliant blue FCF, respectively. The regression coefficients ($n = 15$) for the lines are 0.9993 and 0.9996 for carmoisine and brilliant blue FCF, respectively. Detection limit based on three times the standard deviation of the blank ($3S_b$) was 0.017 and 0.016 $\mu g\,mL^{-1}$ ($n = 10$) for carmoisine and brilliant blue FCF, respectively. The relative standard deviation (RSD) for 0.1 $\mu g\,mL^{-1}$ was 4.14 and 3.30% ($n = 10$), for carmoisine and brilliant blue FCF, respectively.

3.6. Interference studies

In this study, potential interfering ions and dyes were examined for the simultaneous cloud point extraction of 1.0 $\mu g\,mL^{-1}$ of both carmoisine and brilliant blue FCF under the recommended conditions. 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 most of the ions tested do not have significant effect on the determination of both carmoisine and brilliant blue FCF.

4. Application

The proposed procedure has been applied to the determination of carmoisine and brilliant blue FCF in different food samples. The results are given in Tables 2 and 3. According to Table 2, the spiked concentration of carmoisine and brilliant blue FCF can be quantitatively recovered from the food samples by the proposed procedure. These results demonstrate the applicability of the procedure for simultaneous cloud point extraction of carmoisine and brilliant blue FCF in food samples such as jelly, smarties candy and fruity candy.

5. Conclusion

A simultaneous cloud point method has been developed for determination of two dyes in food samples. It was demonstrated that, the proposed procedure is comparable to or even better than most works that are available for monitoring of carmoisine and brilliant blue FCF in mixtures or alone [2,4,6]. In addition the micellar system is an environmental-friendly and simple preconcentration way for the determination of trace amount of carmoisine and brilliant blue FCF prior to spectrophotometric detection. This method gives reasonably low detection limit (ng mL^{-1} levels), wider linearity and also good standard deviations.

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References

[1] M.S. Garcia-Falcon, J. Simal-Gandara, Food Control 16 (2005) 293.
[2] S. Chanlon, L. Joly-Pottuz, M. Chatelut, O. Vittoria, J.L. Cretier, J. Food Compos. Anal. 18 (2005) 503.
[3] N. Dossi, R. Toniolo, A. Pizzariello, S. Susmel, F. Perennes, G. Bontempelli, J. Electroanal. Chem. 601 (2007) 1.
[4] Q.C. Chen, S.F. Mou, X.P. Hou, J.M. Riviello, Z.M. Ni, J. Chromatogr. A 827 (1998) 73.
[5] N. Yoshioka, K. Ichihashi, Talanta 74 (2008) 1408.
[6] S.P. Alves, D.M. Brum, E.C. Branco de Andrade, A.D. Pereira Netto, Food Chem. 107 (2008) 489.
[7] M. Ma, X. Luo, B. Chen, S. Su, S. Yao, J. Chromatogr. A 1103 (2006) 170.
[8] E.C. Vidotti, J.C. Cancino, C.C. Oliveira, M.D.C.E. Rollemberg, Anal. Sci. 21 (2005) 149.
[9] C.C. Wang, A.N. Masi, L. Fernandez, Talanta 75 (2008) 135.
[10] D. Wu, Q. Wu, R. Bian, Y. Wang, Sep. Sci. Technol. 42 (2007) 2693.
[11] M.K. Purkait, S. Das Gupta, S. De, J. Hazard. Mater. B137 (2006) 827.
[12] C.D. Stalikas, Trends Anal. Chem. 21 (2002) 343.
[13] N. Pourreza, Sh. Elhami, Anal. Chim. Acta 596 (2007) 62.
[14] N. Pourreza, S. Rastegarzadeh, A. Larki, Talanta 77 (2008) 733.