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Determination of Erythrosine in Food Samples by CPE-Scanometry as a New Method And Comparison with Spectrophotometric Results

A. Shokrollahi* and F. Zarghampour

Department of Chemistry, Yasouj University, Yasouj, 75918-74831, Iran

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In this study, the trace amounts of erythrosine, as a food dye is determined by cloud point extraction-scanometry (CPE-Scanometry) as a new, facile, available, fast, sensitive, and low cost method. The method is based on the CPE of analyte from aqueous solution, diluting the extracted surfactant-rich phase with ethanol, transfer to Plexiglas® cell and scanning the cells containing the analyte solution with a scanner and measuring the RGB parameters with software written in visual basic (VB6) media. The parameters such as pH of the system, the concentration of the surfactant, equilibration temperature and time were optimized. In addition, the effects of some foreign species were investigated. The linear range for the proposed method and CPE-Spectrophotometry are 0.067-5.330 μg ml^{-1} and 0.030-3.000 μg ml^{-1}, respectively. The results of the proposed method were comparable with those of CPE-Spectrophotometry. The method was successfully applied to the determination of erythrosine in food samples.

Keywords: CPE-Scanometry, Erythrosine, Plexiglas® cell, RGB parameters

INTRODUCTION

Cloud point extraction (CPE) is based on the property that a solute present in aqueous solution of non-ionic surfactant is distributed between two phases. The important advantages of CPE are simplicity, inexpensiveness, having short time, high recovery and enrichment factor, low toxicity and biologically environmentally-friendly and using no organic solvents [1]. Micellar extraction also provides a decrease in the relative detection limit value.

Cloud point extraction procedure has been successfully employed for the preconcentration of compounds in food [2,3], environmental [4], biological samples [5], pharmaceutical samples [6], and dyes [7-10]. This procedure could easily be combined with several analytical methods including spectrophotometry [11], inductively coupled Plasma optical emission spectrometry [12], atomic absorption spectrometry [13], Chromatography [14], graphite furnace atomic absorption spectrometry [15], and paptode [10].

*Corresponding author. E-mail: ashokrollahi@mail.yu.ac.ir

Auxiliary techniques of ultrasonic-assisted cloud point extraction (UA-CPE) [16], microwave-assisted cloud point extraction (MA-CPE) [6], and stirring-assisted cloud point extraction (SA-CPE) [17] are also applicable. Recently, the CPE procedure has been coupled with scanometry as CPE-scanometry, and applied for preconcentration and determination of fast green FCF and violet covasol dyes [18,19]. CPE-scanometry takes advantage of simplicity (handheld scanner and PC), high scanning speed, inexpensive and portable systems, and easily immobilizing of reactants, no need to find λ_{max}, intense archiving of experiences, short response time, limiting the interferences, being able to various simultaneous tests, using of non-transparent and investigation of the reflective properties of the surface.

In the present work, CPE-scanometry is used to preconcentrate and determine trace amounts of erythrosine dye in food and cosmetics samples. Scanometry is used for determining the concentrated dye after CPE. The cells containing the sample solution are scanned with the scanner and then the color of each cell is analyzed with software to

red, green, and blue values. Compared with UV-Vis technique, a considerable accordance is observed at optimal conditions.

Abbaspour *et al*. has introduced the scannometry technique recently [20]. They used commercially available flatbed-scanners for obtaining the images of color solutions. The obtained images were transferred to computer for analyzing and determining the intensity of color solutions. The method was based on the reaction in solution phase, in Plexiglas® cells. The solution was scanned and finally the RGB (red, green and blue) color model was used in color monitoring [21,22]. Recently this method was developed by Shokrollahi *et al*. for determination of acidity constants of indicators [23,24].

Erythrosine (E127), Disodium 2-(2,4,57 tetra iodo-3-oxido-6-oxoxanthine-9-yl) benzoate monohydrate (Fig. 1) is water soluble synthetic colorants and most popularly used as a food coloring agent [25] and a host of other application such as inks [26,27], photographic materials [28], dental plaque [29], bacterial plaque [30], and cancer cells [31]. In addition, it is used in drugs [32], cosmetics [33], and olives [34]. It is notable that erythrosine is highly toxic, causing various types of allergies, thyroid activities, anemia and DNA damage behavior. It is also carcinogenic in nature [35]. For this reason, safety data such as the acceptable daily intake, based on toxicological studies experimental animals and human clinical studies, have been especially determined and evaluated by Food Agricultural Organization (FAO) and World Health Organization (WHO) [36]. The acceptable daily intake of erythrosine is 0.1 mg kg^{-1} body weight based on WHO report [37].

Several methods have been introduced for determination of erythrosine. These methods include Mean Centering of Ratio Spectra [35,38], H-Point Standard Addition [39], Zero-crossing derivative spectrophotometry [40], and electrophoresis with laser induced fluorescence detection [41].

Therefore, a simple, fast, sensitive, and inexpensive method for determination of erythrosine is of practical importance. CPE-Scanometry is a new method for this purpose. However, the main goal of this study is to introduce a coupled technique with the above-of dyes, comparable results with respect to CPE-Spectrophotometry and application of the method for preconcentration and determination of erythrosine in the samples.

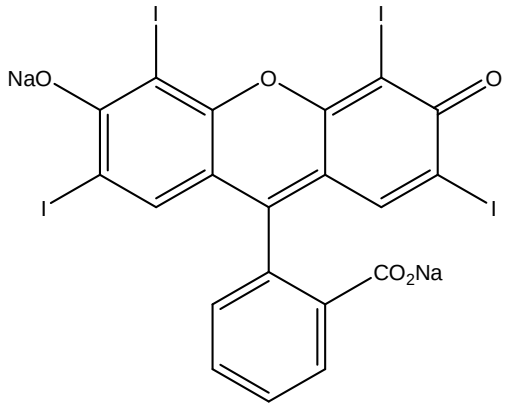

Fig. 1. Molecular structure of erythrosine.

EXPERIMENTAL

Apparatus

The cells (with 1000 µl volume for each of them) were built by using a sheet of Plexiglas®. A Canoscan LiDE 200 flatbed scanner was used for scanning the Plexiglas®sheet. The resolution of the scanner was regulated at 300 dpi. The VB based special software [42] was used to convert the recorded pictures of color of cells to RGB data. A Biohit prolinepipettor 100-1000 µl was used for injecting samples into the cells. A Metrohm (Herisau, Switzerland) digital pH meter Model 827 with a combined glass electrode was used to measure the pH values. A F.A.G thermostat bath (Iran) maintained at the desired temperature was used for the cloud point temperature experiments. Absorbance measurements were made by a JASCO V-530, UV-Vis spectrophotometer (Hachioji, Tokiyo, Japan) equipped with 1-cm quartz cells.

Materials

All chemicals used in this work were of analytical grade. In addition, double distilled water was used throughout the procedure. A 1% (w/v) Triton X-114 (Fluka, Buchs, Switzerland) solution was prepared by dissolving 1.0000 g of Triton X-114 in 100-ml volumetric flask. Erythrosine was purchased from M/s Merck (E. Merck, Darmstadt, Germany). A stock solution of 100 µg ml^{-1} of erythrosine

was prepared and the more diluted solution was prepared from it. Nitrate salts of cadmium, cobalt, nickel, copper, zinc, calcium, and other salts from Merck were of the highest purity available and used without any further purification. Morin was purchased from Sigma-Aldrich (St. Louis, MO). Other chemicals were from Merck. To prepare the desired pH solution, the pH adjustment was carried out by acetic acid/sodium acetate buffer. The surfactant rich phase was diluted with ethanol.

Principles of the Red, Green and Blue (RGB) Color System

The RGB color model is an additive color model in which red, green, and blue lights are added together in various ways to reproduce a broad array of colors. In computing, the color values are often stored as integer numbers in the range 0-255, the range that a single 8-bit byte can offer (by encoding 256 distinct values). In the RGB system, any color is represented in the form of (R, G, B), in which the (0,0,0) and (255,255,255) refer to black and white respectively. Therefore, by increasing the intensity of colors, the color values are decreased. In this system, 16777216 colors could be made. Any color could be described by the following formula:

$$V = R + 256\,G + 256^2\,B$$

where R, G, and B are red, green, and blue values of the main color. For black and white, V is equal to 0 and 16777216, respectively. By using the following flowchart, R, G and B values of V for any color could be extracted:

$$R = V \bmod 256$$

$$G = ((V - R) \bmod (256^2))/256$$

$$B = (V - R - G*256)/(256^2)$$

''Mod'' is a numeric function which returns the remainder when dividing two numbers.

Preparation of Cells Array

In this method, we used the cylindrical cells. These cells were built by creating holes (i.d. 1.5 cm) in the sheet of Plexiglas® (thickness 0.5 cm) using a laser. In order to close the bottom of the holes and make the cells, this holed sheet was stuck to another sheet of Plexiglas® (thickness of 0.1 cm).

In our design, the cells were aligned to 3 columns and 7 rows, giving a total of 21 cells in the Plexiglas®sheet.

Procedure

CPE-scanometry. A typical cloud point experiment was carried out using the following procedure. An aliquot of 15 ml of an aqueous solution containing 2.67 µg ml^{-1} of erythrosine, 0.1% (w/v) of Triton X-114 at pH = 3.50 was prepared. Then, the mixture was heated for 10 min in a thermostatic bath at 40 °C. The separation of two formed phases was carried out by centrifuging for 10 min. The aqueous phase could then be separated completely by a pasteur pipette. The surfactant rich phase was diluted with ethanol to 500 µl, and 450 µl of this solution was injected into the cells. After shaking, the color cells were scanned with the scanner and the obtained images of the color solutions were analyzed to evaluate R, G, and B contents by the developed software (Fig. 2). The values of the RGB colors varied between 39.20 and 155.77 relative to erythrosine concentration. In the scanometry, contrary to spectrophotometry the reflection of color solutions is studied; therefore one of the R, G and B values of color solutions that have the same color as the solution is usually useless, because this value is often higher than 155.77, and does not significantly change during the experiment. In this study, because of red colorful solutions of erythrosine, the red and blue did not change significantly (and was almost greater than 155.77), so the intensities of the green values were chosen. Any effective intensity in the color values (R, G and B) between sample solutions and blanks were plotted *vs*. the considerable parameter.

$$\text{Effective intensity}_R = -\log(R_{sample}/R_{blank})$$

$$\text{Effective intensity}_G = -\log(G_{sample}/G_{blank})$$

$$\text{Effective intensity}_B = -\log(B_{sample}/B_{blank})$$

CPE-UV-Vis. After performing cloud point experiment, using CPE-Scanometry, the surfactant rich phase was

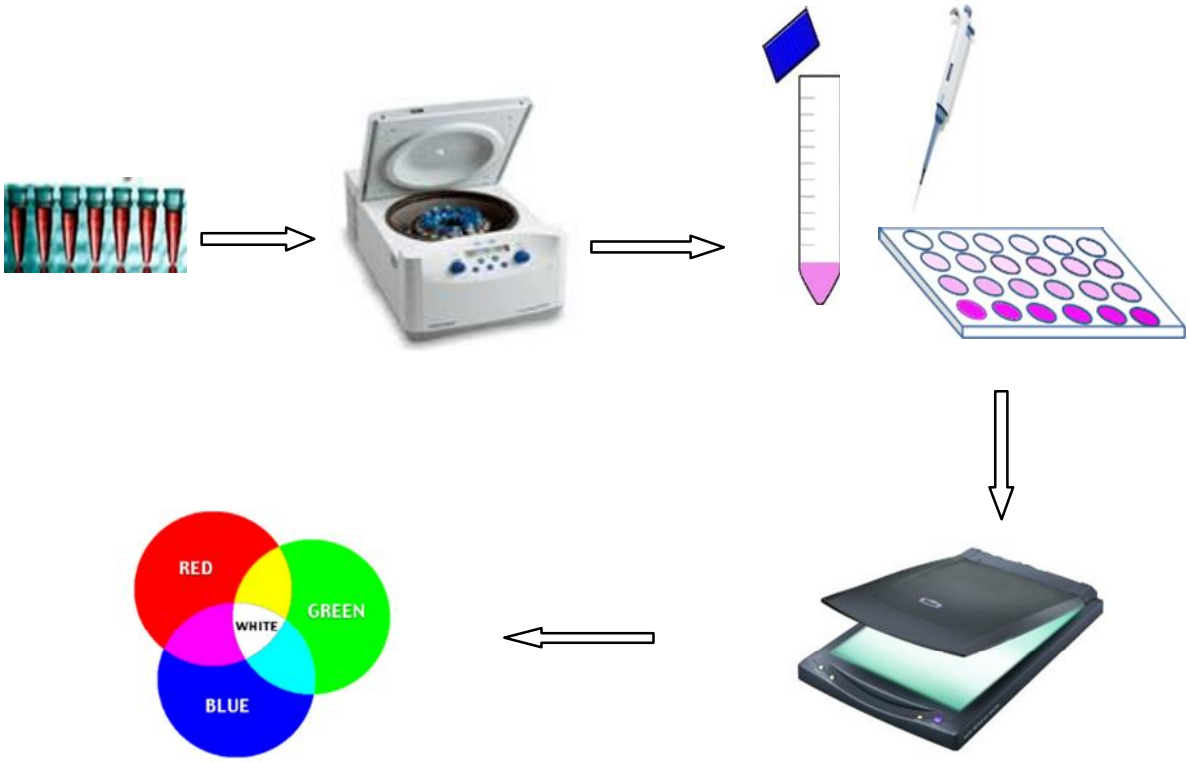

Fig. 2. Schematic of procedure.

diluted with ethanol to 1.0 ml, and the absorption spectra of diluted solution were recorded. A maximum absorption band was at 540 nm (Fig. 3). Therefore, this wavelength was used for subsequent absorbance measurements. To obtain calibration curves, absorbance values were plotted *vs.* analyte concentrations.

Preparation of Real Samples

Fruit jelly and fruit syrup powder. 1.000 g (fruit jelly powder) or 2.000 g (fruit syrup powder) of the sample was transferred to a 250 ml volumetric flask and made volume up to the mark with double distilled water [35]. Then, 3.0 ml of this solution was poured into Falcon tubes and extracted using the proposed method at the optimal conditions.

Fruit drink. 10.0 ml of the sample was transferred to a 50 ml volumetric flask and made volume up to the mark with double distilled water [39]. Then, 2.5 ml of this solution was poured into Falcon tubes and analyzed by the developed procedure.

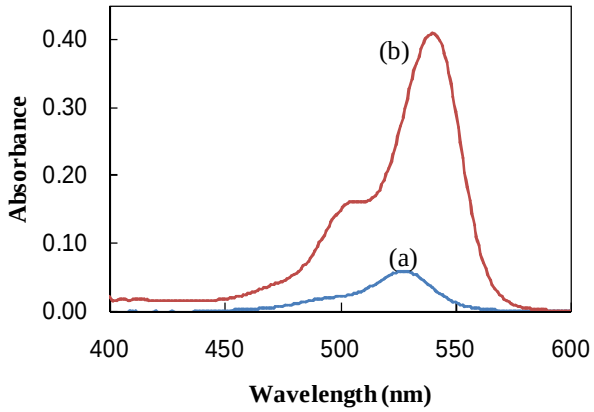

Fig. 3. The absorption spectrum of 1 µg ml^{-1} of erythrosine: without CPE in water (a) and after CPE in ethanol solvent (b).

Rouge Powder Sample

1.000 g of rouge powder in 100 ml volumetric flask was dissolved in a minimum amount of ethanol and made

volume up to the mark with double distilled water [38]. Then, this solution was filtered through filter paper to remove suspended particulate matter. The filtrate was diluted with double distilled water up to standard volume. Then, 2.0 ml of this solution was poured into Falcon tubes and analyzed by the proposed procedure.

RESULTS AND DISCUSSION

The trace amounts of erythrosine as a food dye was determined by CPE-scanometry as a simple, fast, sensitive and inexpensive method. The obtaining results were compared with those obtained from spectrophotometric method. The RGB parameters and absorption spectrum of dyes were recorded after CPE with Triton X-114. The G parameter for 5 µg ml^{-1} of erythrosine was found to be the most sensitive signal without and with cloud point extraction (0.022 and 0.62, respectively), thus this technique can be used as a sensitive method. As obviously seen in Fig. 3, λ_{max} has shifted to red and the light absorption has increased considerably after CPE. Various experimental parameters, such as pH, amount of Triton X-114, time of centrifugation, equilibration temperature and time were studied to obtain an optimized system. These parameters were optimized by the one-at-a-time evaluation method.

Effect of pH

For organic molecules, pH is perhaps the most critical factor regulating the partitioning of the target analyte in the micellar phase. Especially for ionizable species, maximum extraction efficiency is achieved at pH values where the uncharged form of the target analyte prevails. The ionic form of analytes does not interact with the micellar aggregate as strongly as its neutral form.

The effect of pH on the CPE of 2.67 µg ml^{-1} of erythrosine was studied in the range of 2.00-8.00 by addition of hydrochloric acid or sodium hydroxide. Figure 4a shows that at pH 3.50, maximum color intensity can be obtained. In the low pHs, the anionic groups $-COO^-$ change to the uncharged groups -COOH, therefore the neutral form of erythrosine is transformed to the surfactant phase and the extraction efficiency is increased. In the lower pH and extremely acidic medium, neutral dye molecule will probably become cationic by accepting H^+ ions, and

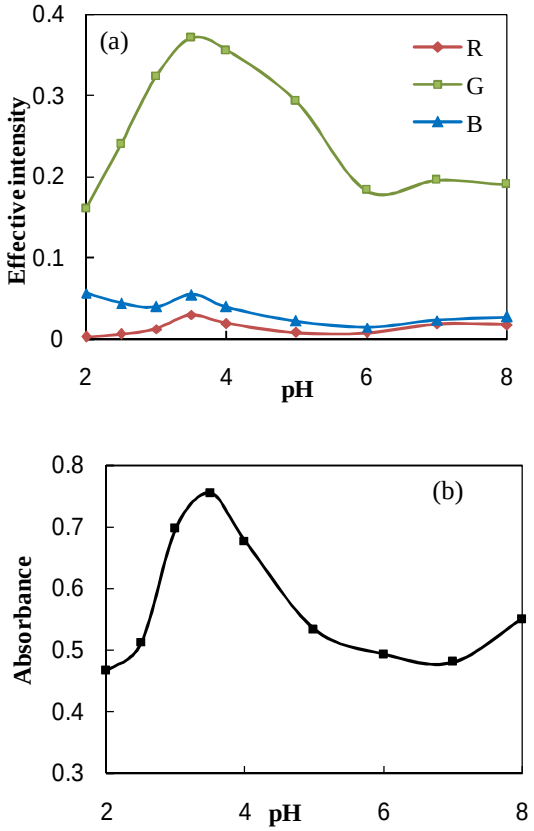

Fig. 4. Effect of the test solution pH on the CPE of erythrosine: against the effective intensity of the solution (a) and absorbance of the solution (b) (conditions: erythrosine, 2.67 µg ml^{-1}; Triton X-114, 0.1% (w/v)).

therefore recovery will be reduced. At higher pH, low acidic pH and especially in alkaline solutions, the anionic form is mostly present and this causes a reduction in the extraction efficiency. A comparison between scanometry and spectrophotometric determination method confirmed that pH 3.50 is the most suitable pH value (Fig. 4b); and it was selected as the optimum pH value and was maintained by adding 2.0 ml of acetic acid/sodium acetate buffer solution. According to the literature [43], two pk_a values were found for erythrosine (3.92 and 5.16 which were attributed respectively to the carboxylic and phenolic groups), the obtaining of optimum pH value can be justified by this.

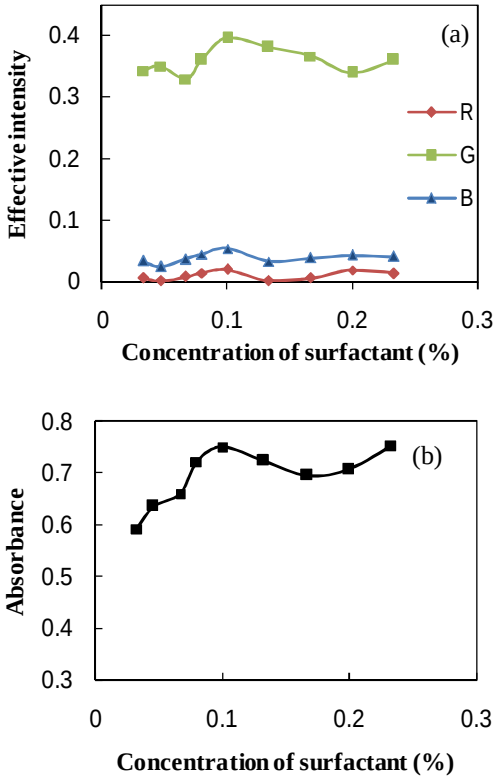

Fig. 5. Effect of Triton X-114 concentration on the CPE erythrosine: against the effective intensity of the solution (a) and absorbance of the solution (conditions: erythrosine, 2.67 µg ml^{-1}; Triton X-114, 0.1% (w/v); pH, 3.5).

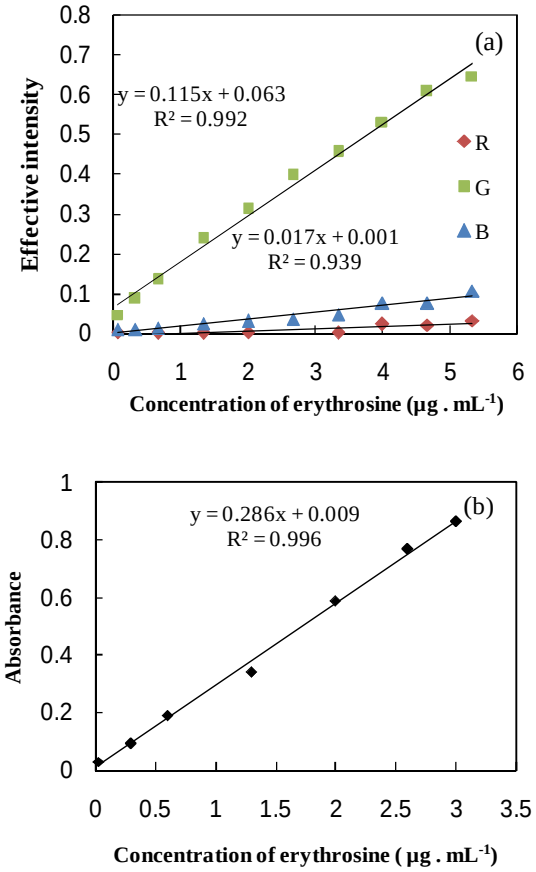

Fig. 6. Calibration graphs for determination of erythrosine by CPE-Scanometry (a) and CPE-UV-Vis (b) at optimum conditions (Triton X-114, 0.1% (w/v); pH, 3.5; temperature of bath, 40 °C; time of bath, 10 min; time of centrifugation, 10 min).

Effect of Triton X-114 concentration

A successful cloud point extraction should be able to maximize the extraction efficiency through minimizing the phase volume ratio ($V_{surfactant\text{-}rich\ phase}/V_{aqueous\ phase}$), so as to improve the preconcentration factor. The effect of Triton X-114 concentration on the extraction of dye was studied in the range of 0.030-0.23% (w/v). As seen in Fig. 5, the highest signal was obtained at 0.1% (w/v) of surfactant. At concentrations less than 0.1% surfactant, extraction is incomplete. No significant change is observed in recovery in 0.1% to about 0.2% surfactant. A slight decrease in the extraction efficiency is duo to remaining very small quantities of Triton X-114 and analyte in the aqueous solution. This phase can be competed with the surfactant-rich phase to draw a little amount of analyte into itself. In addition, at a high concentration of surfactant, the viscosity of the enrichment phase increased and the signal decreased. Therefore, in order to obtain optimum conditions, a 0.1% (w/v) solution of Triton X-114 concentration was selected for further studies**.**

Effects of Equilibration Temperature and Time

Optimal equilibrium temperature and time required to facilitate phase separation and preconcentration efficiency, were investigated. At lower temperature and times, the two phases cannot be formed; however, higher temperature and time cause the dissociation of surfactant-dye assembly while

much higher temperatures may lead to decomposition of the analyte. The effect of equilibrium temperature and time in the range of 25-60 °C and 5-30 min was studied, respectively. It was found that the extraction efficiency reaches to its maximum at 40 °C. Therefore, an equilibration temperature of 40 °C was chosen for the analysis. Maximum extraction efficiency was observed at 10 min. Accordingly, an incubation time of 10 min was chosen to be used in the next experiments in the respective methods.

Effect of Centrifuge Time

Centrifugation can accelerate the equilibration and reduce the time required. The centrifugation time hardly affects the micelle formation, but accelerates the phase separation in the same way as in the conventional separation of a precipitate from its original aqueous environment.

To obtain the maximum efficiency of the method, it is necessary to preconcentrate a trace amount of dye with high sensitivity within a short period. The effect of the centrifugation time on the phase separation was studied in the range of 5-30 min. The best time was obtained at 10 min. At shorter times, the separation was not complete, and longer centrifugation time probably caused partial back extraction to the aqueous phase and a decreased signal.

Study of Interference

The influences of some diverse cations, anions and dyes on the determination of erythrosine were studied. Various amounts of other species were added to a solution containing 2.67 μg ml^{-1} of erythrosine, and the recommended procedure under optimum conditions was applied. An error of less than or equal to ±5.0% in the signal reading was considered as a tolerable value. The results presented in Table 1 show that the selectivity of the CPE-Scanometry is reasonable while low or equal selectivity is obtained with the CPE-UV-Vis method at a same concentration of interferes. As seen, the power of the CPE-Scanometry is considerable in the determination of erythrosine in the presence of some dyes such as Titangelb, Morin and Fluorescein.

Analytical Performance

Under optimum conditions, a linear range of 0.06-5.33 μg ml^{-1} of erythrosine was obtained. The equation of the regression line with and without the preconcentration (for G parameter) was y = 0.115C + 0.063 (Fig. 6a) and y = 0.011C - 0.033 respectively, where C is the concentration of erythrosine in μg ml^{-1}. The detection limit based on three times the standard deviation of the blank ($3S_b$) for each RGB factor was found to be 0.058 (n = 5). The relative standard deviation (RSD) for 2.67 μg ml^{-1} of erythrosine was 0.55% for R (n = 5). As the R and B values do not vary considerably by changing the concentration of dye, the RSD values were calculated only for G value. The enrichment factor, defined as the ratio of the slopes of calibration curves with and without the preconcentration was 10.45 for the proposed method. The preconcentration factor based on the ratio of the initial volume in the centrifuge tube to the final volume of the dissolving solvent is 30.00, where as in CPE-UV-Vis a linear calibration graph in the range of 0.03-3.00 μg ml^{-1} of erythrosine in the initial solution was obtained by applying the optimized conditions. The equation of the regression line with and without the preconcentration was y = 0.286C + 0.009 (Fig. 6b) and y = 0.082C - 0.006 respectively, where C is the concentration of erythrosine in μg ml^{-1}. The detection limit based on three times the standard deviation of the blank ($3S_b$) was 0.022 μg ml^{-1} (n = 5). The relative standard deviation (RSD) for 2.67 μg ml^{-1} erythrosine was 3.27% (n = 5). The enrichment and preconcentration factors for UV-Vis-CPE method were obtained to be 3.49 and 15.00, respectively.

Application to Real Samples

The proposed method was validated by the analysis of erythrosine in food and cosmetics samples. One fruit jelly powder (strawberry), one fruit syrup powder (strawberry), fruit drink (strawberry) and rouge powder were prepared, selected and applied to the determination of concentration of this dye under optimum conditions. An aliquot of the sample solution was prepared according to the preparation of real sample section and analyzed by the procedure described above. The concentration of this dye in the cited real samples was calculated from the standard addition calibration curves. The developed methodology was validated by the recovery studies. Good recoveries (92.36-105.72%) were achieved for all analyzed samples. The results for this study are presented in Table 2.

Table 1. The Effect of Foreign Ions and Dyes on the Determination of 2.67 μg ml^{-1} of Erythrosine by CPE-Scanometry and CPE-UV-Vis Method

Foreign ion/dye	Foreign species (μg ml^{-1})	
	CPE-Scanometry	CPE-UV-Vis
Na^{+}, Ca^{2+}	3300	3300
Cd^{2+}, Ni^{2+}, Pb^{2+}, Mg^{2+}	3300	1000
Cu^{2+}	3300	500
Ba^{2+}, Co^{2+}	500	500
Zn^{2+}	1000	250
Fe^{3+}	10	10
Cl^{-}, ClO_4^{-}, NO_3^{-}, CH_3COO^{-}	3300	3300
Morin	1	0.5
Titangel	1	Interfere
Fluorescein	1	0.5

Table 2. Determination of Erythrosine in Different Samples

Samples	Erythrosine (mg g^{-1})	
	CPE-Scanometry	CPE-UV-Vis
Fruit jelly powder (strawberry)	0.550	0.565
Fruit syrup powder (strawberry)	0.598	0.599
Fruit flavor drink (strawberry)	16.890[a]	16.620[a]
Rouge powder	0.613	0.662

[a]For this real sample the unit of concentration is μg ml^{-1}.

Table 3. Comparative Data for Determination of Erythrosine by CPE-Scanometry and CPE-UV-Vis

Method	Analyte (s)	Reagent	Linear range ($\mu g\ ml^{-1}$)	LOD ($\mu g\ ml^{-1}$)	RSD (%)	Ref.
Mean Centering of Ratio Spectra	Eosin and Erythrosine	Cr(VI)	1.470-8.070	0.498	-	[38]
H-Point Standard Addition	Eosin and Erythrosine	Fe(III)	1.460-6.590	-	-	[39]
electrophoresis with laser induced fluorescence detection	Erythrosine and Other red food Colorants	-	-	0.4 ng ml^{-1}		[41]
Zero-crossing derivative spectrophotometric	Sunset yellow and erythrosine	-	2.000-10.000	-	2.3	[40]
Mean Centering of Ratio Spectra	Eosin and Erythrosine	Fe(III)	0.0527-0.615	0.199	-	[35]
CPE-Scanometry CPE-UV-Vis	Erythrosine	-	0.067-5.330 0.030- 3.000	0.058 0.021	0.55 3.27	This work

CONCLUSIONS

The trace amounts of erythrosine, as a food dye, was determined by CPE-scanometry as a new, facile, fast, sensitive, relative green and inexpensive method and comparable with CPE-UV-Vis technique. The analytical results showed that the method developed could be successfully adopted for the separation and determination of dye with high sensitivity and selectivity. The linear range of the proposed method for determination of erythrosine is wider and the LOD is comparable with CPE-UV-Vis method. The proposed method can be applied for preconcentration and determination of erythrosine in the various food and cosmetics samples.

In Table 3, comparison of this method with some other reported methods for determination of erythrosine is presented. As this table illustrates, the proposed method is comparable with the most previously reported methods and has a lower limit of detection. In addition, it is an inexpensive, simple and rapid method for determination of erythrosine.

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