Green Cloud Point Extraction Coupled for Separation and Determination of Erythrosine in Various Samples Compared to the HPLC Technique

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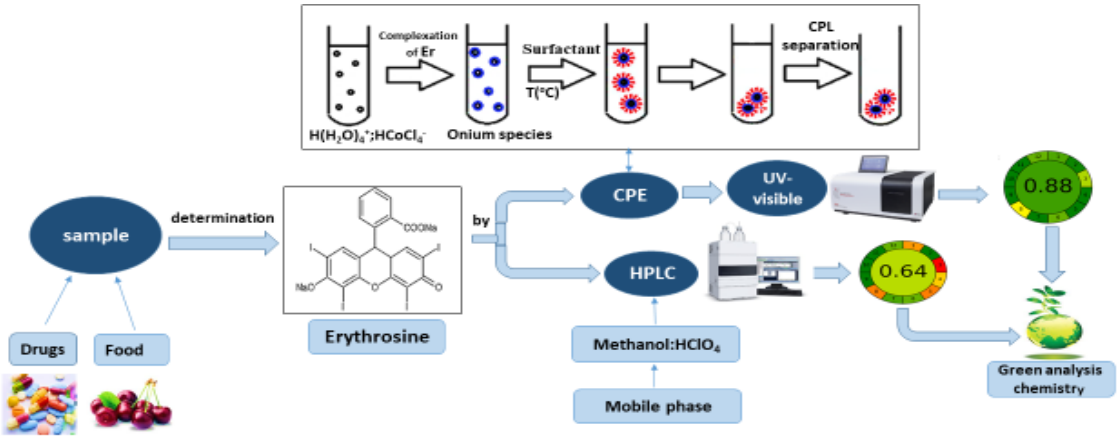
The cloud point extraction (CPE) method using mixed micelles is employed to extract and separate erythrosine. Various conditions affecting the separation process were studied, including the amount of hydrochloric acid, temperature, heating time, Co(II) ion concentration, surfactant volume, and the impact of organic solvents on the dissolution behavior of the cloud point layer. The calibration curve was linear for concentrations ranging from 5 to 1000 μg/mL. The proposed methodologies were validated for drug and food analysis. Statistical analysis compared data from the CPE techniques with those from the HPLC method. The AGREE assessment approach was used to evaluate the environmental sustainability of the methods. It indicated that the CPE method adhered more to green characteristics than the HPLC method.

Keywords: cloud point extraction, onium system, erythrosine, HPLC

Graphical abstract



Introduction

Erythrosine (ER) is a water-soluble synthetic food coloring from the xanthene group of chemicals, also known as disodium 2-(2,4,5,7-tetraiodo-3-oxido-6-oxoxanthin-9yl) benzoate monohydrate. This dye is extensively used in the food, pharmaceutical, and cosmetic industries for its strong dyeing ability and cost-effectiveness. However, overexposure to erythrosine can result in various health issues, including thyroid activity, allergic responses, anemia, and DNA damage due to its toxic nature. To address this, the Food and Agricultural Organization (FAO) and the World Health Organization (WHO) have recommended a daily intake of erythrosine to be around 0.1 mg per kilogram of body weight, based on thorough assessments of potential risks [1, 2].

The identification and separation of synthetic food dyes have become crucial in food processing and manufacturing within the food and beverage industry. Therefore, it is essential to develop a simple, rapid, sensitive, and cost-effective method for determining erythrosine to ensure the safety of food, medicine, and cosmetics, given their harmful health effects.

Various analytical methods, such as mass spectrometry [4], fluorescence [3], capillary electrophoresis [6], high-performance liquid chromatography [5], differential pulse polarography [7], and spectrophotometry [8], are used to analyze dyes. These analytical methods generally involve a pre-concentration process in order to minimize interactions with other components in food samples and ensure that the analyte is present in low concentrations in cosmetics, drugs, and food. Some

classic pre-concentration techniques include liquid-liquid extraction, cloud point extraction [10], and solid phase extraction [9, 11].

In the present study, a highly effective green method was developed for the sensitive and selective spectrophotometric assessment of ER using Co(II) to generate an onium species in an HCl mixture. This method has demonstrated its effectiveness for analyzing ER in the pharmaceutical industry.

Experimental part

Chemicals and reagents

Erythrosine (99.96%), $CoCl_2.6H_2O$ (99.8%), and SDS were performed from Merck, and Triton X-100 and SDS (1% solution) from Alpha Chemika. All solvents (≥99%, for HPLC grade) – 1-butanol, methanol, ethylene glycerol, pentane, ethyl acetate, ethanol, and 2-propanol were purchased from Merck.

The standard Co(II) solution (1 mg/mL) was made by dissolving 0.249 g of $CoCl_2.6H_2O$ in purified water (100 mL). 1% (w/v) sodium dodecyl sulfate SDS solutions were made by dissolving 1.00 g of SDS in 100 mL of purified water. Erythrosine standard solution (100μg/mL) was made by dissolving 0.01 g of ER in distilled water (100 mL). Any additional working solutions were created by progressively diluting with filtered water to the required volume.

Apparatus

A UV-Vis double beam spectrophotometer (Biochrom Libra S60, UK) was used for spectrophotometric studies and absorbance measurement. The thermostated water bath (Hamburg-90, England) maintained a constant temperature during the extraction experiments. Sykam 600 Systems was used for HPLC measurements.

Samples preparation

To determine ER content, various pharmaceutical products, such as capsules, oral suspensions, and treated food samples, were analyzed.

The contents of ten capsules were weighed and finely powdered. The contents of a single capsule (0.1 g) were dissolved in a suitable volume of distilled water while being continuously shaken, and the insoluble particles were filtered out. The filtered solution was then transferred into a 100-mL volumetric flask and diluted with deionized water [12].

For the preparation of oral suspension, an exact volume (2 mL) was dissolved in 100 mL of deionized water [13]. Food samples (cherry syrup, jam, and sweet) were prepared by dissolving a suitable weight of the samples in 50 mL of deionized water. The samples were shaken for 5 minutes to dissolve them thoroughly and then filtered [14].

Standard CPE procedure

In a standard cloud point extraction (CPE) procedure, 10 mL of aqueous solutions containing erythrosine (100 μg/mL) and Co(II) (100 μg/mL) were prepared in an HCl solution. Subsequently, an appropriate amount of either 1% TritonX-100 or a combination of SDS and TritonX-100 was added as the surfactant. The solutions were boiled for a specific time and temperature in an electrostatic water bath. After that, the cloud point layer was removed from the solution and transferred into 5 milliliters of ethanol to dissolve. The measurement was performed using ethanol as a standard at λmax. The absorbance of an ion pair association complexes formed by Co(II) is determined by measuring at the respective wavelengths of maximum absorbance, which are λ_{max} = 541 nm for Triton X-100 and λ_{max} = 488 nm for Triton X-100 + SDS.

Chromatographic parameters used for the HPLC determination of erythrosine:

Parameter	Optimized HPLC conditions
Column	C18
Flow rate	0.2 mL/min
UV	240 nm
Mobile phase	Methanol: $HClO_4$ 75:25
Temperature	25°C
Injection volume	0.001 mL

Results and Discussion

Optimization of the effective parameters of the CPE method

Effect of hydrochloric acid concentration

As in the CPE procedure, the effects of various hydrochloric acid concentrations in the range of 0.1 to 1.0 M were examined using the two surfactants TritonX-100 or a mixture of TritonX-100 and SDS. The results are shown in Figure 1. The results indicate that a concentration of 0.5M HCl with either TritonX-100 or a mix of Triton X-100 and SDS is the optimal condition for enhanced erythrosine extraction by Co(II) as an onium species. This concentration facilitates the rapid formation of the Co(II) chlorination complex and influences the direction of the equilibrium formation of the onium species. Whether the concentration is higher or lower than ideal, it affects the rate of backward dissociation and diminishes the concentration of the extracted onium species [15].

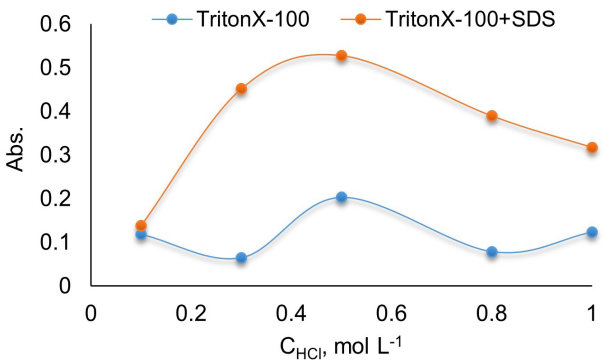


Figure 1. Effect of hydrochloric acid concentration on the formation of the ion association complex.

Effect of temperature

The impact of temperature on the formation of CPE was studied by extracting erythrosine at temperatures ranging from 70 to 95 °C using Triton X-100 or a combination of TritonX-100 and SDS, similar to the standard CPE-Onium procedure. The results are presented in Figure 2.

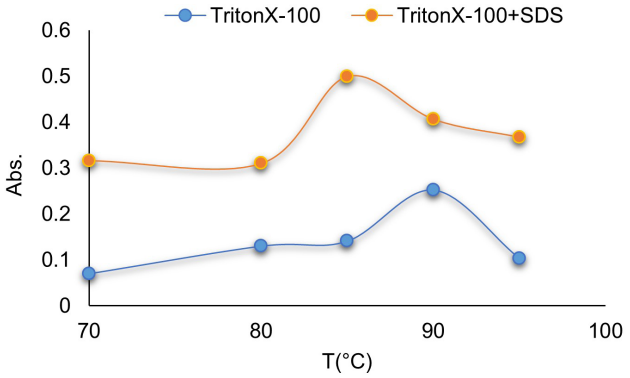


Figure 2. Effect of temperature on the formation of the ion association complex.

The results indicate that the optimal temperature for TritonX-100 and TritonX-100+SDS was 90°C and 95°C, respectively. At this temperature, the liquid ion exchange method resulted in a higher efficiency of erythrosine extraction. This temperature quantitatively explains the energy required for the appropriate aggregation of surfactant micelles to form CPE and the formation of ion-pair associations between erythrosine and $CoCl_4^{-2}$ [16].

Effect of heating time

In the CPE method, extraction of erythrosine onium species occurs by achieving optimal thermodynamic and kinetic equilibrium during a specific heating duration. This drives the complexation process, leading to the formation of micelles and the optimal creation of CPE. Timing is crucial - excessive time reduces extraction efficiency, while suboptimal duration fails to achieve the desired equilibrium [17]. In this study, erythrosine was extracted using the standard CPE-Onium procedure between 10 and 25 minutes for Triton X-100 and 15 and 45 minutes for Triton X-100 + SDS heating times. The results are illustrated in Figure 3. The optimal duration for using TritonX-100 was found to be 15 minutes, while it was 30 minutes when using Triton X-100+SDS.

Effect of Co(II) ion concentration

Erythrosine was extracted from a 10 mL aqueous solution containing various concentrations of Co(II) ions in the presence of 0.5 M of HCl. Figure 4 displays the results of the CPE-Onium procedure, which used either TritonX-100 alone or a combination of TritonX-100 and SDS. The concentration of 100 µg/10 mL was shown to be the most effective amount when using TritonX-100 alone or in conjunction with SDS. This concentration resulted in the most outstanding absorbance value, indicating greater extraction efficiency.

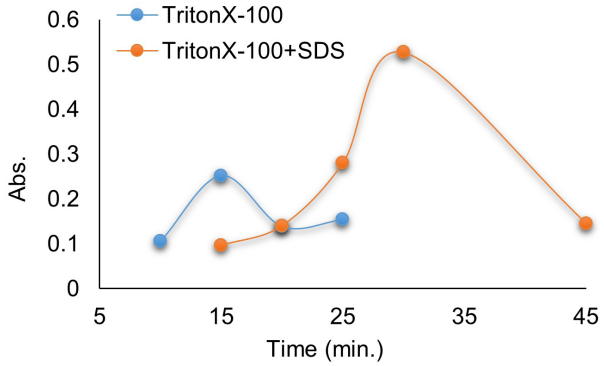


Figure 3. Effect of heating time on the formation of the ion association complex.

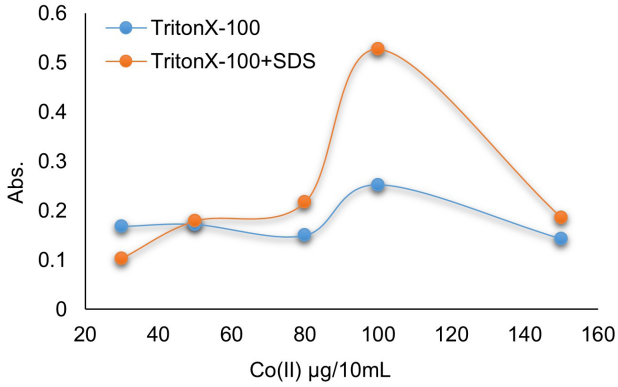


Figure 4. Effect of Co(II) concentration on the formation of the ion association complex.

Effect of surfactant volume

It's essential to use an optimal volume for better extraction efficiency. This volume ensures the required kinetic and thermodynamic equilibrium, leading to the production of the most effective CPE. Failing to reach the optimal critical micelle concentration (CMC) prevents the establishment of CPE equilibrium. On the other hand, exceeding the ideal volume increases diffusion and inhibits CPE formation [19]. In the CPE-Onium procedure, ER was extracted from 10 mL of aqueous solutions under ideal conditions. The extraction involved varying volumes of TritonX-100 (0.1–1.0 mL) and TritonX-100 + SDS (0.2–2.0 mL). The results of this extraction are shown in Figure 5. It was found that 0.8 mL of Triton X-100 and 1.0 mL of Triton X-100+SDS were used for the extraction.

Effect of electrolytes

The CPE-Onium procedure was used under optimal conditions to extract erythrosine from 10 mL of aqueous solutions containing various electrolyte salts. The results are presented in Table 1. The findings illustrate an enhanced effectiveness of extraction when an electrolyte is present in the aqueous solution. This improvement is attributed to the electrolyte's ability to reduce the dielectric constant and polarity of the solution, thereby disrupting the hydration shell of

Co(II) and facilitating the formation of a complex with ER [20].

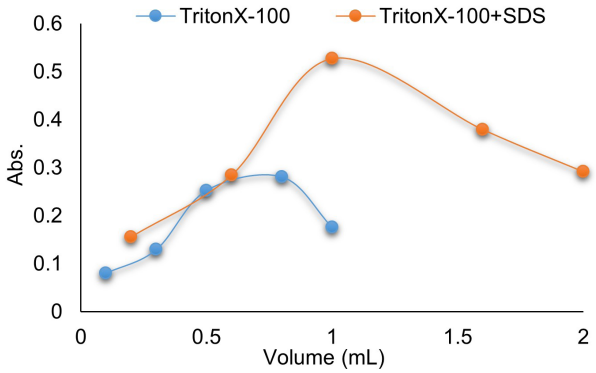


Figure 5. Effect of surfactant volume on the formation of the ion association complex.

Table 1. Impact of electrolyte on the extraction efficiency of ER.

Electrolyte Salt	Abs. at λ_{max} = 541nm
Without	0.280
NH_4Cl	0.251
NaCl	0.359
KCl	0.240
$MgCl_2.6H_2O$	0.289
$AlCl_3$	0.215

Effect of different solvents

The standard method was used to extract erythrosine from 10 mL of aqueous solutions under the best conditions. The resulting CPE was dissolved in various organic solvents. The solvation and/or dielectric effects of the solvents impact the absorption spectra of these solvents. The values for F(D) and Φ(D) were calculated and are presented in Table 2. When the dielectric constant is the sole factor influencing the peak shift, the results show a linear correlation [21,22].

Analytical characteristics of the method

A method combining cloud point extraction with an onium system is used to determine erythrosine spectrophotometrically using Co(II) as a chlorination complex to form onium species. A calibration curve y = 0.001x + 0.0712; R^2 = 0.9996 is generated at 541 nm, the wavelength of maximum absorption, using optimal CPE procedure conditions. The calculated analytical characteristics of the method presented in Table 3.

Table 3. Analytical parameters for the determination of ER by CPE.

Parameter	Value
λ_{max} (nm)	541
Bear's law obey (ppm)	5-1000
Slope	0.001
Intercept	0.017
Quantity Limit (μg mL^{-1})	10.38
R^2	0.9996
Detection Limit (μg mL^{-1})	3.43
Molar absorptivity (L mol^{-1} cm^{-1})	0.869×10^3

HPLC analysis

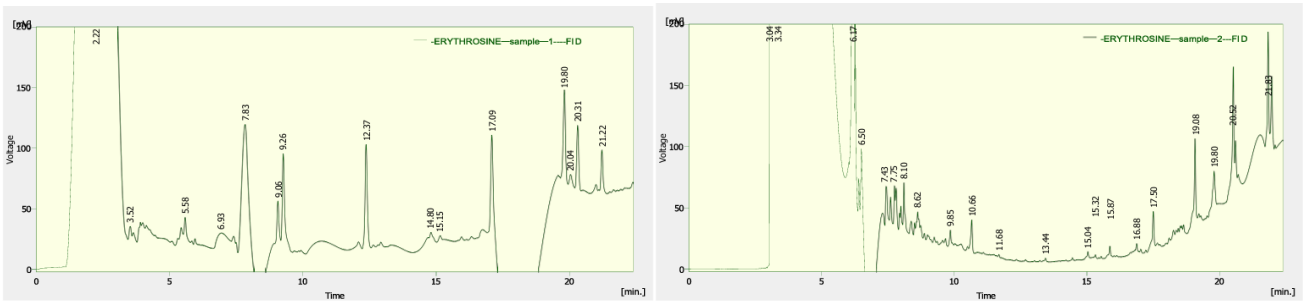
HPLC analysis is utilized to determine erythrosine (ER) levels in pharmaceuticals and food, compared to the CPE-Onium Spectrophotometer method. Figure 6 shows the chromatogram of the standard ER solution of erythrosine (1000 ppm) and sample Ibuprim (Oral suspension). Table 4 details the erythrosine concentration measured by both the CPE-Onium and the HPLC methods.

Table 2. Maximum wavelength and dielectric constant of the organic solvents used to dissolve CPL.

Solvent	λ_{max}(nm)		Dielectric Constant (D)	(D-1/D+1)	Φ(D) = (D-1/D+2)	F(D) = 2(D-1)/(2D+1)
	Triton X-100	Triton X-100 +SDS				
1-butanol	490	491	117.70	0.983	0.975	0.987
Methanol	485	494	32.60	0.941	0.914	0.955
Ethylene glycerol	498	-	37.00	0.947	0.923	0.96
Pentane	498	-	1.84	0.296	0.218	0.359
Ethyl acetate	496	-	6.02	0.715	0.626	0.769
Ethanol	541	488	24.55	0.923	0.863	0.926
2-Propanol	496	-	19.92	0.923	0.887	0.941

Greenness Assessment

The AGREE metrics is a software tool that uses a score range of 0–1 to evaluate how environmentally friendly an approach is. A score of 1 indicates a high level of environmental benefit. The software consists of 12 steps, each covering essential aspects for calculating the greenness score and providing a GAC concept. When tested with the AGREE tool, the method received scores of 0.64 for HPLC and 0.88 for the CPE-Onium method (Figure 7). These scores indicate that the method has environmentally friendly analytical features in line with GAC principles [24].



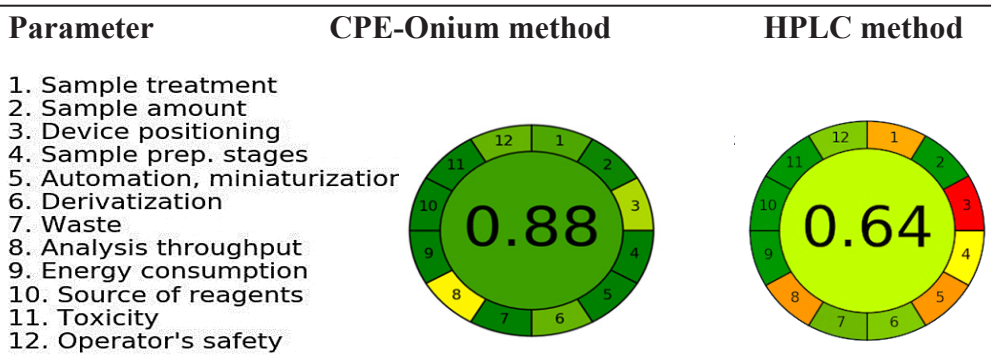
(a) (b)

Figure 6. Chromatograms of (a) ER Standard 1000 ppm and (b) Ibuprim (Oral suspension).

Table 4. Determination of Erythrosine by two methods: CPE-Onium and HPLC.

Name	Company	Manufacture	Measured Concentration	
			CPE – Onium method	HPLC method
Ibuprim (Oral suspension)	*PRISM LIFE-SCIENCES LTD*	India	533.76	533.581
ECHE-OFF (Tablet)	*POZZO*	India	3.001	2.915
Yan (Cherry Juice)	NON GOMC	Armenia	754.151	750.195
Lokman (cherry jam)	MND Kahvaltik gida sanayi Ve Ticaret A.S.	Turkiye	450.24	448.186
Happy pop (Cherry candy)	Alseedawi sweet factory	Kuwait	440.984	441.619
F			1.008484	
P(F<=f) one-tail			0.496832	
F Critical one-tail			6.388233	

Figure 7. Comparison and greenness assessment of the CPE-Onium and HPLC methods.



The Green Analytical Procedure Index provided valuable information on identifying non-green practices. It demonstrated that the CPE-Onium method outperformed the HPLC method. This conclusion was reached because the AGREE chemistry effectively addressed and resolved issues with both methods. The AGREE method is straightforward to implement, considers reagent quantities, and highlights any deficiencies in the approach being studied. Overall, the results from the evaluation tools complemented each other, offering a comprehensive assessment of environmental friendliness and confirming compliance with ecologically sound practices [25].

Conclusions

The study used the CPE approach to determine ER from commercially available food samples such as cherry syrup, jam, candies, and other products. The method is based on creating an onium complex with ER using Co(II) as a chloroanion complex in the presence of surfactants, followed by spectrophotometric analysis. The analytical results indicate that the CPE-Onium method can effectively separate and determine dyes with high sensitivity and selectivity, comparable to the HPLC approach. Additionally, the study used the analytical eco scale, the green analytical process index, and the AGREE evaluation method to rank the provided procedures in terms of greenness.

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