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PRECONCENTRATION AND DETERMINATION OF SAFRANINE T IN ENVIRONMENTAL WATER SAMPLES

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Abstract

A micelle-mediated preconcentration method has been developed for determination of Safranin T at trace amounts, which is widely used in textile industry. It can be evaluated as one of the dangerous chemicals due to its carcinogenicity and toxic properties. The method is based on the cloud point extraction (CPE) of Safranin T at pH 8.0 in the presence of Triton X-114 as nonionic surfactant. The obtained surfactant-rich phase (SRP) was diluted with ethanol and its absorbance was measured at 532 nm. Experimental parameters affecting preconcentration and determination steps have been optimized. The obtained calibration graph was linear in the range of 4-200 $\mu\text{g L}^{-1}$ and correlation coefficient was 0.9838. Detection limit was 1.14 $\mu\text{g L}^{-1}$ and the relative standard deviation for 10 and 100 $\mu\text{g L}^{-1}$ of Safranin T were 2.7 and 1.8 %, respectively. The method was applied for determination of Safranin T in environmental water samples with satisfactory results.

Keywords: cloud point extraction, environmental water samples, Safranin T, spectrophotometry

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1. Introduction

Synthetic organic dyes are commonly used in many industrial areas. Textile industry is one of the most important usage areas. The discharge of highly colored effluents into natural water mediums is not only aesthetically displeasing, but also impedes light penetration, thus upsetting biological processes within streams. The disposal of dyes to aquatic mediums is one of the industry's major problems because such effluents contain a number of contaminants including acid or caustic, dissolved and suspended solids, toxic compounds, heavy metals, and coloring pigments (Gupta et al., 2006; Simsek and Ulusoy, 2016). Out of all these contaminants, color is the first to be recognized factor to human eye and is objectionable to the public for hygienic reasons. In addition, much kind of dyes are toxic to living organisms causing direct destruction of aquatic communities (Bong and Strab, 1974). The entrance of such dyes compounds into the environment causes

heavy hazardous effects (Mousavi and Nekouei, 2011; Vinod et al., 2006). Environmental pollution by dyes is of great public concern. Nearly 20 % of the world's dye production is discharged to water systems by especially textile industries due to the incomplete exhaustion of coloring material and washing operations (Bamonti et al., 2016; Pirillo et al., 2007). Recent studies indicate that dyes are an objectionable type of pollutant because they are generally toxic due to oral ingestion and inhalation, skin and eye irritation, skin sensitization, and carcinogenicity (Garcia-Falcon and Simal-Gandara, 2005; Gharehbaghi and Shemirani, 2012; Khanavi et al., 2012; Pourreza and Elhami, 2007; Pourreza and Zareian, 2009).

The presence of color in aquatic mediums reduces light penetration, which in turn upsets the biological metabolism process. This causes destruction of the aquatic communities' presents in the ecosystem. Environmental regulations have made it mandatory to decolorize dye wastewater prior to

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discharge (Gianotti et al., 2005; Pajootan et al., 2016; Zeid et al., 2012).

Safranin T is a water soluble phenazine dye (Fig. 1) with reddish brown powder, which is mainly used for dyeing tannin, cotton, bast fibers, wool, silk, leather and paper (Bhattacharya et al., 1994). Considering its toxicity to the environment in general and to water bodies in particular though it has wide applications. It is imperative to treat colored effluents for the removal of this dye. The use of Safranin T may cause environmental contamination and affect human health (Acharya et al., 1997; Bhattacharya et al., 1993; Gupta et al., 2011; Zaghibani et al., 2008). Therefore, developing sensitive and simple method for the determination of Safranin T is of great importance and interest in chemical analysis.

The development of new and effective determination methods for trace molecules or ions is so popular research area in analytical chemistry. The main advantages of these type methods are high sensitivity, extremely low detection limit, good selectivity, rapid analysis rate, and inexpensive equipments (Ulusoy et al., 2011).

To our knowledge, there is no method based on spectrophotometric determination after CPE for trace amounts of Safranin T in real samples.

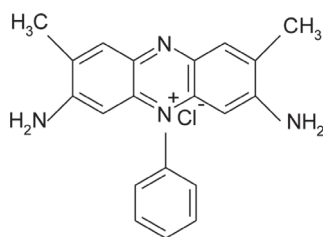


Fig. 1. Molecular structure of Safranin T

Cloud point extraction (CPE) has shown to be an advantageous separation strategy and has been used for the extractive preconcentration, separation and purification of metal ions, metal chelates, biomaterials and organic compounds present in different complex matrixes (Alesso et al., 2012; Bezerra et al., 2005; Perez-Urquiza and Beltran, 2000; Ulusoy et al., 2012). Any substance that binds to the micellar aggregates in the aqueous feed can be extracted from the original solution and concentrated in the small volume of surfactant-rich phase (Paleologos et al., 2005). The dye-surfactant interactions are generally complex in nature. Color of ionic dyes may change in the presence of oppositely charged ionic micelles (Moulik et al., 1989; Tatara et al., 2005). The electrostatic interaction between the dye and the micelle overshadows the hydrophobic or other specific and non-specific interactions between the species (Moulik et al., 1977). Therefore, CPE method can take into account as a useful and simple tool for determination of trace dyes in real samples (Pourreza et al., 2011).

In the present work, a simple and sensitive cloud point extraction method was developed for the determination of Safranin T in real samples. The

method is based on the extraction of Safranin T to Triton X-114 phase in the presence of SDS. Then, the surfactant-rich phase was dissolved in ethanol and analyzed its content of Safranin T by using a spectrophotometer at 532 nm. The method was applied successfully to determine of Safranin T in several water samples.

2. Experimental

2.1. Instrumentation

Absorbance measurements were carried out by a shimadzu model UV-VIS spectrophotometer which has a wavelength accuracy ± 0.2 nm. Acidity of model solutions were determined by a pH meter (Selecta, Spain). Phase separation after preconcentration process was performed by a centrifuge system (Hettich, Germany).

2.2. Reagents

All model solutions and reagents were prepared by using ultra-pure water having a resistivity of 18.2 M Ω .

A stock solution (200 $\mu\text{g mL}^{-1}$) of Safranin T (3,7-diamino-2,8-dimethyl-5-phenyl-, chloride) (Sigma, St. Louis, MO, USA) was prepared by dissolving it in ultra-pure water. Solutions of 5 % (w/v) Triton X-114 and PONPE 7.5 (Sigma) were prepared by dissolving 5.0 g of surfactant in 100 mL of ultra-pure water. The ionic surfactant's solutions (3.0×10^{-3} mol L $^{-1}$ of cetyl pyridinium chloride (CPC) and sodium dodecyl sulphate (SDS) were prepared by dissolving an appropriate amount of chemicals (Sigma) in hot ultra-pure water.

A 0.04 mol L $^{-1}$ of Britton-Robinson (BR) buffer was used to keep the desired pH values. This buffer consists of a mixture of H₃BO₃ (Merck), H₃PO₄ (Merck) and CH₃COOH (Merck) that has been titrated to the desired pH with 0.2 mol L $^{-1}$ NaOH.

2.3. The CPE procedure

An aliquot of solution containing of Safranin T (so that its final concentration would be in the range of 4–200 $\mu\text{g L}^{-1}$), 0.5 mL of 5 % (w/v) of Triton X-114, 1.5 mL of 0.003 mol L $^{-1}$ of SDS, 1.5 mL of 20 % (w/v) of NaCl, and 1.5 mL of pH 8.0 BR buffer, were added to a 50 mL volumetric flask and diluted to the mark with water. This solution was then transferred to a 50 mL falcon tube and placed in a thermostat bath at 50 °C for 10 min. The turbid solution was cooled for easy phase separation for 30 minutes in refrigerator and centrifuged at 4000 rpm for 5 min. Then the aqueous phase was removed by decantation and the surfactant rich phase was diluted with 1.5 mL of ethanol in order to reduce its viscosity prior to spectrophotometric detection at 532 nm. A blank solution was also submitted to the same procedure. Finally, the amount of Safranin T were

determined by using either the directly calibration curve obtained by spectrophotometer or standard addition method.

2.4. Analysis of environmental water samples

Application of the proposed method was carried out by using environmental water samples such as tap water, river water, and industrial wastewaters. Origins of the applied samples can be classified like this:

- tap water from our lab;
- river water from Kızılırmak River (Sivas, Turkey);
- industrial wastewaters from a textile fabric.

All samples were put in clean containers and kept in dark until analysis.

The samples were filtered through Whatman grade No. 40 filter paper and diluted to the mark in a 100 mL of volumetric flask. 20 mL of this solution was treated under the recommended procedure.

3. Results and discussion

The efficiency of CPE depends on anionic, cationic or neutral form of dye in solution. So, chemical structure of Safranin T should keep under control throughout extraction procedure. Experimental variables were investigated and optimized for achieving the highest efficiency and sensitivity in CPE.

Fig. 2 shows the absorption spectra of Safranin T in surfactant-rich phase against reagent blank for five different concentration levels. The obtained results showed that maximum absorbance occurs at 532 nm. The absorbance of Safranin T gradually increases with concentration at 532 nm.

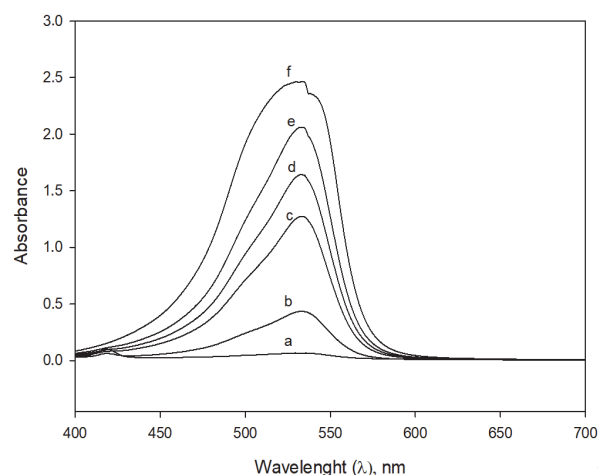


Fig. 2. Absorption spectrum of Safranin T solutions in different concentrations after CPE [a: Blank, b: $20 \mu\text{g L}^{-1}$, c: $60 \mu\text{g L}^{-1}$, d: $100 \mu\text{g L}^{-1}$, e: $120 \mu\text{g L}^{-1}$, f: $160 \mu\text{g L}^{-1}$]

3.1. Effect of pH on CPE

The pH is a critical factor affecting the extractability of organic molecules into the

surfactant-rich phase. The effect of pH on CPE of $100 \mu\text{g L}^{-1}$ of Safranin T was studied in the range of 1–10 by using a buffer series. Fig. 3 shows the influence of pH on CPE of the Safranin T at 532 nm. The maximum signal was obtained by using pH 8.0 buffer. The absorbance values increased until pH 8.0 and then decreased again. The Safranin T dye molecule has two acidic ionization constants as $\text{pK}_{\text{a}1}$:3.0 and $\text{pK}_{\text{a}2}$:8.5, respectively (Guha and Mitta, 1997). It exists as SFH_2^{2+} at low pHs than 3.0 and as SFH^+ in the range of 3–8.5, while it is neutral as SF at higher pHs than 8.5. In our experimental conditions, Safranin T molecules with positive charge form a ion-pair complex with SDS having negative charge. So, the analytical signal increased until pH 8.0 owing to electrostatic interactions. Beyond pH 8.0, it is decreased again because Safranin T is neutral in this range. Therefore, pH 8.0 was chosen for further studies. The used buffer system was prepared by using Britton Robinson series.

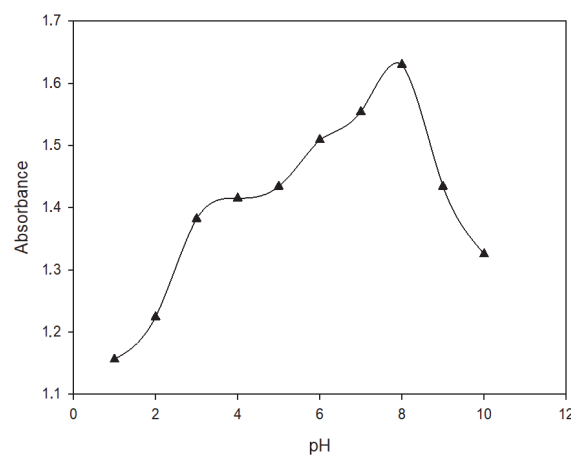


Fig. 3. The effect of pH on CPE

The effective concentration of buffer was also studied and optimized in the range of 0–5 mL for 0.04 M stock solutions. Because of this study, the best signals were obtained with 1.5 mL in the 50 mL of final volume.

3.2. Effect of nonionic surfactant concentration on CPE

A successful CPE should be able to maximize the extraction efficiency through increasing phase volume ratio ($V_{\text{aqueous phase}}/V_{\text{surfactant-rich phase}}$), so as to improve the preconcentration factor. As non-ionic surfactant, Triton-114 and PONPE 7.5 were preferred owing to commercial availability and low costs. In addition, their hazardous properties are so low for human health. Fig. 4 shows changing of absorbance at various surfactant concentrations in the range of 0–0.10 %.

As it can be seen in Fig. 4, analytical signals with Triton X-114 are better than the other surfactant. The best signals were provided by 0.05 % surfactant concentration in the final volume. Extraction efficiency at lower concentrations is so low as

expected due to inadequate transferring of Safranin T molecules to micelle medium.

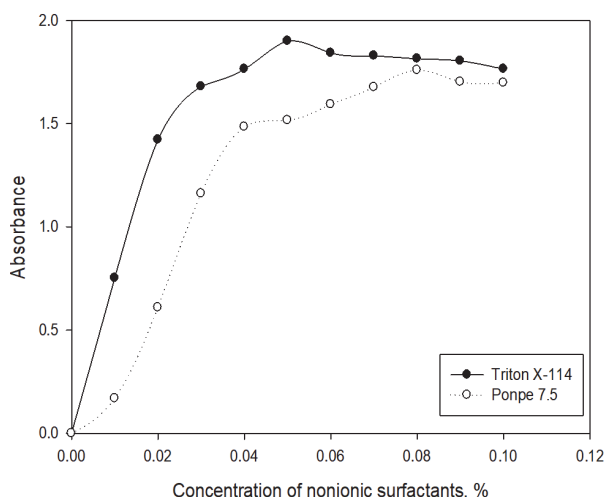


Fig. 4. The effect of nonionic surfactant concentration on CPE

Beyond these concentrations, the analytical signal gradually decreases because of decreasing at solubility of surfactant-rich phase. Therefore, the further studies were followed by a concentration of 0.05 % (v/v) Triton X-114.

3.3. Effect of Ionic surfactant concentration on CPE

In order to obtain highest efficiency in separation and preconcentration of the analyte, mixed micelle-mediated extraction (mixed-MME) system has also been used in CPE. Cloud point (CP) phenomenon is generally observed in non-ionic surfactant solutions (Madrakian et al., 2007).

The extraction of Safranin is carried out by means of the entrance of hydrophobic phenolic rings to surfactant-rich phase. So, organic structure of molecule helps its CPE extraction. Safranin T has already positive charge in the studied pH, a suitable ionic surfactant can increase the extraction efficiency. Cetyl pyridinium chloride (CPC) as cationic surfactant and sodium dodecyl sulfate (SDS) as anionic surfactant were investigated in order to increase the analytical signal.

Two series of model solutions were prepared in the range of $0.1-6.0 \times 10^{-5}$ mol L⁻¹ by using stock SDS and CPC solutions. As predicted, the obtained signals in the presence of SDS are higher than CPC because it facilitates the entrance of Safranin T to micelle media by forming ion association complex. The formed ion-pair complex between SDS and Safranin T is increased the extraction efficiency. As can be seen in Fig. 5, the maximum efficiency was obtained by using 3×10^{-5} mol L⁻¹ SDS. In addition, there is a few articles about together usage of Safranin T-SDS complex for Safranin T removal and SDS determination (Guha and Mittal, 1992; Zaghbani et al., 2008). Results of these studies have been also supported by our experimental data.

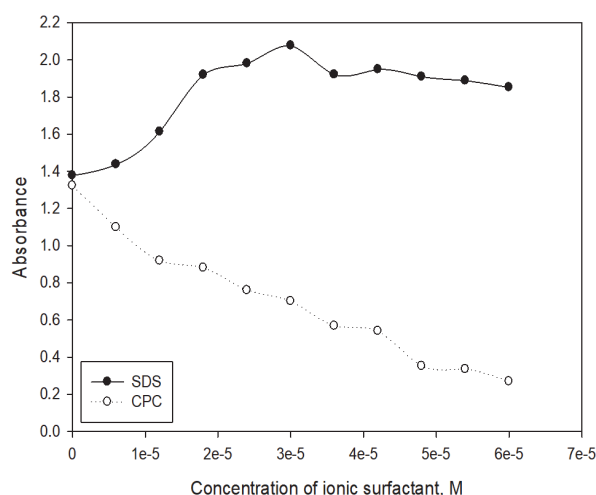


Fig. 5. The effect of ionic surfactant concentration on CPE

3.4. Effect of the incubation temperature and time

Since preconcentration efficiency is affected from equilibration temperature and time, a systematic study was carried out in order to optimize both parameters. The shortest possible extraction time is desired for an ideal procedure. In addition, the high extraction temperatures can be harmful for some sample types. So, it is better to study at low temperatures. The solubilization capability of the micellar system increases with temperature leading to an increase in the dye extraction.

These parameters were studied in the range of 5-50 min and 20-70°C, respectively. It was observed that the formed complex is stable throughout 50 minutes at 50°C. Since it is preferred the shortest time for a fast analysis, ten minute was taken into account as incubation time. So, the solutions were waited at 50°C for 10 minutes in further studies.

3.5. Effects of electrolyte concentration

Cloud point extraction process is effected by some external parameters such as inorganic salts, organic solvents, the other surfactants. Especially, the ionic strength of solutions is strongly effective on cloud point temperature. This is known as salting-out effect (Nascentes and Arruda, 2003). In reality, effluents from textile and dyeing industries would contain a high concentration of salts, which may affect the determination of dye. The effects of NaCl and KCl as ionic salt on CPE of Safranin T were tested in the range of 0.1- 1.0% (w/v). As can be seen in Fig. 6, we couldn't obtain any analytical signal without adding electrolyte. Because the cloud point did not formed, the phase separation could not performed at these conditions. The efficiency of extraction was increased by adding both NaCl and KCl. Although the similar signals were obtained by both electrolytes, signal of analyte in the presence of NaCl is higher than in the presence of KCl. So, a concentration of 0.6 % of NaCl was selected as optimum electrolyte concentration. Of course, the

similar signals can be obtained by using KCl but we preferred NaCl. Electrolytes increased the efficiency of CPE and have not negative effect on CPE even at high concentrations.

The fact that, addition of NaCl electrolyte increased the size of the micelles and aggregation number, thus, enhancing the analyte to be more soluble in the SRP so, more water goes to the dilute phase as a result of salting – out effect.

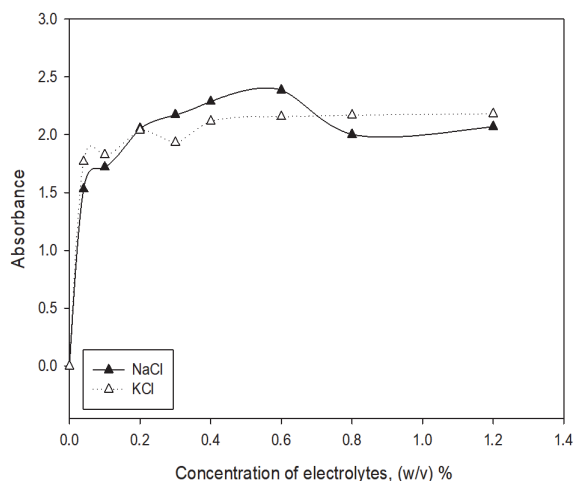


Fig. 6. The effect of electrolyte concentration

3.6. The effect of solvent for surfactant-rich phase

The surfactant rich phase obtained after CPE is viscous for spectrophotometric measurements. So, it needs to dissolve and dilute before analysis. Choosing of the most suitable solvent is so important.

The selected solvent must completely dissolve SRP and it should include any interfering effect on determination step.

Therefore, various solvents like ethanol, methanol, THF, acetonitrile, and water were studied to obtain the maximum extraction efficiency. As can be seen in Fig. 7, the maximum signal was obtained by ethanol. The lowest possible volume of solvent was taken into account in order to obtain the maximum preconcentration factor. So, 1.5 mL of ethanol was used in order to dissolve the SRP before absorbance measurements.

3.7. Interference studies

The influences of foreign (interfering) ions and some dye molecules on the determination of Safranin T were studied and an error of $\pm 5\%$ in the absorbance reading was considered tolerable. Model solutions containing $100 \mu\text{g L}^{-1}$ of Safranin T and different concentration of interfering species were prepared and the developed procedure was applied to test their effects. Similar dyes which have the maximum absorbance about 530 nm and common ions were selected and studied at experiments. The results presented in Table 1 show that the common coexisting species did not have significant effect on the determination of Safranin T.

The proposed method was successively applied for determination Safranin T in water samples. In addition, since Safranin T is a cationic dye and not a azo dye, it is not formed any metallic complex with cations. So, the method can be applied selectively for the determination of Safranin T.

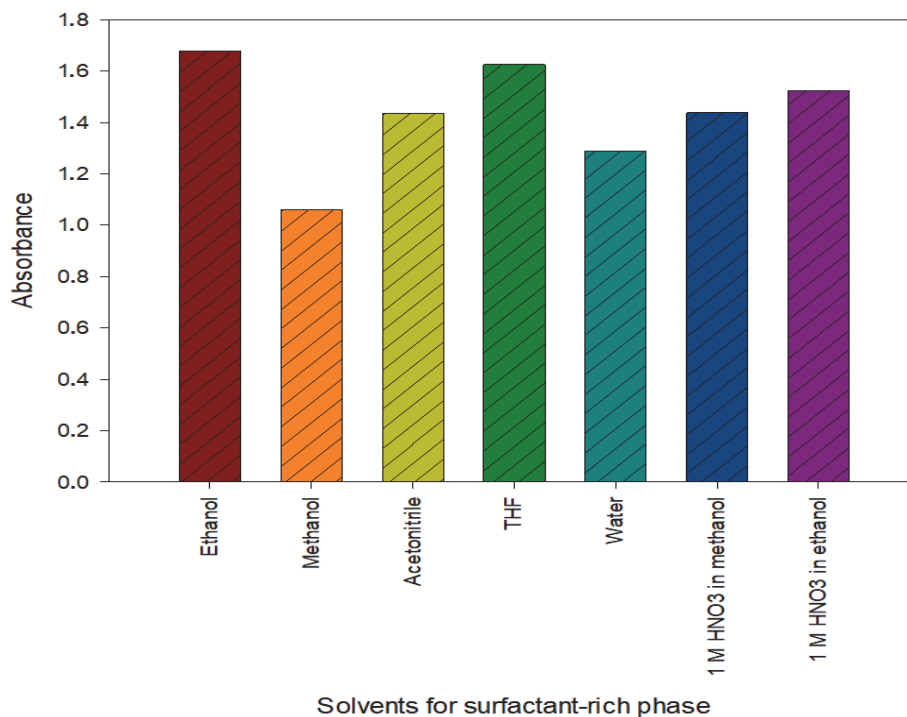


Fig. 7. The chosen of solvent for surfactant rich phase

Table 1. Tolerance limits of foreign ions for determination of 100 µg L⁻¹ Safranin T

<i>Interfering Species</i>	<i>Tolerance limit /[Interfering ion/ Analyte] ratio</i>
K ⁺ , Na ⁺ , NH ₄ ⁺ , (as chloride salts) Cl ⁻ , SO ₄ ²⁻ (as potassium salts)	5000
Ca ²⁺ , Mg ²⁺ , Ba ²⁺ (as nitrate salts)	1000
Cd ²⁺ , Zn ²⁺ , Pb ²⁺ (as nitrate salts) Basic Fuchsin (λ _{max} : 543 nm)	500
Methyl orange (λ _{max} : 507 nm) Pyronine B (λ _{max} : 553 nm) Co ²⁺ , Ni ²⁺ , Cu ²⁺ (as nitrate salts)	300
Rodamine B (λ _{max} : 525 nm) Erythrosin (λ _{max} : 525 nm) Fe ²⁺ (as nitrate salt)	100
Neutral Red (λ _{max} : 533 nm)	20

3.8. Analytical characteristics

Table 2 summarizes the analytical characteristics of the proposed method such as regression equation, linear range, and limits of detection and quantification, repeatability and preconcentration factor. A linear calibration graph in the range of 4–200 µg L⁻¹ of Safranin T in the initial solution was obtained using the optimized conditions. The limits of detection and quantification were 1.14 and 3.87 µg L⁻¹, respectively. The preconcentration factor was calculated by using initial and final volume of samples. The initial volume of solution was 50 mL and final volume was 1.5 mL prior to absorbance measurements. So, this factor was found as 33.3. In addition, enrichment factor was also calculated as proportion of slopes of calibration graphs with and without CPE. As can be seen, the proposed preconcentration method provides important analytical properties like a good enrichment factor as 41.

3.9. Determination of Safranin T in environmental water samples

The accuracy and validity of the proposed method were checked by spiking in various water samples. The collected samples were analyzed according to proposed method.

Repeatability was evaluated by repeating (n:5) the proposed approach for each sample. Results of this study were presented in Table 3. For validation of new developed method, recovery experiments at spiked concentration levels were also added to Table 3.

4. Conclusions

In this study, it was proposed an efficient and sensitive determination method for Safranin T in real samples based on cloud point extraction. The method provides substantial green benefits withusing so limited organic solvents in extraction procedure. The proposed method includes a simple experimental procedure and offers sensitive, selective, and simple approach. The needing equipment for the proposed method can be simply supplied by every routine analysis laboratory.

The extent of extraction is markedly influenced by using mixture of an anionic surfactant (SDS) and a non-ionic surfactant (Triton X-114). The number of methods in literature for determination of Safranin T is so limited due to problems in sensitivity. A few chromatographic were presented for its determination. The methods in literature are generally presented as removal procedures for Safranin T, but the prosed method can determine it at trace levels.

Table 2. Analytical characteristics of the proposed method

<i>Parameters</i>	<i>The Values</i>	
	<i>With CPE</i>	<i>Without CPE</i>
Linear range	4–200 µg L ⁻¹	500–25000 µg L ⁻¹
Slope	0.0122	0.0003
Intercept	0.0184	0.0107
Correlation coefficient (r ²)	0.9838	0.9915
Recovery % (n: 5)	98.7-103.5	99.2-102.5
RSD (%)	2.7 and 1.8 (10 and 100 µg L ⁻¹ , n: 5)	2.5 and 1.6 (1 and 10 mg L ⁻¹ , n: 5)
LOD (µg L ⁻¹)	1.14	140
LOQ (µg L ⁻¹)	3.87	465
Preconcentration factor ^a	33.3	-
Enrichment factor ^b	41	-

^a Preconcentration factor is defined as the ratio of the initial solution volume to the volume of surfactant rich phase

^b Enrichment factor is defined as ratio of slopes of calibration graphs obtained with and without CPE

Table 3. Determination of Safranin T in environmental water

Sample	Added Safranin T ($\mu\text{g L}^{-1}$)	Found		
		Safranin T ($\mu\text{g L}^{-1}$)	Recovery (%)	RSD (%) ³
Tap Water	-	-	-	-
	50.00	50.78±1.45	101.6	2.85
	100.00	102.93±2.52	102.9	2.45
River Water (Kızılırmak River, Sivas, Turkey)	-	-	-	-
	50.00	49.57±1.41	99.1	2.84
	100.00	103.16±2.52	103.2	2.44
Industrial Wastewater ¹	-	9.71±0.28	-	2.98
	50.00	60.51±1.85	101.3	3.05
	100.00	105.12±3.12	95.8	2.96
Industrial Wastewater ²	-	12.24±0.32	-	2.61
	50.00	64.43±1.82	103.5	2.82
	100.00			2.81

¹The sample was taken from the inlet point of wastewater treatment plant; ²The sample was taken from outlet point of textile factory; ³RSD means Relative standard deviation

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