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ARTICLES

Separation, Preconcentration and Spectrophotometric Determination of Rhodamine B in Industrial, Cosmetic and Water Samples by Cloud Point and Solid Phase Extraction[1]

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Abstract—The aim of this study is extraction, preconcentration and spectrophotometric determination of Rhodamine B (**RB**) in aqueous media by developing solid phase extraction (**SPE**) and cloud point extraction (**CPE**) methods. Amberlite XAD-1180 adsorbent and Tergitol NP-7 surfactant were used for SPE and CPE, respectively. Parameters of SPE and CPE which effected quantitative extractions were investigated and optimized. Matrix effects of some ions and dyes were analyzed at the optimum conditions. Developed methods were used to determine RB contents of anti-freeze, lipstick and water samples. The results of both methods demonstrated that the RB was quantitatively extracted and determined. RB contents of solid samples were found between 473 ± 15 and 317 ± 8 μg/g; 472 ± 11 and 312 ± 6 μg/L dye contents were determined for liquid samples. The methods were tested by analysis of spiked samples. Analytical characteristics of the methods were compared with each other and previously reported studies.

Keywords: Rhodamine B, cloud point extraction, solid phase extraction, Tergitol NP-7, Amberlite XAD-1180

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Rhodamine B is an amphoteric, highly water soluble, non-volatile, fluorescent dye of the xanthene class widely used [1] to give fluorescent violet color to soft drinks, paper, leather, ink, food, silk, cotton, acrylic fiber, wool, industrial and cosmetic products [2]. It has a considerably high stability and resistance to photo and oxidative degradation. If swallowed or exposed to human beings and animals it causes irritation to skin, eyes and respiratory system. The carcinogenicity and toxicity of RB over animals and humans have also been proved [3]. The use of RB has been banned and regulated in some countries due to its carcinogenic, mutagenic and toxic effect on all living organisms [4].

Various analytical methods such as CPE [5], SPE [6], molecularly imprinted solid phase extraction (MISPE) [7], liquid-liquid extraction [8] and HPLC [9] were developed for separation and preconcentration of trace level of dye. Among these pretreatment options, adsorption and cloud point extraction appear to have significant potential for the extraction, determination and removal of the dye from waters.

Determination of analytes by the CPE technique is based on the separation of two distinct phases in a solution. At the beginning the solution consists of target species, surfactant molecules and water. When the temperature of the solution rises above the cloud point temperature at which the solution becomes turbid [10], the surfactant molecules become insoluble in the solution to form micelles (also called as surfactant rich phase) [11]. These micelles can bind to analytes and have a very small volume compared to aqueous phase. Removing the phases is simple, and high enrichment factors [12] for the analytes can be gained. This allows determining the dye molecules in aqueous media by using CPE technique.

Solid phase extraction is another technique based on the adsorption of analyte onto a solid phase filled in a column. There are two phases in this technique, i.e. stationary and mobile phases. Aqueous sample containing the target analyte passes through the chromatographic column after providing specific conditions for retention of the analyte on the resin. Retained analyte is eluted from the resin by using appropriate eluents and diluted to desired volume. Thus, gaining effective separation results and high enrichment factors are possible by using SPE technique [13].

Different methods devoted to extraction and preconcentration of Rhodamine dyes have been developed, but the published CPE methods are operated at

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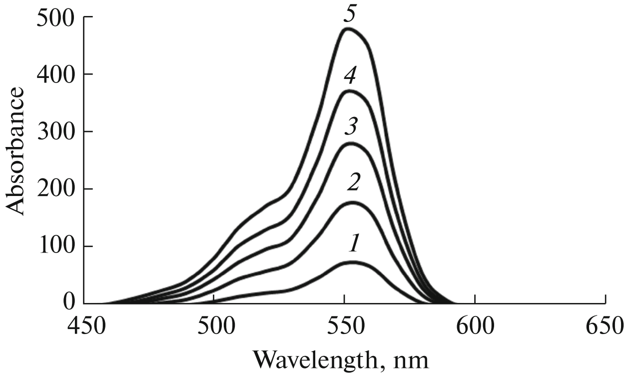

Fig. 1. UV-Vis spectra of Rhodamine B: (*1*), pure dye, (*2*), anti-freeze, (*3*), lipstick, (*4*), after CPE, (*5*), after SPE.

high temperatures. Previously reported studies have higher LOD values and lower preconcentration factors than our methods. RB dye can be determined with characteristics comparable with our methods. Accuracies and precisions of the results of real samples analysis for RB can be evaluated comparatively with concurrent applications of CPE and SPE.

EXPERIMENTAL

Materials. Chemicals and reagents used in experiments were of analytical grade and purchased from Merck (Darmstadt, Germany) and Sigma–Aldrich (MO, USA). These chemicals were used without further purification.

A 1000 mg/L RB stock solution was prepared, and further diluted solutions were prepared daily from this stock solution. Buffer solutions were phosphoric acid buffers (0.1 M) of pH 2 and 3, ammonium acetate buffers (0.1 M) of pH 4–5, phosphate buffer (0.1 M) for pH 7 and 8.

Tergitol NP-7 is a non-ionic alkylaryl polyether alcohol (Sigma–Aldrich, USA) surfactant with a low cloud point of 20°C at 1 wt % and used in CPE without further purification.

Amberlite XAD-1180 is a polystyrene divinylbenzene copolymer purchased from Sigma–Aldrich (MO, USA). A 0.5 g weight of Amberlite XAD-1180 resin was slurried with distilled water in a beaker and poured into the glass column, after which the resin was washed with methanol, water, 1 M HNO_3 in acetone, water, 1 M NaOH, and water, sequentially, in order to eliminate trace metal ions and other inorganic and organic contaminants in the resin.

Apparatus. The UV-Vis spectra were recorded and monitored using a Shimadzu UV-160 A (Kyoto, Japan) model spectrophotometer. For this purpose, the respective UV-Vis absorbance values were recorded at 556 nm which is the maximum wavelength of RB.

The pH of the solutions was measured using a Hanna HI-221 pH meter (RI, USA) and adjusted by appropriate buffer solutions. A Nuve BM 402 (Ankara, Turkey) thermostatic bath was used for temperature adjustments in CPE. The height of the column used in SPE was 15 cm, having a diameter of 1.0 cm with a porous disk.

Real sample preparation. 25 mg of each lipstick sample were accurately weighed and transferred to 50 mL beaker. 25 mL of CCl_4 was added and dissolved by mechanical stirrer for 10 min at room temperature. According to our experimental studies lipstick samples could not be solved any solvents except CCl_4. After complete dissolution of the sample it was transferred into a separatory funnel. Approximately 150 mL of 0.1 M NaOH solution was also added to the separatory funnel and shaken for 10 min. After complete extraction of dye to aqueous medium from organic phase, the samples were filtered through a 0.45 μm PTFE membrane and transferred to beakers. The pH values of these samples were adjusted to 2.5 using phosphate buffer solutions.

Liquid antifreeze and water samples were directly applied to developed methods after filtration through a 0.45 μm PTFE membrane, pH adjustments and necessary dilutions.

Solid phase extraction procedure. 50 mL of each aqueous sample solution containing 2.5 μg RB was prepared. After that, the solutions were adjusted to pH 2.5 and passed through the column gravitationally. RB, retained on the Amberlite XAD-1180, was eluted to final volume of 5 mL with ethanol. The RB concentration in the eluent was determined by UV-Vis spectrophotometry at 556 nm.

Cloud point extraction procedure. 50 mL of test solution containing 2.5 μg RB molecules and 2.5 mL of 2% (w/v) Tergitol NP-7, and 10.0 mL buffer solution was added. The tube was capped. After shaking the solution, it was placed into a thermostatic bath at 25°C for 30 min. After the separation of two phases, the turbid solution was centrifuged at 4000 rpm for

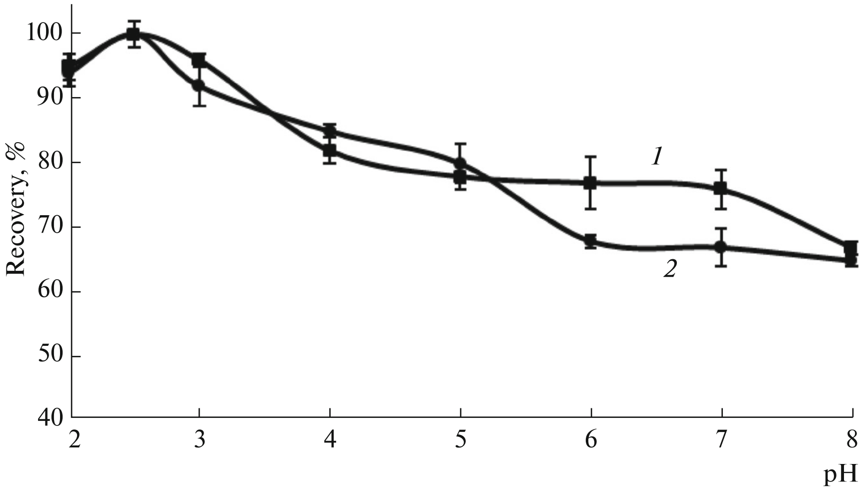

Fig. 2. Influence of pH on the recovery of Rhodamine B for SPE (*1*) and CPE (*2*) methods (*n* = 4).

7 min to separate the surfactant-rich phase containing RB. After centrifugation simple decantation procedure was performed for aqueous phase. The viscous surfactant-rich phase was dissolved with 0.5 mL of ethanol. This solution was transferred to a 2 mL volumetric flask and diluted with deionized water up to 2 mL of final volume. The absorbance of the solution was measured at 556 nm.

RESULTS AND DISCUSSION

UV-Vis spectra of RB dye after SPE and CPE and in pure aqueous solution, lipstick and anti-freeze samples are given in Fig. 1.

Effect of pH. It is the most important parameter for retention of target analyte on the resin and quantitative recoveries for SPE studies. Importance of pH in CPE studies is the most effective parameter for formation of micelles surrounding the dye molecule. Influence of pH for extraction of RB by two methods was investigated between 2 to 8. Experimental results are given comparatively in Fig. 2 with standard deviations. As shown in Fig. 2, quantitative recoveries were obtained for both methods at the pH range of 2–3. Above pH 3 the recovery was not quantitative and decreased with increasing solution pH. Thus, pH 2.5 was chosen as optimum and all subsequent experiments were performed at that pH for both methods.

Influence of variables for solid phase extraction. Sample and eluent flow rates were investigated in the range of 1 to 10 mL/min. The recovery values were given comparatively in Fig. 3a with standard deviations. Extraction of RB was quantitative up to sample flow rates of 2 mL/min, and quantitative recoveries for RB were obtained up to 3 mL/min of eluent flow rate.

Influence of sample volume on the extraction of RB was examined between 25 to 300 mL of sample volume. As it can be seen in Fig. 3b, extraction of RB was quantitative up to 200 mL of sample volume. Further, 200 mL of sample volume was employed to obtain high preconcentration factor. Therefore, a preconcentration factor of 40 could be achieved with 200 mL of the sample volume and 5 mL of final volume.

Different types of solvents and their mixtures were used to investigate the effect of eluent on the recovery of RB. The results are given in Table 1 with standard deviations. Quantitative recovery values were obtained for RB when used 1 M HNO_3 in methanol, ethanol and acetonitrile as eluent. In view of economics and

Table 1. Effect of eluent on the recovery of Rhodamine B (eluent volume: 5 mL, *n* = 4)

Eluent type	Recovery, %[a]
Methanol	76 ± 1
1 M HNO_3 in methanol	95 ± 2
25% Ethanol + 75% H_2O	21 ± 0
50% Ethanol + 50% H_2O	62 ± 0
75% Ethanol + 25% H_2O	85 ± 2
Ethanol	100 ± 1
25% Acetonitrile + 75% H_2O	4 ± 1
50% Acetonitrile + 50% H_2O	47 ± 6
75% Acetonitrile + 25% H_2O	94 ± 4
Acetonitrile	99 ± 1

[a] Mean ± standard deviation.

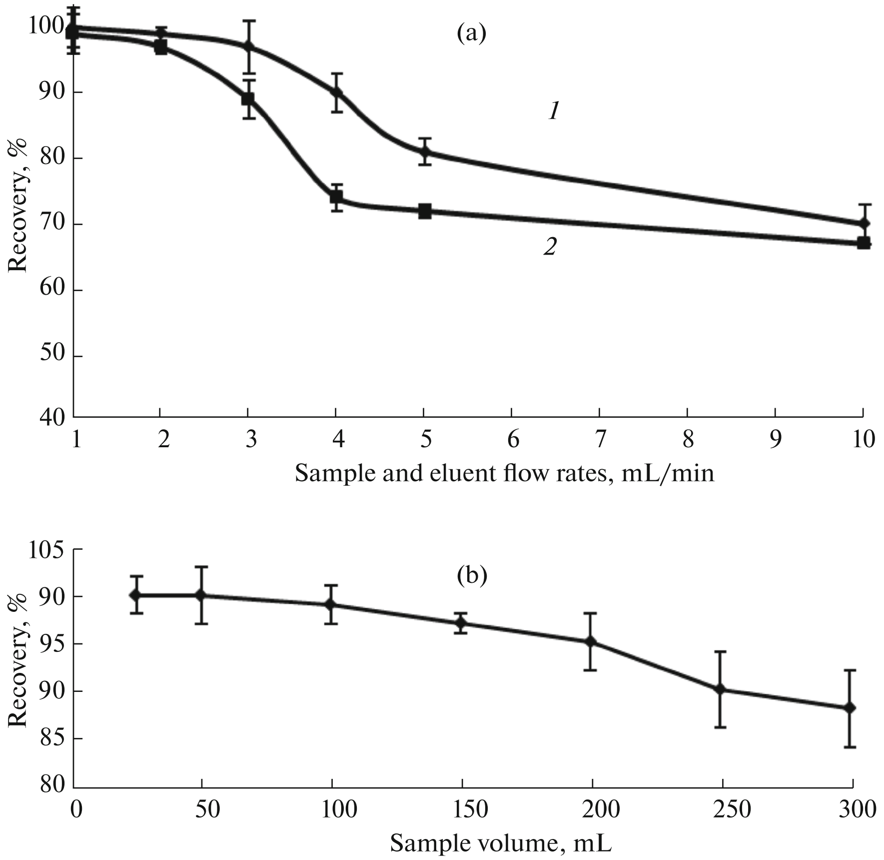

Fig. 3. Effect of sample flow rate (*1*), eluent flow rate (*2*) (a) and sample volume (b) on the extraction of Rhodamine B for SPE method ($n = 4$).

availability of ethanol, 5 mL of ethanol was used as eluent all further applications.

Effect of variables for cloud point extraction. Influence of surfactant concentration on the recoveries of RB dye was studied by varying Tergitol NP-7 concentration of the model solutions. Different surfactant concentrations between 0.05 and 0.60% (w/v) were applied. As given in Fig. 4a, recoveries were not quantitative up to 0.50% (w/v) concentration of surfactant. Recovery values increased with increasing surfactant concentration. Recovery values were constant and quantitative after 0.50% (w/v) Tergitol NP-7 concentration. Therefore, 0.50% (w/v) surfactant concentration was selected and all further experiments were performed applying this surfactant concentration.

The dependences of extraction efficiency upon equilibration temperature and incubation time above the cloud point in the range of 25–45°C and 5–40 min were thoroughly optimized. The results in Fig. 4b showed that an equilibration temperature of 25°C was adequate to achieve quantitative extraction. No considerable changes were observed after 30 min of incubation time; 30 min at 25°C was found optimum.

It was found that the increase of centrifuging rate and time had no considerable effect upon the extraction efficiency and analytical signal. The influence of centrifuge time and rate on CPE was studied in the range of 1–10 min and 1000–4000 rpm, respectively. It was observed that, 4 min centrifuging time and 4000 rpm were adequate to achieve quantitative extraction. No appreciable improvements were observed for longer times and more centrifuge rates. The centrifuging time of 4 min at 4000 rpm was selected for the subsequent experiments.

Effect of sample volume on the recovery of RB was examined by varying the sample volume from 25 to 50 mL. The sample volume did not affect quantitative recoveries in optimum conditions mentioned above. According to maximum sample volume of 50 and minimum final solution of 2 mL, the preconcentration factor of the CPE method was obtained as 25.

Effect of matrix. The effects of matrix ions in spiked samples on the recovery of RB were also investigated. Results are given comparatively in Table 2. There were no interferences in the presence of large amounts of alkaline earth metals, heavy metals, main anions and

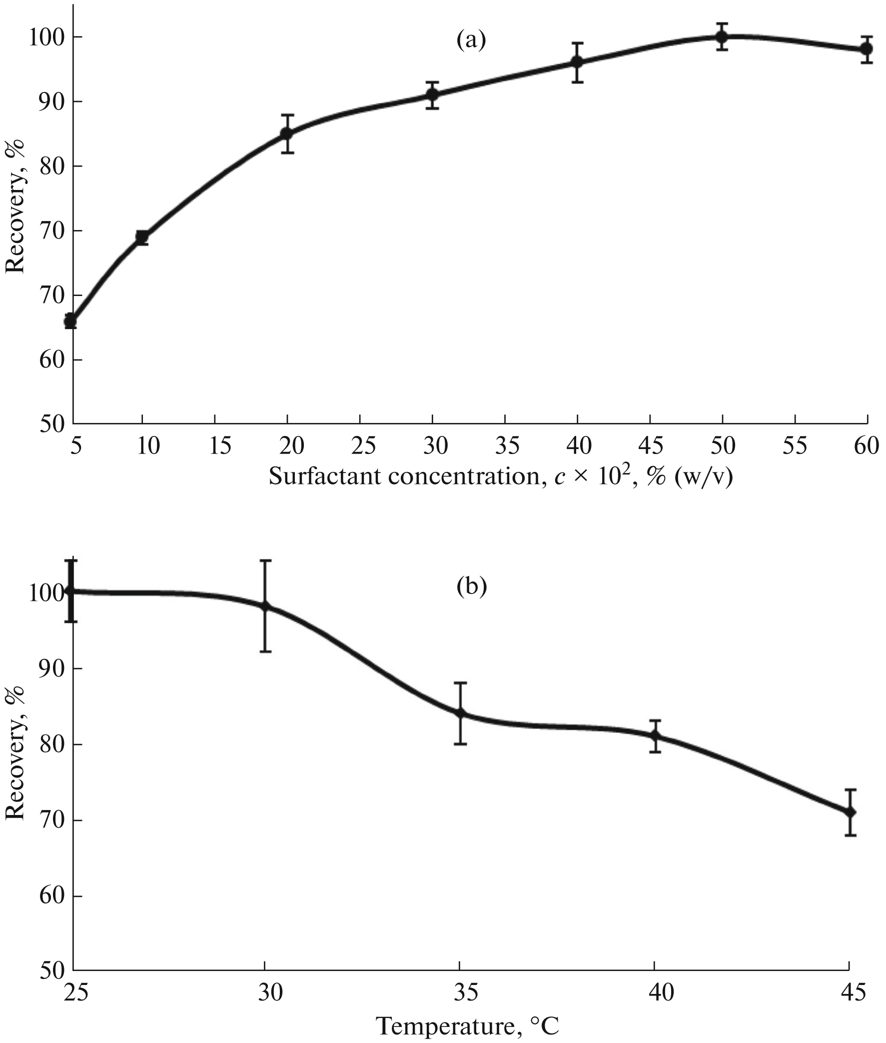

Fig. 4. Effect of surfactant concentration (a) and temperature (b) on the extraction of Rhodamine B for CPE method ($n = 4$).

widely used dyes. Anions, cations and dyes which may exist with RB in real samples were used.

Application to real samples. The methods were successfully applied to different lipstick, anti-freeze and water samples to determine their RB contents. Reliability, repeatability and applicability of the methods were proved by standard addition technique. Accuracies of the methods were performed comparatively with determination of RB contents in real samples. Results are given in Table 3.

Analytical characteristics of the method. Optimal analytical parameters of the proposed methods are given comparatively in Table 4. According to the experimental results, the detection and quantitation limits of the CPE method were lower than those of the SPE method. Preconcentration factor of SPE method was higher than for CPE method. Characteristics of the methods, including pH, eluent and %RSD, were approximately equal. Linear dynamic ranges of the methods obtained were 0.05–5.0 and 0.1–4.0 µg/mL with equations of $A = 0.1973c + 0.0019$ ($R^2 = 0.9998$) and $A = 0.1987c + 0.0012$ ($R^2 = 0.9996$) for CPE and SPE methods, respectively, where A is absorbance unit and c is concentration of RB in µg/mL. Inter- and intra-day precision experiments were performed and results are given in Table 5.

CONCLUSIONS

The analytical parameters of the proposed methods are almost same. In view of high preconcentration factor and %RSD, the SPE method is better than CPE. On the other hand, detection and quantitation limits of the CPE method are slightly better than those of the SPE method. Good results were obtained in recovery and determination studies. Preconcentration

Table 2. Effect of matrix ions and dyes ($n = 4$)

Ion/dye	Added as	Concentration, µg/mL		Recovery, %[a]	
		SPE	CPE	SPE	CPE
Ni^{2+}	$Ni(NO_3)_2 \cdot 6H_2O$	100	100	99 ± 1	98 ± 1
Cd^{2+}	$Cd(NO_3)_2 \cdot 6H_2O$	100	100	100 ± 1	101 ± 1
Pb^{2+}	$Pb(NO_3)_2$	100	100	95 ± 2	98 ± 2
Co^{2+}	$Co(NO_3)_2 \cdot 6H_2O$	100	100	104 ± 1	102 ± 3
Cu^{2+}	$Cu(NO_3)_2 \cdot 5H_2O$	100	100	100 ± 2	99 ± 2
Cr^{3+}	$Cr(NO_3)_3 \cdot 3H_2O$	25	25	106 ± 4	101 ± 2
Al^{3+}	$Al(NO3)_3 \cdot 9H_2O$	50	50	98 ± 2	99 ± 3
Na^+	$NaNO_3$	1000	1000	95 ± 1	99 ± 1
K^+	KNO_3	1000	1000	95 ± 2	97 ± 2
Ca^{2+}	$Ca(NO_3)_2 \cdot 4H_2O$	1000	1000	97 ± 4	99 ± 3
Mg^{2+}	$Mg(NO_3)_2 \cdot 6H_2O$	1000	1000	95 ± 2	96 ± 2
Cl^-	NaCl	1000	1000	94 ± 2	98 ± 1
NO_2^-	$NaNO_2$	100	100	95 ± 2	99 ± 3
SO_4^{2-}	Na_2SO_4	100	100	94 ± 1	96 ± 1
NO_3^-	$NaNO_3$	100	100	97 ± 3	99 ± 2
Sunset yellow	–	0.2	0.2	95 ± 2	101 ± 3
Tartrazine	–	0.2	0.2	94 ± 1	98 ± 2
Methylene blue	–	0.1	0.1	95 ± 2	102 ± 2
Amaranth	–	0.2	0.2	95 ± 2	99 ± 3

[a] Mean ± standard deviation.

Table 3. Recovery studies with analyte addition technique and determination of Rhodamine B contents in real samples ($n = 4$)

Sample	Added, µg		Found, µg[a]		Recovery, %[a]		Content of RB[a]	
	CPE	SPE	CPE	SPE	CPE	SPE	CPE	SPE
Lipstick A	–	–	1.49 ± 0.01	1.49 ± 0.03	–	–	473 ± 15[b]	472 ± 11[b]
	0.75	0.75	2.21 ± 0.05	2.24 ± 0.04	96 ± 2	100 ± 2	–	–
	1.50	1.50	2.90 ± 0.03	2.94 ± 0.04	94 ± 1	98 ± 1	–	–
Lipstick B	–	–	1.12 ± 0.03	1.10 ± 0.06	–	–	317 ± 8[b]	312 ± 6[b]
	0.75	0.75	1.76 ± 0.03	1.76 ± 0.04	85 ± 1	88 ± 2	–	–
	1.50	1.50	2.49 ± 0.02	2.55 ± 0.08	91 ± 1	97 ± 3	–	–
Anti-freeze A	–	–	2.77 ± 0.06	2.77 ± 0.09	–	–	11.1 ± 0.2[c]	11.1 ± 0.4[c]
	1.50	1.50	4.14 ± 0.06	4.13 ± 0.09	91 ± 1	91 ± 2	–	–
	3.00	3.00	5.59 ± 0.05	5.55 ± 0.17	96 ± 1	96 ± 4	–	–
Anti-freeze B	–	–	1.67 ± 0.04	1.67 ± 0.07	–	–	6.7 ± 0.2[c]	6.7 ± 0.3[c]
	1.50	1.50	3.13 ± 0.02	3.12 ± 0.04	97 ± 1	97 ± 1	–	–
	3.00	3.00	4.67 ± 0.07	4.65 ± 0.08	100 ± 2	99 ± 3	–	–
Tap water	–	–	BDL[d]	BDL	–	–	ND[e]	ND
	2.50	2.50	2.51 ± 0.08	2.41 ± 0.07	100 ± 1	96 ± 3	–	–
	2.50	5.00	4.95 ± 0.05	5.09 ± 0.05	99 ± 3	102 ± 1	–	–
Waste water	–	–	BDL	BDL			ND	ND
from textile factory	2.50	2.50	2.48 ± 0.06	2.46 ± 0.09	99 ± 4	98 ± 4	–	–
	5.00	5.00	4.89 ± 0.04	4.86 ± 0.03	98 ± 2	97 ± 1	–	–
Waste water	–	–	BDL	BDL			ND	ND
from leather factory	2.50	2.50	2.51 ± 0.04	2.57 ± 0.07	100 ± 1	103 ± 3	–	–
	5.00	5.00	5.06 ± 0.06	5.16 ± 0.05	101 ± 2	103 ± 1	–	–

[a] Mean ± standard deviation; [b] µg/g; [c] µg/mL; [d] BDL: below detection limit; [e] ND: not determined.

Table 4. Comparison of the characteristics of two methods

Parameter	CPE	SPE
pH	2.5	2.5
Eluent	Methanol	Ethanol
Maximum sample volume, mL	50	200
Preconcentration factor	25	40
Detection limit, μg/L	0.7	1.2
Limit of quantitation, μg/L	1.9	3.2
Linear dynamic range, μg/mL	0.05–5.0	0.1–4.0
RSD, %	<7	<4

Table 5. Intra- and inter-day precision of the methods for determination of Rhodamine B ($n = 4$)

Method	Added, μg	Found, μg	RSD, %	Relative recovery, %
Inter-day				
SPE	2.50	2.46	3.76	98
	5.00	4.78	3.03	96
CPE	1.00	0.97	7.06	97
	2.00	1.93	2.90	96
Intra-day				
SPE	2.50	2.41	2.98	96
	5.00	5.09	1.07	102
CPE	1.00	0.99	5.09	99
	2.00	1.97	1.39	98

Table 6. Performance characteristics of some recent studies on the determination of Rhodamine B

Detection method	Preconcentration method	Duration, min	PF[a]	LOD, μg/L	RSD, %	Reference
UV-Vis	SPE	Unavailable	40	3.14	5.0	[14]
UV-Vis	CPE	30	8.5	1.30	2.4	[4]
Spectrofluorimetry	CPE	15	2.5	0.24	1.46	[15]
UV-Vis	DLLME[b]	5	10	2.39	2.88	[16]
Spectrofluorimetry	CPE	22	2.5	1.40×10^{-2}	–	[3]
UV-Vis	DLLME	18	20	1.93	4.7	[17]
UV-Vis	DLLME	18	20	0.48	4.4	[17]
UV-Vis	SPE	30	40	1.20	5.0	Present study
UV-Vis	CPE	30	25	0.70	6.0	Present study

[a] PF: preconcentration factor; [b] DLLME: dispersive liquid-liquid micro-extraction.

factors, limits of detection and %RSD values of the methods are comparable with other studies. Comparison with the other methods is given in Table 6.

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