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RESEARCH

Simultaneous Preconcentration and Determination of Nile Blue A and Rhodamine B in Aqueous Samples by the Cloud Point Extraction Coupled with the Solution Scanometric Method

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ABSTRACT

Scanometric method has been attended for the study and determination of different analytes. This method coupled with preconcentration methods for improvement the detection limit and applied for the determination of Nile blue and Rhodamine B in binary systems.

In this work cloud point extraction-scanometry (CPE-SC) method was used for preconcentration, simultaneous separation and determination of trace amounts of the rhodamine B (RhB) and Nile blue A (NBA) in aqueous solutions. Some of the advantages of this method are simplicity, cheapness, novelty, rapidity, sensitivity, and safety. Analysis of images obtained from the solution scanning after cloud point extraction of the cited dyes and dilution with proper solvent is done using the RGB program in Visual Basic 6 (VB 6) media. Using three red, green, and blue factors, the RhB and NBA contents were investigated in the aqueous solution. Detection limits of the determination of these dyes were acceptable, and their values were 0.002 and 0.008 µg/mL for NBA and RhB, respectively. The linear ranges of the mentioned method for determining Nile blue A and rhodamine B are 0.01–1.33 µg/mL and 0.01–1.00 µg/mL, respectively.

KEYWORDS

Nile blue A, Rhodamine B, Solution scanometric method, preconcentration, simultaneous determination.

INTRODUCTION

Today, many industrial activities increase environmental contamination, especially through the production of dye pollutants [**1**]. Lately, water pollution and contaminated water have attracted many environmentalists and scientists for water treatment. Water refinery technology is necessary for human and environmental health that is constantly in progress [**2**]. In this research, a method for water treatment is presented, and the amount of the rhodamine B and Nile blue A were determined simultaneously in aqueous media.

Nile blue A ([9-(diethylamino) benzo[*a*]phenoxazin-5-ylidene] azanium sulfate) also is named basic blue 12 and Nile blue sulfate (**Fig. 1a**). It is a nontoxic cationic and fluorescent phenoxazine dye [**3**]. It is a solvatochromic dye [**4**] that was employed for the indication of the neutral acids from the fatty acids [**5**], biological and histological staining [**6,7**], as a proper probe for pH [**8**], a reagent in fingermarks in alcoholic media [**9**] and for intercalating in the main groove of DNA [**10**]. The High affinity of NBA to cancerous cells, optical sensitivity of it to oxygen, and being a photosensitizer cause it to be useful in phototherapy to treat malignant tumors [**11**].

Nile blue A is dangerous for the respiratory system and stimulates the eyes and skin [**12,13**]. According to the above description, the determination of this dye from the environment has notable importance. So far, there has not been a report on the measurement of this dye in the environment.

Rhodamine B is a cationic and tracer dye to determine the flow rate and transportation of the fluorescent molecules. It is a triphenylmethane dye (**Fig. 1b**) applied for enzyme-linked immunosorbent assay, staining fluorescent dye, pot-metal glass, making fireworks and

crackers, and dyeing in textile, paper, paints, and leathers [**14-16**]. Its intense pink color causes wide uses it in the cosmetic, drug, and food industries [**17**].

RhB, in its cationic form, is toxic, and it may cause allergic dermatitis, skin irritation, and mutations [**18, 19**]. Thus, RhB contaminated wastewater treatment has environmental and commercial importance [**20**].

In this way, the content of RhB was determined by UV-Vis spectrophotometry [**21,22**], high-performance liquid chromatography (HPLC) [**23,24**], magnetic solid-phase extraction coupled with fluorescence spectrophotometry [**25**] and HPLC with fluorescence detection (HPLC-FLD) [**26**] in various samples such as lipstick samples, wastewater, soft drink, food, and aqueous samples.

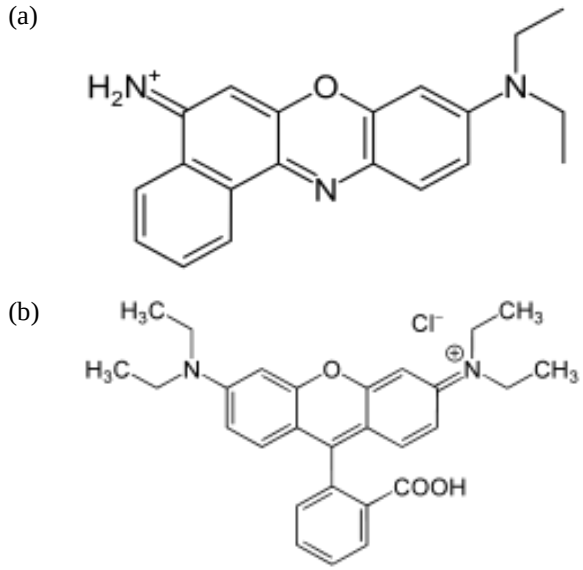

Fig. 1. Chemical structures of (a) Nile blue A and (b) rhodamine B.

The various separation and preconcentration methods such as liquid-liquid extraction [**27,28**], differential spectrophotometry [**29**], ion exchange [**30**], coprecipitation [**31,32**], solid-phase extraction [**33,34**], voltammetry [**35**], CPE-spectrophotometry [**36,37**], CPE-MSPE [**38**] and CPE-scanometry [**39-43**] have been developed in analytical chemistry. One of the applicable separation methods in analytical chemistry is the cloud point extraction approach developed in recent years [**44,45**]. According to the principles of green chemistry, CPE uses water instead of organic solvents because of the very low usage of organic solvents. This approach's other advantages include high efficiency, low cost, speed, simplicity, and low toxicity.

Determination of dyes due to their effect on environmental pollution has attracted special attention [**4648**]. Saving time and cost can determine dyes simultaneously. As you know, a simultaneous determination is one of the important issues in analytical chemistry. Different methods have been employed for the simultaneous determination of dyes in different media and mixture samples [**49-51**].

Scanometry method has been attended for the study and measuring of various analytes [**52-55**]. This method coupled with preconcentration methods to improve the detection limit, and applied for the determination of analytes in single and binary systems [**39-43, 56**].

Considering the mentioned losses, damages, benefits and wide applications in various industries of Nile blue and rhodamine B dyes, it is important to determine trace amounts of them in different samples.

In this research, CPE-Scanometry as a facile, fast, cheap and sensitive method was applied for simultaneous separation, preconcentration, and determination of Nile blue and rhodamine B in aqueous samples.

EXPERIMENTALS

Apparatus and Materials

The cells that contain a sheet of Plexiglas® was built with 1000 µL volume for each [**57**]. A Canoscan LiDE 200 flatbed scanner with 300 dpi horizontal and vertical scan resolution was applied to scan These Plexiglas® cells. Pictures of the scanned cells converse to the RGB data by the special software in visual basic 6 media [58]. In order to adjust the desired pH values, a Metrohm digital pH meter Model 827 (Herisau, Switzerland) was employed with a combined glass electrode. A 100-1000 µL Biohit micropipette was used for the samples injection into the cells. For characterization of the cloud point temperature experiments, was employed an F. A. G. thermostat bath (Iran). For the cloud point extraction was used a Sahand Azma Tajhiz (Iran) centrifuge.

All reagents and chemicals were of analytical grade and used without further purification. All stock solutions were prepared with double-distilled water. Nile blue A, rhodamine B, Potassium nitrate, and citric acid were purchased from Merck Company, Darmstadt, Germany. The nonionic surfactant polyethylene glycol tertoctylphenyl ether (Triton X-114) was obtained from Fluka (Buchs, Switzerland). The stock solutions of NBA and RhB (100 µg/L) were prepared, and the working solutions were prepared from diluting their stock solutions. All cationic and anionic salts were from Merck. An aqueous solution of 1% w/v triton X-114 was prepared by dissolving 1.00 gr Triton X-114 in 100 ml doubly distilled water. The pH adjustment to pH=3.00 as optimum pH was carried out with the citric acid/ citrate buffer solution. Diluting of surfactant rich phase was perfumed by adding ethanol from Merck.

Principles of the RGB color system

In the additive RGB color model, each color is characterized by a specific color intensity of red, green and blue. The intensity range of each color is 0-255. In the RGB system, any color has certain values of R, G and B parameters. In this system, the black and white colors are represented by the ‘0 0 0’ and ‘255 255 255’ RGB values.

16777216 colors can be shown with this model. Each color can be represented by the following equation:

$$V = R + 256\ G + 2562\ B$$

In this formula, R, G and B are red, green and blue values, respectively. The amounts of red, green, and blue values calculated from the following flowchart:

R = V Mod 256

G = ((V - R) Mod (2562)) / 256

B = (V - R - G*256) / (2562)

Where ‘‘Mod’’ is the operation that finds the remainder after division of one number by another.

Preparation of cells array

In this study, cylindrical cavities in the Plexiglas® sheet was used as reaction chambers. The cylindrical cavities (i.d. 1.5 cm, thickness 0.5 cm) were built by a laser beam. Closing the bottom of the holes and building up the cells, this Plexiglas® holed sheet was pathed to another Plexiglas® sheet with a thickness of 0.1 cm. The designed sheet has 4 columns and 5 rows of chambers, totally giving 20 cells in the Plexiglas® sheet.

Theoretical and methodology

A cloud point extraction was carried out according the following procedure. An aliquot of 15 mL aqueous solution containing 0.5 µg mL^{-1} NBA, 1.0 µg mL^{-1} RhB, 0.15% (w/v) of Triton X-114 at pH=3.00 was prepared. Then, the mixture was heated for 25 min in a thermostatic bath at 28℃. The separation of the two formed phases was carried out by centrifuging for 10 min. The aqueous phase could then be separated by a Pasteur pipette. The surfactant rich phase was diluted with ethanol to 500 µL, and 480 µL of this solution was injected into the cell. The color cells were scanned by a scanner, and the obtained images of the color solutions were analyzed to evaluate R, G, and B contents by the developed software. Any effective intensity in the color values (R, G and B) between sample solutions and blanks were plotted vs. the main parameter.

Effective intensity of R= -log (Rsample/Rblank)

Effective intensity of G = -log (Gsample / Gblank)

Effective intensity of B= -log (Bsample / Bblank)

It should be noted that the R, G, or B values, which has the same color as the solution, is often higher than 180, and its amount does not change much during the experiment. In this study, because of the dark blue of the NBA, the green and blue values didn’t change significantly; therefore, the red value was selected as the main parameter for NBA indicator. Also, about RhB, due to its intense pink color, the R and B values are almost constant, so the intensities of the G value were chosen. Optimizing effective factors in this experiment was based on the R and G values as the analytical signals. The schematic diagram of the proposed method is shown in **Fig. 2**.

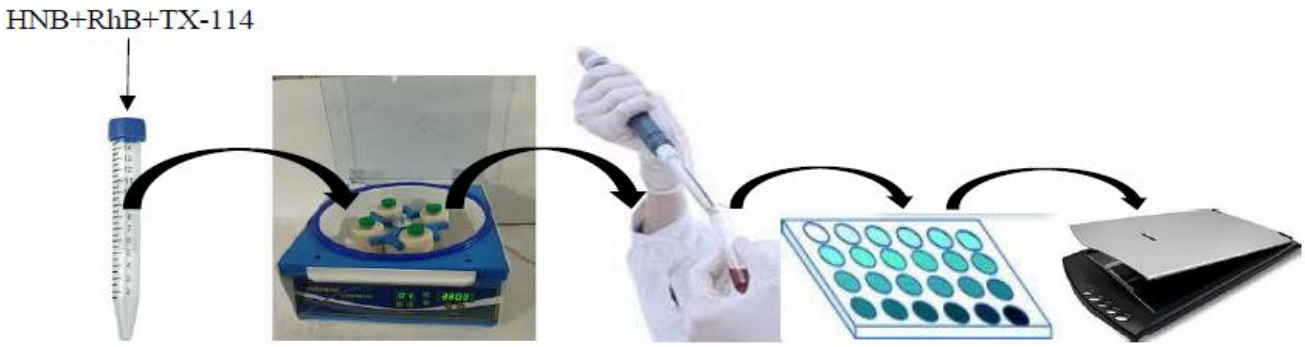

Fig. 2. Schematic diagram of the CPE-Sc method.

In this work, experimental variables such as volumes of extraction and disperser solvents, pH, centrifuge time, and round per minute, and salt addition were assessed and optimized with the aid of one variable at a time optimization methods. The method is based on the CPE of analytes from aqueous solution, diluting the extracted surfactant-rich phase with ethanol, transferring Plexiglas® cell, and scanning the cells containing the analyte solution into a scanner, and measuring the RGB parameters with software written in visual basic (VB 6) media.

RESULTS AND DISCUSSION

The CPE-Scanometry method was developed for simultaneous preconcentration and determination of the NBA and RhB in the aqueous solutions. Various experimental factors such as solution pH, Triton X-114 amount, the equilibria temperature, and the bath and centrifuge times were studied by the one-at-a-time evaluation method to obtain an optimized system. The RGB parameters of two dyes were recorded after CPE with Triton X-114. The G parameter for 1.00 µg mL^{-1} of RhB had the most sensitive signal with cloud point extraction,

and the R parameter for 0.50 µg mL^{-1} of NBA was found to be the most sensitive signal. Thus the method can successfully be used for sensitive simultaneous determination of NBA and RhB. Besides, the effects of some foreign species, including cations, anions, and dyes were investigated. The proposed method was validated to analyze NBA and RhB in water samples.

pH optimization

The pH is one of the most important analytical factors because it affects the extraction efficiency and partitioning of analytes in the micellar phase. Ionizable species can be achieved maximum extraction efficiency when they are in uncharged form, so these species are pH-dependent. Thus, the pH effect on the CPE of the mixture of 1.00 µg mL^{-1} RhB and 0.50 µg mL^{-1} NBA was studied in the pH range from 1.00 to 6.00 by the addition of hydrochloric acid or sodium hydroxide. For obtaining the desired pH for the proposed method, the six solutions were prepared by adding 1.00 µg mL^{-1} RhB, 0.50 µg mL^{-1} NBA, 1.00 mg mL^{-1} Triton X-114, 0.006 mol L^{-1} KCl to a 15.0 ml falcon tube and adding distilled water until it reaches the 15.0 mL mark at different pH. As shown in **Fig. 3**, the maximum color intensity for two target analytes has shown at pH 3.00, so this pH was selected as optimum pH. The ion pairs formation is very important in analytes extraction. According to the structures of these dyes and their acidity equilibrium constants (pK_a (RhB)= 3.70, pK_a (NBA)=9.70), although the dye charge becomes positive at pH=3.00, the presence of salt anion (KCl), can perform ion pair with target analytes and facile their extraction to the organic phase. At lower pH than pH=3.00, increases the positive charge of the dyes NBA and RhB and the sensitivity of the method and extraction efficiency was decreased. Also, at a pH higher than pH=3.00, deprotonation of the hydrogen ion of the hydroxyl group can occur in RhB, and extraction efficiency decreases, though as for acidity constant of NBA, its extraction decreases moderately. Therefore the citric acid/sodium citrate buffer system with the pH=3.00 was selected for further experiments.

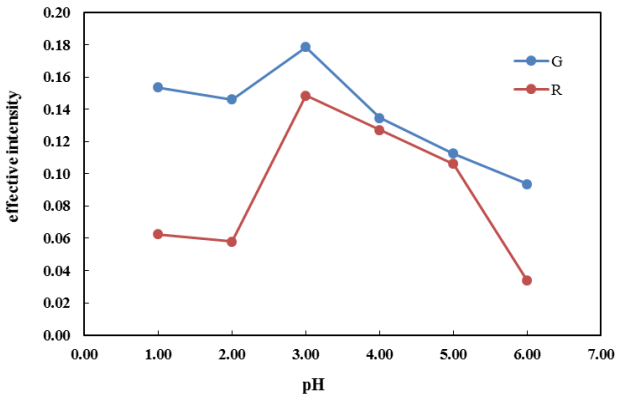

Fig. 3. Effect of pH of the test solution on the CPE of NBA and RhB (conditions: NBA, 0.50 mg L^{-1}; RhB, 1 mg L^{-1}, Triton X-114, 0.13% (w/v); Temperature of the bath, 28 °C; Time of bath, 25 min).

The optimum dose of triton X-114 (surfactant)

The surfactant concentration is one of the important parameters in cloud point extraction. A successful CPE should increase the extraction efficiency via increasing the phase volume ratio (Vaqueous phase/Vsurfactant-rich phase) to improve the preconcentration factor. Because of commercial availability, low toxicity, physicochemical characteristics, low price, the appropriate temperature range of cloud point and its high density in the surfactant-rich phase of triton X-114, it was chosen an efficient phase separating agent. In this study, the effect of Triton X-114 concentrations on the extraction and determination of a mixture of NBA and RhB dyes was investigated in the range of 0.13 - 3.33 (mg mL^{-1}). As you can see in **Fig. 4**, the effective intensity has been increased quickly with increasing Triton X-114 concentration to 1.33 mg mL^{-1}, the effective intensity of dyes increased with very Low or approximately fixed slope. In low concentrations of surfactant, the efficiency of extraction decreases quickly because the extraction was incomplete, and a high dose of it does not affect extraction efficiency because very small amounts of Triton X-114 and analytes remain in the aqueous phase. Thus the optimum dose of Triton X-114 was obtained 1.33 mg $mL^{-1,}$ and this amount was selected for further experiments.

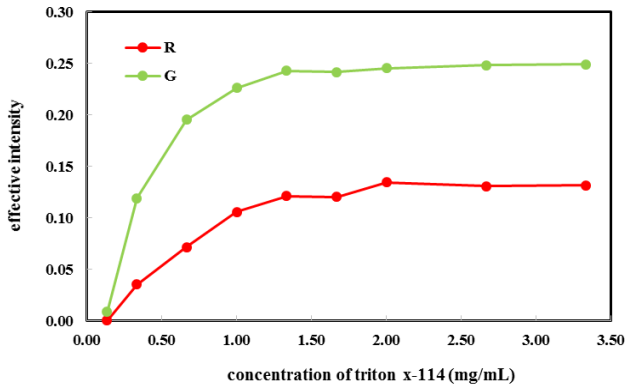

Fig. 4. Effect of Triton X-114 concentration on the CPE of NBA and RhB (conditions: NBA 0.50 µg mL^{-1} RhB 1.00 µg mL^{-1}; pH 3.00; Temperature of bath 28 °C; Time of bath 25 min).

Effects of the equilibration temperature

Temperature is one of the effective parameters in cloud point extraction. The appropriate temperature is closer to the room temperature. The lowest possible equilibration temperature or optimal equilibrium temperature is required for efficient preconcentration and easy phase separation. So, the effect of equilibrium temperature in the range of 25 - 60 ℃ was optimized. For this investigation, the same solutions were prepared at optimum conditions and were placed at the thermostat bath at different temperatures for 10 minutes. Effective intensities of the dyes at different temperatures were recorded by scanning, and the results are presented in **Fig. 5**. As shown in **Fig. 5**, the temperature of 30 ℃ had the highest effective intensity after scanning by a

scanner, so it was selected as the optimum temperature of this assay. At the lower temperatures, the two aqueous and surfactant rich phases can not be formed. The higher temperatures causing the dissociation of the dye – surfactant aggregation, and at the much higher temperatures may decompose the analyte or analytes. However, the selection of the temperature near the room temperature can save energy over the extraction and preconcentration process.

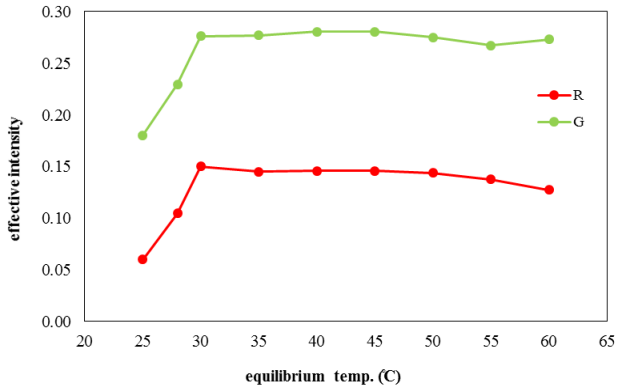

Fig. 5. Effect of equilibration temperature on the CPE of NBA and RhB (conditions: NBA 0.50 µg mL^{-1}; RhB 1.00 µg mL^{-1}; Triton X-114, 1.33 mg mL^{-1}; pH 3.00).

Optimization of equilibration time

The equilibration time has an important role to accesses the separation of the phases and suitable extraction of the analytes. Therefore optimizing this factor is necessary. Thus, the dependence of the good extraction upon equilibration time was studied in the range of 2–35 min. For optimizing the equilibrium time, the same solutions of the cited dyes at the optimal conditions were placed in the thermostat bath. The RGB signals of the solutions from 2 to 25 minutes increase slowly and after 25 minutes decrease. So, equilibrium extraction time (Maximum extraction efficiency) was reached at 25 min, and this time was chosen as the optimum time (**Fig. 6**).

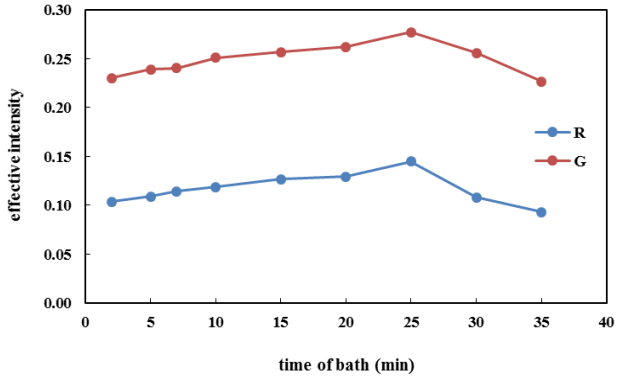

Fig. 6. Effect of equilibration time on the CPE of NBA and RhB (conditions: NBA 0.50 µg mL^{-1}; RhB 1.00 µg mL^{-1}; Triton X-114, 1.33 mg mL^{-1}; pH, 3.00; Temperature of bath, 30 °C).

Optimization of centrifugation time

Centrifugation time has not affected the extraction efficiency, but it is necessary to accelerate phase separation and formation of cloud point solution, causing less extraction time [**59**]. It is necessary to preconcentrate trace amounts of dyes with high sensitivity within a short time to attain maximum efficiency. So, the optimization of centrifuge time and its effect on the CPE and RGB signal of the simultaneous determination of NBA and RhB was investigated within the range of 2-30 min at 2500 rpm. According to **Fig. 7**, the centrifugation time of 10 min had the highest effective intensity and extraction efficiency, so this time was chosen as the optimal centrifugation time. At shorter times than 10 minutes, the separation was not accurate completely, and at longer times, probably back extraction of the analytes to the aqueous phase and cooling down of CPE system decreased the effective intensity and sensitivity.

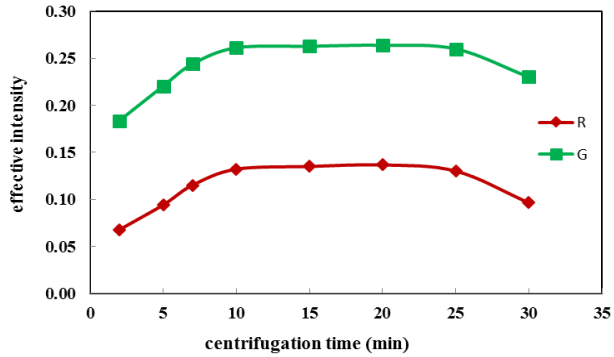

Fig. 7. Effect of centrifugation time on the CPE of mixture of NBA and RhB (conditions: NBA 0.50 µg mL^{-1}; RhB 1.00 µg mL^{-1}; Triton X-114, 1.33 mg mL^{-1}; pH, 3.00; Temperature of bath, 30 °C).

Effect of salt concentration

The addition of salt to the solution can usually affect the extraction efficiency and cloud point of micellar solutions by the salting-out effect [**60,61**] ion-pairing formation. Increasing the concentration of salt in the solution reduces the solubility of the analyte and increases its concentration in the organic phase. Investigating the salt effect on the micellar solutions of the NBA and RhB mixture was performed by adding KCl within the concentration interval of 0.005– 0.400 mol L^{-1} in the solution.

It showed increasing salt to 0.15 mol L-1 causes increasing the effective intensity, and salt reduces the signal slowly (**Fig. 8**). So, this salt concentration was used in further experiments. However, by considering the pH effect, the formation of the ion-pairing between Cl^- and the cationic dye can play an effective role in the extraction of two cited dyes at pH=3.00 in the presence of salt. Higher concentrations could also induce a reduction of diffusion rates of the analytes into the surfactant-rich phase, decreasing extraction efficiency of the NBA and RhB from aqueous phase to organic phase slowly.

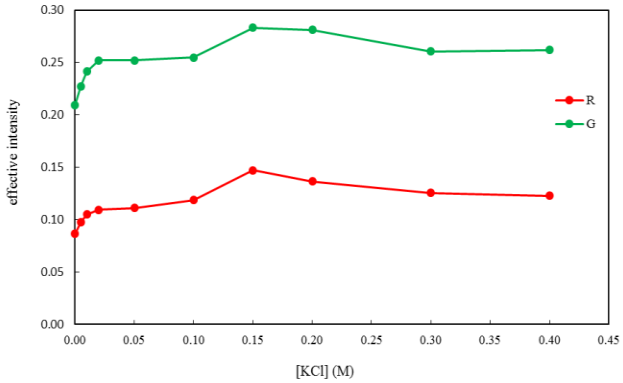

Fig. 8. Effect of salt concentration on the CPE of NBA and RhB (conditions: NBA 0.50 µg mL^{-1}; RhB 1.00 µg mL^{-1}; pH, 3.00; Temperature of a bath, 30 °C; Time of bath, 25 min).

Analytical figures of merit

Under the optimal conditions obtained above, the analytical performance of the proposed method was investigated. Calibration graphs of NBA and RhB were obtained by determining effective intensities of these dyes at different concentrations of standard solutions under the optimal conditions (**Fig. 9**). As shown in these calibration graphs, the linear ranges of the dyes are 0.01 – 1.33 µg mL-1 for the R-value of the NBA and 0.01– 1.00 µg mL^{-1} for G value of RhB. The analytical performance of this method is presented in **Table 1**. Relative standard deviations (RSD, %) for six replicate determinations of NBA and RhB were 8.00% for R-value of NBA and 1.13% for the G value of RhB, indicating good precision of this method the determination of a mixture of NBA and RhB solutions in the real aqueous samples. The limits of detection (LOD) was calculated three times of the standard deviation of the R and G values of the six blanks solutions divided into the slope of the calibration curves of the R-value of the NBA and G value of the RhB to be 0.002 µg mL^{-1} and 0.008 µg mL^{-1}, respectively. By dividing the slopes of the calibration curves before and after preconcentration for each of the analytes were obtained the enrichment factors of determination of NBA and RhB to be 50.87 and 42.65, respectively (**Table 1**). The proportion of the initial volume in the centrifuge tube to the final volume of the dissolving solvent (Ethanol), called the preconcentration factor, was calculated to be 30.00 for both analytes. The analytical performance was shown in **Table 1**.

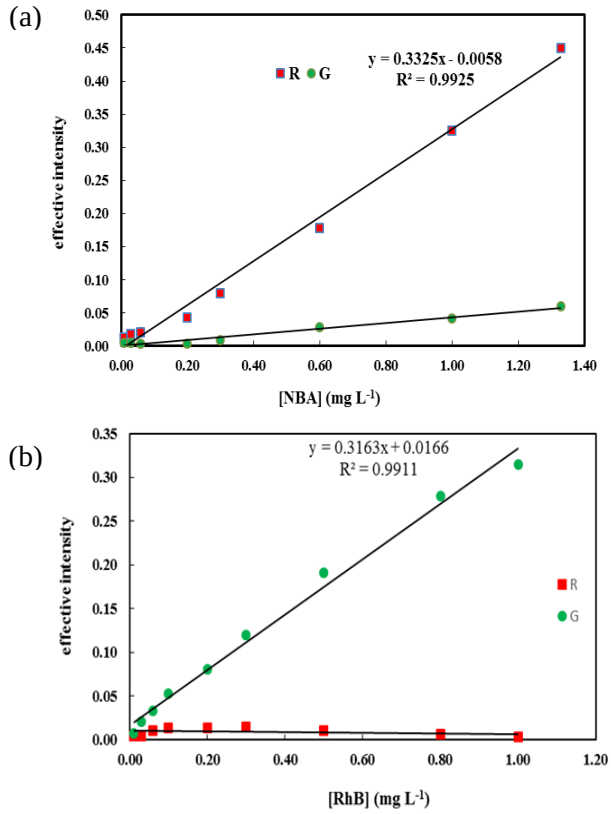

Fig. 9. Calibration graph for determination of (a) NBA and (b) RhB by CPE-Scanometry at optimum conditions (2 ml Triton X-114 (1% (w/v)), pH= 3.00, 28℃, 25 min bath time, 10 min centrifugation time).

Interference study

Under optimal experimental conditions, the selectivity of the simultaneous determination of 0.50 µg mL^{-1} NBA and 1.00 µg mL^{-1} RhB dyes was studied in the presence of the various species (ions and dyes) at different ratios. The results showed a tolerable error of less than 5% for the determination of a mixture of two dyes in the presence of foreign analytes. Common ions and dyes at different volumes were added to the falcon tubes in the presence of the analyte, and it was found that these ions and dyes did not affect the simultaneous determination of NBA and RhB. **Table 2** shows the tolerance limits of interfering ions and dyes in determining a mixture of 1.00 µg mL^{-1} RhB and 0.50 µg mL^{-1} NBA.

Table 1. Analytical figures of merit of the method for determination of NBA and RhB.

Analyte	Main P	Minor P	Calibration equation before CPE	Calibration equation after CPE	EF	Linear range (µg mL^{-1})	LOD (mg L^{-1})	RSD% (n=6)
NBA	R	G	y = 0.0066 C - 0.0008	y = 0.3335 C - 0.0059	50.87	0.01 – 1.33	0.008	8.00
RhB	G	R	y = 0.0074 C - 0.0021	y = 0.3163 C - 0.0166	42.65	0.01 – 1.00	0.002	1.13

Table 2. Tolerance limits of interfering ions and dyes in the determination of a mixture of 1.00µgmL^{-1} RhB and 0.50µgmL^{-1} NBA at optimum conditions.

Interfering ion/dye	Tolerable limit (µg/mL)
K^+, NO_3^-, ClO_4^-, $H_2PO_4^-$, Cl^-, Ni^{2+}, Co^{2+}	1000
Li^+	750
Pb^{2+}, PO_4^{3-}, F^-, Zn^{2+}, Cd^{2+}	500
CO_3^{2-}, Na^+	250
Br^-, Cu^{2+}	100
HCO_3^-, Ca^{2+}	50
Mg^{2+}, SO_4^{2-}, Thymolphtalein,	10
Phenolphthalein, Carmine, Fluorescein	3

Application in real samples

To investigate the applicability of CPE-Scanometry method for NBA and RhB assay, it was applied to extract and determine these dyes in water samples including tap water (Yasouj city), Mehrian river water (Yasouj city), and wastewater of Zardband factory (Yasouj city) at optimized conditions. The proposed method was examined by the recovery studies (**Table 3**). The recovery values were calculated after adding standards in different volumes of 0.000, 0.050, 0.200 and 0.800 µg mL^{-1} NBA and 0.000, 0.030, 0.200 and 0.800 µg mL^{-1} RhB. Recovery percentages from 94.03% to 107.17% for the NBA and from 95.05% to 109.31% are varied for RhB determination. It indicates the CPE-Scanometry method can be used successfully for the simultaneous determination of NBA and RhB in real samples.

Table 3. Simultaneous determination of NBA and RhB and recoveries in water samples.

	Nile Blue A (µg mL^{-1})			Rhodamine B (µg mL^{-1})		
Sample	**added**	**Detected**	**Recovery%**	**added**	**detected**	**Recovery%**
Tap water (Yasouj City)	0.000 0.050	Nd* 0.051 ± 0.003[1]	Nd 101.25	0.000 0.030	Nd 0.030 ± 0.001	Nd 100.05
	0.200	0.199 ± 0.008	99.75	0.200	0.200 ± 0.010	99.81
	0.500	0.502 ± 0.023	100.41	0.800	0.806 ± 0.030	100.48
River water (Mehrian river,	0.000 0.050	Nd 0.050 ± 0.001	Nd 100.99	0.000 0.030	Nd 0.029 ± 0.002	Nd 98.56
Yasouj city)	0.200	0.214 ± 0.012	107.17	0.200	0.210 ± 0.011	105.05
	0.500	0.492 ± 0.028	98.39	0.800	0.795 ± 0.027	99.34
Wastewater of Zardband factory (Yasouj city)	0.00 0.050 0.200 0.500	Nd 0.050 ± 0.002 0.194 ± 0.010 0.470 ± 0.020	Nd 100.40 96.87 94.03	0.00 0.030 0.200 0.800	Nd 0.030 ± 0.003 0.190 ± 0.011 0.874 ± 0.042	Nd 99.18 95.05 109.31

*Detected-Mean ± standard deviation (n= 3).

CONCLUSION

In this paper, CPE-Scanometry is a safe, rapid, easy, novel, portable, inexpensive, and sensitive method for the simultaneous preconcentration, separation, and determination of the trace amounts of Nile blue A rhodamine B dyes. The experimental results demonstrate the cloud point extraction method before solution scanometry is successful in quantitative extraction and preconcentration of NBA and RhB. The extraction process and determination was done with inexpensive instruments with high recoveries acceptable linear range, high precision, and good correlation coefficients. Although, about the removal and degradation of Nile blue have some reports [**62-64**] but there is few reports on determination of it alone [**62**] and not any report in prescence of other analytes according to our search in the literatures. However, rhodamine B was determined by various methods in the different samples. In **Table 4** and **Table 5** the analytical performances of the determination of rhodamine B and Nile blue A in the present work were compared with the previous studies. Although this method has not a very low detection limit, it has a wide linear range. Also, its RSD is comparable to other methods. The selectivity of the method was evaluated by the simultaneous determination of cited dyes in the presence of various analytes, showing a selective assaying method for these dyes. Also, the applicability of the assay was examined at different aqueous real samples.

Table 4. Comparison of the developed methods with other methods for determination of rhodamine B.

Determination method	Matrix	PF	EF	LR (µg L^{-1})	LOD (µg L^{-1})	RSD (%)	Reference
Uv-visible-Spectrophotometry	soft drink, wastewater	40	-	250-3000	3.14	5.0	**[21]**
IL-magnetic Nanoparticles-FS	food samples	25	-	0.40-140.00	60	0.45	**[25]**
Uv-visible spectrophotometry	Water	-	65.50	5-100	1.05	1.3	**[65]**
DLLM-Spectrophotometry	Food and water	330	-	100-3000	2.1	4.0	**[66]**
HPLC-SPE	Food	20	-	0.8-130	0.09	0.66	**[67]**
MISPE	Chili powder	-	110	1.0-20000.0	0.05	3.4	**[68]**
MSPE-HPLC	Food samples	25	-	0.5-150.00	0.08	0.51	**[69]**
CPE-Spectrophotometry	Hand washing liquid soap	8.5	-	5-550	1.3	2.40	**[70]**
CPE-SC (RhB)	Water, wastewater	30	42.65	10-1000	2.0	1.13	**Present work**

Note: PF-Preconcentration factor, EF-Enrichment factor, LR-Linear range

Table 5. Comparison of the developed methods with other methods for determination of Nile blue

Determination method	Matrix	PF	EF	LR	LOD	RSD(%)	Reference
Electrochemical Sensor	Water	-	-	0.03-10 µM	1.21 nM	3	**[62]**
CPE-SC	Water, wastewater	30	50.87	0.01-1.33(µg mL^{-1})	8.0(µg L^{-1})	8	**Present work**

Therefore, this method can be used as an accessible, simple and new method for determination of Nile Blue and Rhodamine B simultaneously in aqueous solutions and wastewaters with good sensitivity. Also the purposed method can be a prelude to image analysis[**71,72**] and smart phone [**73-75**] for studing and determination of the purposed and similar analytes.

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