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Mixed micelle-mediated extraction for simultaneous trace determination of rhodamine B and rhodamine 6G by spectrophotometry coupled with the partial least squares regression

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ABSTRACT

In this work, a novel method has been developed to simultaneously determine trace amount of rhodamine B (RhB) and rhodamine 6G (Rh6G) by spectrophotometry coupled with partial least squares regression. A modified cloud point extraction (CPE) method, which utilized a combination of the nonionic surfactant, Triton X-114, and the anionic surfactant, sodium dodecyl benzene sulfonate (SDBS), as the extractant, was used to preconcentrate RhB and Rh6G from sample solutions. The extraction conditions, such as concentration of surfactants, electrolyte concentration, and pH on the CPE were investigated and optimized using a single-factor method followed by a L_9 (3^4) orthogonal array design (OAD). The maximum absorption wavelengths for RhB and Rh6G are 558 and 533 nm, respectively; linearity is obeyed in the range of 3.4-550 and 4.0-500 ng mL^{-1} with detection limits of 1.2 and 1.8 ng mL^{-1}, and the root mean square errors of prediction (RMSEP) are 9.166 and 11.699 ng mL^{-1}, respectively. The proposed method has been applied successfully to simultaneously determine trace amount of RhB and Rh6G in paprika, hotpot ingredients, and river water samples with the recoveries of 86.8–111.8%.

KEYWORDS

Cloud point extraction;
Rhodamine B;
Rhodamine 6G;
Spectrophotometry;
Partial least squares regression.

INTRODUCTION

Rhodamine B (RhB) and rhodamine 6G (Rh6G) with the chemical structure shown in Figure 1, are derivatives of the synthetic xanthene dyes, both of which have been used for dyeing textile and food stuffs[1,2]. The use of RhB and Rh6G for food coloring has raised serious concerns because of the carcinogenicity, reproductive and developmental toxicity, neurotoxicity, and chronic toxicity towards humans and animals[3,4], thus, has been banned in food stuff by many countries. However, due to their low cost and high effectiveness, these harmful dyes are still used by unethical manufacturers and will probably continue to be used in food coloring in some parts of the world. Moreover, RhB has been unambiguous found in paprika which was far from any anthropogenic addition during its vegetation process[5]. Therefore, a sensitive and reliable method is urgently needed for the simultaneous determination of RhB and Rh6G in various food samples and aquatic environment.

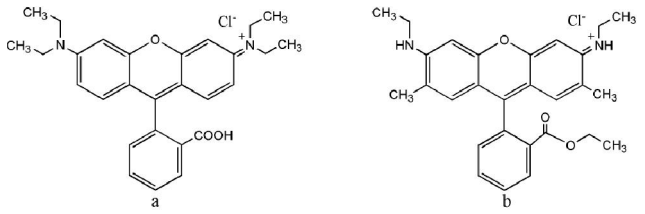

Figure 1 : The chemical structure of RhB (a) and Rh6G (b).

A variety of analytical methods have been proposed for this purpose, such as electrokinetic capillary chromatography[6], high-performance liquid chromatography[7], voltammetric[8], fluorescence spectrophotometry[9,10], and visible spectrophotometry[2,11-13]. The chromatographic methods often require complicated pretreatment procedures, while the spectrophotometric method is not selective and sensitive due to the high-overlapping of their absorption spectra and trace level present in food samples. This motivates us to develop alternative methods for the simultaneous determination of RhB and Rh6G with high sensitivity, selectivity, and simple pretreatment procedure.

In recent years, absorption-concentration matrices have been used in conjunction with chemometric methods for analysis of complex samples such as malachite green and crystal violet[14-17]. Partial least squares (PLS) method plays an important role in solving the problem of closely overlapping absorption spectra[18-21]. As a full spectrum multivariate calibration method, it utilizes a mathematical separation procedure to substitute the traditional chemical separation procedure and shows good predicted results. At the same time, the combination of PLS method and different enrichment techniques, such as dispersive liquid–liquid microextraction (DLLME)[22,23], solid phase extraction (SPE)[24], and cloud point extraction (CPE)[25,26] has been used to simultaneously detect many analytes.

Separation and preconcentration based on CPE are becoming an important and practical application of surfactants in analytical chemistry[27-29]. The technique is based on a property that most nonionic surfactants in aqueous solutions can form micelles and separate into a surfactant-rich phase of a small volume and a diluted aqueous bulk phase when heated to a temperature known as cloud point temperature (CPT). The small volume of the surfactant-rich phase obtained with this methodology permits the design of extraction schemes which can lead to a large preconcentration factor and therefore, be especially suitable for the trace level analysis.

In this work, we combine the advantages of the PLS methodology and CPE procedure for simultaneous spectrophotometric determination of trace amount of RhB and Rh6G. In view of the cationic nature of RhB and Rh6G, a combination of a nonionic surfactant, Triton X-114, with an anionic surfactant, sodium dodecyl benzene sulfonate (SDBS), was used for the mixed micelle-mediated cloud point extraction, which is based on the fact that the increased interactions between the cationic dyes and anionic-nonionic mixed micelles via not only hydrophobic but also electrostatic forces could enhance the extraction efficiency[30,31]. The parameters which affect the extraction efficiency were optimized by using a single-factor method and then a L_9 (3^4) orthogonal array design (OAD) to achieve high extraction efficiency for both of RhB and Rh6G extraction. The applicability of presented method for the analysis of food and industrial waste water samples was also investigated. To the best of our knowledge, this study is the first report describing the application of CPE-PLS spectrophotometric method for simultaneous determination of RhB and Rh6G in real samples.

EXPERIMENTAL

Reagents

All chemicals used in the experiments were of analytical grade and used without further purification. Double distilled water was used throughout the experiments. Standard stock solutions of RhB and Rh6G at a concentration of 100.0 μg mL^{-1} were prepared by dissolving appropriate amounts of rhodamine B and rhodamine 6G (obtained from Shanghai Chemical Reagent Company, China) in water, respectively. Working standard solutions were prepared by stepwise dilution of stock standard solutions prior to use. Aqueous 5% (v/v) solution of Triton X-114 was prepared by dissolving 5.0 mL of Triton X-114 (obtained from Amresco, USA) in 100 mL water. SDBS solution (4.00 g L^{-1}) was prepared by dissolving 4.00 g of SDBS in 1000 mL volumetric flask and dilute to the mark with water.

Instrumentation and software

In this work, a PerkinElmer (Lambda 45) spectro-

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photometer with 10 mm quartz cells was used for UV–vis spectra acquisition. A Professional Meter PP-15 pH-meter, equipped with a combined glass calomel electrode, was employed for the pH adjustments. The spectral data were imported to the Matlab environment and all programs were run on an ASUS computer with core i3 as central processing unit (4 Gb RAM).

General procedure

An aliquot (8.0 mL) of standard or sample solution containing no more than 5.5 μg of RhB and 5.0 μg of Rh6G was transferred into a 10 mL graduated centrifuge tube. Then, 0.2 mL of 4.0 g L^{-1} of SDBS, 1.3 mL of 5% (v/v) of Triton X-114, and 64.4 mg (1.1 mmol) sodium chloride were added to the tube, and diluted to the mark with water. The pH of the solution was adjusted to 2.6 using 0.1 mol L^{-1} hydrochloric acid and 0.1 mol L^{-1} sodium hydroxide. The resultant solution was shaken and kept in a thermostated water bath for 25 min at 55 ºC. After being centrifuged for 5 min at 4000 rpm, the mixture was cooled in an ice bath in order to increase the viscosity of the surfactant-rich phase and the aqueous phase was easily decanted by simply inverting the tube. The surfactant-rich phase was dissolved and diluted to 0.5 mL with ethanol, then transferred into a 0.5 mL quartz cell. The spectra were recorded in the wavelength region of 450–650 nm with a resolution of 1 nm against a reagent blank. Because the amount of RhB and Rh6G in 10.0 mL sample solution is measured after preconcentration by CPE in a final volume of 0.5 mL, the solution is concentrated by a factor of 20.

In addition, high-performance liquid chromatography (HPLC) (Agilent 1100 Series, USA) was also used to detect RhB and Rh6G in real samples according to Ding et al.[7], with a C18 column (150 mm × 2.1 mm) and a fluorescence detector. An isocratic elution using 45% of acetonitrile with 55% of 0.1% (w/v) of phosphate buffer was adopted as the mobile phase. The detection was performed at an excitation wavelength of 535 nm for RhB and 515 nm for Rh6G, and an emission wavelength of 580 nm for RhB and 554 nm for Rh6G, respectively.

Preparation of real samples

Paprika, and hotpot ingredients samples were collected from a local market. After homogenized by a disintegrator, 1.000 g of each sample was put into a 15.0 mL conical tube and extracted by ultrasonic vibration for 10 min with 10.0 mL double distilled water. After centrifuged at 4000 rpm for 5 min, the supernatant was taken and filtered through a 0.45 μm membrane. The extraction process was repeated in triplicate and the supernatants were amalgamated into a volumetric flask and diluted to 50.0 mL with double distilled water. River water was collected from Xiangjian River where close to a printworks. The impurities of collected samples were removed by filtration through 0.45 μm filter film. All the as-prepared samples were stored at 4 ºC in the dark and analyzed within a week.

RESULTS AND DISCUSSION

As shown in Figure 2, the absorption spectra of RhB and Rh6G in surfactant-rich phase overlap with each other. Simultaneous determination of these two dyes is simply impossible with conventional methods due to the similarity of their absorption spectra. This challenge could be overcome by using the PLS regression. In the PLS regression, the "mathematical separation" replaces tedious "chemical separation", which therefore allows for the direct simultaneous determination of RhB and Rh6G in complex matrices even in the presence of other unknown analytes. Because the absorption spectra of RhB and Rh6G, which were recorded after CPE with Triton X-114 and SDBS, show the maximum absorption bands at 558 and 533 nm for

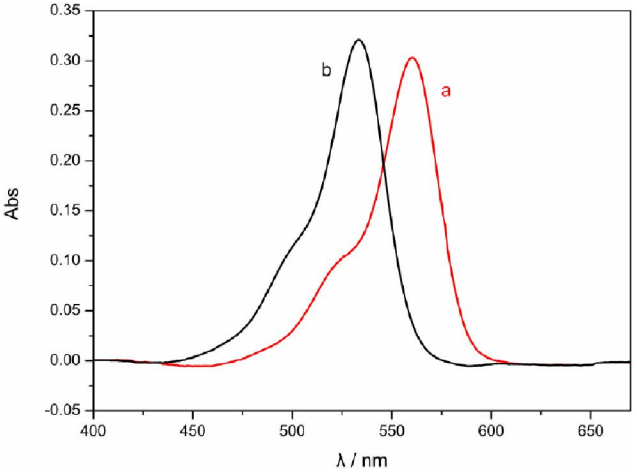

Figure 2 : The absorption spectrum of 150 ng mL^{-1} RhB (a) and 140 ng mL^{-1} Rh6G (b) after cloud point

RhB and Rh6G, respectively, the absorbances at these two wavelengths were used to evaluate the optimum conditions for the mixed micelle-mediated CPE.

Optimum conditions for cloud point extraction

In order to achieve maximum extraction efficiency, several parameters affecting the CPE of RhB and Rh6G, such as pH, surfactant type and concentration, salt concentration, equilibration temperature and time, were optimized by a combination of single-factor method and OAD L_9 (3^4).

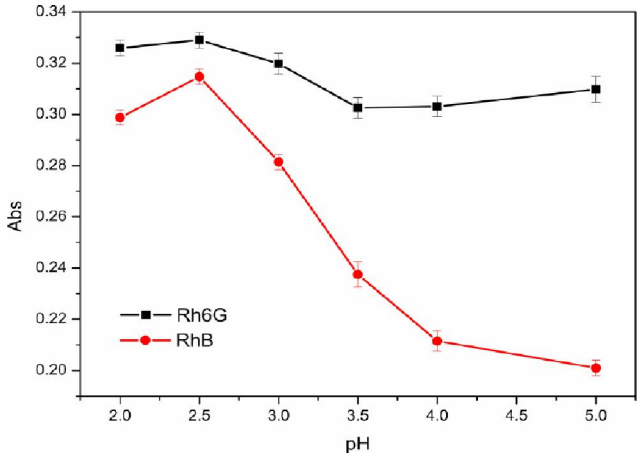

Figure 3 : The effect of pH on the absorbance of 150 ng mL^{-1} RhB and 140 ng mL^{-1} Rh6G after cloud point extraction with mixed micelles.

Effect of pH

The pH of solution is an important factor during CPE process involving analytes that possess acidic or basic moieties, as it can alter the ionic form of the analytes. In this step, the effect of pH on the amount of extracted RhB and Rh6G was investigated in the pH range of 2.0–5.0. As can be seen in Figure 3, the optimal pH is 2.5, in which the two dyes are predominantly in their cationic form, resulting in an enhanced interaction between the cationic analytes and the anionic mixed micelles, and consequently, greater extraction efficiency. Thus, pH 2.5 was chosen for the subsequent experiments.

Effect of the surfactant species and concentration

Mixed micelle-cloud point extraction was chosen for extraction of the target analytes because RhB and Rh6G are cations and highly soluble in aqueous solution in pH 2.5 solution, leading to poor extraction efficiency in CPE. Consequently, the three anionic surfactant, SDBS, sodium dodecyl sulfate (SDS), and lauryl sodium sulfate (SLS), were individually tested in this study to form the ion-pairs before CPE, combined with a nonionic surfactant, Triton X-114 or polyethylene glycol 400 (PEG 400). As shown in Fig. 4, the RhB-SDBS and Rh6G-SDBS ion-pairs can transfer effectively into the aggregates of Triton X-114, leading to higher extraction efficiency. Therefore, the combination of Triton X-114 and SDBS was used for the mixed micelle extraction in this work.

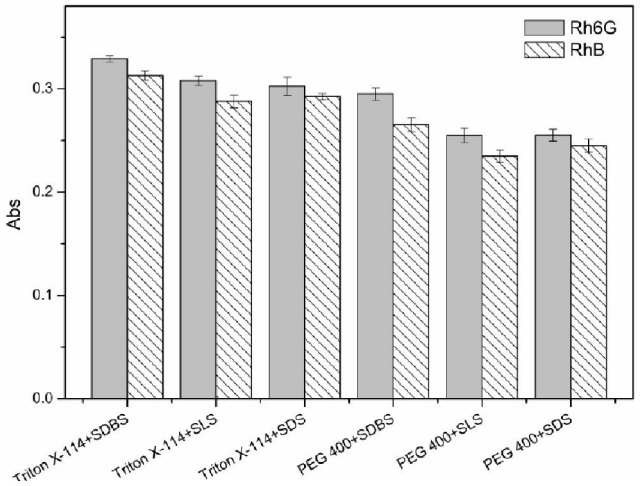

Figure 4 : Absorbance comparison of RhB and Rh6G after preconcentration and extraction by different combination of non-surfactant and anionic surfactant extractants. The concentrations of RhB and Rh6G were as the same as that in Figure 2.

The effect of SDBS concentration in the range of 0–1.80 g L^{-1} on the extraction of RhB and Rh6G was investigated. As can be seen in Figure 5, the absorbances of RhB and Rh6G increased with increasing SDBS concentration up to 0.06 g L^{-1} for RhB and 0.08 g L^{-1} for Rh6G, respectively, then decreased slowly at higher concentrations. Therefore, the optimum SDBS concentrations for RhB and Rh6G were selected as 0.06 g L^{-1} and 0.08 g L^{-1}, respectively.

The effect of Triton X-114 concentration was also investigated in the range of 0.10–0.80% (v/v) at a fixed level of SDBS. Figure 6 demonstrates that the measured absorbances of both RhB and Rh6G increased with increasing the concentrations of Triton X-114 up to 0.60% (v/v) for RhB and 0.70% (v/v) for Rh6G, then decreased with a further increase of the surfactant concentration. Therefore, the optimum Triton X-114 concentrations for RhB and Rh6G were selected as

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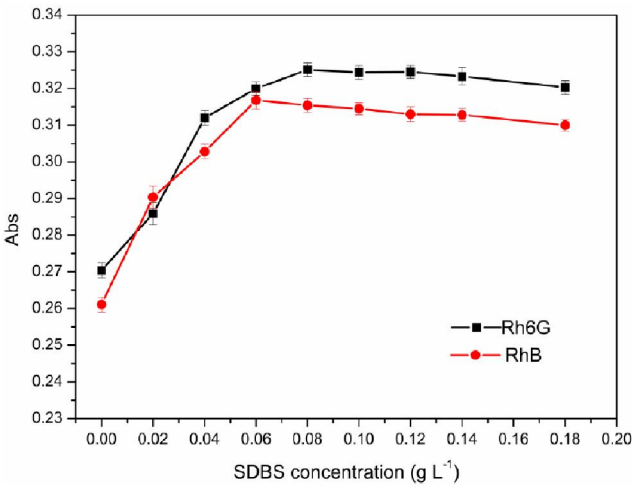

Figure 5 : The effect of SDBS concentration on the absorbance of 150 ng mL^{-1} RhB and 140 ng mL^{-1} Rh6G after cloud point extraction with mixed micelles

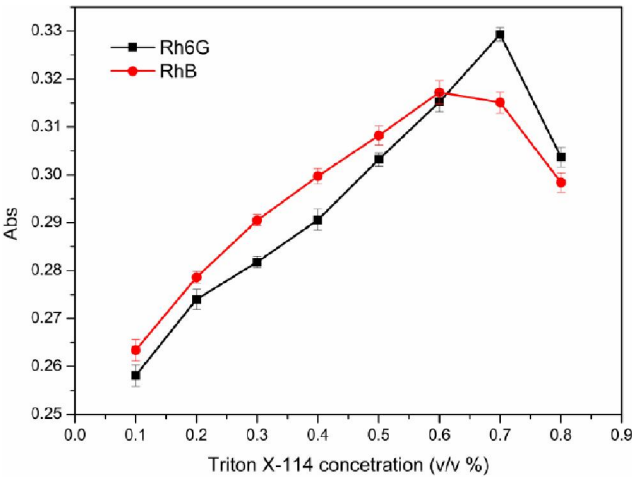

Figure 6 : The effect of Triton X-114 concentration on the absorbance of 150 ng mL^{-1} RhB and 140 ng mL^{-1} Rh6G after cloud point extraction with mixed micelles.

0.60% (v/v) and 0.70% (v/v), respectively.

Effect of salt types and concentrations

In this study, three salting-out electrolytes (NaCl, $CaCl_2$ and Na_2SO_4) were individually added to the mixed micellar solutions to investigate their effects on the CPE. The results reveal that NaCl produced effective separation and lower CPT than Na_2SO_4 and $CaCl_2$ at the same concentration, both to RhB and Rh6G. So, NaCl was further investigated in the concentration range of 0.02–0.12 mol L^{-1}. An increase in the concentration of NaCl up to 0.10 mol L^{-1} increased the absorbance and above this value, no significant change was observed for the two analytes. Thus, a concentration of 0.10 mol L^{-1} was selected as optimum.

Effects of equilibration temperature and incubation time

It is desirable to employ the lowest possible equilibration temperature and the shortest incubation time, which compromise the efficient separation of the phases and the completion of the extraction. Therefore, the effect of equilibration temperature (30–60 ºC) was studied. It was found that 55 ºC is adequate for both analytes. The dependence of extraction efficiency upon equilibration time was also studied for a time interval of 5–45 min. Maximum extraction efficiency was observed at 55 ºC after 25 min, accordingly, an incubation time of 25 min was set in this study.

Orthogonal array design for further optimizing CPE conditions

Until now, the "best" CPE separation conditions were obtained by use of the single-factor method (i.e., varying one parameter at a time while keeping the others constant). Because OAD method uses a simultaneous multivariate optimization approach to obtain a global rather than a local optimum, the orthogonal array design L_9 (3^4) was carried out in order to optimize further the experimental conditions for CPE based on the previous optimized results. The design involved four factors, the pH (P), the concentrations of anionic (A) and nonionic (N) surfactants, and the salt (S) concentration, each at three levels (shown in TABLE 1). The results of the OAD experiment can be statistically treated by direct observation analysis to evaluate the effect or the importance of a given factor[32, 33].

As can be seen in TABLE 1, the level difference of factor P was the largest, indicating that pH was the most important factor of those considered. The concentration of nonionic surfactant (N) was also important, but the NaCl concentration (S) of less importance. Thus, from the OAD experiments, two possible extraction condition combinations, A_3-S_1-P_3-N_2 (denoted as scheme A) and A_3-S_3-P_3-N_2 (scheme B), seemed optimal for RhB; and two for Rh6G, that is, A^*_2-S^*_2-P^*_3-N^*_1 (scheme C) and A^*_2-S^*_3-P^*_3-N^*_2 (scheme D). To pursue the best extraction conditions, the four schemes were tested experimentally. After performing each scheme in triplicate, scheme B (SDBS 0.08 mol L^{-1}, NaCl 0.11 mol L^{-1}, pH 2.6 and Triton X-114 0.60%) and scheme D (SDBS 0.08 mol L^{-1}, NaCl 0.11 mol L^{-}

TABLE 1 : The treatment of factors and their levels, and the range analysis of rhodamine B and rhodamine 6G on absotibance corresponding to the L_9 (3^4) orthogonal experiment

no	Influencing factors and levels								Abs (n=3)	
	SDBS concentration (gL^{-1})		NaCl concentration ($molL^{-1}$)		pH		Triton X-114 concentration (v/v %)			
	RhB (A)	Rh6G (A*)	RhB (S)	Rh6G (S*)	RhB (P)	Rh6G (P*)	RhB (N)	Rh6G (N*)	RhB	Rh6G
1	0.04(1)	0.06(1)	0.09(1)		2.4(1) 2.6		0.55(1)	0.65(1)	0.2750	0.3144
2	0.04(1)	0.06(1)	0.10(2)		2.5(2)		0.60(2)	0.70(2)	0.2947	0.3127
3	0.04(1)	0.06(1)	0.11(3)		2.6(3)		0.65(3)	0.75(3)	0.3040	0.3168
4	0.06(2)	0.08(2)	0.09(1)		2.5(2)		0.65(3)	0.75(3)	0.2921	0.3019
5	0.06(2)	0.08(2)	0.10(2)		2.6(3)		0.55(1)	0.65(1)	0.2977	0.3344
6	0.06(2)	0.08(2)	0.11(3)		2.4(1)		0.60(2)	0.70(2)	0.3007	0.3305
7	0.08(3)	0.10(3)	0.09(1)		2.6(3)		0.60(2)	0.70(2)	0.3126	0.3275
8	0.08(3)	0.10(3)	0.10(2)		2.4(1)		0.65(3)	0.75(3)	0.2924	0.3053
9	0.08(3)	0.10(3)	0.11(3)		2.5(2)		0.55(1)	0.65(1)	0.2932	0.3067
k_1	0.291	0.315	0.293	0.315	0.289	0.317	0.289	0.319		
k_2	0.297	0.322	0.295	0.317	0.295	0.307	0.303	0.324		
k_3	0.299	0.313	0.299	0.3180	0.305	0.326	0.296	0.308		
Best level level	A_3	A^*_2	S_3	S^*_3	P_3	P^*_3	N_2	N^*_2		
R	0.008	0.009	0.006	0.003	0.016	0.019	0.014	0.016		

[1], pH 2.6 and Triton X-114 0.70%) resulted in higher spectrophotometer responses for RhB and Rh6G, respectively. In order to reach a same extraction condition for simultaneous extraction of RhB and Rh6G, 0.65% (v/v) of Triton X-114 was chosen and the responses has no significant difference from scheme B and scheme D. Therefore, the most favorable condition combination for the mixed micellar cloud point extraction of RhB and Rh6G was: pH 2.6, 0.65 % (v/v) of Triton X-114, 0.08 g L^{-1} of SDBS, and 0.11 mol L^{-1} of NaCl.

Individual calibration

Calibration curves were constructed for the indi-

TABLE 2 : Composition of synthetic mixtures and predicted values by PLS model and statistical parameters for the system

Sample	Synthetic samples (ng mL^{-1})		Prediction (ng mL^{-1})		Recovery (%)	
	RhB	Rh6G	RhB	Rh6G	RhB	Rh6G
1	8.5	20.0	7.3	21.5	85.9	107.5
2	17.0	120.0	16.3	117.9	95.9	98.3
3	34.0	180.0	37.2	175.0	109.4	97.2
4	85.0	12.0	87.4	10.5	102.8	87.5
5	119.0	140.0	120.4	146.3	101.2	104.5
6	136.0	400.0	155.0	387.4	114.0	96.9
7	170.0	160.0	166.4	169.8	97.9	106.1
8	204.0	160.0	219.7	166.9	107.7	104.3
9	306.0	240.0	319.4	243.6	104.4	101.5
10	425.0	300.0	429.5	331.3	101.1	110.4
AR					102.1%	101.4%
RPE					4.66%	5.67%
RMSEP					9.166	11.699

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vidual standard solutions of RhB and Rh6G at the optimum extraction conditions and wavelength of 558 and 533 nm, respectively. The calibration curves were linear in the range of 3.4–550 ng mL^{-1} and 4.0–500 ng mL^{-1} with the equations of $A = 0.0019\ c + 0.0199$ (r^2 = 0.9995, n = 10) and $A = 0.0021\ c + 0.0303$ (r^2 = 0.9987, n = 10) for RhB and Rh6G, respectively (where A is the absorbance and c is the concentration of analytes in the unit of ng mL^{-1} in aqueous phase). The limit of detection, defined as LOD=$3S_b/m$ (where LOD, S_b and m are the limit of detection, standard deviation of the blank, and slope of the calibration graph, respectively), was found to be 1.2 and 1.8 ng mL^{-1} for RhB and Rh6G, respectively.

PLS Method

The first step in the simultaneous determination of RhB and Rh6G by PLS involves constructing the calibration matrix. A number of 25 mixtures were selected as the calibration set, and the concentrations of RhB and Rh6G in their linear range were randomly designed to obtain more information from the calibration procedure. Under these conditions, the calibration models were obtained and then validated by a 10 synthetic mixture set containing RhB and Rh6G in randomly selected proportions. The concentration of each sample was then predicted and compared with the known

concentration of this reference sample. In this work, 10 synthetic test samples were analyzed with the presented method. The results obtained are given in Table 1. In order to select the number of factors in the PLS algorithm, a cross-validation method, which leaves out one sample at a time, was used[34]. The prediction error was calculated for each analyte for the prediction set, which are the samples not participating in the construction of the model. This error was expressed as the prediction residual error sum of squares (PRESS), which is defined as follows:

$$PRESS = \sum_{i=1}^{n} (\hat{c}_i - c_i)^2 \quad \textbf{(1)}$$

where n is the number of samples in the prediction set, c_i is the actual concentration in the ith sample, and $\hat{c}_i$ is its estimated value. *PRESS* was calculated for the first variable, which built the PLS modeling in the calibration step. After that, another latent variable was added for the model building and the *PRESS* was calculated again. These calculations were repeated for 1-25 latent variables, which were used in the PLS modeling. This procedure was repeated for each element. In order to find the smallest model (fewest number of factors), the F-statistic was used to carry out the significance determination. The PLS modeling for each element had a different number of factors. The optimum factors for RhB and Rh6G to be 4 and 5, respectively.

Using the data in TABLE 2, the average recovery (*AR*) of RhB and Rh6G in mixtures can be calculated as:

$$AR(\%) = 100 \times \sum_{i=1}^{n} (\hat{c}_i / c_i) / n \quad \textbf{(2)}$$

The prediction error of RhB and Rh6G in the mixtures, defined as the relative predictive error (*RPE*) of the predicted concentrations, can be calculated as:

$$RPE(\%) = 100 \times \left[\sum_{i=1}^{n} (\hat{c}_i - c_i)^2 / \sum_{i=1}^{n} (c_i)^2 \right]^{0.5} \quad \textbf{(3)}$$

The root mean square error of prediction (*RMSEP*) can be calculated as:

$$RMSEP = \left[\sum_{i=1}^{n} (\hat{c}_i - c_i)^2 / n \right]^{0.5} \quad \textbf{(4)}$$

The calculated results of *AR*, *RPE* and *RMSEP* for PLS are also show in TABLE 2.

Interference study

The effect of different ions and dyes on the simultaneous determination of 150 ng mL^{-1} RhB and 140 ng mL^{-1} Rh6G by this method were investigated. An ion or a dye was considered as interferent, when it caused a variation in the absorbance of the analyte greater than ± 5%. The results presented in TABLE 3 show the good selectivity of the procedure. The three common dyes, sudan I, methylene blue, and allura red, were also tolerable up to 50, 20 and 10-fold of the analytes, respectively.

Applications and validation

The reliability and applicability of the developed method was examined by the simultaneous determination of RhB and Rh6G in paprika, hotpot ingredients,

TABLE 3 : Tolerance limit of foreign species

Foreign species	Tolerance ratio
Na^+, K^+, Cu^{2+}, NH_4^+, Ni^{2+}, Mg^{2+}, Pb^{2+}, Cd^{2+}, Ca^{2+}, Zn^{2+}, Hg^{2+}, Cl^-, I^-, NO_3^-, CO_3^{2-}, SO_4^{2-}, PO_4^{3-} and CH_3COO^-	1000
Fe^{3+}, Al^{3+}	800
Co^{2+}, Cr^{3+}, Mn^{2+} and NO_2^-	500
Sudan Ⅰ	50
Methylene blue	20
Allura red	10

TABLE 4 : Determination of RhB and Rh6G in real samples

Sample	Added (ng mL^{-1})		Found±SD (ng mL^{-1})		Recovery (%)		By HPLC Found±SD (ng mL^{-1})	
	RhB	Rh6G	RhB	Rh6G	RhB	Rh6G	RhB	Rh6G
Paprika	0.0	0.0	26.8±1.3	BDL	-	-	25.2±1.2	BDL
	0.0	250.0	31.6±1.8	249.4±2.1	-	99.8		
	250.0	0.0	278.4±12.5	BDL	100.6	-		
	50.0	50.0	76.7±6.1	43.4±2.5	99.8	86.8		
	120.0	120.0	150.3±4.2	106.4±4.5	102.9	88.7		
	250.0	250.0	281.3±6.8	236.3±12.4	101.8	94.5		
Hotpot ingredients	0.0	0.0	27.4±1.6	BDL	-	-	26.4±1.3	BDL
	0.0	250.0	28.1±2.2	247.3±10.2	-	98.9		
	250.0	0.0	289.5±15.3	BDL	104.8	-		
	50.0	50.0	78.6±2.6	50.4±2.9	102.4	100.8		
	120.0	120.0	155.1±2.3	125.2±3.3	106.4	104.3		
	250.0	250.0	293.5±5.3	262.5±11.2	106.4	105.0		
River water	0.0	0.0	BDL	BDL	-	-	BDL	BDL
	0.0	250.0	BDL	251.8±4.2	-	100.7		
	250.0	0.0	258.5±11.2	BDL	103.4	-		
	50.0	50.0	56.1±2.7	47.4±4.3	112.2	94.8		
	120.0	120.0	133.4±1.3	134.1±3.9	111.1	111.8		
	250.0	250.0	248.4±2.1	250.2±11.2	99.4	100.1		

[a] standard deviation.; [b] below the detection limit.

TABLE 5 : Performance comparison of the detection of RhB and Rh6G with other methods.

Technique	Analyte	Linear range (ng mL^{-1})	LOD (ng mL^{-1})	R^2	References
Voltammetric method	RhB	4.78-956.06	2.93	0.9942	[8]
Fluorophotometry	RhB	0.765-478.03	0.239	0.9995	[10]
CPE-fluorophotometry	RhB	0.0467-100	0.014	0.999	[9]
CPE-UV visible[a]	RhB	5-550	1.3	0.9982	[11]
CE[b]-UV visible	RhB	1200-59800	300	-	[6]
DLLME-UV visible	Rh6G	5-900	2.39	0.9988	[2]
DLLME-UV visible	RhB	5-100	1.05	0.9993	[13]
SPE-UV visible	RhB	250–3000	3.14	0.9996	[12]
SPE-HPLC	RhB	2-50	0.5	0.9950	[7]
		50-1000		0.9999	
	Rh6G	0.5-20	0.1	0.9940	
		20-400		0.9994	
CPE-UV visible-PLS	RhB	3.4-550	1.2	0.9995	This work
	Rh6G	4.0-500	1.8	0.9987	

[a] UV visible spectrophotometry; [b] Capillary electrophoresis

and river water samples. The accuracy of the method and the effect of the matrix of the real samples were assessed by the recovery experiments from samples spiked with the known amounts of analytes. TABLE 4 summarizes the results obtained for real samples. The recoveries are in the range of 86.8–111.8%, which indicates that the PLS model is able to predict the concentrations of RhB and Rh6G in real samples.

The presence of RhB and Rh6G in all studied samples was also validated by HPLC. A good correlation between predicted values and HPLC results was obtained (shown in TABLE 4). In addition, a comparison of this method with others[2,6-13] for detecting RhB and/or Rh6G was summarized in TABLE 5 and the result indicates that this method has lower detection limit or wider linear range than most of the reported methods, and above all, it can be applied to simultaneous determination of both RhB and Rh6G in a cost-effective and time-saving fashion.

CONCLUSIONS

In this study, we have developed a new method for the simultaneous detection of trace amount of rhodamine B and rhodamine 6G. The method employs mixed micelle cloud point extraction and the PLS regression methodology. The results demonstrate the effectiveness of the cloud point extraction system in quantitatively extracting and preconcentrating rhodamine B and rhodamine 6G. Without tedious pre-separation procedures and expensive instrumentation, this method has been successfully used to determine rhodamine B and rhodamine 6G in paprika, hotpot ingredients, and river water with low prediction errors and acceptable recoveries, revealing the applicability of the method for real sample analysis.

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