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Separation/Analysis Rhodamine B by Anion Surfactant/Ionic Liquid Aqueous Two-Phase Systems Coupled with Ultraviolet Spectrometry

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Abstract

A novel method for the separation/analysis rhodamine B has been described. The ionic liquid (1-tetradecyl-3-methylimidazolium bromide)/anion surfactant (sodium dodecyl sulfate)/NaCl two-phase systems (ATPSs) is presented as a simple, rapid and effective sample pretreatment technique coupled with ultraviolet spectrometry for analysis rhodamine B in soft drink. The effects of parameters on the ATPSs extraction of rhodamine B such as amount of surfactant, ionic liquid and salt, pH, temperature, stabilization and centrifugal time have been studied in details. Under the optimized conditions, the linear range of calibration curve for rhodamine B was 0.05 - 7.0 µg·mL^{-1} and the detection limit was 3.2 ng·mL^{-1}. The phase equilibrium and the mechanism of phase separation for ATPSs have been discussed. This method has been applied to the determination of rhodamine B in soft drink.

Keywords

1. Introduction

Rhodamine B is an triphenylmethane dye (**Figure 1**) used in industrial fields, such as textile and foodstuff industry [1] [2], which is harmful if swallowed by human beings and animals, and causes irritation to the skin, eyes and respiratory tract [3]. In many countries, rhodamine B has been banned in food products due to the ha-
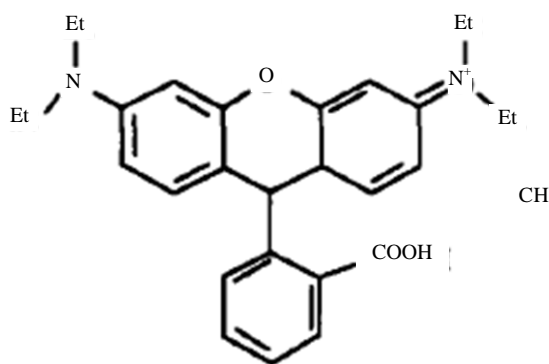
Figure 1. The chemical structure of rhodamine B.

zardous nature and harmful effects [2]. So it has been considered worthwhile to make efforts to develop a simple method for the determination of rhodamine B in real samples.

The direct determination of trace rhodamine B in real samples may not be possible because of low concentrations and matrix interferences, so the preliminary preconcentration and sample clean-up steps are necessary. The methods of preconcentration separation contain high performance liquid hromatography (HPLC) [1]-[4], liquid-liquid extraction (LLE) [5], cloud point extraction (CPE) [6], solid phase extraction (SPE) [3] and so on. LLE is widely applied for the trace metals determination due to its inexpensive cost and easy operation. However, the extraction solvents of LLE are volatile, inflammable and toxic organic solvents which are easy to pollute the environment.

The aqueous two-phase systems (ATPSs) are a kind of LLE technique which is a simple and environmentally friendly separation system because traditional volatile solvents are not used in the whole process. ATPSs are usually composed of two polymers [7], polymer/salt [8], two surfactants [9] or RTILs/salt [10], which have been used in separation and purification of various biological products [11], metal ions [12], dyes [13], drug molecules [14]. However, 1) the high viscosity rich phase may make following determination difficult [14] in the most of polymers—ATPSs; 2) the phase equilibrium processes in surfactants—ATPSs are relatively time-consumming [9]; 3) the RTILs-ATPSs were required a great quantity of ionic liquid and salt [10]. There are the synergistic effect between surfactants and ionic liquids (RTILs) due to their unique characteristics [15] [16]. RTILs and surfactants could form an ordered molecular assembly which not only own the inherent of surfactant but also possess many advantages of RTILs, and achieve the integration of their nature [16]. These features of ordered molecular offer numerous opportunities for the modification of existing extraction processes and for the development of new extraction processes. The ATPSs based on 1-butyl-3-methylimidazolium tetrafluoroborate ([Bmim] [BF$_4$])/cationic surfactant (NPTABr) have been reported [17]. But, the ATPSs based on RTILs/anion surfactants seem to be lacking.

In this work, ATPSs based on RTILs (1-tetradecyl-3-methylimidazolium bromide)/anionic surfactant (sodium dodecyl sulfate, SDS)/NaCl were established for separation/analysis trace rhodamine B in real samples coupled with ultraviolet spectrometry at 554 nm. The ATPSs showed a wide range of temperature, quick phase separation with low concentration of the RTILs/salt, which have been successfully applied to the determination of rhodamine B in real samples with satisfactory results.

2. Experimental

2.1. Instrumentation and Reagents

Ultraviolet spectrophotometer; pHS—25 pH meter (Shanghai, China); Fourier transform infrared spectrometer; High-speed centrifuge (feige); electronic balance (Beijing); magnetic stirring apparatus (Shanghai); conductivity meter (Shanghai).

A standard rhodamine B stock solution of 100.0 μg·mL^{-1} was prepared. The solution was diluted to 10.0 μg·mL^{-1}. Series of HAc-NaAc buffer solutions, pH 3.0 to 8.0, 5% (w/v) solution of SDS and 3% solution of RTILs were prepared. All the chemicals were of analytical reagent grade and all solutions were prepared in deionized water.

2.2. Procedure

2.2.1. Synthesis Method of RTILs

Briefly, 1-tetradecyl-3-methylimidazolium bromide [C_{14}mim][Br] was synthesized by adding equal amount (0.05 mol) of n-methylimidazole and 1-bromotetradecane to a 250.0 mL round bottom flask fitted with reflux condenser. The flask and its content were stirred and heated at 110˚C for 3 h. Subsequently, the outcome was washed three times with diethyl ether in a separation funnel. Finally, the collected ionic liquid was heated at 40 ˚C under vacuum last for 4h to remove the solvent.

2.2.2. Phase Diagrams [18]

The 0.3% (W/V) RTILs aqueous solution and 0.3% SDS solution were prepared with 2.0% NaCl solution. Samples were prepared by mixing stock-solutions of RTILs and SDS in test tubes at different ratios. The tubes were then immersed in a water bath at temperature T = 20˚C, 40˚C, 60˚C for about 5 min until phase equilibrium state was attained.

2.2.3. ATPS Extraction Procedure

1.0 mL standard solutions (10.0 μg·mL^{-1}) of rhodamine B, 2.0 mL pH = 3.8 buffer solution, 1.0 mL 20% NaCl, 0.6 mL 3.0% RTILs and 1.0 mL 5.0% SDS were transferred into 10.0 mL centrifuge tube. The mixtures were diluted to the mark with water and shaken thoroughly. Separation of the phases was achieved by centrifugation at 4000 rpm for 4 min. For the separation of two phases, the RTILs/SDS-rich phase was mixed with 1.0 mL acetate buffer (pH 3.8) and then diluted to 5.0 mL with distilled water. Then the absorbance was measured by ultraviolet spectrometry against a reagent blank.

2.2.4. Sample Preparation

According to literature [5].

  Determination of critical micell concentration (cmc).

  According to literature [19].

3. Results and Discussion

3.1. Effect of Temperature on ATPSs

Phase diagrams of aqueous two-phase systems (ATPSs) composed of RTILs, SDS and salt were constructed at different temperatures, which could reflect the two-phase region of ATPSs clearly.

  The effect of temperature on phase equilibrium of RTILs/SDS/NaClt ATPSs were examined at T = 20˚C, 40˚C, 60˚C, respectively. When the temperature increased from 20˚C to 60˚C, the two-phase region wasn't changed and the ATPSs could be formed with n_{RTILs}:n_{SDS} = (2.7:1) - (3.3:1) at 20˚C - 60˚C. According to phase diagram, the room temperature (20˚C) was chosen for the following experiments.

3.2. Effect of the Composition of ATPSs on Extraction Efficiency

The extraction efficiency of rhodamine B (E%) could be controlled and optimized with a judicious choice of RTILs/SDS/NaCl ATPSs.

3.3. Effect of SDS Concentration on the Extraction Efficiency

The amount of SDS affecting the extraction efficiency of rhodamine B was investigated (**Figure 2**). E% was increased gradually with the increase of SDS and achieved maximum when the volume of SDS approached to 1.0 mL, then decreased. The ATPSs could not form with the amount of 5.0% SDS more than 1.1 mL. So the amount of SDS in the ATPSs was 1.0 mL.

3.4. Effect of RTILs Concentration on the Extraction Efficiency

The effect of the amount of RTILs was investigated on the extraction efficiency of rhodamine B and the results were illustrated in **Figure 3**. As it could be seen, E% was going up with the RTILs in the range from 0.5 - 0.6 mL, and the maximum appeared at the 0.6 mL, and then decreased. This was because the aqueous two-phase
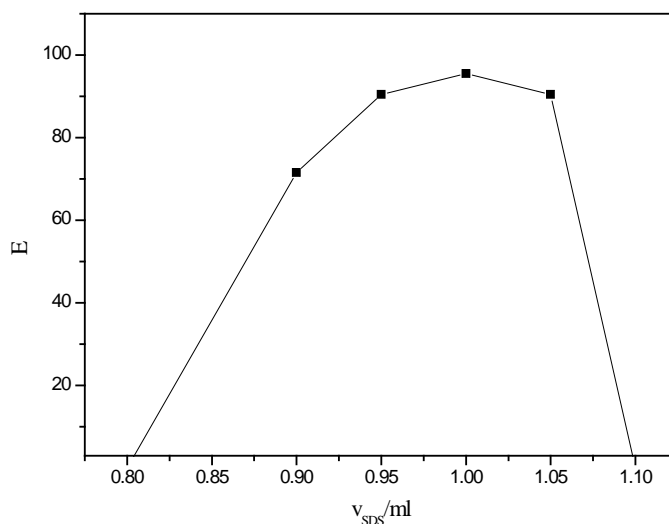
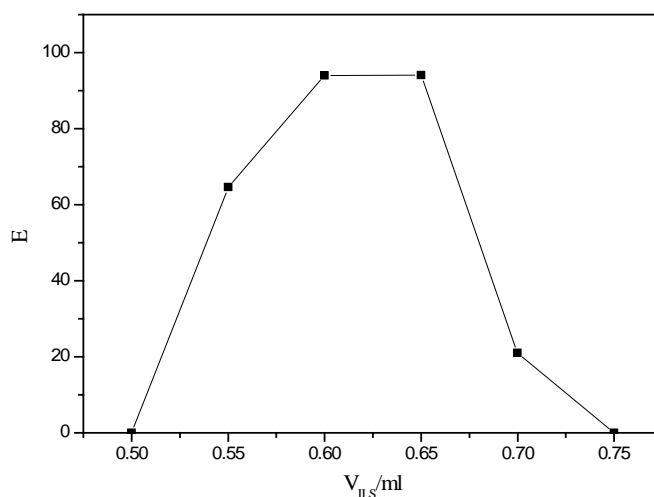**Figure 2.** The effect of the amount of surfactant on E%.

Figure 3. The effect of the amount of RTILs on E%.

could not formed with the amount of 3.0% RTILs lower than 0.5 mL or more than 0.75 mL. In this work 0.6 mL 3.0% (w/v) RTILs was used.

3.5. Effect of pH on the Extraction Efficiency

The pH plays a unique role on ATPSs formation and subsequent extraction. The ATPSs could not formed at pH < 3.0 or pH > 4.4. The effect of pH on the extraction efficiency was studied within the pH range 3.0 - 4.2. E% was at the maximum and wasn't changed when the pH value was in the range of 3.0 - 4.2. In the paper, pH 3.8 was chosen for the following experiments.

3.6. Effect of Salt Concentration on the Extraction Efficiency

The mixture of SDS and RTILs aqueous solution was a homogeneous solution at room temperature. The ATPSs was formed after salt was introduced into the above mixture. **Figure 4** showed the effects of NaCl concentration on the partition behavior of rhodamine B. The system contains 0.6 mL 3.0% RTILs and 1.0 mL 5.0% SDS, ATPSs could be formed when 20% NaCl amount was over 0.7 mL. The experiment results was shown that the extraction efficiency at the maximum and wasn't changed when the amount of 20% NaCl was in the range of 1.0

- 1.2 mL, But the extraction efficiency was quickly decreased when the amount of the salt was beyond 1.2 mL. So the amount of NaCl in the ATPSs was 1.0 mL.

3.7. Effect of Stabilization Time and Centrifugal Time

It was important to select an appropriate stabilization time and centrifugal time that guaranteed the achievement of equilibrium in the ATPSs. The solution was shaken finely for several seconds; the two-phase could be separated by centrifugalization. 4 min centrifugal time was found to be sufficient to establish good extraction efficiency. Thus, 4 min was selected for the subsequent experiments.

3.8. Effect of Foreign Ions

The interference of different foreign substrates were discussed in the determination of 1.0 $\mu g \cdot mL^{-1}$ rhodamine B. The tolerance limits were determined for a maximum error of ±5% and the results from these studies are collected in **Table 1**. The results were collected in **Table 1** which was shown the good selectivity of the procedure.

3.9. Analytical Parameters

The calibration graph for the determination of rhodamine B was obtained under the best experimental conditions.
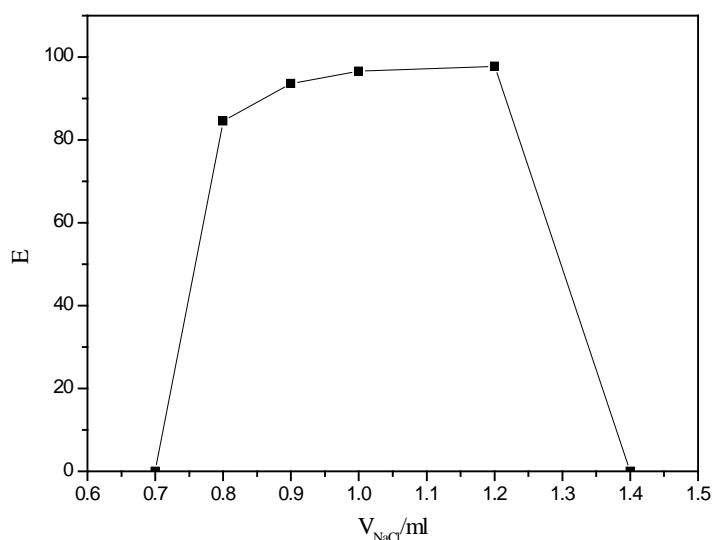

Figure 4. The effect of salt concentration on the extraction efficiency.

Table 1. Tolerance limits of foreign ions for the determination of rhodamine B (results within 5% error).

Interferences ions	Tolerance ratio (w_1/w)
K^+, Na^+	1000
Cu^{2+}	1000
Pb^{2+}	1000
Cl^-, $C_2O_4^{2-}$	1000
Ca^{2+}	100
Sunset yellow	1000
Lemon yellow	1000
Carmine	50

The results was shown a good linear relationship in the range of 0.05 - 7.0 $\mu g \cdot mL^{-1}$. The equation of the analytical calibration curves was $A = 0.52545c - 0.00528$ (c: $\mu g \cdot mL^{-1}$), $r^2 = 0.9936$. The detection limit (DL) of rhodamine B was determined to be 3.2 $ng \cdot mL^{-1}$. The relative standard deviations (R.S.D.) was 3.8% (n = 4, c = 1.0 $\mu g \cdot mL^{-1}$).

3.10. Compared with Some Recent Studies on the Determination of Rhodamine B

Table 2 compares the characteristic data of the present method with those reported in literatures. Generally, the detection limit obtained by the present method is comparable to those reported method, and the range of linearity and selectivity are better than them.

3.11. Samples Determination

The proposed method was applied for the determination of rhodamine B in soft drink. The standard addition method was used and the analytical results and the recovery are presented in Table 3. The average recoveries of the proposed method were 94.0% - 102.2%.

3.12. Discussion of Mechanism of Phase Separation

The mechanism of the ATPSs (RTILs/SDS/NaCl) on separation rested with two factors: 1) The synergistic effect between the RTILs and SDS; 2) the effect of salting-out.

3.12.1. The Synergistic Effect between the SDS and RTILs

The synergistic effect between surfactants and ionic liquids could be reflected on the change of cmc between the RTILs and surfactants. Evaluating the cmc by the measurement of electric conductivity is workable .The plots of electrical conductivity in the different concentration of the solution were showed in Figure 5. The RTILs or RTILs/SDS concentration at which the micellization starts was evident from the change in the slope of the plot. The inflection point was taken as the cmc. The results were listed in Table 4.

As can be seen from Table 4 that the cmc of RTILs, SDS, and SDS/RTILs were 2.5 $mmol \cdot L^{-1}$, 8.4 $mmol \cdot L^{-1}$ and 0.4 $mmol \cdot L^{-1}$, respectively. In this work, the molar ratio of RTILs and SDS was 1:3.4. So, the cmc of the clint model should be 5.0 $mmol \cdot L^{-1}$. There was discrepancy for the cmc of the mixed solution (SDS/RTILs) in determination value and the clint model value, which demonstrated that there was the synergistic effect between SDS and RTILs. A new ordered molecular assembly was formed.

Table 2. Characteristic performance of some recent studies on the determination of rhodamine B.

Method	detection limit ($ng \cdot mL^{-1}$)	range of linearity ($ng \cdot mL^{-1}$)	Interferences of dyes	References
CE-UV-vis	1.3	5 - 550	8 - 15	[3]
SPE-UV-vis	3.14	25 - 300	1 - 5	[4]
SPE-HPLC	250	1000 - 10,000	-	[2]
ATPSs-UV-vis	3.2	5 - 7000	50 - 1000	This work

Table 3. Determination results of soft drink.

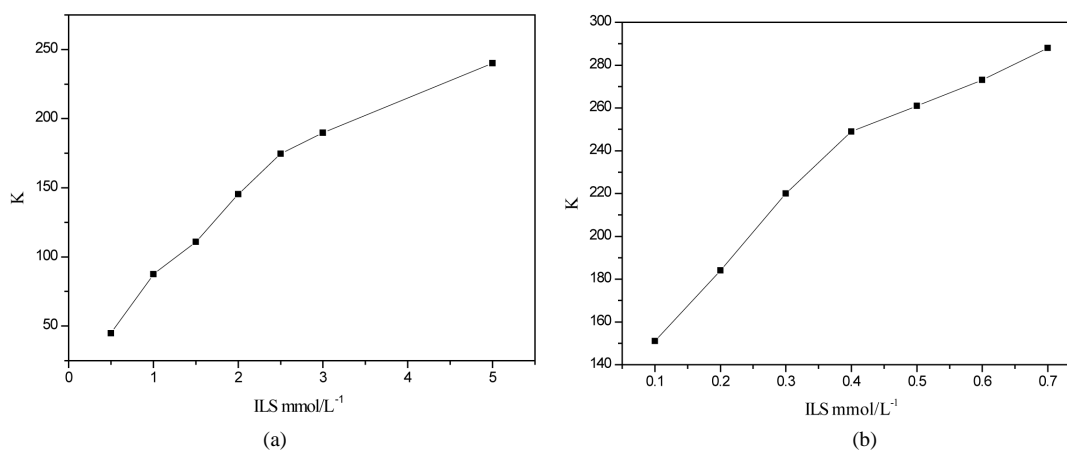| Sample	Added ($\mu g \cdot mL^{-1}$)	Found ($\mu g \cdot mL^{-1}$)	Recovery (%)
	0	ND	-
	0.30	0.29	96.7%
Soft drink	0.50	0.47	94.0%
	0.70	0.70	100.0%
	0.90	0.92	102.2%

Figure 5. The plots of electrical conductivity in the different concentration. (a) Electrical conductivity vs the concentration of RTILs; (b) Electrical conductivity vs. the concentration of RTILs/SDS.

Table 4. cmc of the different medium.

Medium	cmc (mmol/L)	
	Determination value	Clint model value
RTILs	2.5	
SDS	8.4 [20]	
RTILs/SDS	0.4	5.0[d]

[d]Calculate by Clint model [21].

3.12.2. The Effect of Salting-Out

The mechanism of RTILs/salt ATPSs formation is under the label of salting-out [10]. When the kosmotropic salt solutions were added in aqueous RTILs solutions, the water molecules close to these ions would be in an elec-tro-constriction state because of their water structuring nature, and their role as solvents to RTILs was reduced [22]. In other words, the formation of RTILs-ATPSs may be considered to be a competition between the hydro-philic polymer and the inorganic salt for the water molecules. The competition is won by the inorganic ions be-cause of their stronger affinity for the water. Compared with RTILs, the molecular assemblies (RTILs/SDS) was more hydrophobic, the water molecules away from the molecular assemblies to the inorganic salt more easier than only RTILs, so the molecular assemblies could form the aqueous two-phase system easier than RTILs.

4. Conclusion

The ATPSs consisting of RTILs and SDS are an excellent strategy for extraction of rhodamine B from real sam-ples. This novel extraction technique can be employed in combination with ultraviolet spectrometry as a viable for the quantitative determination of rhodamine B in real samples. The method is characterized with simplicity, rapidity, high selectivity and low cost.

Acknowledgements

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Abbreviations

cmc: critical micell concentration
SDS: sodium dodecyl sulfate
RTILs: room temperature ionic liquids
ATPSs: aqueous two-phase systems

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