Determination of Rhodamine B Pigment in Food Samples by Ionic Liquid Coated Magnetic Core/Shell Fe₃O₄@SiO₂ Nanoparticles Coupled with Fluorescence Spectrophotometry

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Abstract: The Ionic liquids (IL) 1-octyl-3-methylimidazole hexafluorophosphate ([OMIM]PF₆) was selected from three ILs along with (1-butyl-3-methylimidazole hexafluorophosphate ([BMIM]PF₆), 1-hexyl-3-methyl-imidazole hexafluoro-phosphate ([HMIM]PF₆), were coated by Fe₃O₄@SiO₂ nanoparticles with core-shell structure to prepare magnetic solid phase extraction agent (Fe₃O₄@SiO₂@IL) and establish a new method of magnetic solid phase extraction (MSPE) coupled with Fluorescence spectrophotometry for separation/analysis of Rhodamine B. The results showed that Rhodamine B was adsorbed rapidly by Fe₃O₄@SiO₂@[OMIM]PF₆ and eluted by ethanol. Under the optimal conditions the linear range, detection limit (DL), correlation coefficient (R) and relative standard deviation (RSD) were found to be 0.40-140.00 µg L⁻¹, 0.06 µg L⁻¹, 0.9993 and 0.45% (n=3, c=10.00 µg L⁻¹), respectively. The pre-concentration factor of the proposed method was 25-fold. The Fe₃O₄@SiO₂ NPs can be used repeatedly for 10 times. This method has been applied to the determination of Rhodamine B in food samples.

Keywords: Rhodamine B, IL-magnetic Nanoparticles, Magnetic SPE, Fluorescence Spectrophotometry

1. Introduction

Rhodamine B (RhB), a red pigment, is widely used as colorant in textiles, dyeing, paper and printing [1]. RhB is harmful if swallowed by human and animals, and causes irritation to the skin, eyes and respiratory tract [2], [3], [4], and has been considered and used as color additive in foodstuffs. Because of the low price and bright color of rhodamine B, some traders use it as a food additive illegally [5], harming the health of consumers [6]. Thus, due to this, it was considered worthwhile to make efforts to develop a simple method for the determination of RhB in different samples. Various techniques have been developed for the determination of RhB to date, including ultraviolet spectrophotometry [7], fluorescence spectrophotometry [8], capillary chromatography [9], high-performance liquid chromatography (HPLC) [10]. However, direct determination with spectrophotometry is difficult owing to low concentration of RhB in natural samples. So that, it is important to develop a new method for determination/separation procedure of RhB.

Magnetic solid phase extraction (MSPE) is a technique that uses a magnetic material as absorbent matrix in solid-phase extraction technique [11]. In MSPE procedures, the magnetic adsorbents are exposed in the sample solution to adsorb the analyte and then collect the analyte by an external magnetic field, which greatly simplifies the SPE procedure and enhances extraction efficiency [12]. To date, MSPE extraction agent is mainly Fe₃O₄ nanoparticles (NPs) with specific chemical functional group modified on the surface to
achieve concentration of the targeted analyte. Numerous organic polymers and inorganic polymers have been used to modify Fe₃O₄ NPs. Gao et al. used polyaniline loaded Fe₃O₄ (PANIs/Fe₃O₄) as sorbents for magnetic solid-phase extraction fluoroquinolones in honey samples [13]. Cao et al. used ionic liquid coated Fe₃O₄@graphene (IL/Fe₃O₄@graphene) as sorbents for magnetic solid-phase extraction nitrobenzene compounds in environmental water samples extraction [14]. Liu et al. used magnetic core-sporerous shell microspheres with C₁₈-functionalized interior pore-walls (C₁₈-Fe₃O₄@mSiO₂) as adsorbent measured the residue of cephalosporins in milk [15]. However, it has not been reported for determination of RhB using Fe₃O₄@mSiO₂@IL.

In the present work, we have prepared magnetic solid phase extraction agents, Fe₃O₄@mSiO₂@IL through self-assembly. This extraction agent combines the advantages of the ILs and the magnetic nanoparticles (MNP). Compared with the previously reported works "[16], [17], [18]", this adsorbent based MSPE providing a rapid, and efficient sample preparation process, which enables the treatment of large volume samples in a short period of time. A novel MSPE method coupled with Fluorescence spectrophotometry was therefore established for separation/analysis of RhB from food samples.

2. Experimental

2.1. Equipment and Reagents

FTIR spectra were measured with a Bruker Tensor 27 spectrometer (Bruker Company, Germany). Samples were pressed into KBr pellets and recorded at the frequencies from 4000 to 400 cm⁻¹ with resolution of 4 cm⁻¹. A Tecnai 12 TEM (Philips, Netherlands) was used to obtain micrographs of the MNPs. Chromatographic separation of analytes was achieved with Fluorescence spectrophotometry.

All chemicals and reagents were at least of analytical reagent grade, unless otherwise stated. RhB standards were purchased from Shanghai Chemical Reagent Corporation, (China). A standard stock solution was prepared by dissolving 10.0 mg of each standard in 100 mL of ethanol and stored in dark at 4°C. N-methylimidazole (Darui Fine reagent grade, unless otherwise stated. RhB standards were dissolved 10.0 mg of each standard in 100 mL of ethanol (China). A standard stock solution was prepared by dissolving 10.0 mg of each standard in 100 mL of ethanol.
3. Results and Discussion

3.1. Characterization of the MNPs

The synthesized Fe₃O₄@SiO₂@ILsMNPs were characterized by FT-IR spectroscopy, thermo-gravimetrical analysis and magnetic characterization.

Figure 1. was shown the FTIR spectra of Fe₃O₄ (curve a), Fe₃O₄@SiO₂ (curve b), Fe₃O₄@SiO₂@[BMIM]PF₆ (curve c), Fe₃O₄@SiO₂@[HMIM]PF₆ (curve d) and Fe₃O₄@SiO₂@[OMIM]PF₆ (curve e). Compared the curves of a and b, a peak of 580 cm⁻¹ was assigned to Fe-O-Fe stretching vibration; the strong peaks of 1200-1000 cm⁻¹ correspond to the Si-O-H and Si-O-Si stretching vibration; which indicated that SiO₂ had been successfully loaded on the surface of Fe₃O₄. In the curve c, d and e, the peaks at 2920 cm⁻¹ and 2850 cm⁻¹ corresponded to the C-H stretching vibration; the peaks at 1575 cm⁻¹ corresponded to the characteristic absorption of imidazole groups in the Fe₃O₄@SiO₂@ILs; in addition, the peak of 840 cm⁻¹ was attributed to the P-F stretching vibration in the Fe₃O₄@SiO₂@ILs [28], which indicated that ILs had been successfully immobilized on the surface of Fe₃O₄@SiO₂.

Thermo-gravimetric analysis (TGA) was conducted in a nitrogen atmosphere, and the heating rate employed was 5°C min⁻¹ all cases from 25 to 1000°C. Figure 2 presents the TGA of Fe₃O₄@SiO₂ (a) and Fe₃O₄@SiO₂@IL (b). The figure, illustrates a weight loss of 2.7% at 240°C corresponding to the water content. Compared curve a and b, an additional weight loss of 6.6% is observed from 200 to 650°C which is due to the ILs coating.

3.2. Optimization of Adsorption

The factors affecting the adsorption process of RhB such as pH, temperature and solution volume were studied and the adsorption behavior of Fe₃O₄@SiO₂@IL on RhB was compared with that of MNPs.

3.2.1. Effect of pH

As shown in Figure 3, the adsorption efficiency of RhB was varied with the pH, which was between 3.0 to 12. It could be concluded that the adsorption (retention) efficiency of RhB on IL MNPs was above 90.0%. It reached the highest value 94.44% when pH was 3.0. As the dissociation constant (pKa) of RhB is 3.2, the major existence form of RhB was molecule form at pH 3.0, the hydrophobicity of molecule form was stronger than its ionic form, Therefore, pH 3.0 was selected for the subsequent assays.
3.2.2. Effect of Adsorption Temperature and Time

The adsorption efficiency of RhB on IL MNPs and MNPs at different temperatures (5.0-60°C) were studied (Figure 4). The adsorption efficiency of RhB on Fe₃O₄@SiO₂@IL was higher than that on MNPs and was always above 70% from 0.5°C to 15.0°C and was above 85% from 0.20°C to 50.0°C. The experiment was carried out at room temperature.

3.2.3. Effect of the Sample Volume

The adsorption efficiency of RhB varied with the increase of sample volume. The amount of RhB was fixed at 50.0 µg and the volume of the sample solution increased from 10.0 mL to 90.0 mL. The adsorption efficiency of RhB was above 89% from 10.0 to 80.0 mL and remain steady, and 77.85% when sample volume was 90.0 mL. So the largest sample volume allowed was 80.0 mL (Figure 6).

3.3. Adsorption Capacity

The adsorption capacity is defined as the maximum amount of RhB adsorbed per gram of IL MNPs. The adsorption capacity of RhB on IL MNPs was studied (Figure 7). When the concentration of RhB was 50.0 µg/mL (volume: 50 mL), the adsorption of RhB for 0.0225g IL MNPs reached the maximum. The adsorption capacity for IL MNPs was calculated as 105.80 mg/g.

3.4. Optimization of Elution

3.4.1. Selection of Eluent

In this work, different eluents were investigated. The order of elution efficiency was ethanol>methanol> cetyltrimethyl ammonium bromide (CTAB)> sodium dodecyl sulfonate (SDS) > NaOH (0.1 M) > HCl (0.1 M). So ethanol was adopted as the eluent. Figure 8.
3.4.2. Eluent Volume

The effect of ethanol volume was evaluated on the elution of RhB. The results in Figure 9 described that quantitative elution (above 90%) was observed as the volume of ethanol was higher than 3.0 mL. The pre-concentration factor is 25-fold. The optimum volume of ethanol was chosen at 3.0 mL.

3.4.3. Elution Time

The elution process was completed within 10.0 min, and the elution efficiency did not change with a stable elution efficiency of 89.29% thereafter. The elution time of 10.0 min for RhB was adopted (Figure 10).

3.5. The Reusability of Fe₃O₄@SiO₂@IL

In order to investigate the recycling of the Fe₃O₄@SiO₂NPs, they were washed with 2mL ethanol for twice after each MSPE run and subsequently assembled with IL. Each re-prepared adsorbent was used for MSPE. The experimental results are shown in Figure 11. It was clear that no obvious loss of the adsorption capacity occurred after ten times of recycling.

3.6. Evaluation of Interferents

With a relative error of less than ±5%, the influence of some interferents that food samples contain on the determination of RhB in the presence of foreign substances was investigated. The tolerance limit for various foreign substances is in Table 1. The results indicated that the majority of these substances in samples had no remarkable interference on the (RhB) determination.

| Interferent                        | Tolerance ratio |
|------------------------------------|-----------------|
| SO₂⁻, Br⁻                          | 400             |
| Citrate, glucose                   | 230             |
| NO⁻, Zn²⁺, Cu²⁺, phenol            | 90              |
| Safranin, Bright yellow, Sunset Yellow | 18             |
| Allura Red, Congo red              | 17              |
| Rhodamine 6G                       | 8               |
| Allura Red                         | 8               |
| Congo red                          | 2               |

3.7. Analytical Performance of the Method

Under the optimum conditions, the linear calibration curve was obtained in the concentration range of 0.40-140.00 µg L⁻¹. The equations of calibration graph is I (peak area) =
from real samples. In summary, we have prepared FeOx/IL MNPs as adsorbent, for MSPE of. The magnetic nano-adsorbent is prepared by a self-assembly technique, which is very simple and mild. Compared with traditional SPE, this MSPE based on the FeOx/IL MNPs as an SPE adsorbent is fast, and the adsorbent can be easily separated from the sample solution. Moreover, this adsorbent has a high extraction capacity and high enrichment factors and is able to treat large-volume samples in a short period of time. The hydrocarbon chains of ILs on the surface of the adsorbent can provide adsorption sites for Rhodamine B other organic pollutants through π–π and hydrophobic interactions. The proposed method for the analysis of Rhodamine B is satisfactory. Thus, this adsorbent may also find potential application in the extraction and analysis of other analytes.

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4. Conclusion

In this work, IL MNPs was synthesized as magnetic solid phase extraction adsorbent to pre-concentrate/separate RhB

### Table 2. Determination results of the samples.

| Sample          | Added (µg kg\(^{-1}\)) | Found (µg L\(^{-1}\)) | Recovery (%) |
|-----------------|------------------------|-----------------------|--------------|
| Chili powder    | 0                      | 5.46                  | —            |
|                 | 6.00                   | 5.01                  | 103.5        |
|                 | 60.00                  | 49.50                 | 97.0         |
|                 | 102.00                 | 100.9                 | 101.8        |
| Hot pepper      | 0                      | 4.03                  | —            |
|                 | 50.00                  | 4.03                  | 98.0         |
|                 | 102.00                 | 100.5                 | 100.7        |
|                 | 0                      | ND                    | —            |
| Chinese prickly ash | 6.00              | 5.02                  | 100.7        |
| prickle ash     | 60.00                  | 50.4                  | 100.8        |
|                 | 103.00                 | 101.2                 | 100.1        |

### Table 3. Comparison with the results in other literatures.

| Method       | LR (µg/L) | LOD (µg/L) | RSD (%) | Ref     |
|--------------|-----------|------------|---------|---------|
| SPE-UV       | —         | 3.14       | 5.0     | [30]    |
| SPE-HPLC     | 1000-10,000 | 250      | 2.87    | [31]    |
| HPLC–CL      | 1.5-82.1  | 1.50       | 4.0     | [32]    |
| MSPE-Fluorescence | 0.40-140.00 | 0.06   | 0.45    | This method |

#### 3.9. Comparison of the Proposed Method with Relevant Literature

The comparison of current work with some other methods on the determination of RhB (Table 2) reveals that this method is either comparable or has rather pronounced advantages over them. Moreover, it is obvious that the present work has high sensitivity, wide linear range, and easy operation.

Table 3 listed the linear range and the limit of detection for the analysis of RhB in real samples obtained by the reported methods, such as solid phase extraction coupled with UV spectrophotometry, solid phase extraction coupled with high performance liquid chromatography, and HPLC–CL method. Compared with other reported methods, the method adopted in the present work obviously had a satisfactory linear range and limit of detection.
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