

Determination of Rhodamine B in Food Samples by Fe₃O₄@ Ionic Liquids-β-Cyclodextrin Cross Linked Polymer Solid Phase Extraction Coupled with Fluorescence Spectrophotometry

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Abstract Fe₃O₄@Ionic liquids β-cyclodextrin polymer(Fe₃O₄@ mono-6- deoxy-6- (1-ethyl- imidazolium)-β-cyclodextrin iodide polymer, ILs-β-CDCP) was prepared. A novel method based on Fe₃O₄@ILs-β-CDCP solid phase extraction coupled with fluorescence spectrophotometry for Rhodamine B (RhB) determination, was investigated. Results were shown that RhB was adsorbed on Fe₃O₄@ILs-β-CDCP and eluted with sodium dodecyl sulfate (SDS) (1.0%) rapidly. Different parameters, such as pH, adsorption time and volume, eluent volume and time were studied. This method introduced linearity for RhB between 0.01–9.00 μg/mL⁻¹, the limit of detection was 5.2 ng/mL⁻¹, correlation coefficient (R) was >0.9987 and the relative standard deviation (RSD) was 3.1% (*n* = 3, *c* = 4.00 μg/mL). The mechanism of adsorption of RhB on Fe₃O₄@ILs-β-CDCP was studied through the FTIR analysis and the inclusion constant of Fe₃O₄@ILs-β-CDCP-RhB. This method was applied successfully for determination of RhB in real samples with satisfactory results.

Keywords Rhodamine B · Fe₃O₄@ Ionic liquids-β-cyclodextrin polymer · Magnetic solid-phase extraction · Fluorescence spectrophotometry

Introduction

Rhodamine B (RhB) a pigment, is widely applied as color material in textiles and printing industries [1]. RhB is dangerous for human, causes skin and respiratory tract irritation [16, 22, 23], and has been banned for used as color additive in food stuffs. Some merchants use it in food mono-factories [11] so that harming people health [2]. Therefore, improving an easy method for determination of RhB in various samples is important. Different methods were applied such as ultraviolet spectrophotometry [18] fluorescence spectrophotometry [4], capillary chromatography [8] and HPLC [19] for determination of RhB. Among these methods, fluorescence spectrophotometry has the advantages of lower-cost analysis, easier operation and better accuracy. Thus, direct separation through spectrophotometry is not easy due to the little amounts of RhB in real samples. so, in this work magnetic solid phase extraction (MSPE) of (Fe₃O₄@ILs-β-CDCP) coupled with fluorescence spectrophotometry was introduced to determine (RhB) in food samples.

MSPE is a process depend on using magnetic absorbents for separation of various analytes from high amounts of sample. The magnetic absorbent was mixed with sample then analyte adsorbed by magnetic agents. The analyte-magnetic agents was separated from the sample through an external magnetic field after elution with the perfect eluent. Now a days, MSPE absorbent is exactly focused on Fe₃O₄ nanoparticles (NPs) with special functional groups. Various polymers were used to modify Fe₃O₄NPs [6, 13].

Ionic liquids (ILs) are a type of organic salts that had special physio-chemical properties like fair stability and hydrophobic properties [7, 9], IL/Fe₃O₄@ graphene were used as sorbents for MSPE nitrobenzene polymers in water extraction [3].

Ionic liquids coated MNPs were used as adsorbent in separation of aromatic hydrocarbons in water [10], separation of

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RhB in food using an ionic liquid β -CDCP SPE -HPLC, determination of Allura Red in food by Ionic liquid β -CDCP SPE -HPLC [15]. Ionic liquid coated $\text{Fe}_3\text{O}_4@ \text{SiO}_2$ nanoparticles were used accompanied with HPLC determination of RhB in food samples [5]. However, it was not mentioned for determination of RhB by $\text{Fe}_3\text{O}_4@ \text{ILs-}\beta\text{-CDCP}$.

In this study, MSPE agents were prepared, these agents have the property of the ionic liquids and (MNPs). Here MSPE adsorbent providing a fast and satisfactory preparation of sample, that applied for separation of high samples concentration in a few minutes. A novel method of MSPE coupled with fluorescence spectrophotometry for determination of (RhB) in food samples was introduced.

Experimental

Apparatus and Chemicals

Chemicals were offered through reagent grade, others mentioned. Standards solutions were brought from Reagent Corporation, Shanghai (China).

N,N-dimethylformamide, sodium hydroxide, hydrochloric acid, potassium iodide, methanol, acetonitrile, acetone, 1-ethylimidazole (Beijing Aipuxilong Biotechnology Co. Ltd., Beijing, China),

Centrifuge (Anke Scientific Instrument Factory, Shanghai), timing multifunctional oscillator (Guohua Co., Ltd., China), digital constant temperature water-bath (Guohua Co., Ltd., China).

A F-4500 spectrophotometer (Hitachi, Japan) was used for all the fluorescence measurement, with excitation and emission slits at 5.0 nm, $\lambda_{\text{ex}} = 330 \text{ nm}$ and 1-cm quartz cell.

Procedure

The synthetic procedure for $\text{ILs-}\beta\text{-CDCP}$ was prepared as in literature [14, 26, 27].

Synthesis of $\text{Fe}_3\text{O}_4@ \text{ILs-}\beta\text{-CDCP}$

First (3.0 g) of $\text{ILs-}\beta\text{-CDCP}$ were dissolved in 15 mL DMF and 30 mL in 70 °C in distilled water for 15 min, then 1.6 mL HDL under nitrogen. The mixture was put through stirring for 24 h. The product was gathered by filtering, and 200–300 mL of acetone was added and filtered and left under a continuous stirring for 24 h in 50 °C.

secondly (4.0 g) of $\text{ILs-}\beta\text{-CDCP}$ was added to 10 g of $\text{Fe}_3\text{O}_4 \cdot 7\text{H}_2\text{O} + 10 \text{ g FeCl}_3 \cdot 6\text{H}_2\text{O} + 200 \text{ mL}$ deionized water and ammonia solution 25 mL in 50 °C under nitrogen for 30 min, temperature was increased to 90 °C, The mixture was washed with deionized water using magnet and dried in vacuum for 24 h in 50 °C to obtain $\text{Fe}_3\text{O}_4@ \text{ILs-}\beta\text{-CDCP}$.

Adsorption/Elution

At room temperature, the sample solution after treatment and the pH buffer were added into a tube, and then distilled water was added to 40.0 mL. 0.1 g $\text{Fe}_3\text{O}_4@ \text{ILs-}\beta\text{-CDCP}$ were added into the tube, then solution was shaken for 15.0 min and centrifuged. 3.0 mL of Sodium dodecyl sulfate (SDS) (1%) was added into the used $\text{Fe}_3\text{O}_4@ \text{ILs-}\beta\text{-CDCP}$ as eluent. The mixture was ultrasonically vibrated at room temperature. Supernatant solution was separated via A F-4500 fluorescence spectrophotometer.

Sample Preparation

An amount of 5 g of hot pepper, chili powder and Chinese prickly ash were sensitively weighed into the beakers respectively, eluted in 90.0 mL of ethanol. Then evaporated at 60 °C, finally diluted with deionized water in 100.0 mL flask and put in darkness to be detected.

Results and Discussion

Characterization of $\text{Fe}_3\text{O}_4@ \text{ILs-}\beta\text{-CDCP}$

Infrared Spectroscopy

Fig. 1, showed the FTIR spectrum of (a) $\text{ILs-}\beta\text{-CDCP}$, (b) $\text{Fe}_3\text{O}_4@ \text{ILs-}\beta\text{-CDCP}$. Characterization data were as follows:

(A) $\text{ILs-}\beta\text{-CDCP}$ (IR/KBr, cm^{-1}): 1118, 1326, 3127 cm^{-1} . The peak of 1326 cm^{-1} due to C = C vibration; 3127 cm^{-1} peak was attributed to C-N vibration, which proved the $\text{ILs-}\beta\text{-CDCP}$ synthesis.

(B) $\text{Fe}_3\text{O}_4@ \text{ILs-}\beta\text{-CDCP}$ (IR/KBr, cm^{-1}): 1118, 1326, 3127 cm^{-1} . The peak of 1118 cm^{-1} were due to amide N-H absorption effect; the peak of 1326 cm^{-1} correspond to the absorption of band I, II and III of amide; in addition, the peak of 3127 cm^{-1} was attributed to C = O vibration, which indicated HDI and $\text{ILs-}\beta\text{-CDCP}$ reaction and confirmed $\text{Fe}_3\text{O}_4@ \text{ILs-}\beta\text{-CDCP}$ formation.

Scanning Electron Microscopy

The microscopic morphological structures which applied by SEM to differentiate and compare the external features of $\text{Fe}_3\text{O}_4@ \text{ILs-}\beta\text{-CDCP}$. Fig. 1, 2, showed the SEM micrographs of $\beta\text{-CDCP}$ (a), $\text{ILs-}\beta\text{-CDCP}$ (b) and $\text{Fe}_3\text{O}_4@ \text{ILs-}\beta\text{-CDCP}$ (c). When (a) with (b) and (c) was compared, $\text{ILs-}\beta\text{-CDCP}$ showed a spongy, fluffy porous and relatively coarse surface while $\text{Fe}_3\text{O}_4@ \text{ILs-}\beta\text{-CDCP}$ (C) showed a different surface similar to layer shape, and more bright with macro pores. The modification of Fe_3O_4 made the polymer's surface change.

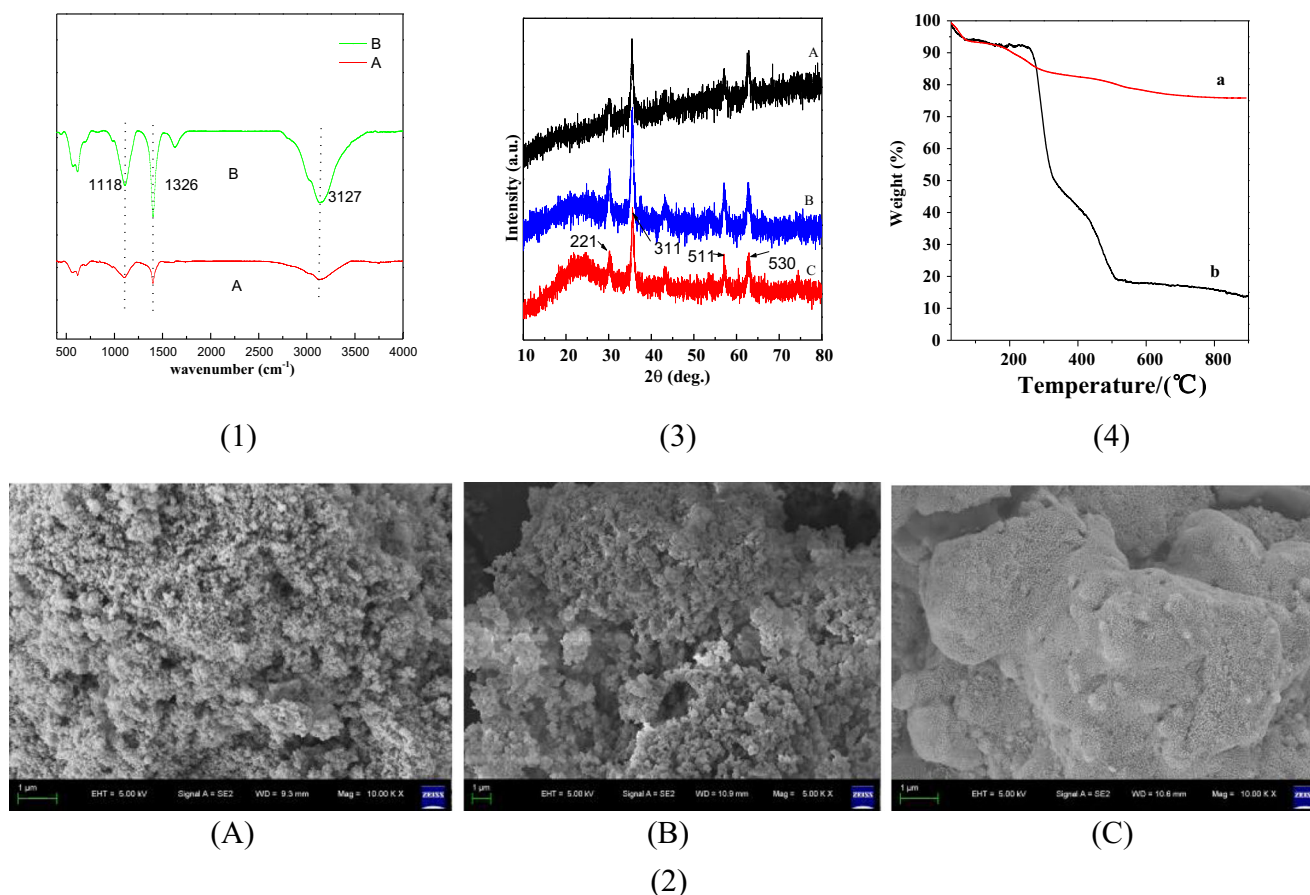


Fig. 1 illustrated: *1.1.* FTIR spectra of **a** ILs-β-CDCP, **b** Fe₃O₄@ ILs-β-CDCP. *1.2.* SEM micrographs of β-CDCP **a**, ILs-β-CDCP **b** and Fe₃O₄@ILs-β-CDCP **c**. *1.3.* XRD Patterns of Fe₃O₄ **a**, ILs-β-CDCP **b**, Fe₃O₄@ILs-β-CDCP **c**. *1.4.* TGA curves of **a** ILs-β-CDCP and **b** Fe₃O₄@ILs-β-CDCP

Characterization by X-Ray Diffraction

The XRD spectra of Fe₃O₄, ILs-β-CDCP and Fe₃O₄@ILs-β-CDCP are shown in Fig. 1. 3. The four peaks appeared at 2 θ of Fe₃O₄ and ILs-β-CDCP 30.03°, 37.01°, 58.00°, 63.00°. Fe₃O₄@ILs-β-CDCP had the same peaks as Fe₃O₄, which proved that the crystal shape of Fe₃O₄ particles were not altered through modifying of Fe₃O₄@ ILs-β-CDCP.

Thermo Gravimetric Analysis

Thermo gravimetric analysis (TGA) revealed the weight loss process of the materials, which indicated the difference between the ILs-β-CDCP(a) and Fe₃O₄@ILs-β-CDCP (b). In this paper, TGA was conducted in a nitrogen atmosphere, and the heating rate employed was 5 °C min⁻¹ all cases from 28 to 1000 °C (Fig. 1.4). The experimental results could be concluded that (1) the ILs-β-CDCP(a) showed a mass loss of about 1.5% after heating to 200–300 °C corresponding to the water content; (2) for the Fe₃O₄@ILs-β-CDCP(b), an additional weight loss of 5.4% was observed from 300 to 500 °C

due to the decomposition of ILs. This observation suggested that the ILs had been located on the surface of β-CDCP.

Optimization of Adsorption

Effect of pH

The pH value is not only affecting the existing RhB, but also can change the density of the negative charge on the surface of Fe₃O₄@ ILs-β-CDCP. Thus, it was necessary to investigate the pH value effect, the adsorption efficiency of RhB, was varied with the pH Fig. 2a. It could be concluded that the extraction efficiency of RhB by Fe₃O₄@ ILs-β-CDCP was above 91.0% when the pH was of 7.0. It reached the highest value 94.1% when pH was 8.0. This due to the fluorescence intensity of RhB

which was increased with pH increasing before 8.0.

Effect of Adsorption Temperature and Time

The extraction efficiency of RhB, on Fe₃O₄@ILs-β-CDCP at various temperatures (5.0–50.0 °C) were investigated in

Fig. 2 Effect of pH(a), Temperature(b), Extraction time(c), Sample volume(d) on extraction efficiency of $10 \mu\text{g mL}^{-1}$ rhodamine B

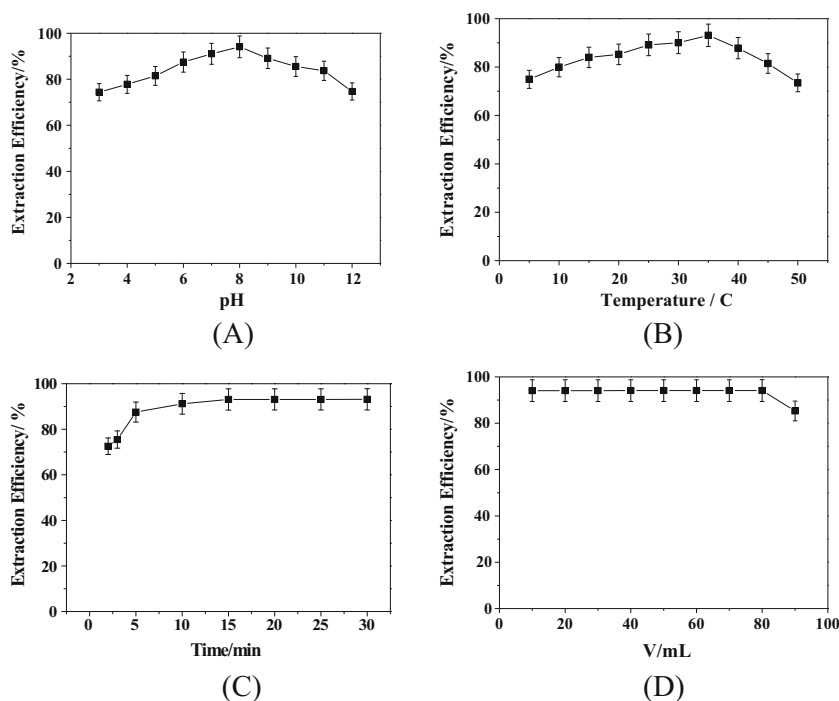


Fig. 2b. The efficiency of extraction of RhB, by $\text{Fe}_3\text{O}_4@ \text{ILs-}\beta\text{-CDPCP}$ was higher than 90% from 25.0 °C to 50.0 °C. The process was carried out at 25.0 °C.

The extraction process was finished in 15.0 min, the adsorption efficiency of RhB remained almost stable (93.0%) Fig. 2c, so 15.0 min was adopted as the extraction time for (RhB).

Effect Sample Volume

The extraction efficiency of RhB, varied with the increase of sample amount, the amount of the sample increased from 10.0 to 90.0 mL. The efficiency of adsorption of (RhB), was above 94% from 10.0 to 80.0 mL and went down slightly when volume was higher than 80.0 mL. Fig. 2d. Therefore the volume allowed was 80.0 mL.

Adsorption Capacity

The capacity of adsorption is known as highest volume of RhB which adsorbed per gram of the ($\text{Fe}_3\text{O}_4@ \text{ILs-}\beta\text{-CDPCP}$). The capacity of adsorption of RhB, on $\text{Fe}_3\text{O}_4@ \text{ILs-}\beta\text{-CDPCP}$ was studied through changing RhB concentration in aqueous samples before MSPE procedure. When the concentration of RhB was $60.0 \mu\text{g mL}^{-1}$, the adsorption of RhB reached the maximum. The capacity of adsorption for $\text{Fe}_3\text{O}_4@ \text{ILs-}\beta\text{-CDPCP}$ was found to be 10.90 mg g^{-1} .

Optimization of Elution

Selection of Eluent

Various eluents were studied in this work, their elution efficiency was ordered as follow: sodium dodecyl sulfonate (SDS) > cetyltrimethyl ammonium bromide (CTAB) > methanol > ethanol > NaOH (0.1 M) > HCl (0.1 M). So (SDS) was adopted.

Effect of Eluent Volume

Effect of (SDS)(1.0%) volume on the elution efficiency of RhB was studied. The results showed that it was over 94% when sodium dodecylsulfonate (SDS) was over than 3.0 mL. So the volume 3.0 mL was selected for (SDS).

Effect of Elution Time

The elution efficiency was increasing along with time until it reached 94.2% at 15.0 min, then it did not change and the elution process was finished thereafter. Elution time of 15.0 min was adopted for (RhB).

Reuse of $\text{Fe}_3\text{O}_4@ \text{ILs-}\beta\text{-CDPCP}$

The $\text{Fe}_3\text{O}_4@ \text{ILs-}\beta\text{-CDPCP}$ MNPs were washed with 2.0 mL of ethanol two times after each MSPE procedure. The reusability of $\text{Fe}_3\text{O}_4@ \text{ILs-}\beta\text{-CDPCP}$ was applied via efficiency of

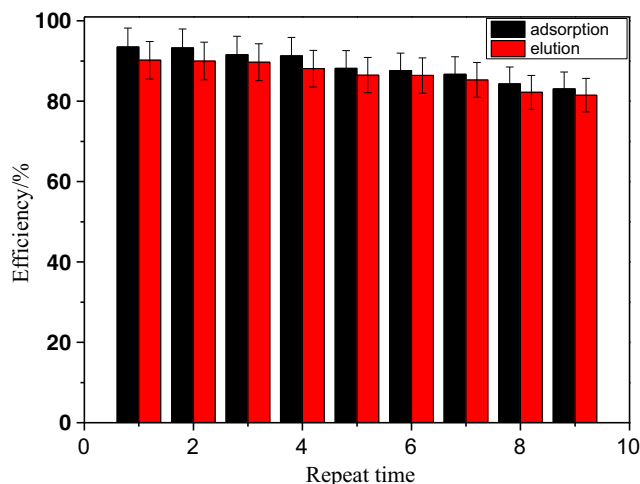


Fig. 3 Effect of reuse of Fe₃O₄@ ILS-β-CDCP in extraction and elution

adsorption and elution Fig. 3. The Fe₃O₄@ ILS-β-CDCP material could be reused nine times with efficiency of adsorption efficiency higher than 84% for Rhodamine B.

Effect of Interferents

The effect of interferents which food samples may contain on separation of RhB in the availability of interferents was investigated. The limit of tolerance for different interferents was 400 for SO₄²⁻, Br⁻, 230 for Citrate and phenol, 90 for NO₃⁻, 18 for Zn²⁺, Cu²⁺, glucose, 17 for Safranin T, Allura Red, Congo red, 15 for Bright yellow, Sunset Yellow and 2 for Rhodamine 6G. The results showed that most of the foreign material in samples had no interference in RhB determination.

Analytical Performance

Under optimum conditions described above, The proposed method introduced wide linearity within the concentration range of 0.10–9.00 μg mL⁻¹ with a correlation coefficient (R) >0.9987. Low detection limit 5.2 ng mL⁻¹ was found.

Sample Analysis

This method was introduced to separate RhB in hot pepper, chili powder and Chinese prickly ash Table 1. RhB amount was 5.46 μg kg⁻¹ in chili powder, the recovery rate of RhB was from 99.0% to 100.8%. and it was 4.33 μg kg⁻¹ in hot pepper the recovery rate of RhB from 97.0% to 100.7%.

No RhB was detected in Chinese prickly ash, the recovery rate of (RhB) was from 100.5% to 100.2%.

Table 1 Separation results of the samples

Sample	Added (μg kg ⁻¹)	Found (μg kg ⁻¹)	Recovery (%)
Chili powder	0	5.46	—
	5.00	5.01	100.2
	50.00	49.50	99.0
	100.00	100.9	100.8
Hot pepper	0	4.33	—
	4.00	4.06	97.0
	40.00	44.03	98.0
	100.0	100.5	100.7
Chinese prickly ash	0	ND	—
	5.00	5.02	100.5
	50.00	50.4	100.3
	100.00	101.2	100.2

Adsorption Mechanism of Fe₃O₄@ ILS-β-CDCP for Rhodamine B

The mechanism of adsorption of RhB on Fe₃O₄@ILS-β-CDCP was studied through the FTIR analysis and the inclusion constant.

FTIR Analysis

The FTIR spectrum for Fe₃O₄@ILS-β-CDCP and rhodamine B are important to study the mechanism of adsorption of rhodamine B on this polymer [24].

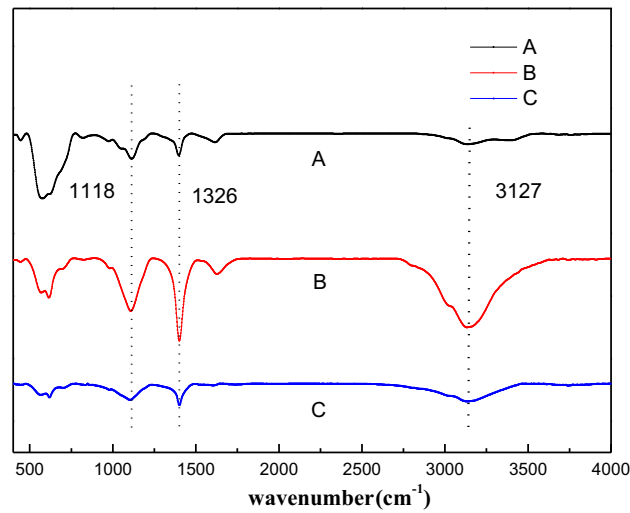
FTIR spectrum for rhodamine B, the Fe₃O₄@ILS-β-CDCP and Fe₃O₄@ ILS-β-CDCP - rhodamine B was studied (Fig. 4. 1). The intensity of the σ_{C=C} peaks from 1118 to 1326 cm⁻¹ in imidazole ring of Fe₃O₄@ ILS-β-CDCP -rhodamine B, are so high than those of the Fe₃O₄@ ILS-β-CDCP. The ILS bond interactions play a great role in the Fe₃O₄@ ILS-β-CDCP - rhodamine B inclusion [21]. The σ_{O-H} peak (3127 cm⁻¹) in the Fe₃O₄@ ILS-β-CDCP -rhodamine B increased, which composes that the hydroxyl part in rhodamine B is enclosed in the Fe₃O₄@ ILS-β-CDCP- rhodamine B.

Inclusion Constant

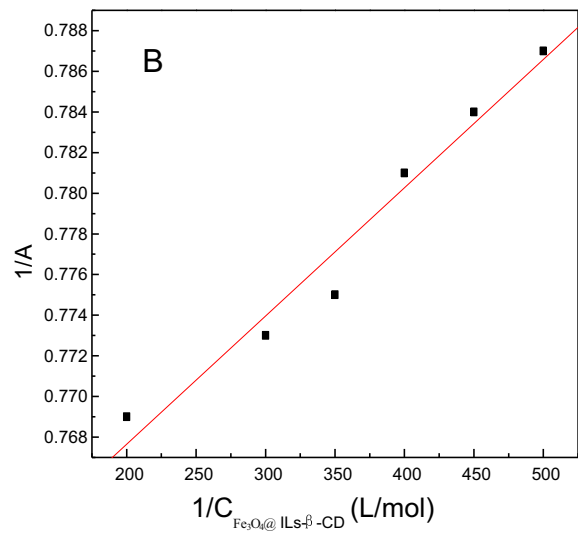
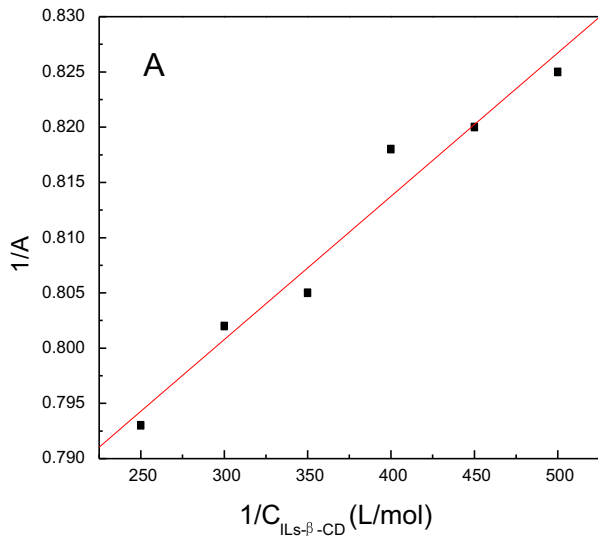
The inclusion constant K shows inclusion properties of host-guest molecules. The higher K was, the more stable inclusion complex was. K is calculated according to Benesi-Hildebrand- equation (double reciprocal plot) [20].

In this work, the inclusion constants of the monomers of two kinds of polymers (ILS-β-CDCP and Fe₃O₄@ILS-β-CDCP) and RhB were obtained.

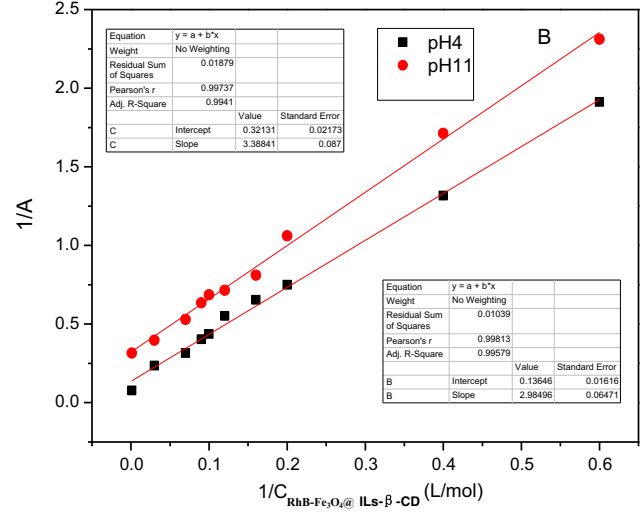
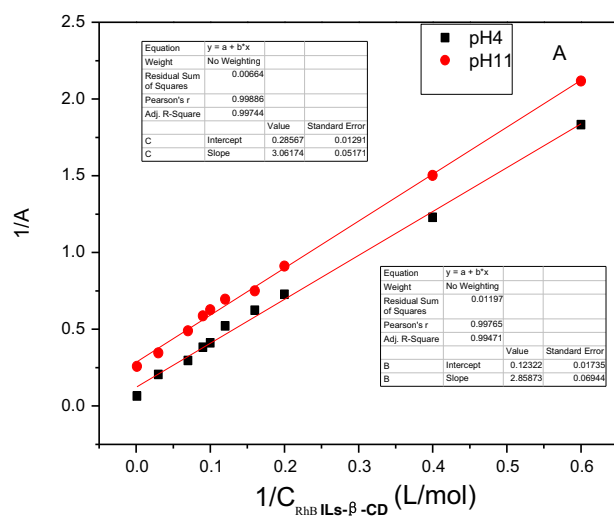
The double reciprocal plots for them were studied (Fig. 4 2). The two of them showed a satisfactory linearity and correlation coefficients of 0.9827 for ILS-β-CDCP and 0.9838 for Fe₃O₄@ILS-β-CDCP. It was concluded that both ILS-β-



(1)



(2)



(3)

Fig. 4 (1) FTIR spectra of **a** rhodamine B, **b** ILs-β-CDCP, and **c** Fe₃O₄@ILs-β-CDCP-rhodamine B. (2) Double reciprocal plot of the of ILs-β-CDCP- RhB (**a**) and Fe₃O₄@ILs-β-CDCP- RhB (**b**). (3) Double reciprocal plot of the of ILs-β-CDCP- RhB (**a**) and Fe₃O₄@ILs-β-CDCP- RhB (**b**) at pH: 4.0 and 11:0

CDCP and Fe₃O₄@ILs-β-CDCP with rhodamine B at the ratio of 1:1. The inclusion constant (K) of Fe₃O₄@ILs-β-CDCP- RhB inclusion complex was 1.18×10^4 L/mol, which was higher than 5.87×10^3 L/mol for ILs-β-CDCP- RhB. It indicated that the inclusion ability of Fe₃O₄@ILs-β-CDCP towards RhB was stronger than that of ILs-β-CDCP. This was due to the adsorption efficiency of Fe₃O₄@ILs-β-CDCP which was better than that of ILs-β-CDCP.

The inclusion constants of ILs-β-CDCP- rhodamine B and Fe₃O₄@ILs-β-CDCP- rhodamine B inclusion complexes were studied at (pH = 4.0 and pH = 11.0) and results were displayed in (Fig. 4 3), the values of R² were nearly 1 and it was form the inclusion complexes with rhodamine B at the ratio of 1:1.

On the these basis, it could be concluded that: (1) the inclusion constants of Fe₃O₄@ILs-β-CDCP-rhodamine B were always greater than that of ILs-β-CDCP- rhodamine B, which is reflected that the inclusion ability of Fe₃O₄@ILs-β-CDCP towards rhodamine B was stronger than that of ILs-β-CDCP; (2) the inclusion constants of Fe₃O₄@ILs-β-CDCP-rhodamine B were stable in different pH. It was consistent with the effect of pH on the adsorption efficiency and explained the adsorption efficiency was associated with the formation of inclusion complexes. These two all declared that the presence of ionic liquid enhanced the inclusion ability of Fe₃O₄@ILs-β-CDCP with rhodamine B and was conducive to adsorption of rhodamine B.

Comparison with Other Method

Table 2, listed the linear range and the limit of detection for separation of (RhB)in real samples obtained by the reported methods, such as HPLC, SPE-UV spectrophotometry, SPE-HPLC, MSPE-HPLC and HPLC–CL method. Compared with other reported methods, the method adopted in the

Table 2 Comparison with the results in other literatures

Method	Limit of detection	Linear range	Ref.
SPE-UV	7.46 μg/L	0.02–51.00 μg/mL	[17]
SPE-HPLC	1.9 μg/mL	0.02-8 μg/mL	[12]
HPLC-CL	7.8 μg/L	20–1400 μg/L	[25]
MSPE-HPLC	0.08 μg/mL	0.50–150.0 μg/mL	[5]
Fluorescence - spectrophotometry	5.2 ng/mL	0.1–9.0 μg/mL	Proposed method

present work obviously had a satisfactory linear range and limit of detection.

Conclusion

In this work, Fe₃O₄@ILs-β-CDCP was synthesized as magnetic solid phase extraction material to separate (RhB), in food samples. The proposed method for the analysis of Rhodamine B was proved to be satisfactory.

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Compliance with Ethical Standards

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Conflict of Interest Authors have a financial relationship with the organizations that sponsored the research. And the organizations are listed as follows:

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- (2) For Almojtaba Bakheet.

He is the current PhD students, declares that he has no conflict of interest.

Ethical Standards – Animal Rights and Informed Consent The research proposed in this article does not contain any studies with human or animals subjects. There are no ethical issues with human or animal subjects in our studies.

Informed Consent Not applicable.

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