Choline Proline Ionic Liquid-Functionalized $Fe_3O_4@SiO_2$ Nanoparticle Magnetic Solid Phase Extraction Coupled with High-Performance Liquid Chromatography for Analysis of Allura Red in Lipstick Sample

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Synopsis

A separation/analysis of allura red based on magnetic solid-phase extraction–high-performance liquid chromatography was developed. The extractant was prepared by functionalizing choline proline ionic liquid on $Fe_3O_4@SiO_2(Fe_3O_4@SiO_2@[Ch][Pro])$. Influences of experimental variables including extraction condition (material amount, pH, time, temperature, ionic strength, and sample volume) and elution conditions (eluent selection, amount, time) were evaluated. Under the optimal conditions, good linear calibration curves were obtained in the range of $0.02-5.0 \mu g/mL$, and limits of detection was 9.0 ng/mL. The proposed method was successfully applied for the determination of allura red in lipstick sample.

INTRODUCTION

Dyes are often used as colorants in foods, medicines, and cosmetics. Cosmetics have been widely welcomed by consumers in recent years. Lipsticks can change the lip color; this intuitive feeling is more attractive to consumers (1). Allura red is a dye commonly used in lipsticks; as a synthetic sulfonated azo red dye (Figure 1), compared with natural pigments, it has the advantages of good stability and low cost, but the molecular structure contains an azo group (-N = N-), which may cause adverse effects on human health (2). And the dye in lipstick is easy to be eaten by consumers, and excessive intake of allura red can cause carcinogenic diseases, allergies, multiple sclerosis, and brain damage (3). Therefore, it is necessary to determine the allura red of lipstick. At present, the commonly used methods for determining allura red are UV spectrophotometry (2)

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Figure 1. Structure of allura red.

and liquid chromatography (4). Because the matrix in lipsticks is complex and cannot be measured directly, it is important to develop effective sample pre-preparation methods.

Magnetic solid phase extraction (MSPE) is an extraction technology based on magnetic or magnetizable materials as solid absorbents. In the MSPE procedure, the magnetic adsorbent is dispersed in the sample solution to adsorb the analyte, and then, the analyte is collected by an external magnetic field, which greatly simplifies the traditional solid phase extraction and improves the extraction efficiency (5). MSPE is mainly based on the surface modification of Fe₃O₄ nanoparticles; SiO₂ is often used to modify Fe_3O_4 nanoparticles, and because of its high porosity and high surface area, it has a good adsorption of target analytes (6). However, the material also has its limitations; unlike chemical adsorption process, physical adsorption is limited in terms of porosity and surface area, especially the blockage of oil and organic macromolecules in the real sample, leading to a decrease in the adsorption rate of the analyte (7). Therefore, it has become a research focus in recent years to functionalize Fe₃O₄@SiO₂ nanoparticles to improve the adsorption. Some adsorbents such as Fe₃O₄@SiO₂@MOFs (8), Fe₃O₄@ $SiO_2@ILs$ (9), and $Fe_3O_4@SiO_2@GO$ (10) are used in the separation analysis of target analytes. Choline amino acid ionic liquids ([Ch][AA] have attracted attention in recent years because of their advantages of nontoxicity, good biocompatibility, and natural degradation. The positively charged choline cations in its structure showed a good electrostatic interaction with anionic target analytes. However, there are few reports on the modification of $Fe_3O_4@SiO_2$ nanoparticles with choline amino acid ionic liquids.

The aim of this work was to develop a method which was based on $Fe_3O_4@SiO_2@[Ch]$ [Pro], followed by magnetic solid-phase extraction—high-performance liquid chromatography to determine allura red in real samples. $Fe_3O_4@SiO_2@[Ch][Pro]$ was selected as an extractant, which was with good biological affinity and environmentally friendly, and no organic solvent that was of great importance from the perspective of green chemistry was consumed in the entire extraction process.

EXPERIMENTAL

REAGENTS

Anhydrous iron trichloride, ferrous sulfate heptahydrate, polyethylene glycol 400, ammonia, anhydrous ethanol, tetraethyl orthosilicate, choline chloride, proline (Pro), sodium hydroxide, hexamethylene bis-isocyanate, potassium bromide, dimethyl sulfoxide, and acetone were purchased from Sinopharm Chemical Reagent Co. Ltd. (Beijing, China).

INSTRUMENT

The following instruments were used in this study: a constant temperature oscillator (Guohua Electric Co., Ltd. Jintan, China), infrared spectrometer (Bruker, Germany), LC1220 high-performance liquid chromatography (Agilent Technologies, Santa Clara, CA), and DZF-6020 vacuum drying oven (Shanghai Jinghong Experimental Equipment Co., Ltd., Shanghai, China).

SYNTHESIS OF FE₃O₄@SIO₂

First, FeCl₃ (0.02 mol), FeSO₄·7H₂O (0.01 mol), and polyethylene glycol 400 (30 mL, 10%) were dissolved into 75 mL of deionized water at 80°C. Next, 50 mL of NH₃ aqueous was added under vigorous stirring and nitrogen protection. Then, the obtained magnetic nanoions were separated by a magnet and rinsed three times with deionized water; the product was dispersed with 240 mL of ethanol, 60 mL of distilled water, and 15 mL of ammonia water, and sonicated for 20 min. After sonication was completed, 1.7 mL of tetraethyl orthosilicate was added, and the mixture was stirred at 60°C for 12 h. Finally, it was washed twice with water and ethanol to obtain Fe₃O₄@SiO₂ (11).

SYNTHESIS OF CHOLINE PROLINE IONIC LIQUID ([CH][PRO])

Referring to the synthesis method of [Ch][AA] reported in the literature, [Ch][AA] was obtained by ion exchange and neutralization reaction, and the brief steps are as follows: first, [Ch]OH was obtained by passing [Ch]Cl through a ion exchange column. Then, [Ch][Pro] solution was obtained by a simple neutralization reaction between [Ch]OH and Pro. Last, the solution was dried to obtain [Ch][Pro] (12).

SYNTHESIS OF FE₃O₄@SIO₂@[CH][PRO]

A mixture of $Fe_3O_4@SiO_2$ (0.2 g), [Ch][Pro] (0.4 g), and dimethyl sulfoxide (40 mL) was dispersed ultrasonically for 1 h in a 250-mL three-necked flask and transferred to a water bath and stirred at 70 °C for 2 h; then, 1.0 mL of hexamethylene diisocyanate was added, and stirring was continued for 48 h. After the reaction, the product was separated with a magnet, and washed with distilled water and anhydrous ethanol three times. Finally, the product was dried at room temperature for 48 h to afford $Fe_3O_4@SiO_2@[Ch][Pro]$.

EXTRACTION PROCEDURE

The extraction of allura red was carried out in a 10-mL tube. A mixture of 1.0 mL of 100 μ g/mL allura red standard solution and 2.0 mL buffer (pH = 5.0) was diluted with

deionized water to a final volume of 10.0 mL 8.0 mg $Fe_3O_4@SiO_2@[Ch][Pro]$ nanoparticles was added in the tube and stored at room temperature for 5 min, and the nanoparticles were separated in a magnetic field and washed with 6.0 mL of KBr/acetone (KBr: acetone = 2:1, v:v; KBr: 2 mol/L). Finally, nanoparticles were separated again with an external magnet, and 20.0 µL of the upper solution was taken for HPLC determination.

CHROMATOGRAPHIC CONDITION

The allura red was separated in an Apollo C_{18} (150 × 4.60 mm, 5 micron, Shanghai Evans & Trade Co., Ltd., Shanghai, China); the mobile phase was a mixture of 0.02 M ammonium acetate and methanol (70:30, V/V). The flow rate was 1 mL/min, and the injection volume was 20 µL. The detector was programmed at 502 nm.

RESULTS AND DISCUSSION

CHARACTERIZATION OF FE3O4@SIO2@{CH}{PRO}

Figure 2 showed the FT-IR spectra of $Fe_3O_4@SiO_2$, [Ch][Pro], and $Fe_3O_4@SiO_2@$ [Ch] [Pro](curves a, b, c). From Figure 2, (i) the peaks around 582 cm⁻¹ and 1,042 cm⁻¹ in a



Figure 2. FT-IR spectra. (A) Fe₃O₄@SiO₂, (B) [Ch][Pro], and (C) Fe₃O₄@SiO₂@[Ch][Pro].



Figure 3. SEM image (A) Fe₃O₄@SiO₂ and (B) Fe₃O₄@SiO₂@[Ch][Pro].

curve were attributed to Fe-O and Si-O, respectively, which proved the successful synthesis of $Fe_3O_4@SiO_2$. (ii) In curve b, the N-H stretching of amino acids and the O-H stretching of choline correspond to broad peaks around 3,400 cm⁻¹, and the characteristic peaks (carboxylate anion stretching) of amino acid ionic liquids appeared around 1,590 cm⁻¹. (iii) There were stretching vibration peaks of Fe-O and Si-O at 582 cm⁻¹ and 1,042 cm⁻¹ in curve c, and the characteristic of amino acid ionic liquids appeared (1,590 cm⁻¹). These FT-IR data indicated that $Fe_3O_4@SiO_2@[Ch][Pro]$ have been successfully synthesized.

Figure 3 was an SEM image (a, b) of $Fe_3O_4@SiO_2$ and $Fe_3O_4@SiO_2@[Ch][Pro]$. In Figure 3A, it can be clearly observed that SiO_2 was successfully wrapped on Fe_3O_4 . Compared with Figure 3A, the particles on the surface of $Fe_3O_4@SiO_2$ became larger in Figure 3B, which proved that [Ch][Pro] was successfully functionalized on the surface of $Fe_3O_4@SiO_2$.

OPTIMIZATION OF EXTRACTION CONDITIONS

Effect of sorbent amount. The influence of sorbent amount on the extraction efficiency was evaluated; different amounts of $Fe_3O_4@SiO_2@[Ch][Pro] (4.0-12.0 mg)$ were used for the same procedures, and the result is shown in Figure 4. From Figure 4, it can be seen that by increasing the amount of $Fe_3O_4@SiO_2@[Ch][Pro]$ from 4.0 to 8.0 mg, the extraction rate of allura red was slightly increased. The extraction efficiency reached a maximum after 8.0 mg of sorbent; therefore, 8.0 mg of $Fe_3O_4@SiO_2@[Ch][Pro]$ was selected to obtain higher extraction efficiency.

Effect of pH. pH was a significant factor affecting the existing form of allura red during the extraction process. The effect of pH on the extraction of allura red was studied in the range between 4.0 and 10.0. It can be seen from Figure 5 that allura red had higher extraction efficiency under acidic conditions. The reason for this phenomenon might be that allura red exists in the form of $R-SO_3^-$ anion under acidic conditions, which exist as electrostatic force with choline cation of $Fe_3O_4@SiO_2@[Ch][Pro]$. When pH was > 7, the extraction rate gradually decreased, and the reason was attributed to the increase in OH⁻ concentration, which competed with $R-SO_3^-$ to adsorb on the nanoparticles (13). Therefore, pH 5.0 was selected in the following experimental step.



Figure 4. Effect of $Fe_3O_4@SiO_2@[Ch][Pro]$ amount on extraction efficiency. Experimental conditions: 2.50 µg/mL allura red and 2.0 mL buffer (pH = 5.0).



Figure 5. Effect of pH on the extraction rate. Experimental conditions: 2.50 μ g/mL allura red, 8.0 mg Fe₃O₄@SiO₂@[Ch][Pro], and 2.0 mL buffer.

Effect of temperature. The effect of temperature on extraction was investigated from 5°C to 45°C. It was found that the temperature had little effect on the extraction efficiency of allura red, and the extraction rates were all greater than 92% at selected temperatures. So the following experiment was performed at room temperature.

Effect of extraction time. The contact time between analyte and adsorbent was an important factor affecting the adsorption process. The effect of the extraction time in range of 1-25 min was investigated. As shown in Figure 6, the extraction process started immediately, and the extraction rate increased sharply to 96% within 5 min, and then remained stable, so in this experiment, the extraction time was chosen as 5 min.

Effect of ionic strength. The ionic strength may affect the adsorption of allura red by magnetic nanoparticles, so 0-6.0% (w/v) of NaCl was used as the electrolyte model to investigate the effect of ionic strength on the adsorption. The results were shown in Figure 7. When NaCl was not added, the extraction efficiency of allura red was the largest. As the concentration of NaCl in the system increased, the extraction efficiency decreased. The reason for this phenomenon may be that the extraction was dominated by electrostatic force, Cl⁻ and allura red competed for adsorption by choline cations, leading to a reduction in the extraction rate. Therefore, no NaCl added was chosen in this experiment.

Effect of sample volume. The effect of sample volume on the extraction efficiency of allura red was explored in the range of 10.0–70.0 mL; the results were shown in Figure 8. From



Figure 6. Effect of time on extraction efficiency. Experimental conditions: 2.50 μ g/mL allura red, 8.0 mg Fe₃O₄@SiO₂@[Ch][Pro], and 2.0 mL buffer (pH = 5.0).



Figure 7. Effect of ionic strength on extraction efficiency. Experimental conditions: 2.50 μ g/mL allura red, 8.0 mg Fe₃O₄@SiO₂@[Ch]{Pro], and 2.0 mL buffer (pH = 5.0).



Figure 8. Effect of sample volume. Experimental conditions: 10 µg allura red, 8.0 mg Fe3O4@SiO2@[Ch] [Pro], and 2.0 mL buffer (pH = 5.0).

Figure 8, with the increase in sample volume, the extraction rate of allura red reduced. The extraction rate was lower than 85.0% when the sample volume was more than 40 mL. Therefore, the system allowed a maximum sample volume of 40 mL.

Adsorption (extraction) capacity. The adsorption (extraction) capacity of Fe₃O₄@SiO₂@[Ch] [Pro] was investigated by changing the concentration of allura red. The results are shown in Figure 9. As can be seen from Figure 9, when the concentration of allura red in the system reached 40.0 μ g·mL⁻¹, the adsorption (extraction) reached saturation. The adsorption (extraction) capacity of Fe₃O₄@SiO₂@[Ch][Pro] for allura red was 65.4 mg/g.

OPTIMIZATION OF ELUTION CONDITIONS

Eluent selection. The effect of different eluents on the elution rate of allura red was studied; the results are shown in Figure 10A and B. As shown in Figure 10A, the elution rate of allura red by common organic solvents (ethanol and acetone) was very low. For 2 mol/L NaCl, KCl, and KBr, the elution efficiency of allura red can reach 53.21, 52.14, and 65.94%, respectively. The reason for this phenomenon might be that the elution rate was greatly affected by anions, and the ionic radius of Br⁻ is larger than that of Cl⁻; there was an electrostatic effect between Br⁻ and choline cations in the solution, so part allura red was eluted. As shown in Figure 10B, when 2 mol/L KBr solution was mixed with acetone



Figure 9. Adsorption (extraction) capacity. Experimental conditions: 8.0 mg Fe3O4@SiO2@[Ch][Pro] and 2.0 mL buffer (pH = 5.0).



(A) Eluent selection.



(B) Effect of potassium bromide/acetone. 2.50 µg/mL allura red

Figure 10. (A) Eluent selection. (B) Effect of potassium bromide/acetone, and 2.50 µg/mL allura red.

in different volume ratios, it showed strong elution ability for allura red, and the elution rate can reach 98%. This phenomenon may be caused by the synergistic effect of acetone and potassium bromide. On the one hand, acetone and water molecules can form hydrogen bonds, and the hydrogen bonding force was greater than the Van der Waals force, so acetone was attracted by the water molecules, and the water molecules repel the dye. KBr was ionized in water to produce ions, which attracted to hydrogen ions and hydroxide ions in water, separating acetone from water. On the other hand, allura red was eluted by Br^- and dissolved in acetone. Therefore, 2 mol/L KBr/acetone (2:1, v/v) was finally selected as an eluent.

Effect of eluent volume. The effect of eluent volume in the range of 2.0–10.0 mL on the elution rate of allura red was evaluated, and the result is shown in Figure 11. As the eluent volume increased, the elution rate gradually increased. When the eluent volume was 6.0 mL, the maximum value was reached; therefore, the optimal eluent volume for this experiment was 6.0 mL, and the pre-enrichment factor of allura red was 6.7 times (sample volume/eluent volume).

Effect of eluent time. The elution time was investigated in the range of 2.0–10.0 min; the results showed that the elution can be completed within 2 min, and the elution efficiency was greater than 97%, so the optimal elution time was chosen as 2 min.

*Reuse times of Fe*₃O4@SiO2@{Ch}{Pro} The reuse times of Fe₃O₄@SiO₂@[Ch]{Pro} were investigated, and the result is shown in Figure 12. From Figure 12, it could be reused



Figure 11. Effect of eluent volume, and 2.50 μ g/mL allura red.



Figure 12. Reuse times of $Fe_3O_4@SiO_2@[Ch]{Pro}$, and 2.50 µg/mL allura red.



Figure 13. HPLC chromatograms. (A) Allura red standard solution (5 μ g·mL-1), (B) sample before extraction, and (C) sample after extraction.

14 times, and the extraction efficiency was lower than 85.0% after being eluted 14 times, which proved that the material can be reused.

ANALYTICAL APPLICATION

The HPLC chromatogram of allura red standard solution (curve a) and samples before and after extraction (curve b,c) are shown in Figure 13. From Figure 13, the retention time (t_R) values for allura red were 11.2 min, and the concentration of the allura red was significantly increased after extraction.

Linear relationship and detection limit. The linear equation, linear correlation coefficient, linear range, relative standard deviation, and detection limit of the method in this article are listed in Table I.

Comparison with other methods. The developed method was compared with other methods reported in the literature for extraction and determination of allura red (Table II). From Table 2, it can be seen that this method has a wide linear range, low detection of limit, and short extraction time.

Real sample analysis. To evaluate the applicability of the proposed method, two commercial lipsticks were extracted using the method and analyzed by HPLC. The analysis results are shown in Table III. From Table 3, it can be known that allura red was measured in one of the lipstick samples, and the content of allura red in the lipstick was 16.78 mg/ kg. The recovery rate of allura red was 88.05–104.26%, which proved that the method can be used to determine the content of allura red in lipstick.

Enter Relationship and Detection Entite			
Parameter	Allura red		
Linear regression equation	S = 4485152c + 240565		
Linear range (µg/mL)	0.02-5.0		
Correlation coefficient (r^2)	0.9997		
Limit of detection (ng/mL)	9.0		
RSD (%)	3.8		

Table I	
Linear Relationship and Detection	Limit

Table II				
Comparison of Methods for Determination of Allura Red				

Method	Extraction agent	Linear range (µg/mL)	Detection limit (ng/mL)	Extraction time (min)	Reference
MSPE-LC-MS	NH ₂ -LDC-MP	0.05-5.0	9.0	-	(14)
M-dSPE-LC-MS	MSPD	0.005 - 1.0	1.0	-	(15)
MSPE-HPLC	Fe ₃ O ₄ -NH ₂ @GO	0.005-1.5	2.0	15	(16)
CSPE-UV	T-1180	0.4-8.0	1.0	120	(17)
CPE-UV	Mixed micelles	0.02-1.4	8.0	50	(18)
MSPE-HPLC	Fe ₃ O ₄ @SiO ₂ @IL	0.02-5.0	9.0	5	This work

Analytical Results of Allura Red in Real Sample						
Sample	Added (mg/kg)	Found (mg/kg)	Recovery (%)	RSD (%)		
	0.00	16.78	/	3.4		
Lipstick (I)	7.50	23.38	88.05	2.7		
	15.00	30.79	93.40	1.2		
	0.00	ND	/	/		
Lipstick (II)	7.50	7.07	94.27	3.8		
	15.00	15.64	104.26	2.1		

 Table III

 Analytical Results of Allura Red in Real Sample

CONCLUSION

In this work, an MSPE method was developed for pre-concentration of allura red for determination by HPLC. The sorbent was prepared by functionalizing choline proline ionic liquid on $Fe_3O_4@SiO_2$. The method had simple operation steps, short extraction process time, and high extraction efficiency. This method was used to determine the content of allura red in lipsticks, with high sensitivity and good accuracy.

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