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A novel molecularly imprinted polymer for simultaneous extraction and determination of sudan dyes by on-line solid phase extraction and high performance liquid chromatography

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ABSTRACT

A novel molecularly imprinted polymer was synthesized with attapulgite employed as matrix, which is simple and time-saving. In this method, sudan I was chosen as template molecule, 2-vinylpyridine as functional monomer and ethylene glycol dimethacrylate as cross-linking agent, respectively. The imprinted polymer was characterized by the infrared spectroscopy and transmission electron microscopy. Then the selectivity experiments were performed on sudan dyes and the recognition coefficients for sudan I, sudan II, sudan III and sudan IV were 2.9, 1.9, 1.9 and 2.3, respectively. As the packing material of solid-phase extraction, the imprinted polymer has been applied to on-line concentration of the four sudan dyes in samples from Yellow River water, tomato sauce and sausage. The corresponding analytical methods to determine these sudan dyes have been developed. The limits of detection for these sudan dyes were in the range of 0.01–0.05 ng mL^{-1} for Yellow River water, 1.0–3.0 ng g^{-1} for tomato sauce and 0.8–3.0 ng g^{-1} for sausage.

1. Introduction

Molecularly imprinted polymers (MIPs) are a kind of synthetic materials with artificially generated binding sites to recognize a target molecule in preference to other compounds with similar structures, and they have been extensively used in catalysis [1], sensors [2], drug carriers [3], artificial antibodies [4], high performance liquid chromatography (HPLC) [5], solid-phase extraction (SPE) and solid-phase microextraction (SPME) [6]. The common methods for preparing MIPs suitable for SPE include bulk polymerization, precipitation polymerization and suspension polymerization. Although the MIPs prepared by conventional methods exhibit high selectivity, they still suffer some intrinsic limitations, such as the heterogeneous distribution of the binding sites, the deep embedded binding sites in bulk and poor site accessibility for the template molecule [6]. In order to overcome these defections, MIPs based on various novel assisted-matrixes have been prepared, including silica [7], multi-walled carbon nanotubes [8], chitosan [9], Fe_3O_4 magnetic nanoparticles [10], and quantum dot [11].

Attapulgite (ATP), a kind of natural one-dimensional nanosilicate clay with reactive –OH groups on its surface, has been widely used as an environmental absorbent [12], catalyst [13], rheological control agent [14], etc. Because of its unique morphology, surface properties, and low price, ATP has drawn extensive attention in the preparation of polymer/ATP nanocomposites. In our previous work [15], we had firstly synthesized MIP prepared by attapulgite as matrix aiming at selective SPE of diethylstilbestrol. However, the synthetic approach was complex and time-consuming, which restricted its wide applications.

Sudan I, sudan II, sudan III and sudan IV (sudan I–IV, see Fig. 1) are azo dyes considered to be genotoxic carcinogen, and they impose a potential risk on public health once they enter the food chain [16], so they are illegal as additives in foodstuffs according to both the Food Standards Agency and the European Union [17]. Unfortunately, these dyes are still being used to enhance the appearance of products such as chilli, tomato sauces, salami, olive oil, and many other frequently eaten foodstuffs [18], which should be monitored to ensure the food safety. The European Commission has set the detection limits for these sudan dyes at 0.5–1.0 μg g^{-1} since 2003 [19,20]. In this work, the limits of detection for these substances could reach the range of 0.8–3.0 ng g^{-1} for tomato sauce and sausage samples.

In view of the complexity of matrix and the low levels of sudan dyes containing in real samples, some pretreatment steps, such as liquid-solid extraction [21], pressurized liquid extraction [17], the MIP-SPE [22] and cloud point extraction [23], have been applied for the purpose of isolation or enrichment.

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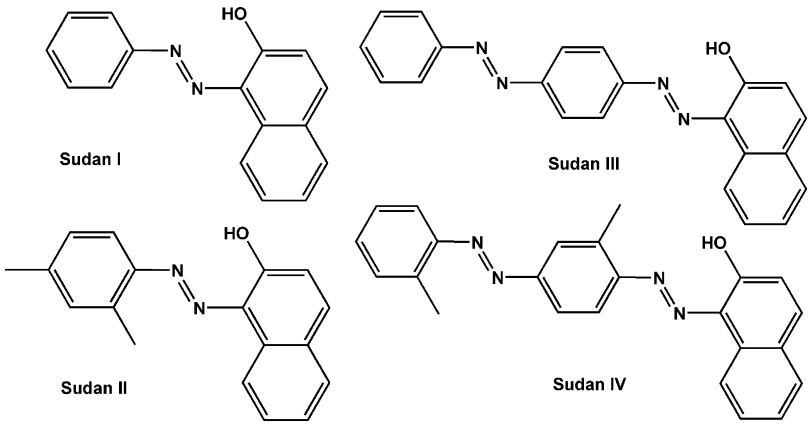

Fig. 1. Structures of sudan dyes.

The approach to synthesize sudan I MIP using ATP as matrix was developed in this work, which is more simple and time-saving approach compared with the one mentioned in our previous study [15]. Additionally, the MIP-SPE column based on sudan I MIP was prepared and used for the simultaneous on-line SPE to determine sudan I–IV. Under this condition, the π–π and the hydrophobic interactions between the analytes and the sorbent occurred simultaneously in the MIP-SPE column during the sample preparation [22,24]. After the optimization of sample pH, loading flow rate, loading time and ionic strength to on-line MIP-SPE, the corresponding analysis methods for sudan dyes in Yellow River water, tomato sauce and sausage samples were established. The results indicated that sudan I MIP using ATP as matrix exhibited the excellent recoveries for the four dyes.

2. Experimental

2.1. Materials and chemicals

Attapulgite (ATP) was provided by Gansu ATP, (Gansu, China), which was dried in vacuum at 110 °C for 48 h before use. Methacrylic acid (MAA), ethanol, acetonitrile and acetic acid were from Tianjin Guangfu Fine Chemical Research institute (Tianjin, China). Ethylene glycol dimethacrylate (EGDMA), 3-methylacryloxypropyltrimeth-oxysilane (MATMS), and 2-vinylpyridine (2-VP) were purchased from Alfa Aesar (Beijing, China). Azo-bis-isobutyronitrile (AIBN) and 4-tertbutylphenol (TP) were obtained from Chemistry Reagent Factory of Chinese Fuchen (Tianjin, China). Sudan I–IV were purchased from Sinopharm Chemical Reagent Co. Ltd (Shanghai, China). Toluene and dimethylformamide (DMF) (Tianjin Chemical Reagent Co., Tianjin, China) were purified with CaH_2 stirring overnight and distilled under reduced pressure to remove the trace water. All the chemicals mentioned above were of analytical grade. Ultra pure water used throughout the whole experiments was obtained from the MILLI-Q (Millipore, Bedford, MA, USA) purification system. HPLC-grade acetonitrile was from Dima Technology (RichmondHill, USA).

The chromatographic analytical system consisted of a Model 210 HPLC pump and a UV detector (Varian Prostar). All separations were carried out on a C_{18} column (Dikma Technologies, 250 × 4.6 mm). The UV–vis detector was operated at 500 nm.

2.2. Preparation of MIP sorbent

The method for preparing MIP is shown in Fig. 2. And in this experiment, molar ratio among template molecule, functional monomer and cross-linking agent was determined according to literatures [22,25]. The whole experiment in detail was performed as the following procedure.

First, MATMS was assembled onto the surface of the attapulgite nanofibrillar clay [26]: 1.0 g ATP and 0.8 mL MATMS were dispersed into 50 mL dried toluene with ultrasonic agitation for 30 min and then the mixture was refluxed for 8 h with N_2 protection. After cooling to room temperature, the product, methylacryloxypropyl modified attapulgite (M-ATP), was filtered and thereafter thoroughly washed with ethanol and dried in a vacuum at 40 °C overnight.

Second, sudan I (24.8 mg, 0.1 mmol) was dissolved in 25 mL acetonitrile. Under stirring, 2-VP (42 μL, 0.4 mmol) and EGDMA (375 μL, 2 mmol) were added. Then M-ATP (500 mg) was dispersed in the solution. The polymerization was initiated by adding 30 mg AIBN under N_2 protection at 55 °C, and then kept stirring for 8 h. After that, the polymer was collected and washed with ultra pure water and acetonitrile. Then template molecules were removed by Soxhlet extraction in acetonitrile–acetone solution 70:30 (v/v) for 48 h. For comparison, non-imprinted polymer (NIP) was also prepared in the similar manner described as above, except for the absence of sudan I.

The obtained products were characterized with Nicolet Nexus 670 Fourier transform infrared (FTIR) (MN, USA) spectrometer and JEM1200EX transmission electron microscope (TEM) (Tokyo, Japan).

2.3. Rebinding test and selectivity evaluation

In order to investigate the binding capacity of the MIP in acetonitrile, static absorption experiments were performed as follows: 20 mg imprinted materials was added into a flask and 10 mL solution of sudan I with different concentration was also added. After being shaken for 24 h in the dark at room temperature, the solutions were centrifuged, filtered, and then determined by HPLC. The data of the static absorption experiment were further processed according to the Scatchard equation (1) [27] to estimate the binding parameters of the MIPs.

$$\frac{Q}{C_{free}} = \frac{(Q_{max} - Q)}{K_d} \quad (1)$$

Here Q is the amount of sudan I bound to MIP at equilibrium, Q_{max} is the maximum binding capacity, C_{free} is the equilibrium concentration of sudan I and K_d is the dissociation constant, respectively.

Twenty milligrams of the MIP or NIP was equilibrated with 10 mL sudan I–IV or TP (4-tert-butylphenol) solution (prepared with acetonitrile) with concentration of 200, 50, and 5 μg mL^{-1} to evaluate the selectivity of the imprinted sorbent. The mixture was shaken for 24 h at room temperature (about 18 °C) to facil-

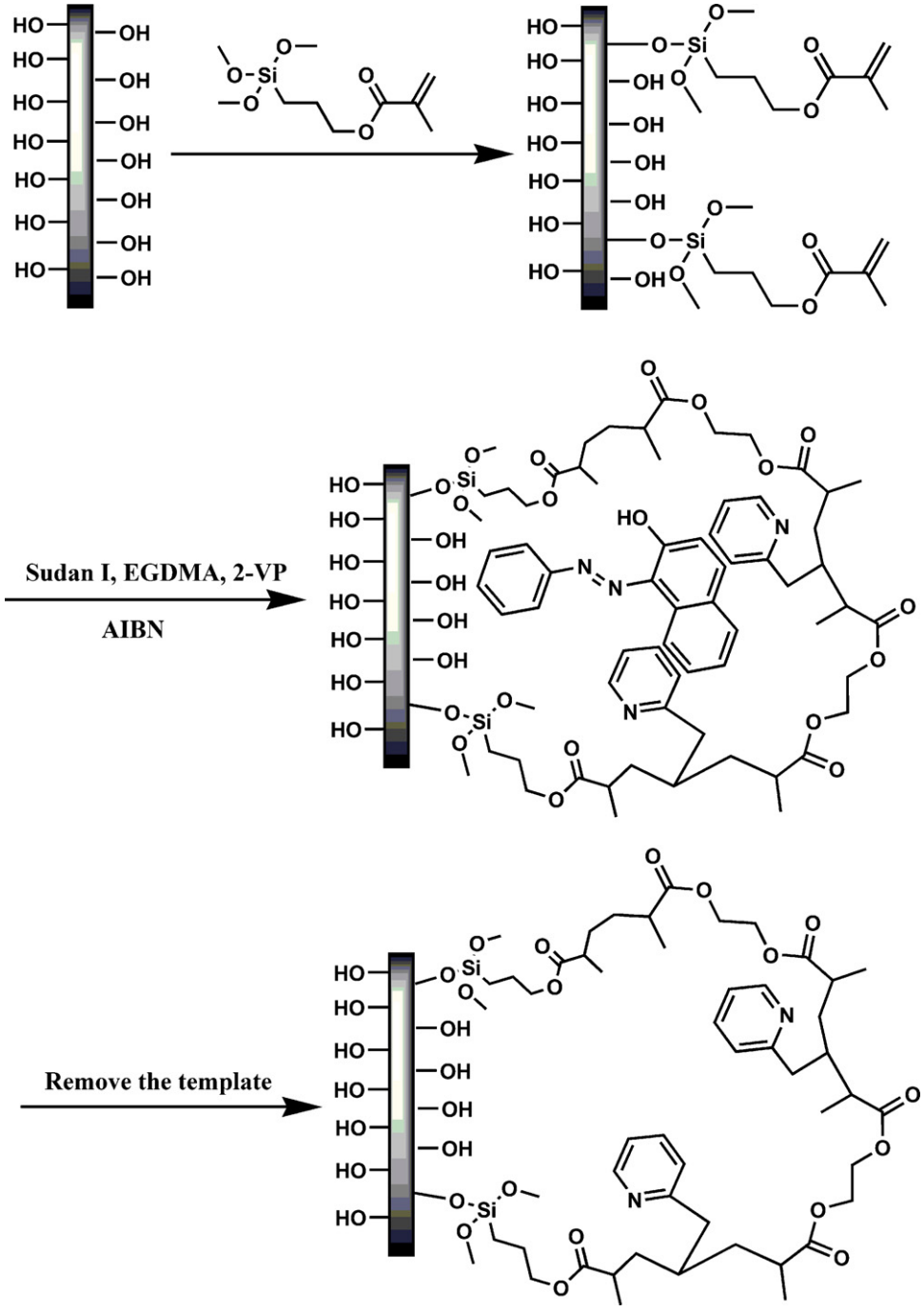

Fig. 2. Preparation procedure of molecularly imprinted polymer.

itate adsorption of these compounds onto the MIP sorbent. The concentrations of free analyte were determined by HPLC.

Recognition coefficient (α) was used to evaluate the recognition ability, which was calculated according to the following formula (2) [28]. In theory, when α is greater than 1, it indicates that the MIP has selectivity to the analyte.

$$\alpha = \frac{S_{MIP}}{S_{NIP}} \quad (2)$$

where S_{MIP} and S_{NIP} represent the amount of analyte bound to MIP and NIP sorbent, respectively.

2.4. On-line SPE-HPLC determination of sudan I–IV

In order to evaluate the applicability of the MIP for on-line SPE-HPLC determination of sudan I–IV, a SPE column packed with 80 mg MIP was prepared and placed in a six-port valve loop. The configuration of on-line SPE coupled with HPLC was assembled according to literatures [29]. Hereon, Waters 510 pump was used to load samples.

The MIP-SPE column was pretreated with acetonitrile and pure water before being used. The extraction was performed by passing sudan dyes aqueous solution through the MIP-SPE column using Waters 510 pump when the six-port injector valve set to “LOAD”

position. As a result, sudan mixtures were preconcentrated by the MIP-SPE column. Simultaneously, the mobile phase was directly driven by HPLC pump through the analytical column to obtain a flat baseline. Then, the injector valve switched to "INJECT" position and the adsorbed sudan dyes were eluted by the HPLC mobile phase at a flow rate of 1.0 mL min^{-1} for 1.5 min at room temperature (about 18 °C). The mobile phase consisted of 10% A (ultra pure water) and 90% B (acetonitrile–acetone mixture (90:10, v/v)). Before loading the next sample, the loading system was purged using ultra water and acetonitrile at the flow rate of 4 mL min^{-1} for 1 min.

2.5. Determination of sudan I–IV in samples

2.5.1. Determination of sudan I–IV in Yellow River water

The water sample was filtrated through a 0.22 μm filter firstly. Then 100 mL water sample was loaded on the preconditioned MIP-SPE column at flow rate of 5 mL min^{-1}. Later, the MIP-SPE column was washed by 8.0 mL methanol and 8.0 mL ultra pure water at a flow rate of 4.0 mL min^{-1} before desorbing the analytes for further analysis.

2.5.2. Determination of sudan I–IV in tomato sauce and sausage samples

Extraction of the dyes from 2.0 g spiked or nonspiked tomato sauce was carried out in a 10 mL centrifuge tube followed by adding 2 mL acetonitrile and water solution (50:50, v/v). The mixture was shaken (QL-861 vortex shaker, Jiangsu Haimen Kylin-Bell Lab Instruments, Haimen, China) for 2 min to extract dyes. The extract was carefully decanted to the other centrifuge tube, and the residues were extracted twice again. The extract was pooled together, filtrated through a 0.22 μm filter and diluted to 25 mL with water. Then it was loaded on the preconditioned MIP-SPE column.

Extraction of the dyes from sausage samples was performed in the similar steps described as above.

3. Result and discussion

3.1. Preparation of MIP

The selection of a suitable template is mainly driven by the similarity to the analytes [30], and a good template of these analytes should possess the general structure of the molecule. As to the four sudan dyes, they have very similar structures, and sudan I was chosen as the template of MIP to simultaneously extract these four dyes.

In our previous work [15], the hyperbranched aliphatic polyester was firstly grafted onto the ATP by the polycondensation of the 2,2-bis(hydroxymethyl)-propionic acid so that more hydroxyl groups could appear on the surface of ATP. Afterwards, the vinyl functional group was introduced by the esterification between methacrylate and the hydroxyl modified on the materials. It was obvious that these steps were time-consuming and not conducive to widely use. While in this work, we developed a more simple approach to synthesize sudan I MIP. The vinyl functional group was easily modified onto the surface of ATP with MATMS by silane coupling reaction, and then the modified ATP was directly used as the matrix of MIP, which was time-saving, economic and beneficial to the development of ATP matrix based MIP.

3.2. Characteristics of molecular imprinting polymers

FTIR spectra of ATP, M-ATP and MIP are shown in Fig. 3. The characteristic peak of ATP was around 1640 cm^{-1}, corresponding to the hydrated bonds of ATP. The absorbance band of C=O at 1713 cm^{-1} in M-ATP spectrum shows the MATMS had been grafted

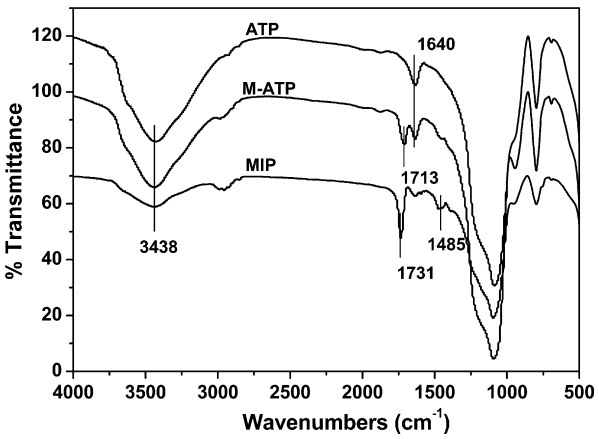

Fig. 3. FT-IR spectra of ATP, M-ATP and MIP.

onto the surfaces of the ATP. The obvious increase of carbonyl group at 1731 cm^{-1} and the presence of the bands at about 1485 cm^{-1} of C–N demonstrate that the EGDMA and 2-VP had polymerized to the surface of M-ATP.

The TEM images of ATP and MIP are shown in Fig. 4. There are significant layers present on the surface of ATP, which indicated MIP has been successfully prepared.

3.3. Rebinding test and selectivity evaluation of the imprinted sorbent

The MIP materials have higher binding capacity to sudan I (Fig. 5), and the two distinct linear portions in Scatchard analysis indicate a fact that two classes of binding sites exist in the imprinted polymer: one exhibits high selectivity or affinity with high binding energy, while another has low affinity with low binding energy. From the slope and intercept of the straight line, the K_{d1} and Q_{max1} of the higher affinity binding sites can be calculated to be 0.031 mmol L^{-1} and 0.297 mmol g^{-1}, respectively. Similarly, the K_{d2} and Q_{max2} values of the lower affinity binding sites are 0.215 mmol L^{-1} and 0.425 mmol g^{-1}, respectively.

The α of MIP to sudan I–IV were greater than 1 (Table 1). It is clear that MIP can recognize not only the template molecule (sudan I) but also structurally related compounds (sudan II–IV). Four sudan dyes have similar adsorption behavior on MIP. In binding process, specific recognition sites with respect to template generated on the surface of MIP led to high binding ratio for the four dyes. However, the α for TP was around 1, indicating the MIP had no specific site to the compound with significantly different structure. These results demonstrate the applicability of sudan I as template for MIP preparation. Meanwhile, It can been seen that α for sudan I–IV were increasing with the decreased concentration of each dye, which can attribute to the nonspecific adsorption of sudan I–IV on the MIP reduced at lower concentration.

3.4. On-line MIP-SPE

3.4.1. Separation and desorption conditions

Firstly, the separation conditions of standard mixture without the on-line SPE were optimized. When the mobile phase was acetonitrile: water (90:10, v/v), all of the sudan dyes could be baseline separated. However, under this identical separation conditions, no chromatographic peaks were detected when the on-line SPE and HPLC system was coupled together, which showed that desorption of analytes from the MIP adsorbent were particularly slow. Hence 10% acetone was added into acetonitrile to improve the desorption of four sudan dyes. At last, mobile phase consisted of 10%

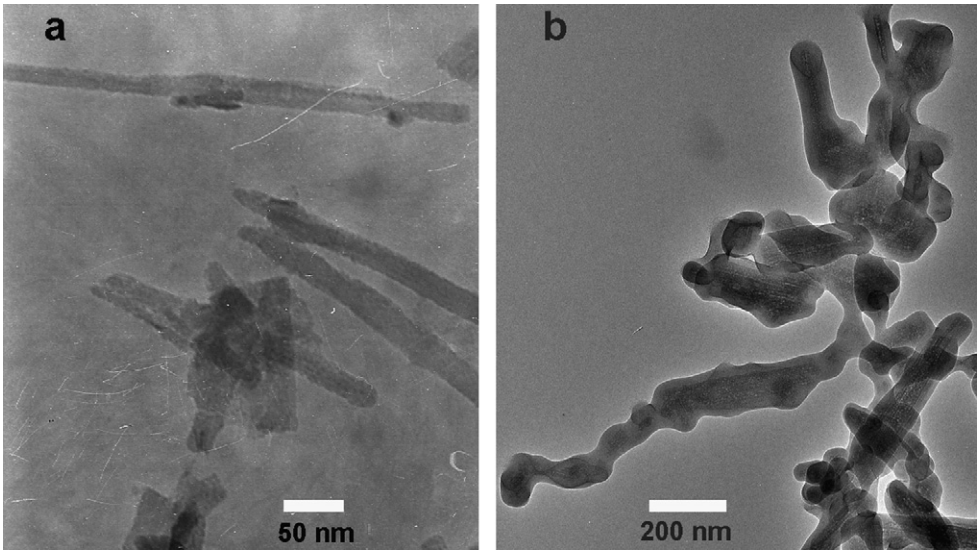

Fig. 4. TEM of ATP (a) and MIP (b).

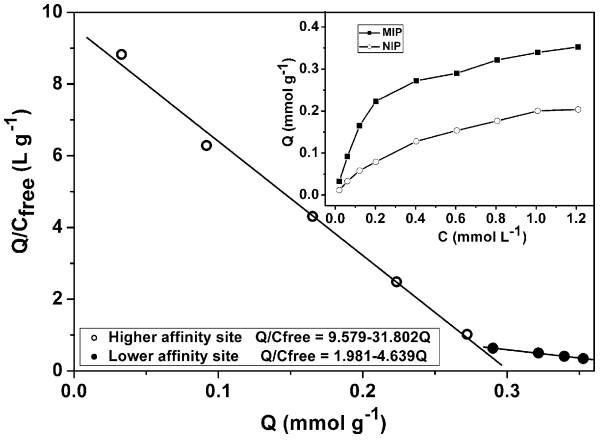

Fig. 5. Adsorption isotherm and Scatchard analysis of MIP (each point in the isotherm was the average values of three replicates; the RSDs for all points were lower than 3.2%). 20 mg of each material was suspended in 10 mL sudan I–IV solution with different concentrations and the adsorption was kept for 24 h.

A (ultra pure water) and 90% B (acetonitrile:acetone (90:10, v/v)) was used and all chromatographic peaks corresponding to analytes were obtained by baseline separation.

The time for on-line desorption of analytes was optimized by using the mobile phase for different time, which indicated that 1.5 min was enough to desorb all extracted analytes and longer time was not necessary. The MIP-SPE column was conditioned and cleaned for further loading using ultra pure water and acetonitrile at the flow rate of 4 mL min^{-1} for 1 min, respectively.

3.4.2. Sample pH

pH value is essential not only for achieving high capacity, but also for bringing forward the selectivity of the polymer in aqueous media. The effect of pH was evaluated by preparing a Britton–Robinson buffer. A pH range of 2–9 was studied. As shown in Fig. 6a, sudan I and sudan II were retained well on MIP-SPE column in acidic and neutral solution, and sudan III and sudan IV retained in neutral solution. It might be due to the physical–chemical properties of MIP materials and the target compounds. It has been known that there are many electron-rich groups (pyridine rings) on the surface of MIP, which resulted in multiple adsorption mechanism such as π–π conjugate bonding, electrostatic and hydrophobic interactions present simultaneously and improved the adsorption ability of materials further. Sudan I–IV were retained well on the MIP-SPE column in neutral solution, which implied that π–π conjugate bonding and hydrophobic interactions between sudan I–IV and MIP played a key role in the adsorption.

3.4.3. Flow rate of loading sample

The flow rate of loading sample has influence on the rebinding efficiency. Usually, the flow rate of loading sample was optimized by keeping the total sample solution volume constant. Herein the optimization of flow rate was conducted with the constant sample loading volume (30 mL) at flow rate 1.0–6.0 mL min^{-1}. Fig. 6b shows that when flow rate exceed 5 mL min^{-1}, the adsorption of sudan I and sudan IV sharply reduced, which means higher speed ($\geq$5 mL min^{-1}) is not conducive to these two dyes to be fully adsorbed onto the MIP-SPE column. However, the loading flow rate had little effect on adsorption of sudan II and sudan III. In order to maximize the efficiency of assay, the flow rate of loading sample was chosen as 5 mL min^{-1}.

Table 1
Recognition coefficient of MIP to sudan dyes from static adsorption test (each point in the isotherm was the average values of three replicates; the RSDs for all points were lower than 2.6%. The unit of S_{MIP} and S_{NIP} were mg g^{-1}).

Analytes	200.0 μg mL^{-1}			50.0 μg mL^{-1}			5.0 μg mL^{-1}		
	S_{MIP}	S_{NIP}	α	S_{MIP}	S_{NIP}	α	S_{MIP}	S_{NIP}	α
Sudan I	39.13	13.49	2.9	17.33	5.56	3.1	3.15	0.95	3.3
Sudan II	28.19	14.84	1.9	10.98	5.37	2.0	2.26	0.98	2.3
Sudan III	22.65	11.92	1.9	11.66	6.02	1.9	2.02	0.63	3.2
Sudan IV	30.24	13.15	2.3	15.26	6.24	2.4	2.92	1.01	2.9
TP	13.88	15.42	0.9	7.91	7.12	1.1	1.27	1.27	1.0

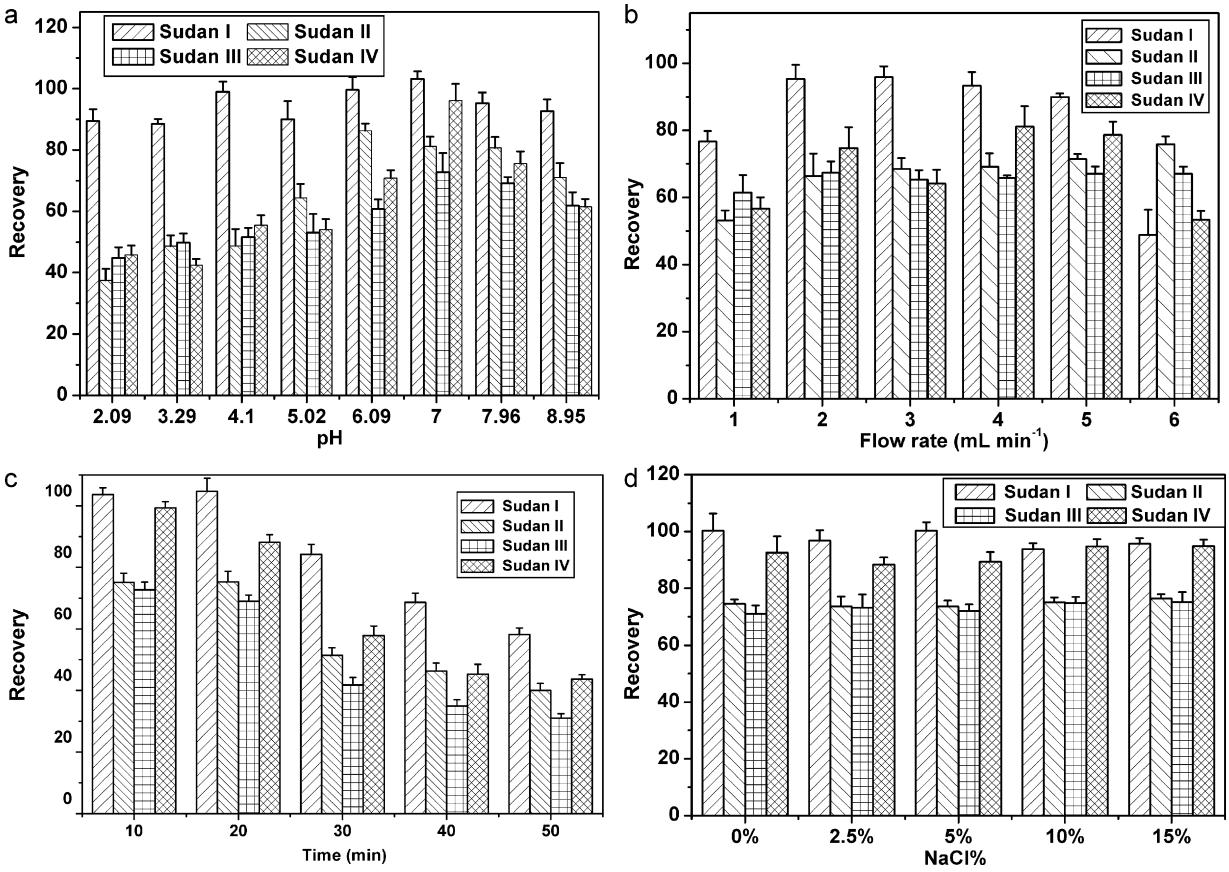

Fig. 6. (a–d) Effects of pH on peak area of sudan dyes, (b) flow rate of loading sample solution on peak area of sudan dyes, (c) loading time on extraction efficiency of sudan dyes, (d) salt concentration on peak area of sudan dyes. Tests were carried out with 1.0 ng mL^{-1} sudan dyes aqueous solution.

3.4.4. Time of loading sample

The flow rate of loading sample fixing at 5 mL min^{-1}, the time of loading sample (1 ng mL^{-1}) was evaluated to reach the optimal condition. Fig. 6c illustrates the extraction efficiency of analytes obtained from the different loading time. It can be seen that the extraction efficiency of sudan II–IV were significantly reduced after 20 min. So the 20 min was chosen for the following experiments. The corresponding breakthrough amount on per gram of MIP materials was about 26.2, 18.8, 17.5 and 22.0 μg for sudan I–IV, respectively.

3.4.5. Ionic strength

Effects of different ionic strength were investigated under different NaCl concentration (0–15%, w/v). It can be seen from Fig. 6d, the response for four investigated dyes slightly increased with the increase of ion strength. The reason could be attributed to variations of the solubility of these dyes in water. With an increase of salt concentration, the affinity of organic dyes to water phase would decrease and lead to higher adsorption efficiency. However, high salt concentration is unfavorable for on-line SPE system and it needs more time to wash the residual salt in desorption procedure. So there was no NaCl added to the sample matrix in further investigation.

3.4.6. Selectivity of SPE column

Based on the above optimization results, the on-line selectivity of MIP was evaluated. Chromatograms of the sudan I–IV through MIP-SPE column and NIP-SPE column pretreated are shown in Fig. 7. It was obvious that the retention times of sudan I–IV from NIP-SPE coupled HPLC system were shorter than those from MIP-SPE system, which can be ascribed to the poor retention of sudan I–IV on NIP-SPE pretreatment column. Recognition coefficient α (α = peak area from MIP-SPE column/peak area from NIP-SPE column) for sudan I–IV could be calculated to be 1.59, 2.24, 1.74 and 3.49, respectively. The α were different with those obtained according to static adsorption experiments, which implied that different interaction mechanism between the compounds and sorbents existed under different solvent conditions.

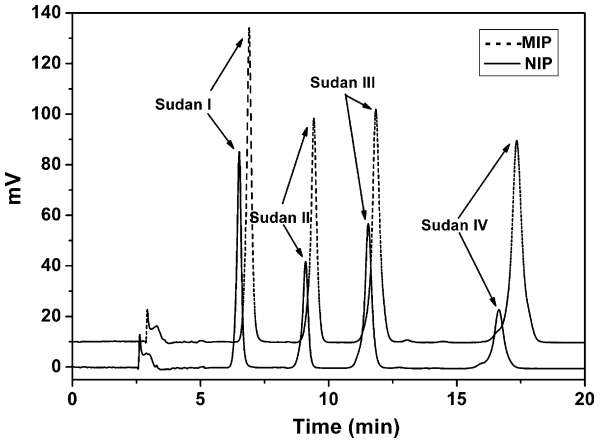

Fig. 7. Chromatograms of the sudan I–IV on MIP-SPE column and NIP-SPE column. 100 mL sudan dyes aqueous solution (5 ng mL^{-1}) was loaded on the SPE at a flow rate of 5 mL min^{-1}.

Table 2
Performance of on-line SPE HPLC method in sample from Yellow River water.

Analytes	Linear range (ng mL^{-1})	Enrichment ratio	LODs (ng mL^{-1})	LOQs (ng mL^{-1})	RSD (%, n = 3)				
					Intra-day		Inter-day		
					0.1 ng mL^{-1}	20 ng mL^{-1}	0.1 ng mL^{-1}	1 ng mL^{-1}	20 ng mL^{-1}
Sudan I	0.05–20	2930	0.01	0.02	3.4	2.3	2.8	2.2	1.9
Sudan II	0.10–20	1770	0.05	0.08	3.8	2.9	3.2	3.8	2.6
Sudan III	0.10–20	1690	0.05	0.08	2.9	4.6	3.4	3.4	2.9
Sudan IV	0.08–20	2240	0.04	0.06	3.3	3.7	3.3	3.1	3.2

Table 3
Performance of on-line SPE HPLC method in tomato sauce and sausage samples.

		Sudan I	Sudan II	Sudan III	Sudan IV
Tomato sauce	Linear range (ng g^{-1})	5–1000	10–1000	10–1000	5–1000
	Enrichment ratio[a]	141	98	101	121
	LODs (ng g^{-1})	1.0	3.0	3.0	2.5
	LOQs (ng g^{-1})	3.0	8.0	8.0	4.0
Sausage	Linear range (ng g^{-1})	4–1000	10–1000	10–1000	5–1000
	Enrichment ratio[a]	151	103	106	122
	LODs (ng g^{-1})	0.8	3.0	3.0	1.5
	LOQs (ng g^{-1})	2.0	6.0	6.0	3.0

[a] Enrichment factor refers to the enrichment to 6 mL extraction solution.

3.5. Analytical method validation

3.5.1. Determination of sudan I–IV in Yellow River water

Because the sudan dyes can be enriched in biont, it is necessary to determine these dyes in environmental water sample. The samples were extracted as described in Section 2.5.1. The linear range, enrichment ratio, reproducibility (RSD), limits of detection (LODs), limits of quantification (LOQs) were calculated and summarized in Table 2. The LOD and LOQ were obtained from the diluted samples and the signal-to-noise ratio (S/N). The LOD value was the sample concentrations (S/N = 3) and LOQ value was the sample concentrations (S/N = 10). As can be seen, both good precision and low LODs indicated that MIP materials were suitable for extraction of the four sudan dyes in water sample. In addition, accuracy of the method was determined by calculating the recoveries in the extraction of real samples spiked at different concentration levels (0.1, 1.0 and 20 ng mL^{-1}) based on triple repeatability. Recoveries of all analytes were 88.5–101.2% in Yellow River water sample.

3.5.2. Determination of sudan I–IV in tomato sauce and sausage

The proposed methods were applied to the analysis of four kinds of sudan dyes in two kinds of tomato sauce and sausage samples. Table 3 showed the linear range, enrichment ratios, LODs and LOQs of the methods to tomato sauce and sausage samples. The mean recoveries were above 85.5% and RSDs were around 3.0% in these samples. The results suggested that this method was feasible in the determination of sudan dyes in tomato sauce and sausage products. Besides, the effect of SPE column has not significantly decreased although it has been used for as many as 300 times.

Chromatograms of the sudan I–IV standard addition in tomato sauce (12.5 ng g^{-1}), and tomato sauce are shown in Fig. 8. Only sudan I was found in the first kind tomato sauce, and the content was 16.2 ng g^{-1}.

3.5.3. Comparison of methods

Until now, these sudan dyes in a variety of foodstuffs were determined by traditional liquid-phase extraction (LPE) [31], LPE-SPE [32], ionic liquids extraction (ILE) [33] and off-line MIP-SPE [18,22] coupled with HPLC or HPLC with mass spectrometry (MS). The LODs of these methods are shown in Table 4. During these reported methods, only LPE-HPLC was used for tomato sauce sample. As could been seen, the methods developed in this work was superior to the one reported in literature [31]. Meanwhile, the LODs of the proposed method in this work were in the same order of magnitude with the most methods reported. It showed the sudan I MIP using ATP as matrix was feasible in the determination of these sudan dyes in real samples.

Table 4
Comparison of LODs (ng g^{-1}) of sudan dyes with different methods.

Method	Sudan I	Sudan II	Sudan III	Sudan IV
LPE-HPLC [31]	25[a]	26[a]	31[a]	42[a]
LPE-SPE-HPLC-MS [32]	4.6	4.0	4.8	4.2
ILE-HPLC [33]	8.0	7.5	7.0	8.2
Off-line MIP-SPE-HPLC [18]	<750	<750	<750	<750
Off-line MIP-SPE-HPLC [22]	1.3	1.3	1.4	1.6
On-line MIP-SPE-HPLC (this work)	≤1.0	≤3.0	≤3.0	≤2.5

[a] ng mL^{-1}.

For further verifying the feasibility of the new method, the same sample was analyzed with Chinese national standard (GB/T 19681-2005) and the proposed method synchronously. The results showed that no significant difference existed between both methods.

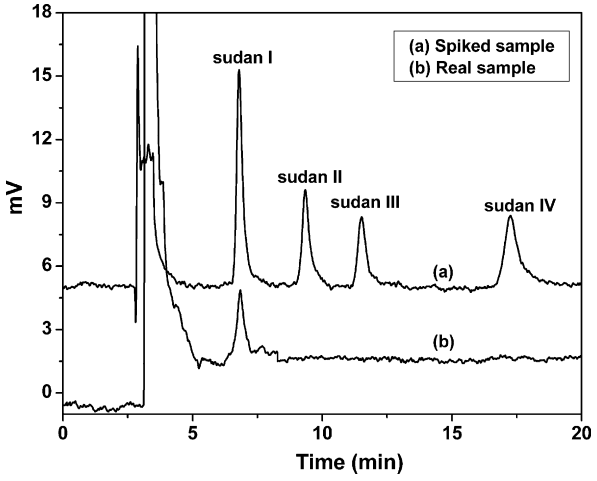

Fig. 8. Chromatograms of the sudan I–IV standard addition in tomato sauce (each 12.5 ng g^{-1}, a), and real tomato sauce (b).

4. Conclusion

In this paper, sudan I imprinted material with on-line SPE coupled with HPLC has been prepared successfully with ATP as the matrix. The MIP not only has high specific recognition selectivity for the template, but also has compatibility to the other three sudan dyes due to the highly similar structures. Therefore, the MIP was used to simultaneously on-line extract sudan I–IV in samples from Yellow River water, tomato sauce and sausage. The proposed methods showed the sudan I MIP was feasible in the determination of these sudan dyes in real samples.

Without complex and time-consuming hyperbranched treatment, the approach developed to synthesize MIP here exhibited the same performance with those reported in the previous studies. It can be anticipated that MIP using ATP as matrix will achieve further development by simplifying the synthesis approach.

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