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Selective Solid-Phase Extraction of Sudan I in Chilli Sauce by Single-Hole Hollow Molecularly Imprinted Polymers

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ABSTRACT: A new single-hole hollow molecularly imprinted polymer (SHH-MIP) was prepared by multistep seed swelling polymerization using Sudan I as template molecule and successfully applied to selective solid-phase extraction (SPE) of Sudan dyes in chilli sauce samples. The polymers possessed high specific surface area obtained by nitrogen adsorption and good thermal stability without decomposition lower than 380 °C by thermogravimetry analysis. Much higher binding capacity was exhibited than with irregular solid MIP prepared by bulk polymerization, because most of the binding sites were located in the surface of the polymers, facilitating template removal and mass transfer. Accordingly, the SHH-MIP was employed as SPE adsorbent for chilli sauce analysis and offered high recoveries for Sudan I in the range of 87.5−103.4% with the precision of 1.94−5.33% at three spiked levels. The SHH-MIP with high selectivity and high stability was demonstrated to be potentially applicable for high selective preconcentration and determination of trace Sudan dyes in complicated samples.

KEYWORDS: *Sudan dyes, molecularly imprinted polymers, solid-phase extraction, emulsifier-free emulsion polymerization, chilli sauce*

INTRODUCTION

Sudan dyes (Sudan I, II, III, and IV) are phenyl-azoic derivatives with an orange-red appearance and extensively used to color waxes, oils, petroleum, solvents, and polishes. However, they may pose an increased risk of cancer for humans and have been classified as category 3 carcinogens.[1,2] Additionally, illegally, Sudan I, one of the most frequently used Sudan dyes, has also been adopted for coloring various foodstuffs, such as particular brands of curry powder and chilli powder. The use of Sudan I in foods is now banned worldwide according to both the Food Standards Agency (FSA) and the European Union (EU).[3] Consequently, the development of highly effective and practicable methods for selective determination of Sudan dye in foodstuffs is urgently required.

Until now, the most widely used method for Sudan dyes is liquid chromatography,[4,5] approved by the European Commission. Liquid chromatographic methods associated with different detectors are performed for the determination of Sudan dyes, such as ultraviolet (UV),[6] mass spectrometry (MS),[7,8] and diode array detection (DAD).[9] Besides, many other methods have been developed, for example, capillary electrophoresis,[10] plasmon resonance light scattering,[11] and enzyme-linked immunosorbent assay.[12] Meanwhile, owing to the complexity of sample matrices and low levels of analytes, sample pretreatment and enrichment processes have become the crucial steps in the analytical procedures.

Among various pretreatment procedures, solid-phase extraction (SPE) was universally used for the concentration of Sudan dyes in foodstuffs.[13,14] In a SPE procedure, the adsorbent was a most significant factor for obtaining high extraction efficiency. Recently, a highly selective material of molecularly imprinted polymers (MIP) with specific molecular recognition property for template molecules has attracted great attentions in SPE as adsorbent.[15−17] The MIP-based SPE has been increasingly applied for the analysis of Sudan dyes, such as molecularly imprinted (MI) SPE for the detection of Sudan I in food matrices[18] and for the simultaneous extraction and determination of Sudan dyes (Sudan I, II, III, and IV) in samples from Yellow River water tomato sauce and sausage by online SPE and HPLC,[19] and all four dyes in sausage samples were also determined by MISPE combined with ultrasound-assisted dispersive liquid−liquid microextraction.[20] Although the prepared MIP have exhibited high selectivity, high stability to harsh chemical and physical conditions, and excellent reusability, they also present some problems, such as incomplete template removal, low binding capacity, and slow mass transfer.[21]

To solve those problems, the combining of molecular imprinting technology with hollow polymer preparation methods has been proposed. The main advantages recognized up to now in the use of hollow polymers arise from their controllable hole structure, which favors mass transfer. Herein, single-hole hollow (SHH) MIP were prepared by multistep seed swelling polymerization, and they were successfully applied as SPE sorbents for selective preconcentration of Sudan I in chilli sauce samples. The polymers were fully characterized and evaluated by scanning electron microscopy (SEM), transmission electron microscopy (TEM), nitrogen adsorption, and thermogravimetry (TG) analysis. The molecular recognition specificity was investigated in detail. For comparison, several MIP were prepared by multistep seed

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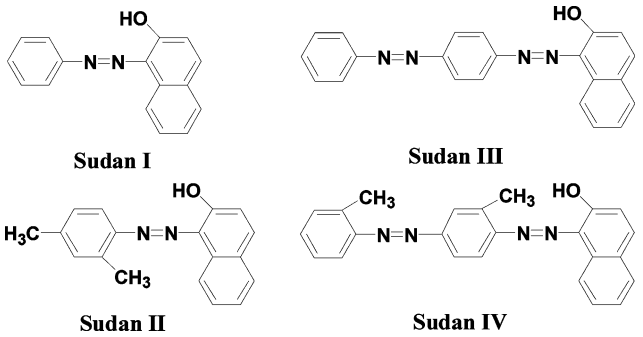
swelling polymerization using polystyrene seeds obtained by emulsifier-free emulsion polymerization and dispersion polymerization, respectively, and bulk polymerization, and the structural analogues including Sudan II, III, and IV were also studied for selectivity test.

EXPERIMENTAL PROCEDURES

Reagents and Chemicals. Sudan dyes (Sudan I, II, III, and IV) were purchased from Shanghai Chemical Reagents Co. (Shanghai, China). Their structures are shown in Scheme 1. Styrene, divinylbenzene (DVB), and methacrylic acid (MAA) were purchased from Sigma-Aldrich (Shanghai, China) and distilled under vacuum prior to use to remove stabilizers. Benzoyl peroxide (BPO) and 2,2′-azobis(isobutyronitrile) (AIBN) were purchased from Shanghai Chemical Reagents Co. and recrystallized in methanol prior to use. Dibutyl phthalate (DBP) as swelling reagent and sodium dodecyl sulfate (SDS) were supplied by Tianjin Reagent Plant (Tianjin, China). Other affiliated chemicals were all obtained from local suppliers (Yantai, China). All solvents and chemicals were of analytical grade and used without further purification unless otherwise specified.

Scheme 1. Chemical Structures of Sudan I, II, III, and IV



Synthesis of Carboxylated Polystyrene Particles by Emulsifier-free Emulsion Polymerization. Micrometer-sized, monodisperse carboxylated polystyrene seed particles were prepared by emulsifier-free emulsion polymerization similar to a reported procedure[22] with minor modification. Sodium bicarbonate (0.15 g) was dissolved in distilled water (100 mL) in a 250 mL three-necked, round-bottom flask. The mixture was magnetically stirred under a nitrogen atmosphere. Acrylic acid (1 mL) in styrene (5 mL) was added to the above solution. After the solution was heated to 65 °C, potassium persulfate (0.1 g) in water (50 mL) was then poured into the mixture; the polymerized reaction was heated to 70 °C for 24 h under a nitrogen atmosphere. The obtained carboxylated polystyrene particles were purified by repeated centrifugation and washed with ethanol and water (1:1, v/v).

Preparation of Sudan I Imprinted Single-Hole Hollow Polymers. The Sudan I imprinted single-hole hollow polymers were prepared by multistep seed swelling polymerization based on the steps below. Polystyrene of 0.4 g was weighed into 100 mL of distilled water of 0.25% SDS solution in a 250 mL three-necked, round-bottom flask. In the first step, swelling was accomplished by ultrasonication treatment by adding 1.5 mL of DBP to the homogeneous emulsion for 24 h. Subsequently, the second-step swelling was carried out by adding 5−10 mL of toluene, 688 μL of MAA, and 496 mg of Sudan I. After swelling for another 24 h, 8 mL of DVB and 80 mg of initiator AIBN were added into the mixture solution. The polymerization was undertaken with magnetic stirring in a nitrogen atmosphere at 60 °C for 18 h. The product was further aged at 70 °C for 2 h. Then the polymers were obtained by centrifugation and rinsing using anhydrous ethanol three times to wash off residues. Afterward, the polymers were washed by methylene chloride (good solvent for polystyrene) in a Soxhlet extractor for 12 h to remove the linear polymer polystyrene. Finally, Sudan I imprinted single-hole hollow polymers (SHH-MIP) were fabricated through removal of Sudan I templates using a solvent mixture of methanol/acetic acid (9:1, v/v). The products were dried in a vacuum oven at 40 °C to obtain the MIP material.

For comparison, nonimprinted single-hole hollow polymers (SHH-NIP) were also prepared by using the same procedures and conditions, only without the addition of Sudan I molecules.

Characterization. The morphologies of the SHH-MIP were observed by SEM (JSM 5600 LV, operating at 20 kV) and TEM (JEM-1230, operating at 100 kV). All samples were sputter-coated with gold before SEM analysis, and those used for TEM were prepared by placing several drops of the particle suspension on the carbon-coated copper grid and allowing them to dry.

Nitrogen adsorption experiments were used to measure the specific surface area of polymers. The work was operated on AUTOSORB 1 (Quantachrome Instruments, Germany). The samples were degassed in a vacuum at 300 °C prior to adsorption measurements. The thermostability and purity were evaluated by TG (Mettler Toledo).

Estimation of Molecular Recognition Properties. To evaluate the binding capacity of the SHH-MIP obtained, a static adsorption test and a dynamic adsorption test for Sudan I were carried out in acetonitrile solutions. The procedures were carried out as follows: 20 mg of polymer particles was put into a 5 mL flask containing 2.0 mL of Sudan I acetonitrile solutions of various concentrations. After 24 h of shaking at room temperature, the samples were centrifuged and the supernatant solutions were collected, the concentrations of which were determined using HPLC-UV (Skyray Instrument Inc., China). The binding amount (Q) of Sudan I was calculated by subtracting the free concentrations from the initial concentrations. The maximum binding capacity (Q_{max}) and dissociation constant (K_d) were estimated by processing with the Scatchard equation[23]

$$Q/C_e = (Q_{max} - Q)/K_d$$

where Q is the amount of Sudan I adsorbed to polymer at equilibrium, C_e is the free Sudan I concentration at equilibrium, K_d is the dissociation constant, and Q_{max} is the apparent maximum amount that can be bound. K_d and Q_{max} can be obtained from the slope and intercept of the linear curve plotted in Q/C_e versus Q, respectively.

Meanwhile, the dynamic adsorption test was performed by monitoring the temporal amount of Sudan I in the solutions.

Selectivity experiments were conducted by using Sudan (II, III, and IV) that are structurally similar to Sudan I. A C_{18} column of 150 mm × 4.6 mm i.d. (Arcus EP-C_{18}, 5 μm, 4.6 × 150 mm column, Waters) was used as the analytical column. HPLC conditions optimized for the Sudan dyes were as follows: column C_{18} (Arcus EP-C18, 5 μm, 4.6 × 150 mm column, Waters); mobile phase, acetonitrile/water (95:5, v/v); flow rate, 1.0 mL min^{-1}; room temperature; UV detection at 478 nm for Sudan I and at 520 nm for Sudan II, III, and IV; injection volume, 20 μL.

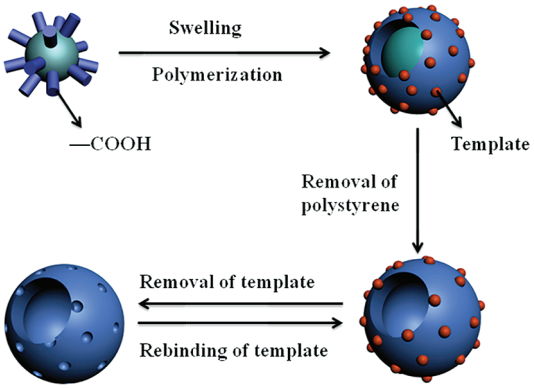
MISPE Procedure. The MIP-SPE column was prepared by packing 400 mg of the SHH-MIP into an empty PTFE cartridge. The cartridge was conditioned sequentially with 5 mL of methanol and 3 mL of *n*-hexane at 1 mL min^{-1}, and then the cartridge was loaded with the prepared samples above at a certain flow rate. After loading, the column was washed with 5 mL of methanol/acetic acid (9:1, v/v) at 1 mL min^{-1}. Finally, all of the fractions from the sample elution steps were collected and then evaporated to dryness at 40 °C under a stream of N_2 gas.[24] The residues were dissolved in 1 mL of acetonitrile for HPLC analysis.

Analysis of Chilli Sauce Samples. Chilli sauce samples were used to demonstrate the applicability of the SHH-MIP-SPE for the preconcentration of Sudan I from complicated matrices. Chilli sauce samples were prepared as follows: 10 g of chilli sauce was weighed into a small beaker, 50 mL of *n*-hexane was added to dissolve the sample, the mixture was filtered, and the residue was washed with *n*-hexane several times; *n*-hexane was collected to concentrate by using a rotary evaporator. Then the extraction solution was concentrated for analysis by HPLC-UV. For spiked tests, appropriate amounts of Sudan I standard were added into the chilli sauce sample and extracted from the matrix through the overall procedure.

RESULTS AND DISCUSSION

Preparation of Sudan I Imprinted SHH-MIP. The preparation process of the SHH-MIP is schematically illustrated in Scheme 2. The construction of Sudan I imprinted single-hole hollow polymers includes the synthesis of carboxylated polystyrene particles, the preparation of solid MIP, and the removal of polystyrene and then the used template molecules. Hollow polymer microspheres are most commonly fabricated by coating the surface of the substrate particles with charged polyelectrolytes or reactive polymers, followed by removal of the core particles using chemical etching.[22] Generally speaking, the polystyrene seeds with relatively large size were easy for the next swelling step, but small polystyrene seeds with high specific surface area can ensure that the most binding cavities are at the interior and exterior surfaces of the SHH-MIP.[25] For comparisons, polystyrene seeds were prepared by emulsifier-free emulsion polymerization and dispersion polymerization, respectively. Then the two different polystyrene seeds were used in a multistep swelling polymerization for swelling and pore-forming. The strong hydrogen-bond interactions between the carboxyl groups at the surface of the polystyrene seeds and the functional monomers will thus drive the selective polymerization at the surface of the polystyrene seeds. A consecutive two-step procedure including swelling and polymerization was carried out at 60 °C for 18 h and at 70 °C for 2 h, followed by dissolving polystyrene seeds with dichloromethane to form a single hole in the highly cross-linked polymer shell. As a result, hollow MIP with a single hole were obtained, that is, SHH-MIP.

Scheme 2. Schematic Illustration of the Preparation Process of SHH-MIP



Optimization of Polymerization Conditions for Preparation of the SHH-MIP. To make the SHH-MIP have good selectivity and adsorption effect, the ratio of template molecular and functional monomer, the type of cross-linkers, and the type and amounts of porogen were taken into consideration.

It is widely accepted that the strength of the interactions between template and monomer significantly affects the affinity of MIP. A suitable functional monomer can give the MIP higher binding capacity and selectivity. MAA was chosen as the functional monomer to interact with Sudan I, due to its unique characteristics of high capability to act as the hydrogen-bond donor and acceptor and good suitability for ionic interactions. Besides, to obtain good specific absorption, the ratios of template molecule and functional monomer were investigated. On the basis of sequence experiments, the optimal molar ratio was set at 1:4 for Sudan I to MAA.

In addition, cross-linkers have profound influences on selectivity and binding capacity of MIP. The commonly used cross-linkers of DVB and EGDMA were tested in fabricating the structure of polymers, to maintain stable cavity configuration. After extensive experiments, DVB with an appropriate amount was chosen.

Porogenic solvent also plays an important role in polymerization. Toluene was chosen, which acted as not only porogen but also a good dissolving solvent, especially in the noncovalent interaction system. Toluene has an aprotic property and low polarity, which not only will not influence the bonding strength between functional monomer and template but also will endow the morphology of the polymers.

Characterization of the SHH-MIP. The prepared SHH-MIP were characterized by SEM and TEM, nitrogen adsorption experiments, and TG analysis. The SEM and TEM images of polystyrene and SHH-MIP are shown in Figure 1. Carboxylated polystyrene seeds with a uniform size of 500 nm were prepared by emulsifier-free emulsion polymerization (Figure 1A), and a uniform size of 970 nm was obtained by dispersing polymerization (Figure 1B). It is exciting that the SHH-MIP could be prepared by both methods. As seen from Figure 1C, the polymers prepared by multistep swelling polymerization with polystyrene seeds of emulsifier-free emulsion polymerization (SHH-MIP-E) (Figure 1A) obviously have a big-hole in the microsphere; this image reveals a relatively uniform size distribution with a mean diameter of about 1−2 μm. The

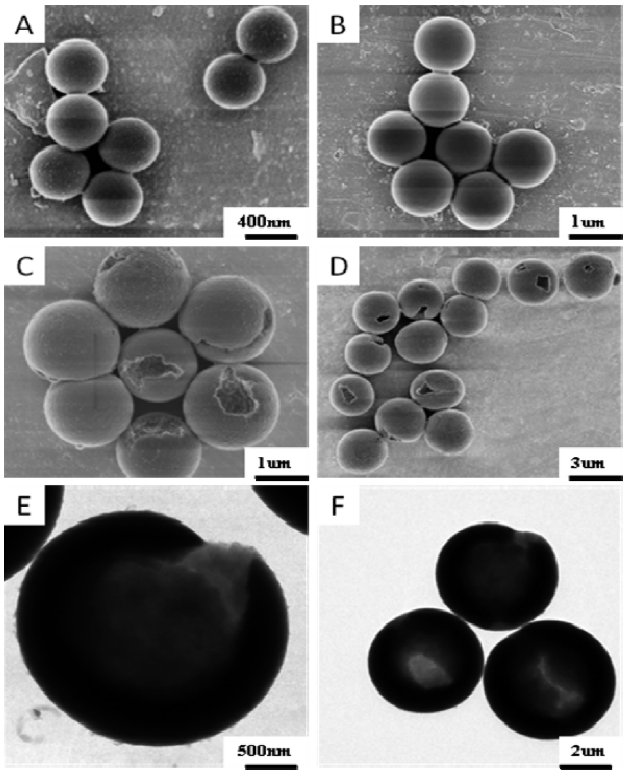


Figure 1. SEM images (A−D) and TEM images (E, F) of polystyrene seeds and SHH-MIP: (A) polystyrene seeds prepared by emulsifier-free emulsion polymerization; (B) polystyrene seeds prepared by dispersing polymerization; (C) SHH-MIP prepared by multistep swelling polymerization with (A) seeds; (D) SHH-MIP prepared by multistep swelling polymerization with (B) seeds; (E) TEM image of (C); (F) TEM image of (D).

polystyrene seeds were eluted by dichloromethane, and then a huge spherical cavity was formed. The hollow interiors and open holes can simultaneously be observed by TEM (Figure 1E), which is obviously different from the shell formed by DVB. On the other hand, the SHH-MIP prepared by multistep swelling polymerization but with polystyrene seeds of dispersing polymerization (SHH-MIP-D) (Figure 1B) obviously have a big hole in the spherical particles, too; however, the particle sizes of the MIP range from 2 to 4 μm (Figure 1D), larger than that of the prepared SHH-MIP shown in Figure 1C. The TEM image of Figure 1F shows a similar structure to that of Figure 1E but with lower volume percent of the cavity. From the above SEM and TEM images, it is clear that the prepared MIP have a single hole at the highly cross-linking polymer shells and a hollow structure by dissolving the polystyrene out of the hole with dichloromethane. The micrometer-sized single-hole hollow microspheres are much smaller than the MIP produced by the traditional methods.

For the SHH-MIP, the pore structure and surface area were studied using the nitrogen adsorption method, and the corresponding results are listed in Table 1. As can be seen, the SHH-MIP present much higher specific surface areas than the MIP from bulk polymerization (MIP-B); the SHH-MIP-E using seeds of emulsifier-free emulsion have higher ones than seeds of dispersion (SHH-MIP-D). Meanwhile, the specific areas of MIP are larger than those of the corresponding NIP, suggesting more additional binding sites are produced by eluting the template molecule of MIP. The results indicated the SHH-MIP with larger specific surface areas might result in higher binding capacity.

Table 1. Specific Surface Area (m^2 g^{-1}) of the MIP and Corresponding NIP

polymer	MIP	NIP
SHH-MIP-E[a]	345.8	272.5
SHH-MIP-D[b]	280.7	205.9
MIP-B[c]	102.6	64.6

[a]SHH-MIP prepared by multistep swelling polymerization with polystyrene seeds obtained by emulsifier-free emulsion polymerization. [b]SHH-MIP prepared by multistep swelling polymerization with polystyrene seeds obtained by dispersing polymerization. [c]MIP prepared by bulk polymerization.

TG analysis is based on continuous recording of mass changes of a sample of material, as a function of a combination of temperature with time and, additionally, of pressure and gas composition. This is possibly because the temperature range over which a mixture of compounds melts is dependent on their relative amounts.[26] Figure 2A shows the TG curve of three MIP prepared by different methods. The three MIP had similar TG curves except for the residues. With the temperature increasing from 50 to 100 °C, their weight loss was mainly due to the loss of absorbed water. In the range of 100−390 °C, the rate of weight loss for MIP is very slow, whereas a high rate of weight loss is presented at temperatures ranging from 390 to 490 °C. The peak temperatures of MIP prepared by emulsifier-free emulsion polymerization, dispersion polymerization, and bulk polymerization were 458.04, 456.20, and 450.97 °C, respectively, and the residue amounts were 19.04, 27.58, and 27.06%, respectively. The weight loss might result from the dissolving of MIP. Hence, the prepared SHH-MIP were fully demonstrated to occupy good thermal stability at lower than 380 °C. These can also be clearly explained from Figure 2B.

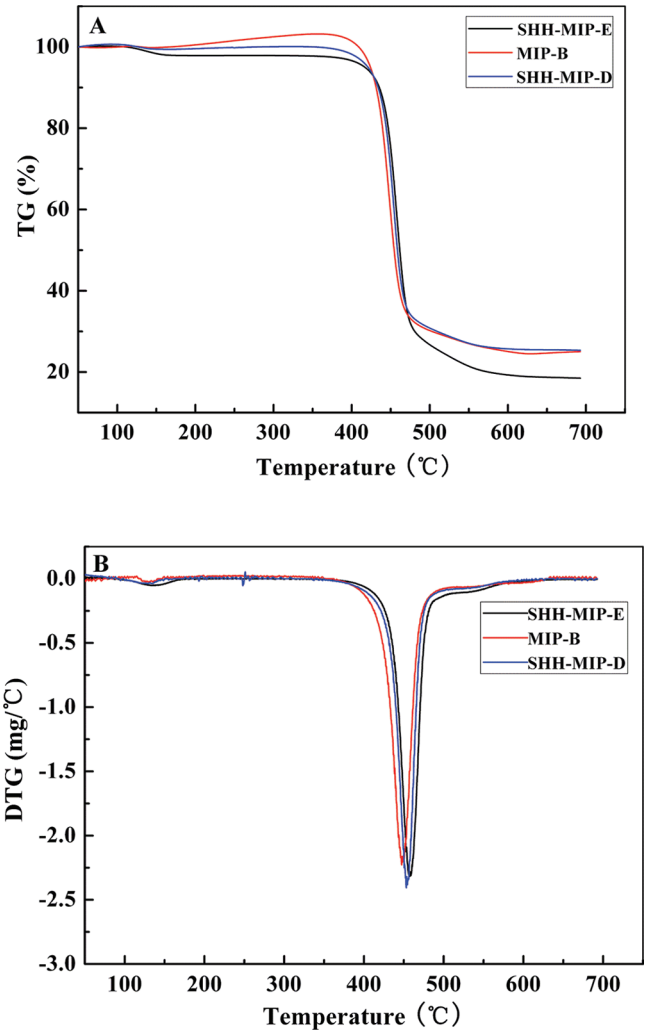


Figure 2. (A) TG and (B) DTG curves of three different MIP: SHH-MIP-E, SHH-MIP prepared by multistep swelling polymerization with polystyrene seeds obtained by emulsifier-free emulsion polymerization; SHH-MIP-D, SHH-MIP prepared by multistep swelling polymerization with polystyrene seeds obtained by dispersing polymerization; MIP-B, MIP prepared by bulk polymerization.

Estimation of Molecular Recognition Properties of the SHH-MIP. Figure 3A shows the binding isotherms of Sudan I onto the SHH-MIP and NIP. The curves were obtained by plotting the saturated adsorption amounts with equilibrium concentrations of the Sudan I in acetonitrile solution. As seen, the adsorption capacity of Sudan I increased quickly with the increase of initial concentration. At equilibrium concentrations higher than 5.0 mmol L^{-1}, adsorption of MIP became stable and its recognition sites were almost saturated. NIP had the same trend as MIP with lower saturated adsorption amounts. Thus, adsorption isotherms are important for describing how adsorbates interact with adsorbent.[15] More importantly, as seen from the figure, MIP1 prepared by emulsifier-free emulsion polymerization using DVB as cross-linker and toluene (5 mL) as porogen, as shown in Table 2, possessed the highest binding capacity among the four SHH-MIP, much higher than that of the corresponding control NIP. This can be explained Figure 1C,E and Table 1; that is, larger

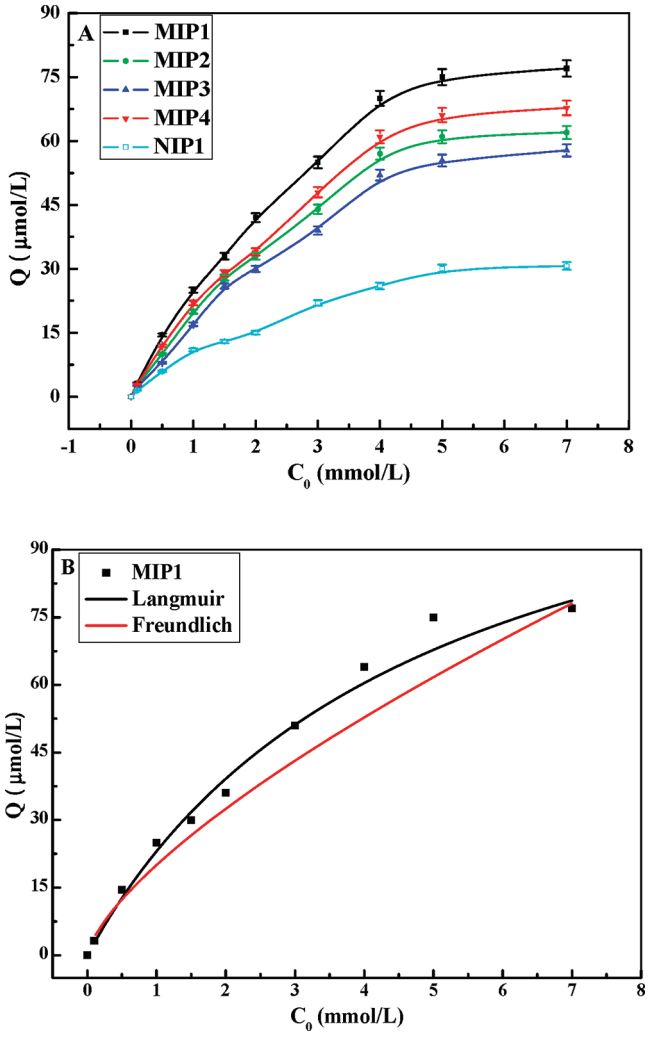


Figure 3. (A) Binding isotherms of four MIP for Sudan I and MIP1 corresponding to NIP in acetonitrile. Experimental conditions: $V = 2.0$ mL; mass of polymer, 20 mg; adsorption time, 24 h. (B) Comparison of Langmuir and Freundlich isotherm models for Sudan I adsorption onto MIP1.

specific surface area results in higher binding capacity. Also, MIP1 obtained the highest imprinting factor of 2.51 (Table 2), indicating it possessed excellent selectivity for template molecule, because a large number of binding sites were located in the proximity of the interior surface in favor of template access and removal through the single-hole hollow structure.

Scatchard analysis was employed to further analyze the binding isotherms. According to the Scatchard equation, the plot for Sudan I adsorbed to MIP was obtained (data not shown). There were two apparent sections within the plot that can be considered as two straight lines. The results implied that there were two types of binding sites for MIP, and the rebinding sites were mainly dependent on hydrogen bonding. Consequently, the K_d and Q_{max} of the four kinds of SHH-MIP were calculated. The adsorption isotherm constants and corresponding condition parameters are shown in Table 2. The results further confirmed that MIP1 possessed the optimal molecular adsorption properties.

As for the sorption isotherm models, Freundlich and Langmuir isotherm constants for adsorption of Sudan I onto the four MIP are displayed in Table 3. It can be observed that the Langmuir isotherm model yielded a better fit than those by the Freundlich model, for correlation coefficients (R^2) above 0.99. With MIP1 as an example, Figure 3B shows the equilibrium data for Freundlich and Langmuir isotherms, further confirming that the Langmuir isotherm model is suitable to all of the adsorptions in this study.

Table 3. Isotherm Model Constants for the Four MIP

isotherm model	constant	MIP1	MIP2	MIP3	MIP4
Langmuir	R^2	0.998	0.998	0.996	0.996
	Q_m	106.38	101.01	99.01	102.04
	K	0.376	0.309	0.264	0.349
Freundlich	R^2	0.983	0.984	0.986	0.985
	K_f	26.00	21.37	18.99	23.24
	$1/n$	0.710	0.722	0.728	0.718

Dynamic binding experiments were performed to estimate the molecular recognition properties of the MIP prepared by three different methods. Sudan I could easily diffuse into the recognition sites of SHH-MIP through the hollow surface cavities, leading to higher binding capacity and faster mass transfer, as can be seen from Figure 4. For the solid MIP prepared by bulk polymerization, template removal was still difficult, attributed to the large volume. Therefore, the binding capacity was much lower than that of the SHH-MIP. When the two kinds of SHH-MIP were compared, those lower sized nanopores were more in favor of template removal and mass transfer. As a result, the SHH-MIP-E using polystyrene seeds obtained by emulsifier-free emulsion polymerization had higher binding capacity and faster mass transfer than SHH-MIP-D using polystyrene seeds obtained by dispersion polymerization. A time of 60 min was needed to reach adsorption equilibrium for all three MIP (Figure 4). In the same adsorption time of 60 min, the binding capacity of SHH-MIP-E was remarkably higher than that of the traditional MIP-B.

To further investigate the competitive recognition ability of the MIP, three Sudan dyes as structural analogues were used. As seen from Figure 5, for all MIP, the binding capacities for Sudan I are much higher than that of the three references. The binding capacities for Sudan II were higher than the other two

Table 2. Effects of Polystyrene Seeds, Cross-Linker, and Toluene Amount on the Adsorption Isotherm Constants for the SHH-MIP

polymer	polystyrene seeds	cross-linker	toluene (mL)	I^a	R^2_1	$K_{d1}{}^b$	$Q_{max1}{}^c$	R^2_2	K_{d2}	Q_{max2}
MIP1	emulsifier-free	DVB	5	2.51	0.997	1.56	77.30 ± 1.48	0.908	4.27	154.53 ± 4.13
MIP2	dispersion	DVB	5	2.02	0.968	1.79	66.96 ± 0.81	0.862	5.71	145.09 ± 3.98
MIP3	emulsifier-free	DVB	10	1.88	0.926	1.91	62.06 ± 2.12	0.883	4.44	112.49 ± 4.56
MIP4	emulsifier-free	EGDMA	5	2.21	0.949	1.50	64.17 ± 2.76	0.869	5.41	152.65 ± 4.34

[a]Imprinting factor. [b]Dissociation. [c]Total amount of the immobilized ligand.

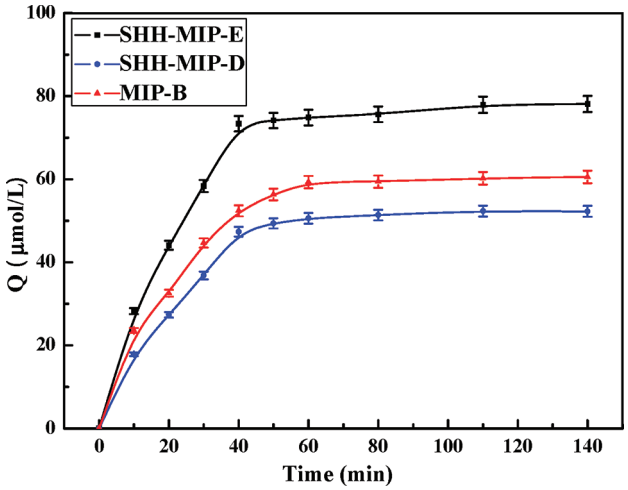


Figure 4. Kinetic uptake of Sudan I onto the different MIP. Experimental conditions: V = 2.0 mL; C_0 = 40 mg L^{-1}; mass of polymer, 20 mg.

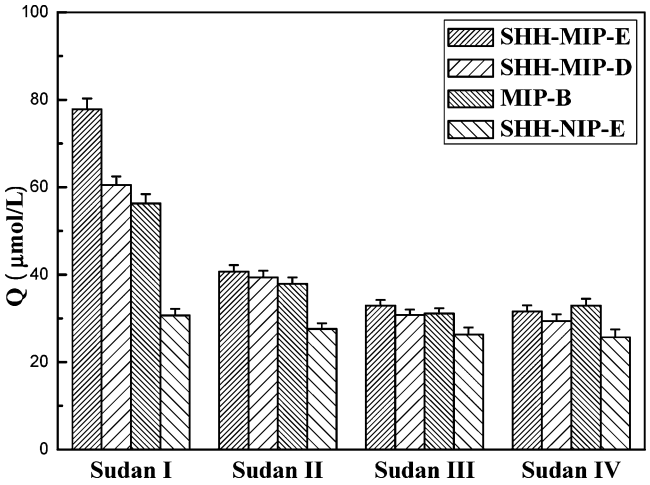


Figure 5. Binding capacities of different MIP for Sudan I and its structural analogues Sudan II, III, and IV.

as its structure is more similar to that of Sudan I. The SHH-MIP had similar selectivity when compared to MIP-B because the same functional monomers and the same template to functional monomer ratio were used during the preparation of those two kinds of MIP. Therefore, the binding cavity is still the same, which resulted in the same selectivity but improved mass transfer and binding capacity. The obtained SHH-MIP could selectively recognize the delicate difference of Sudan I from its analogues. The obtained SHH-MIP supplied an attractive SPE sorbent to selectively preconcentrate Sudan I.

Application of the SHH-MIP-SPE to Chilli Sauce Samples. To evaluate the potential application of SHH-MIP for selective preconcentration of Sudan I in real samples, chilli sauce samples spiked with Sudan I at 1, 10, and 100 mg kg^{-1}, respectively, were passed through the SHH-MIP filled SPE column, whereas MIP-B, SHH-NIP-E, and a traditional aluminum oxide cartridge were used for comparison. Validation of the SHH-MIP-SPE method was performed by examining the recoveries of spiked chilli sauce samples. The precision of the method was evaluated by calculating the relative standard deviation (RSD) of the extraction at the three different concentration levels using the optimized procedures. The results are shown in Table 4. As seen, MIP-B, SHH-NIP-E, and commercial aluminum oxide exhibited low recoveries for Sudan I; in contrast, satisfactory recoveries were obtained for SHH-MIP. By choosing SHH-MIP-E and SHH-MIP-D as sorbents, the recoveries of Sudan I ranged from 89.7 to 102.0% and from 87.5 to 103.4%, with precisions of 1.94−4.73 and 2.66−5.33%, respectively. This demonstrated the potential applicability of the SHH-MIP-SPE for highly effective preconcentration, separation, and accurate quantification of Sudan dyes in real samples.

Table 4. SPE Recovery Comparisons Using Different Sorbents for Sudan I under Optimal Conditions

	1 mg kg^{-1}		10 mg kg^{-1}		100 mg kg^{-1}	
sorbent	recovery[a] (%)	RSD[b] (%)	recovery	RSD	recovery	RSD
SHH-MIP-E	102.0	4.73	95.0	4.22	89.7	1.94
SHH-MIP-D	103.4	5.33	91.4	4.63	87.5	2.66
MIP-B	90.4	5.46	77.3	4.61	59.9	1.44
SHH-NIP-E	63.2	6.95	53.3	1.21	36.2	0.35
aluminum oxide	89.0	3.46	87.7	2.63	84.5	2.12

[a] n = 5. [b] Relative standard deviation, n = 5.

Figure 6 presents the chromatograms of Sudan I from chilli sauce samples. As seen from Figure 6a, the endogenous Sudan I was not detected, and it was extremely difficult to detect the Sudan I without performing the extraction preparation process because of the low concentration level and severe interferences from complicated matrices. Whereas after the SHH-MIP-SPE, the matrix effects were drastically reduced (Figure 6b,c), and the spiked Sudan I was remarkably concentrated as shown in Figure 6c, which was attributed to the fact that the SHH-MIP have highly selective recognition and adsorption capacity.

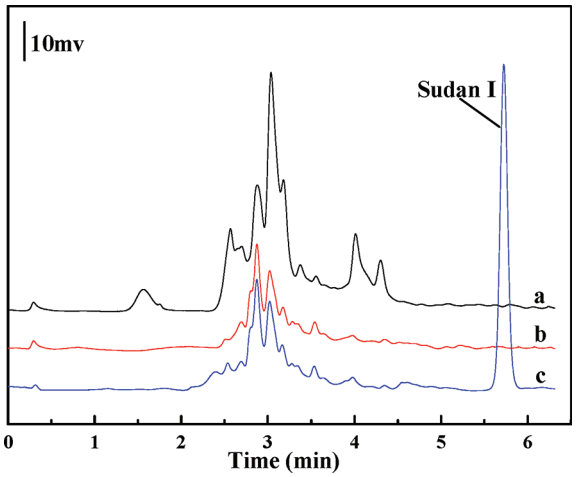


Figure 6. HPLC-UV chromatograms of chilli sauce samples: (a) without extraction; extraction with SHH-MIP-E without spiking (b) and spiked with 10 mg L^{-1} Sudan I standard (c).

In the chilli sauce sample, the developed SHH-MIP-SPE could attain favorable limits of detection (LOD) of 3.3 and 5.0 μg kg^{-1} for SHH-MIP-E and SHH-MIP-D, respectively, based on the signals as 3-fold the baseline noise.

Much excellent work about the preparation of Sudan MIP and applications as SPE sorbent for the separation and

Table 5. Comparison of Recovery and LOD for Sudan Dye Determination Using MIP

template molecule	polymerization method	real sample	recovery (%)	LOD (μg/kg)	ref
Sudan I	multistep swelling	chili sauce	87.5−103.4	3.3; 5.0	this work
Sudan I	bulk	chili powder	70		18
Sudan I	bulk	tomato sauce		1.0−3.0	19
Sudan I	sol−gel	chilli powder	80.3−94.0	1.2	6
Sudan IV	sol−gel		89.1−95.2	0.01	27
phenylamine−naphthol	suspension	catsup products	86.3−102.9	2	28
aniline−naphthol	suspension	egg yolk	87.2−103.5	2.4	2
phenylamine−naphthol	suspension	egg yolk	94.1−102.5	9	29
phenylamine−naphthol	suspension	sausage	86.3−107.5	1−5	20

preconcentration of Sudan dyes has been reported, so some, such as bulk,[18,19] sol−gel,[6,27] and suspension[2,20,28,29] polymerization, methods were summarized and compared with the present work, and the results are listed in Table 5. As can be seen from the table, as a new material, the single-hole hollow MIP is an ideal candidate for SPE sorbent.

In summary, this work presents the attempt of preparing single-hole hollow MIP as selective sorbent for the preconcentration and determination of Sudan I in chilli sauce samples. The obtained SHH-MIP displayed excellent characteristics, such as high specific surface area, good thermal stability, high binding capacity, and fast mass transfer for Sudan I, owing to the single-hole hollow structure with imprinting effect. The developed method can be expected to have strong potential in the routine monitoring of food quality control and market surveillance to ensure food supply safety. Besides, the SHH-MIP-E has an average particle size of only several micrometers and, thus, can be extended for use as highly selective packing sorbents for HPLC columns. Furthermore, molecular imprinting combined with single-hole hollow polymers opens a new window of interest to the exploration of functionalized hollow polymers and provides new opportunities in the applications involved in the highly selective recognition of targeted species and, therefore, the high-efficiency enrichment of trace analytes from complicated matrices.

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