



# Molecularly imprinted polymer coated solid-phase microextraction fibers for determination of Sudan I–IV dyes in hot chili powder and poultry feed samples

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## ABSTRACT

In this research, a novel strategy was developed to prepare molecularly imprinted polymer (MIP) coated solid-phase microextraction fibers on a large scale with Sudan I as template and stainless steel fibers as substrate. More than 20 fibers could be obtained in one glass tube, and the efficiency and coating repeatability were enhanced remarkably in contrast with the yield of only one fiber in our previous works. The obtained MIP-coated stainless steel fibers were characterized by homogeneous and highly cross-linked coating, good chemical and thermal stabilities, high extraction capacities, and specific selectivities to Sudan I–IV dyes. Based on the systemic optimization of extraction conditions, a simple and cost-effective method based on the coupling of MIP-coated SPME with high-performance liquid chromatography was developed for the fast and selective determination of trace Sudan I–IV dyes in hot chili powder and poultry feed samples. The limits of detection of Sudan I–IV dyes were within 2.5–4.6 ng g<sup>-1</sup>, and the spiked recoveries were in the range of 86.3–96.3% for hot chili powder sample and 84.6–97.4% for poultry feed sample.

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## 1. Introduction

Sudan I–IV dyes are phenyl-azoic derivatives extensively used as colourants in chemical industries and household products. They may lead to genotoxic cancer and mutation for human and are categorized as class 3 carcinogens [1]. Unfortunately, these dyes are still illegally used as additives in foodstuffs to enhance the appearance of products, particularly in chili-containing foods (such as chili powder) and poultry feeds. Therefore, a simple, accurate, and practicable method for the monitoring of Sudan I–IV dyes in foodstuffs is required urgently. Traditionally, Sudan dyes were analyzed by high performance liquid chromatography (HPLC) coupled with ultraviolet–visible (UV) detection [2–5], mass spectrometric (MS) detection [6–9], chemiluminescence detection [10], and electrochemical detection [11]. Owing to the complexities of sample matrixes and the low levels of Sudan dyes, sample pretreatment such as widely used liquid extraction (LE) [2,4,8,10,12], liquid–liquid extraction [13], pressurized liquid extraction [14], and solid-phase extraction (SPE) [15–17] is crucial for the separation and enrichment of Sudan dyes prior to HPLC analysis. However, these methods suffer from several disadvantages such as the large

consumption of organic solvent, tedious procedures, or low enrichment factors.

Solid-phase microextraction (SPME), pioneered by Belardi and Pawliszyn [18] in 1990s, is a novel, simple, time-saving, and solvent-free pretreatment technique which integrates sampling, separation, enrichment, and injection into single one operation. The key of SPME is the coating on a silica fiber, so the development of SPME was always centered on the investigation of new coatings, and some commercial coatings and lots of home-made materials were exploited to improve the applicabilities of SPME for different kinds of compounds [19–30]. Recently, molecularly imprinted polymers (MIPs), acclaimed as the artificial antibody, have become one of hot research fields for SPME coating materials due to their unique characteristics of predetermined and specific selectivity, easy preparation, and good chemical stability.

The application of MIP in SPME was reported with different manners such as in-tube MIP–SPME [31], MIP-coated fiber [32–35], and MIP monolith fiber [36–38], which has been summarized by Martin-Esteban in a review paper [39]. In our group, the effort was poured into the coating of MIPs on silica and stainless steel fibers through chemical-bonding and co-polymerization strategy [40–42]. However, the preparation yield and efficiency became an important problem yet to be solved, which impose restrictions on the further commercial application of MIP-coated SPME fibers. When two or more silylated fibers were inserted simultaneously into one vessel such as glass tube for co-polymerization, the adher-

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ing of these fibers together was difficult to avoid and would result in the failure of coating. Therefore, only one MIP-coated fiber could be obtained in one glass tube with a total consumption of about 20–30 mL polymerization solution. For the preparation of a batch of MIP-coated fibers, lots of glass tubes, polymerization solutions, and manual labour were wasted, and the repeatabilities of MIP coating thickness and morphology among different tubes were affected.

In this study, a simple strategy was developed to achieve the preparation of MIP-coated SPME fibers on a large scale. With Sudan I as template and stainless steel fibers as substrate, more than 20 MIP-coated fibers could be obtained in single one glass tube, and the preparation yield, efficiency, and coating repeatability were enhanced remarkably. A simple, sensitive, and cost-effective method based on the coupling of MIP-coated SPME with HPLC–UV was developed for the fast and selective determination of trace Sudan I–IV dyes in hot chili powder and feed samples. As we know, this is the first report concerning the improvement of home-made MIP-coated SPME fiber productivity and the application of SPME method in Sudan dyes pretreatment.

## 2. Experimental

### 2.1. Chemicals and reagents

Sudan dyes including Sudan I–IV were obtained from JinChun Reagent Corporation (Shanghai, China). The mixed and individual stock solutions of four Sudan dyes were prepared with the concentration of  $10 \text{ mg L}^{-1}$  in hexane, and the further solutions with lower concentration were prepared by the serial dilution of the stock solutions. All solutions were stored at  $4^\circ\text{C}$  in a refrigerator. Phenol and 1,2-diphenyldiazene were obtained from JinChun Reagent Corporation. Acrylamide (AA), 4-vinylpyridine (4-VP), trimethylolpropane trimethacrylate (TRIM), and ethylene glycol dimethacrylate (EGDMA) were obtained from Sigma–Aldrich (St. Louis, MO, USA). Methacrylic acid (MAA) and azo(bis)-isobutyronitrile (AIBN) were obtained from Damao Reagent Plant (Tianjin, China). Vinyltriethoxysilane (VTEOS) was purchased from Shengda Fine Chemical Industry Corporation (Beijing, China). The HPLC-grade methanol and acetonitrile were obtained from Merck (Darmstadt, Germany). Water was double distilled. All other reagents were of analytical grade. All solutions used for HPLC mobile phase were filtered through  $0.45\text{-}\mu\text{m}$  nylon filter membranes. The stainless steel fibers ( $316 \text{ L}$ ,  $200 \mu\text{m}$  O.D.) were obtained from an ironware factory.

### 2.2. Fiber preparation

The surface treatment and the silylation of stainless steel fibers were according to our previous work [41]. MIP-coated stainless steel fibers were prepared by the multiple bulk co-polymerization method which was summarized in our previous paper [42], but improved in this study. Briefly,  $93.2\text{-mg}$  Sudan I,  $127.5\text{-}\mu\text{L}$  MAA, and  $18.4\text{-mL}$  acetone were added into a ground conical flask for 12-h swirling at  $5^\circ\text{C}$ , and then  $28.9\text{-mg}$  AIBN and  $1.92\text{-mL}$  TRIM were added in and mixed adequately. Subsequently,  $2\text{-mL}$  above pre-polymerization solution was transferred into a small glass tube and deoxygenized with nitrogen gas for 5 min. Meanwhile, 20 silylated stainless steel fibers were spaced and fixed parallelly with a toroidal adhesive note, and then immersed into above solution. The tube was sealed immediately, and the polymerization was performed in a thermostatic water bath (Senxin, Shanghai, China) at  $60^\circ\text{C}$ . After 2 h, the fibers were pulled out from the solidified polymer in the tube, and a thin layer of MIP was coated on the fiber surface. Moreover, after 12-h condition in  $60^\circ\text{C}$  in a drying

oven (Senxin, Shanghai, China), these fibers were immersed again into another fresh pre-polymerization solution, and were coated repeatedly through identical procedures mentioned above until the total repeated coating times of 10. Finally, the MIP-coated fibers were repeatedly soaked in  $10 \text{ mL}$  of  $10\%$  (v/v) acetic acid solution in methanol for 30 min to remove Sudan I template molecules, until Sudan I could not be detected in the soaking solution by HPLC. After washing with methanol for 3 times and drying with nitrogen gas, these MIP-coated fibers were ready for following studies. For comparison, non-imprinted polymer (NIP)-coated fibers were prepared simultaneously following the identical procedures except the addition of Sudan I template. The obtained MIP and NIP coatings were scraped from the fiber top to obtain a uniform length of  $10 \text{ mm}$ .

### 2.3. Coating characterization

The morphological evaluations during fiber preparation were performed with a 50iPol polarizing microscope (Nikon, Japan), and the coating thickness was measured with a scale eyepiece which was calibrated with a  $0.01 \text{ mm}$  slide micrometer. The scanning electron micrography was obtained with an S-3400N-II scanning electron microscope (Hitachi, Japan). The infrared absorption spectrum of MIP coating was obtained with an IR-prespige-21 FT-IR spectrometer (Shimadzu, Japan). The thermogravimetric analysis over  $50\text{--}600^\circ\text{C}$  was performed with an STA-409 PC thermogravimetric analyzer (Netzsch, Selb/Bavaria, Germany) with the heating rate of  $10^\circ\text{C min}^{-1}$ .

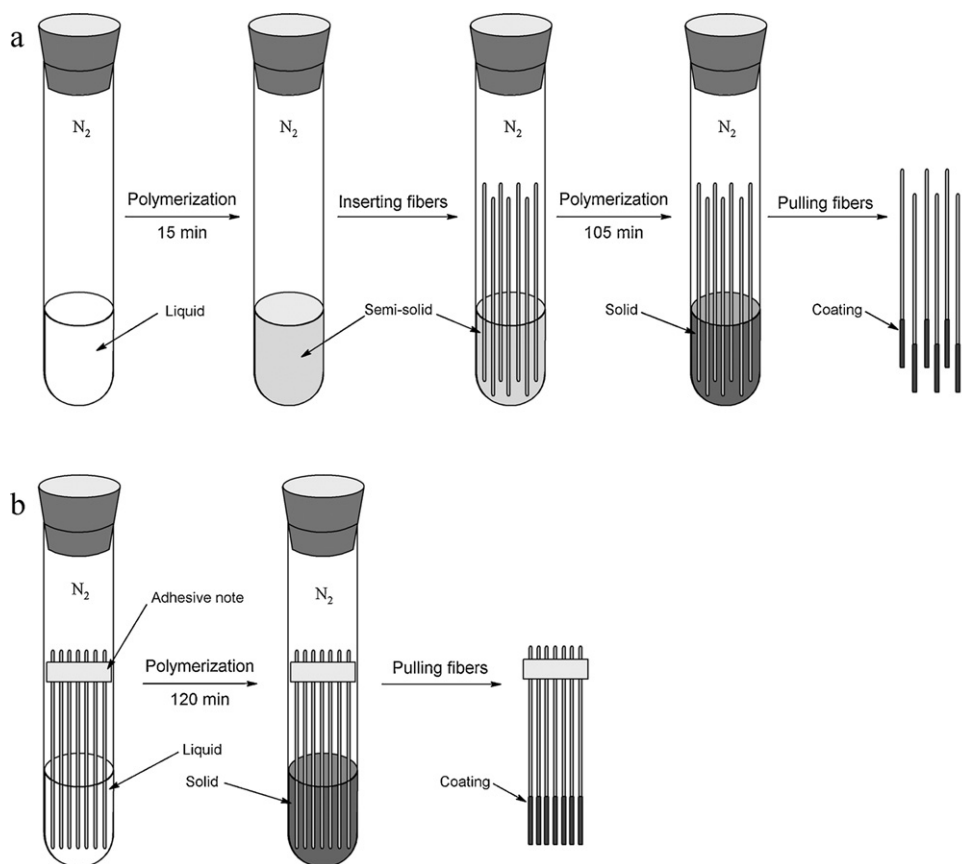
### 2.4. SPME procedures and HPLC analysis

Three milliliter of standard solution or sample solution was added into a  $5\text{-mL}$  glass vial with a Teflon magneton, and then one MIP- or NIP-coated stainless steel fiber was immersed into the solution for 30-min extraction at the stirring rate of 750 rpm. Subsequently, the fiber was pulled out and rinsed with hexane for 30 s, and then was immersed into a SPME–HPLC coupling device (Supelco, Bellefonte, PA, USA) for 10-min desorption with about  $60\text{-}\mu\text{L}$  methanol. After desorption, the fiber was pulled out and immersed into methanol for 10-min condition to be ready for the next extraction.

The chromatographic analysis was performed with a C-10ATvp HPLC (Shimadzu, Japan), an ultraviolet detector, and a SPME–HPLC coupling interface. The column was Dikma  $\text{C}_{18}$  ( $250 \text{ mm} \times 4.60 \text{ mm}$  I.D.,  $5 \mu\text{m}$  packing, Beijing, China). The mobile phase consisted of  $1\%$  formic acid solution and acetonitrile ( $10:90$ , v/v) at a constant flow rate of  $1.0 \text{ mL min}^{-1}$ , and the separation time for real samples was about 55 min. Detection wavelength was set at  $520 \text{ nm}$ . The quantification of Sudan dyes was carried out by the peak area measurement using external standard calibration.

### 2.5. Sample pretreatment

Hot chili powder and poultry feed samples were selected for the valuation of Sudan I MIP-coated stainless steel fibers, which were obtained from a supermarket and a feed corporation, respectively.  $0.5 \text{ g}$  of chili powder or feed sample was weighed into a  $25\text{-mL}$  ground conical flask and  $5 \text{ mL}$  of hexane was added. The mixture was extracted for 10 min under ultrasonication, and then filtrated. The obtained filtrates were quantified to the volume of  $10 \text{ mL}$  with hexane. For the spiking analysis,  $0.5 \text{ g}$  of sample was mixed with  $12.5$ ,  $50$  and  $125 \mu\text{L}$  of  $1.00 \text{ mg L}^{-1}$  Sudan dyes mixed standard solution, prior to above extraction procedures. The spiking concentrations were achieved with three levels of  $25.0$ ,  $100$  and  $250 \text{ ng g}^{-1}$ , respectively.



**Fig. 1.** Schematic representation of two modified strategies for MIP-coated fiber preparation. (a) Inserting of many fibers into semi-solid polymer after 15-min polymerization; (b) inserting of array of many fibers fixed parallelly with an adhesive note into liquid solution before polymerization.

### 3. Results and discussion

#### 3.1. Sudan I MIP-coated SPME fiber preparation

In our previous works [40,41], it was found that only one MIP-coated fiber could be obtained in one polymerization glass tube with a total consumption of about 20–30 mL polymerization solution. This problem was caused by that the adhering of fibers together in liquid solution was difficult to avoid when many fibers were placed into one tube.

In this contribution, we presented two modified strategies to enhance producibility and to achieve fiber preparation on a large scale. For the first modified strategy shown in Fig. 1(a), the liquid polymerization solution in a glass tube was first heated at 60 °C for 15 min to be converted into semi-solid status, and then many silylated stainless steel fibers could be inserted simultaneously into the semi-solid polymer at regular intervals. Refilled with nitrogen gas, the tube was sealed and the polymerization was continued until the total polymerization time of 2 h. Through this method, more than 20 fibers could be obtained in one tube. However, it was found that the MIP coatings on these fibers were thin, inhomogeneous, and

had low-coverage. These problems were probably resulted from the incomplete chemical bonding between the silylated fiber and the polymer. Therefore, different fiber-inserting times (counted after polymerization), including 0, 5, 15, 30, 45, and 60 min, was tested to study the mechanism behind the coating procedure. As shown in Table 1, the results indicated that the thickness, uniformity, and coverage of MIP coating declined with the delay of fiber-inserting time, and no coating could be obtained when this time was over 30 min. It could be concluded that the chemical-bonding based coating mainly proceeded during first 15 min in liquid solution, and it would be hindered in semi-solid or solid polymer. Therefore, the silylated fibers should be inserted into the polymerization solution prior to the polymerization.

As shown in Fig. 1 (b), another simple strategy was developed, in which an array of many fibers fixed parallelly within an adhesive note was inserted into the tube before the polymerization to guarantee sufficient chemical bonding. By this method, more than 20 fibers could be obtained in only one tube when a toroidal adhesive note was utilized, and the MIP coatings were homogeneous and dense with the average thickness of 2.4 μm (RSD of 4.3%).

**Table 1**  
Effect of fiber-inserting time (counted after polymerization) to MIP coating thickness and morphology.

Fiber-inserting time (min)	0	5	15	30	45	60
Polymerization solution status	Liquid	Liquid	Semi-solid	Solid	Solid	Solid
Coating thickness (μm)	2.5	1.9	0.8	–	–	–
Coating uniformity	Very good	Good	Bad	–	–	–
Coating coverage (%)	100	100	~25	–	–	–

“–” No MIP coating was obtained on the surface of stainless steel fiber.

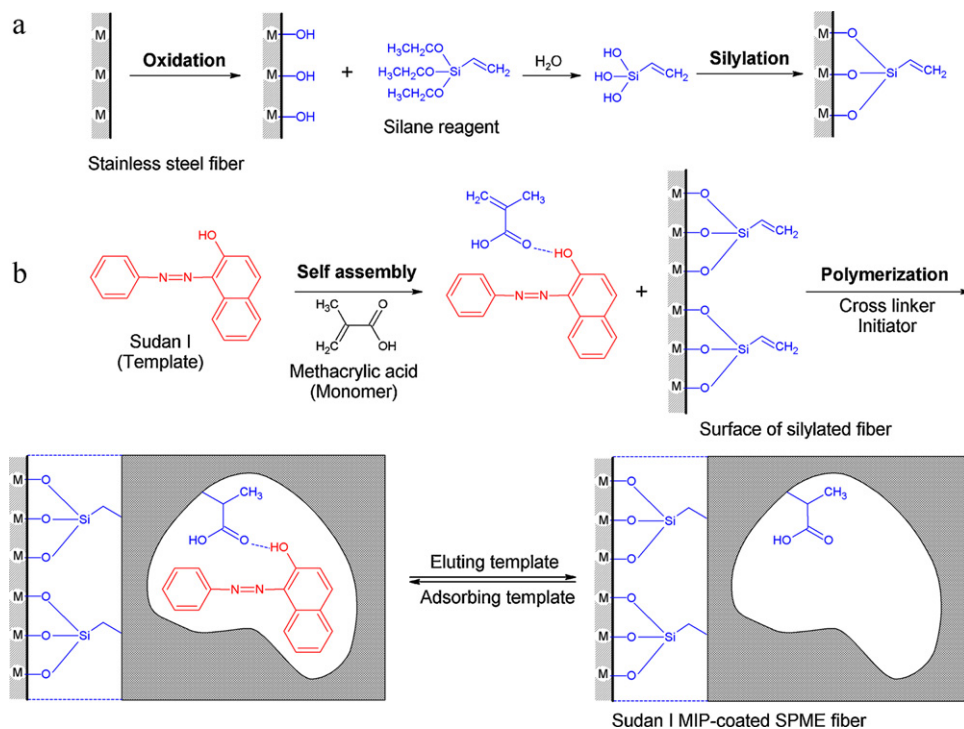


Fig. 2. Schematic representation of Sudan I MIP-coated stainless steel fiber preparation.

The simplified preparation scheme of Sudan I MIP-coated stainless steel fiber is shown in Fig. 2. It is well-known that the polymerization conditions such as solvent, monomer, cross-linker, and component proportions are crucial for the preparation feasibility and the MIPs performance. These conditions, applied for MIP-1 to MIP-9 preparation (see Table 2), were optimized with the criterion of extraction capacity and selectivity of MIP coating. As shown in Table 2, MIP-4, MIP-5, and MIP-6 were found with low extraction amounts and poor selectivities, and it was indicated that monomer and cross linker played important roles in MIP preparation. The conditions for MIP-2 were proved to be optimal because of its highest selectivity coefficient of 5.8 (defined as the ratio of extraction amount of Sudan I with MIP coating to that with NIP coating).

A batch of Sudan I MIP-coated fibers were prepared at a large scale through the fiber preparation strategy in Fig. 1(b) and under the polymerization conditions of MIP-2, and 200 fibers were obtained in 10 glass tubes successfully. After repeating coating procedures for 10 times, the average thickness of MIP coating was

19.8  $\mu\text{m}$  with RSD of 3.7% among 10 tubes and 2.4% among 20 fibers in one tube, and the fiber preparation repeatability was enhanced remarkably in contrast with 6.1–6.7% reported in our previous works [40,41].

### 3.2. MIP-coated fiber characterization

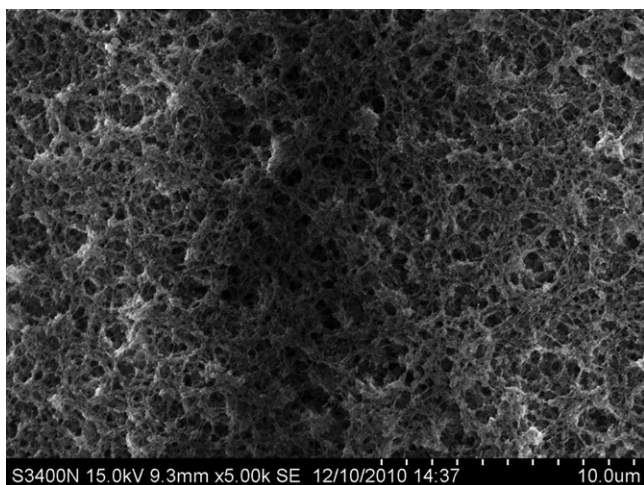
The scanning electron micrograph of Sudan I MIP-coated stainless steel fiber was obtained under the magnifications of 5000 $\times$ . As shown in Fig. 3, a highly cross-linked and porous structure was observed remarkably and the pore diameter was about 1  $\mu\text{m}$ . It would be advantageous to the fast adsorption and desorption of analytes, and could provide a guarantee of sufficient extraction performance of MIP coating with the thickness of only 19.8  $\mu\text{m}$  for the analysis of trace Sudan dyes. Compared with previous reported MIP-coated silicon fibers [42], remarkable changes in coating thickness (about 19–25  $\mu\text{m}$  on silica fibers) and surface topography have not been found with the MIP coating on the stainless steel fiber. It demonstrated that stainless steel fibers just

Table 2  
Optimization of polymerization conditions for Sudan I MIP coating preparation.

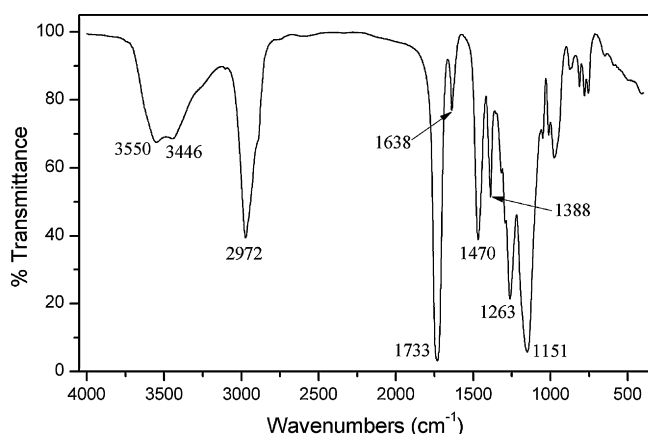
	Solvent	Monomer	Cross linker	Volume ratio of monomer plus cross linker to solvent	Molar ratio of template to monomer	Extraction <sup>a</sup> amount with MIP coating (ng)	Extraction amount with corresponding NIP coating (ng)	Selectivity coefficient <sup>b</sup>
MIP-1	Benzene	MAA	TRIM	1:9	1:4	1.43	0.29	4.9
MIP-2	Acetone	MAA	TRIM	1:9	1:4	1.28	0.22	5.8
MIP-3	Hexane	MAA	TRIM	1:9	1:4	1.09	0.29	3.8
MIP-4	Benzene	4-VP	TRIM	1:9	1:4	0.38	0.32	1.2
MIP-5	Benzene	AA	TRIM	1:9	1:4	0.67	0.25	2.7
MIP-6	Benzene	MAA	EGDMA	1:9	1:4	0.96	0.30	3.2
MIP-7	Benzene	MAA	TRIM	1:6	1:4	1.26	0.28	4.5
MIP-8	Benzene	MAA	TRIM	1:9	1:1	1.14	0.33	3.5
MIP-9	Benzene	MAA	TRIM	1:9	1:8	1.34	0.31	4.3

<sup>a</sup> Extraction conditions: 100  $\mu\text{g L}^{-1}$  Sudan I in hexane of 0.15 mL was extracted with MIP- or NIP-coated stainless steel fiber for 120 min, desorption was performed in methanol of 0.15 mL for 30 min, injection volume for HPLC analysis was 10  $\mu\text{L}$ .

<sup>b</sup> Selectivity coefficient was defined as the ratio of extraction amount of Sudan I with MIP coating to that with corresponding NIP coating.



**Fig. 3.** Scanning electron micrograph of Sudan I MIP-coated stainless steel fiber under 5000 $\times$  magnification.



**Fig. 4.** Infrared (KBr pellet) spectra of Sudan I MIP coating.

worked as a non-fragile and rigid substrate and would not bring considerable effect on the characteristics and performance of MIP coating.

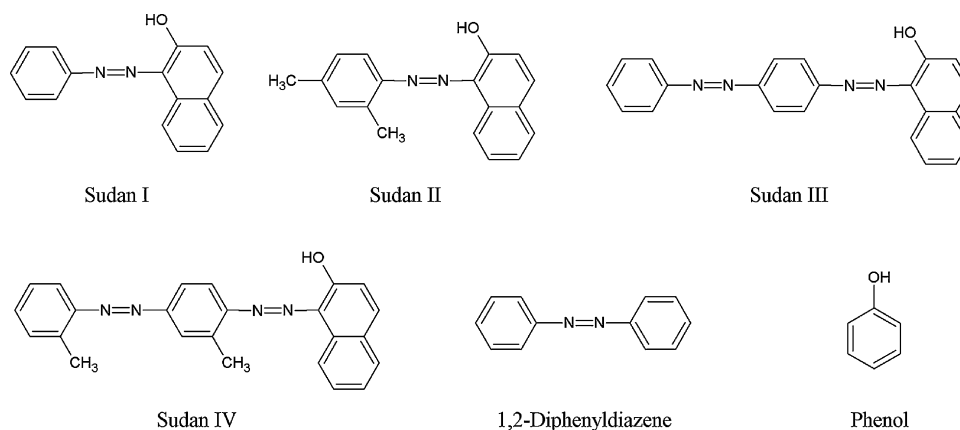
The infrared spectra of Sudan I MIP coating was shown in Fig. 4, and the main functional groups of Sudan I MIP coating could be found with corresponding infrared absorption peaks. Four strong infrared absorption peaks of 3550, 2972, 1733, and 1470  $\text{cm}^{-1}$  were

found, which were attributed to hydroxyl groups, methyl groups, carbonyl groups, and methyl groups, respectively. Simultaneously, the presence of residual C=C bonds in MIP coating was indicated with a minor peak around 1638  $\text{cm}^{-1}$ , which was crucial for the chemical bonding between MIP layer and layer during the repeated coating procedures [42]. These characteristic groups belong to MAA monomer and TRIM cross-linker used in Sudan I MIP coating preparation shown in Fig. 2.

The thermogravimetric analysis was performed to investigate the thermal stability of Sudan I MIP coating. The results indicated that an obvious mass loss occurred at about 260  $^{\circ}\text{C}$  and the fastest mass loss occurred at 371  $^{\circ}\text{C}$ . A soaking test was performed to observe the chemical stability of Sudan I MIP coating, in which 12 MIP-coated stainless steel fibers were immersed into methanol, ethanol, acetonitrile, acetone, chloroform, ethyl acetate, benzene, toluene, hexane, 10% (v/v) acetic acid solution, 0.1  $\text{mol L}^{-1}$  hydrochloric acid, and 0.1  $\text{mol L}^{-1}$  sodium hydroxide for 24 h, respectively. Subsequently, these fibers were rinsed with ethanol or water, dried with nitrogen gas, and then observed with a microscope. Notably, all MIP coatings retained good surface qualities without any cracking, dropping, or dissolving events.

### 3.3. Selectivity

As shown in Fig. 5, Sudan I–IV dyes and the reference compounds of 1,2-diphenyldiazene and phenol were used to investigate the selectivities of Sudan I MIP-coated fiber, and the NIP-coated fiber was used as comparison. The solutions of above compounds were prepared individually with the concentration of 25  $\mu\text{g L}^{-1}$  to avoid competitive adsorption. The results were shown in Fig. 6, and specific selectivities were found with the MIP-coated fiber to Sudan I–IV with the selectivity coefficients of 5.6, 4.8, 4.3, and 3.0, respectively. It is well-known that the selectivity of MIPs is based on the complementarities of size, shape, and functional groups between analyte and the recognition cavity in MIPs. Therefore, a higher structure similarity of Sudan II to Sudan I template resulted in its higher selectivity coefficient than those of Sudan III and IV. In contrast, noteworthy differences in the extraction amounts of 1,2-diphenyldiazene and phenol were not observed between the MIP- and NIP-coated fibers, due to their significant structure differences from Sudan I. It indicated that the non-specific adsorption mechanism played a key role in MIP coating when analytes differed from the template molecule distinctly.



**Fig. 5.** Chemical structures of Sudan I–IV dyes, 1,2-diphenyldiazene, and phenol.

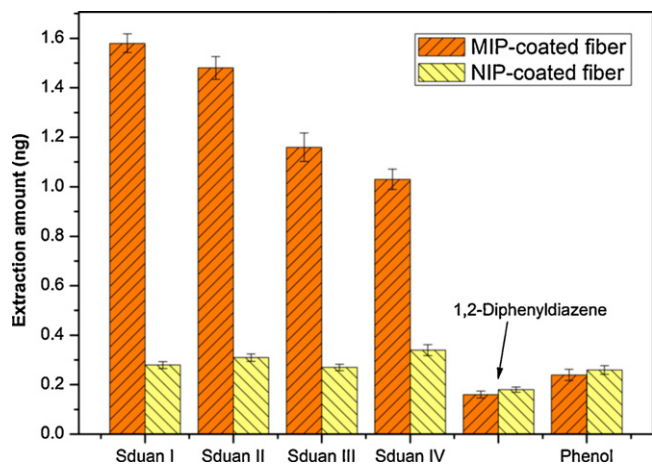


Fig. 6. Extraction amounts of Sudan I–IV dyes, 1,2-diphenyldiazene, and phenol with MIP- or NIP-coated fibers at  $25 \mu\text{g L}^{-1}$  level.

### 3.4. Extraction capacity

A series of Sudan I–IV dyes mixed standard solutions of  $0.5\text{--}500 \mu\text{g L}^{-1}$  were used to perform the extraction capacity study of Sudan I MIP-coated fiber. High extraction capabilities were observed with the MIP coating to Sudan I and its structural analogues of Sudan II–IV, resulting from the tailor-made recognition cavities in MIP coating. As the template, the extraction amount of Sudan I was larger than those of Sudan II–IV. When the concentration was up to  $150 \mu\text{g L}^{-1}$ , the extraction was close to saturation and the extraction capacities of Sudan I–IV were found to be about 6.4, 6.0, 5.0, and 4.7 ng, respectively. Moreover, the reproducibility of fiber-to-fiber extraction was studied with ten MIP-coated stainless steel fibers for the extraction of  $25.0 \mu\text{g L}^{-1}$  mixed standard solution, and RSDs of 2.2–4.8% for extraction amounts of four Sudan dyes were obtained satisfactorily.

### 3.5. Optimization of SPME conditions

The extraction solvent is crucial to the MIP-coated fiber SPME method, and conventional organic solvents including hexane, benzene, toluene, ethyl acetate, chloroform, acetone, acetonitrile, and methanol were selected for the solvent optimization.  $25.0 \mu\text{g L}^{-1}$  Sudan I–IV dyes mixed standard solutions in above solvents were prepared and extracted with the MIP-coated fibers. As shown in Fig. 7, the results indicated that higher extraction amounts of four Sudan dyes could be achieved when non-polar solvents such as

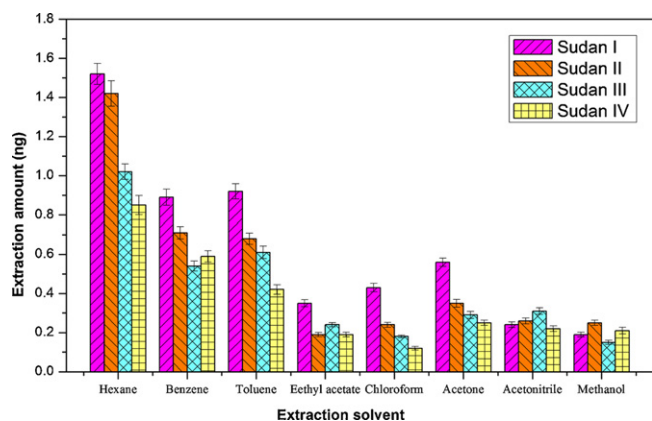


Fig. 7. Extraction amounts of Sudan dyes at  $25 \mu\text{g L}^{-1}$  level in different extraction solvents.

hexane and benzene were used. In contrast, dissatisfactory results were found with polar solvents such as acetonitrile and methanol. These results were basically consistent with our previous works [40,41], and the main cause probably lied in the interference of polar solvent to the hydrogen-bonding based recognition of MIP coating. Therefore, hexane was finally adopted through following investigations and that would be in favor of the MIP coating selectivity.

The optimizations of extraction time and desorption time were performed with  $25.0 \mu\text{g L}^{-1}$  Sudan I–IV dyes mixed standard solution in hexane. For extraction time, it was observed that the extraction amounts of four Sudan dyes reached equilibrium quickly after 30 min. This quick adsorption was mainly caused by two factors. Firstly,  $19.8\text{-}\mu\text{m}$  thin thickness of MIP coating could guarantee the sufficient accessibility of selective recognition sites. Secondly, the highly cross-linked and porous morphological structure of MIP coating was advantageous to the diffusion speed of analytes into the MIP coating. For desorption time, above factors were also beneficial to the quick desorption of analytes in the MIP coating. Therefore, it was indicated that about 75–90% of the adsorbed Sudan I–IV dyes could be quickly desorbed within 1 min, and desorption reached equilibrium quickly after 5 min only. Through following studies, extraction time of 30 min and desorption time of 10 min were selected finally.

The optimization of stirring speed was performed at 0, 100, 250, 500, 750, and 1000 rpm, and the extraction amounts of four Sudan dyes were all enhanced with the increase of stirring speed and arrived at equilibrium around 750 rpm.

### 3.6. Linearity, limit of detection, and precision

The linearity of Sudan I MIP-coated SPME–HPLC method was studied with a series of Sudan I–IV dyes mixed standard solutions under above optimal extraction conditions, and the linear ranges of  $0.5\text{--}100 \mu\text{g L}^{-1}$  were achieved with the correlation coefficient of 0.9991, 0.9979, 0.99864, and 0.9983 for Sudan I–IV, respectively. The limits of detection (LOD) was calculated with signal-to-noise ratio of 3, and 0.14, 0.26, 0.21, and  $0.29 \mu\text{g L}^{-1}$  were obtained for Sudan I–IV, respectively, which were much lower than the LODs of  $0.5\text{--}1.0 \mu\text{g g}^{-1}$  recommended by the European Commission since 2003 [43,44]. The method precision was monitored with  $25.0 \mu\text{g L}^{-1}$  Sudan I–IV dyes mixed standard solution and the RSDs of extraction amounts of Sudan I–IV were 3.1, 3.9, 5.1, and 4.8% ( $n = 10$ ), respectively.

### 3.7. Real sample analysis

The hot chili powder and poultry feed samples were selected for the validation of practical applicability of Sudan I MIP-coated SPME–HPLC method. However, four Sudan dyes were all not detected in two samples, so the spiking recovery analysis was performed at three levels of 25.0, 100, and  $250 \text{ ng g}^{-1}$ . The results demonstrated that trace Sudan I–IV dyes could be monitored with MIP-coated SPME–HPLC method and traditional UV detector, as shown in Fig. 8. To our knowledge, this is the first report about the application of SPME in Sudan dyes pretreatment. As shown in Table 3, the recoveries of four Sudan dyes were in the range of 86.3–96.3% for hot chili powder sample and 84.6–97.4% for poultry feed sample.

Moreover, LODs of Sudan dyes in hot chili powder and poultry feed samples were studied, and  $2.5\text{--}4.6 \text{ ng g}^{-1}$  were obtained for four dyes, which was much lower than the reported LODs of 400–1100, 25–50, and  $3\text{--}24 \text{ ng g}^{-1}$  with only liquid extraction (LE) pretreatment and LC–MS or LC–MS/MS detection [8,45,46], as shown in Table 4. After the liquid extraction of solid or pasty samples, further pretreatment such as SPE or MIP–SPE would effectively

**Table 3**

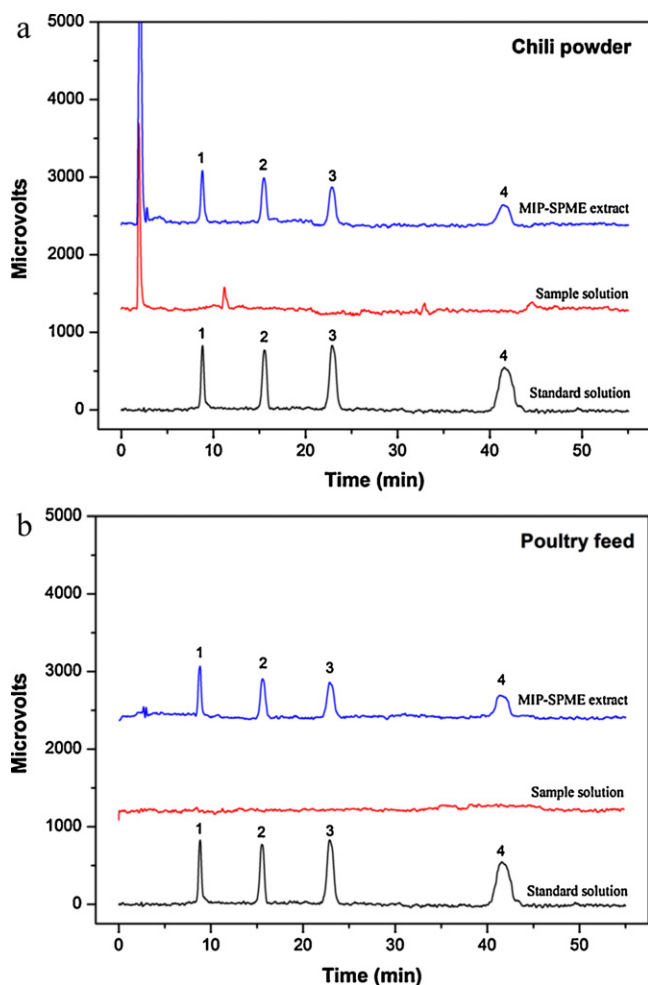
Limits of detection (LOD) and recoveries of Sudan I–IV dyes in spiked hot chili powder and poultry feed samples.

Compound	Hot chili power								Poultry feed							
	LOD <sup>a</sup> (ng g <sup>-1</sup> )	25.0 ng g <sup>-1</sup>		100 ng g <sup>-1</sup>		250 ng g <sup>-1</sup>		LOD (ng g <sup>-1</sup> )	25.0 ng g <sup>-1</sup>		100.0 ng g <sup>-1</sup>		250 ng g <sup>-1</sup>			
		Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)		Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)		
Sudan I	2.5	88.3	5.7	95.8	4.3	96.3	3.9	2.8	89.3	6.2	93.6	4.6	97.4	2.6		
Sudan II	3.1	85.6	6.4	94.3	5.7	96.2	5.2	3.6	87.4	7.3	94.5	6.3	95.9	2.9		
Sudan III	3.7	87.4	4.5	95.1	4.8	94.8	3.4	4.1	86.4	5.7	93.6	4.6	95.3	4.2		
Sudan IV	4.6	86.3	7.2	94.3	5.3	92.6	4.8	4.5	84.6	7.1	92.4	5.8	94.2	3.8		

<sup>a</sup> LOD was calculated with signal-to-noise ratio of 3.**Table 4**

Comparison of LODs of Sudan I–IV with different sample preparation and detection methods.

Matrix	Sample preparation method	Detection method	LOD (ng g <sup>-1</sup> ) of Sudan I–IV	Ref.
Hot chili tomato sauce	LE	LC–ESI–Q–TOF–MS	900, 400, 900, 1100	[45]
Dried chili and curry	LE	LC–APCI–MS	20, 50, 50, 50	[46]
Chili tomato sauce; chili sauce	LE	LC–ESI–MS/MS	4, 3, 5, 11; 11, 3, 9, 24	[8]
Eggs	LE–SPE	HPLC–UV	4.6, 4.0, 4.8, 4.2	[12]
Tomato sauce; sausage	LE–MIP–SPE	HPLC–UV	1.0, 3.0, 3.0, 2.5; 0.8, 3.0, 3.0, 1.5	[17]
Catsup products	Ultrasonic extraction (UE)–MIP–SPE	HPLC–UV	2.0, 4.0, 5.0, 7.0	[47]
Eggs	MIM–MSPD–DLLME <sup>a</sup>	HPLC–UV	2.4, 2.3, 3.1, 6.1	[48]
Hot chili power; poultry feed	UE–MIP–SPME	HPLC–UV	2.5, 3.1, 3.7, 4.6; 2.8, 3.6, 4.1, 4.5	Present work

<sup>a</sup> Molecularly imprinted microsphere based matrix solid-phase dispersion coupled with dispersive liquid–liquid microextraction.**Fig. 8.** Chromatograms of 0.2 mg L<sup>-1</sup> Sudan I–IV dyes mixed standard solution, 25.0 ng g<sup>-1</sup> spiked chili powder and poultry feed sample solutions, and extracts of spiked sample solutions with MIP-coated SPME method. (1) Sudan I, (2) Sudan II, (3) Sudan III, (4) Sudan IV; injection volume for standard solution and spiked sample solutions: 10  $\mu$ L.

enhance the LODs of Sudan dyes even with traditional HPLC–UV detection. In contrast with widely studied SPE–HPLC [12] and MIP–SPE–HPLC methods [17,47], LODs of four Sudan dyes were at the same levels, but the consumptions of time, labour, and solvent would be reduced with the MIP-coated SPME–HPLC method. Promisingly, Sudan I MIP-coated SPME–HPLC method would provide a selective, sensitive, and efficient solution for the trace analysis of Sudan dyes in various samples.

#### 4. Conclusions

A novel and simple strategy was developed to prepare MIP-coated stainless steel SPME fiber on a large scale with remarkably enhanced efficiency and coating repeatability, by which about 20 MIP-coated fibers could be obtained in single one glass tube. The obtained Sudan I MIP-coated fibers possessed high selectivities and extraction capacities to four Sudan dyes, and a simple, sensitive, and cost-effective method based on the coupling of MIP-coated SPME with traditional HPLC–UV was developed for the fast and selective determination of trace Sudan I–IV dyes in hot chili powder and poultry feed samples. Notably, the solving of preparation yield and efficiency of MIP-coated SPME fiber would promote the further commercial application of MIPs in SPME method.

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