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Synthesis and Characterization of a Molecularly Imprinted Silica Gel Sorbent for the On-Line Determination of Trace Sudan I in Chilli Powder through High-Performance Liquid Chromatography

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A highly selective imprinted polymer was synthesized by a surface molecular imprinting technique in combination with a sol−gel process. The imprinted polymer was evaluated by FT-IR and static and kinetic adsorption experiments. The results showed that the imprinted sorbent exhibited good recognition and selective ability, offered a faster kinetics for the adsorption and desorption of Sudan I than the non-imprinted sorbent, a saturated binding capacity (Q_{max}) that reached 33.47 mg g^{-1}. The prepared sorbent was applied for the determination of trace Sudan I through on-line solid-phase coupled with high-performance liquid chromatography (SPE-HPLC). With a loading flow rate of 1.5 mL min^{-1} for sampling 50 mL, an enrichment factor of 1266 was achieved. The detection limit (S/N = 3) was 1.2 ng L^{-1}, and the peak area precision (RSD) for five replicate detections of 0.01 μg L^{-1} Sudan I was 3.66%. The Sudan I in the chilli powder from the local market was determined at three levels (0.25, 0.5, and 1.0 ng g^{-1}) with recoveries ranging from 80.31 to 94.02%.

KEYWORDS: Sudan I; molecular imprinting; sol−gel; on-line solid-phase extraction; high-performance liquid chromatography

INTRODUCTION

Sudan azo-dyes are synthetic colorants known also as Sudan I−IV (**Figure 1**). As one of the important azo dyes, Sudan I (1-phenylazo-2-hydroxynaphthol, CAS Registry No. 842-07-09) is routinely used in many fields such as household commodities, the textile industry, and for coloring solvent and floor polishes. Sudan I is not a permitted food color additive under the *Colors in Food Regulations* (1995) (*1*), and its presence is not permitted in foods for any purpose at any levels. However, for many years it had been employed as an additive in foods by some merchants, particularly in those containing chilli powders, because of its intense red-orange color (*2*), and it was also found in a number of relishes, chutneys, seasonings, sauces, and ready meals. The genetic toxicity of some azo-dyes has been confirmed and, among organic colorants, most of the azo-dyes are recognized to be carcinogens. The European Community (2003/460/EC) and many other countries did not allow Sudan I as an additive in foods because it has been proved to cause tumors in the liver or bladder of rats, mice, and rabbits,

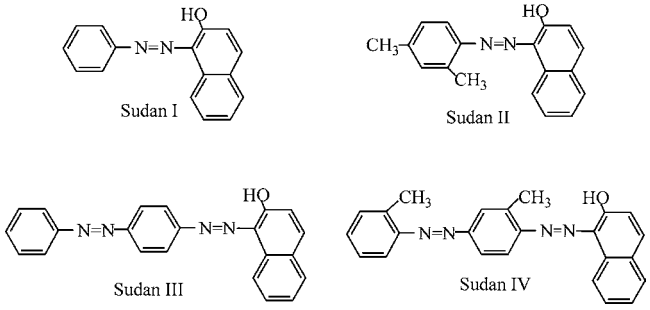

Figure 1. Chemical structures of Sudan I and structurally related compounds Sudan II–IV.

it is also considered a possible genotoxic carcinogen and mutagen to human, and it is classified as a category 3 carcinogen by the International Agency for Research on Cancer (IARC) (*3*−*5*). Now, the presence of Sudan I and other Sudan dyes has been found in food products and caused panic among customers in China and Europe. For these reason, accurate and reliable analytical methods for the determination of Sudan I in foods are required for the assurance of consumer health.

Several analytical methods have been developed to determine the presence of this compound based on gas chromatography,

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liquid chromatography with ultraviolet−visible (UV), fluorometric or on-line electrogenerated BrO^-−luminol chemiluminescence detection, mass spectrometry (MS), and liquid chromatography (LC)/MS/MS (*6*−*9*). However, when the content of Sudan I is trace in foods, it is difficult to detect or very expensive instruments (LC/MS/MS) are needed to reveal it. Consequently, the development of a simple, rapid, inexpensive, and sensitive analytical method for the determination of trace Sudan I in foods is of particular significance and necessity.

Molecular imprinting technology is a synthetic approach to imitate natural molecular recognition, and it has been proved to be an efficient method to produce functionalized materials that have the ability to recognize the specific template from a mixture of closely related compounds. The application of molecularly imprinted polymer (MIP) has been numerously reported during the past few years (*10*−*20*). It has become increasingly attractive in many fields, such as chiral separation, chemical sensors, or immunoassay-like analysis as synthetic antibody. However, one of the most exciting applications of this imprinted functionalized material is as sorbent for solid-phase extraction (SPE) (*14*−*21*).

Recently, molecularly imprinted sol−gel materials have been extensively studied because they have been verified to be much more specific toward the target species compared to the traditional imprinted method (*22*−*25*); most of these materials exhibit high affinity and selectivity but poor site accessibility to the target molecules. Surface molecular imprinting is one of the important types of molecular imprinting. The imprinted polymer not only possesses high affinity and selectivity but also can avoid above problems with mass transfer. The development of a sol−gel process combined with surface imprinting technology has been reported (*26*−*28*).

In this study, a new molecularly imprinted amino-functionalized silica gel polymer was prepared by combining a surface imprinting technique with a sol−gel process using Sudan I as template, 3-aminopropyltriethoxysilane as functional monomer, tetraethoxysilicane as cross-linker, and silica gel as support material in acetonitrile solvent. The imprinted polymer was applied as sorbent for the determination of trace Sudan I in chilli powder by on-line SPE-HPLC. The factors affecting preconcentration and separation of the analytes are discussed in detail to establish a simple and sensitive method as a potential analytical strategy to monitor the illegal addition of Sudan I in foods.

MATERIALS AND METHODS

Materials and Reagents. Silica gel (80−100 mesh, Qingdao Ocean Chemical Co., Qingdao, China) was used as the support material to prepare the imprinted functionalized polymer. Sudan I−IV (Shenyang Chemical Factory, Shenyang, China), 3-aminopropyltriethoxysilane (APTES), and tetraethoxysilicane (TEOS) (Wuhan University Chemical Factory, Wuhan, China) were used in this study. Ethanol and chloroform were supplied by Tianjin Chemical Factory (Tianjin China). Acetonitrile, acetone, and other chemicals were purchased from Merck (Darmstadt, Germany). All reagents were of the highest available purity and at least of analytical grade. Doubly deionized water (DDW; 18 $M\Omega\ cm^{-1}$) obtained from a Water Pro water system (Labconco Corp., Kansas City, MO) was used throughout the experiments.

Instrumentation. The high-performance liquid chromatographic system consisted of two LC-10ATVP pumps and a Shimadzu SPD-10AVP ultraviolet detector (Shimadzu, Kyoto, Japan). All separations were achieved on an analytical reversed-phase Shimadzu VP-ODS column (4.6 mm × 150 mm long) at a mobile flow rate of 1.0 mL min^{-1} under gradient elution conditions at a 30 °C column temperature. Mobile phase A was 0.1% formic acid water solvent (v/v)/acetonitrile (85:15, v/v); mobile phase B was 0.1% formic acid acetonitrile solvent (v/v)/acetone (80:20, v/v). The gradient mobile phase consisted of 25% A and 75% B for 10 min and then was linearly varied to 100% B, maintained for 22 min, and finally returned to the initial condition for 8 min; the column was flushed with B for 30 min (*29*). Class-vp software was used to acquire and process spectral and chromatographic data. The UV detector was operated at 480 nm.

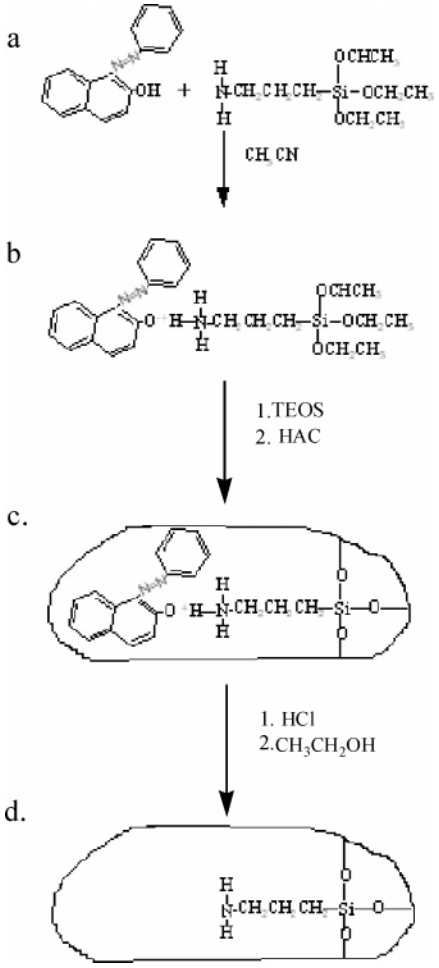

Figure 2. Schematic representation of the molecularly imprinted sol–gel polymer used in this study.

A model FIA-3100 flow injection system (Vital Instruments, Beijing, China) was used for on-line solid-phase extraction preconcentration. Tygon pump tubes were used for delivering the sample solution. Small-bore (0.5 mm i.d.) PTFE tubes were adapted for all collections, which were kept at the shortest possible length to minimize the dead volume. FT-IR spectra (4000−400 cm^{-1}) in KBr were recorded using a Vector 22 spectrometer (Bruker). A Cary 50-Bio UV spectrometer (Victoria, Australia) was also used in this study.

Preparation of Imprinted Polymer. To activate the silica gel surface, 8 g of silica gel was mixed with 60 mL of 33% (v/v) methanesulfonic acid and refluxed under stirring for 8 h. The solid product was recovered by filtration, washed with DDW to neutral, and dried under vacuum at 70 °C for 8 h (*28*). To prepare the imprinted silica gel polymer, 0.5 g of Sudan I was dissolved in 12 mL of acetonitrile and mixed with 1.8 mL of APTES. The mixture was magnetically stirred for 30 min, and then 0.5 g of activated silica gel and 1.8 mL of TEOS were added. After 20 min of stirring, 1.2 mL of 1.0 mol L^{-1} HAC was added. The mixture began to cohydrolyze and co-condense after 10 min of stirring and then was incubated for 10 h at 60 °C. The product was filtered, washed with ethanol, and dried in a vacuum oven at 100 °C for 8 h. Thus, the activated silica gel surface was grafted with the complex. The polymer was extracted with 50 mL of ethanol and 15 mL of 1.0 mol L^{-1} HCl under magnetic stirring for 2 h to remove Sudan I (**Figure 2**). The product was isolated by filtration, washed with ethanol, and then neutralized with 0.1 mol L^{-1} NaOH

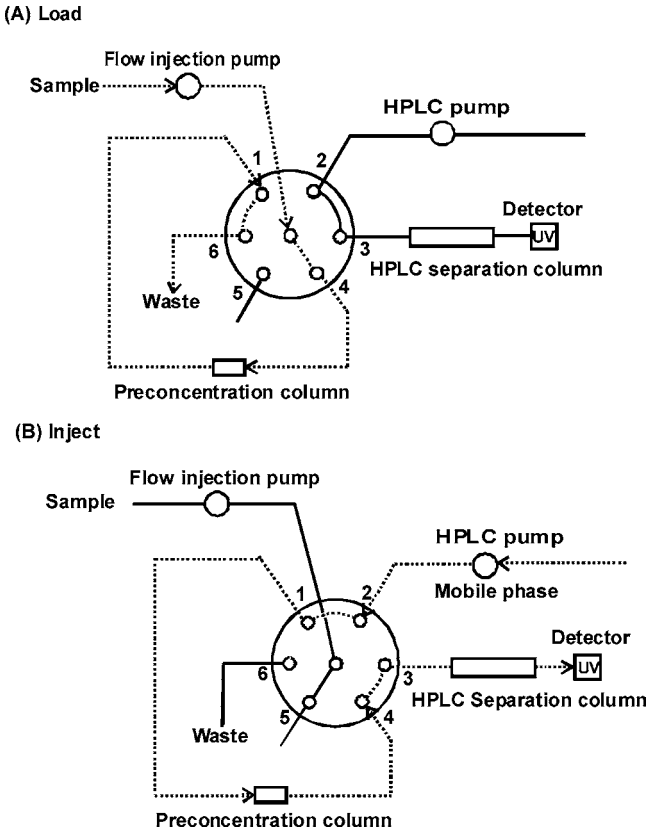

Figure 3. Schematic diagram of the on-line solid-phase extraction preconcentration coupled with HPLC. HPLC injector valve position: (**A**) load; (**B**) inject.

and washed with pure water. Finally, the polymer was dried under vacuum at 80 °C for 12 h. The washed imprinted material was checked to be free of Sudan I by UV spectrometry at 480 nm. For comparison, the non-imprinted functionalized silica gel polymer was also prepared following the same procedure, but without the addition of Sudan I.

Static Adsorption Test. To measure adsorption capacity, 20 mg of imprinted or non-imprinted polymer was equilibrated with 10 mL of ethanol solution containing Sudan I at various concentrations. The mixtures were mechanically shaken (200 times/min) for 1.5 h at room temperature with a horizontal shaker and then separated centrifugally (5000 rpm) for 5 min. The supernatants were measured for the unextracted Sudan I by UV spectrometry at 480 nm and the adsorption capacity (Q) calculated. Selective recognition studies were performed by adsorbing Sudan I and structurally related compounds, Sudan II−IV at the 80 mg L^{-1} level with imprinted and non-imprinted polymer. The supernatants were measured for the unextracted Sudan II, III, and IV at 495, 504, and 515 nm, respectively. The competitive property of imprinted and non-imprinted polymer toward the mixture of Sudan I and Sudan II−IV at 20 mg L^{-1} was determined by HPLC.

Uptake kinetics of Sudan I by the imprinted functionalized silica gel polymer was also examined. Twenty milligrams of the polymer was added to the 10 mL of 80 mg L^{-1} of Sudan I ethanol solution; the mixture was then mechanically shaken (200 times/min) for 5, 30, 60, 120, 180, and 240 min at room temperature and then separated centrifugally (5000 rpm) for 5 min, respectively. The supernatants were measured for the unextracted Sudan I by UV spectrometry.

Procedures for the On-Line SPE-HPLC Determination of Sudan I Using the Imprinted Sorbent. To evaluate the applicability of the imprinted functionalized silica gel sorbent for on-line SPE-HPLC determination of trace Sudan I in foods, a cylindrically shaped SPE microcolumn (1.5 cm × 4 mm) packed with 70 mg of the imprinted functionalized silica gel sorbent was prepared. A schematic diagram for the on-line SPE preconcentration coupled with HPLC for determination of trace Sudan I in chilli powder is shown in **Figure 3**. First, the sample solution was introduced onto the solid-phase extraction microcolumn at a flow rate of 1.5 mL min^{-1} for 33 min while the HPLC injector valve was in the load position, so that the Sudan I were preconcentrated by the sorbent-packed precolumn and the unwanted water went to waste (W) (**Figure 3A**). Second, the analytes adsorbed on the solid-phase extraction microcolumn were eluted in the backflush mode by the HPLC mobile phase at a flow rate of 1.0 mL min^{-1} into the chromatographic separation column for 1 min by switching the HPLC valve from the "load" to the "inject" position (**Figure 3B**). As such, the sample band in the microcolumn was compressed into a narrow band before entering the analytical column and the band broadening effect was reduced. Third, the HPLC injector valve was turned to the load position for the next sample preconcentration while the analytes were separated in the chromatographic separation column to improve sample throughput. In this way, a complete cycle of the on-line SPE preconcentration and HPLC separation of the Sudan I lasted for 70 min. Chromatograms were recorded and stored on the hard disk of the computer. The peak areas were calculated at 480 nm and used for data evaluation.

Preparation of Samples. To check the accuracy of the developed on-line SPE-HPLC using the imprinted functionalized silica gel sorbent, the red chilli powder spiked with Sudan I was used, which was free of Sudan I before spiking. Briefly, 2.0000 g of commercial red chilli powder was weighed into a 100 mL conical flask, spiked with 1.0 mL of standard solution (0.5, 1.0, or 2.0 μg L^{-1}) containing 0.5, 1.0, and 2.0 ng of Sudan I, and dissolved in 30 mL of acetonitrile solution. The mixture was mechanically shaken (180 times/min) for 2 h at room temperature and then separated centrifugally (5000 rpm, 15 min). After the supernatants had been filtered with a 0.2 μm filter again, 15 mL of the filtrate was transferred into a 50 mL calibrated flask and diluted to the mark with DDW for on-line enriching.

RESULTS AND DISCUSSION

Characteristics of Imprinted and Non-imprinted Sorbent and Activated Silica Gel by FT-IR Spectra. To ascertain the presence of APTES in the functionalized silica gel sorbent, FT-IR spectra of activated silica gel and non-imprinted and imprinted amino-functionalized silica gel sorbent are compared in **Figure 4**. The observed features around 1057 and 971 cm^{-1} indicated Si−O−Si and Si−O−H stretching vibrations, respectively. OH vibration reflected at 3419 and 1638 cm^{-1} indicated that it had adsorbed water (*26*). The bands around 792 and 465 cm^{-1} resulted from Si−O vibrations. Imprinted and non-imprinted sorbent showed similar locations and appearances of the major bands. Characteristic features of the imprinted and non-imprinted sorbents when compared with activated silica gel were a N−H bond around 1561 cm^{-1}, a C−H bond around 2933 cm^{-1}, and a CH_2−N bond around 1413 cm^{-1}. These results suggested that $-NH_2$ had been grafted onto the surface of activated silica gel after modification, so the APTES had been combined with the surface of the functionalized silica gel sorbent and it might have reacted with Sudan I in the imprinted material.

Evaluation of Static Adsorption. To measure adsorption capacity, 20 mg of Sudan I imprinted or non-imprinted polymer was equilibrated with 10 mL of Sudan I in a range of concentrations (20−140 mg L^{-1}) dissolved in ethanol. The mixture was mechanically shaken for 1.5 h at room temperature and then separated centrifugally. The supernatant was measured for the unextracted Sudan I by UV spectrometry. The isothermal adsorptions are plotted in **Figure 5**.

It was shown that the adsorption capacity of molecularly imprinted or non-imprinted polymer toward template molecules increased with increasing Sudan I initial concentration. Obviously, the adsorption capacity of the imprinted polymer was higher than that of the non-imprinted polymer; the adsorption capacity of imprinted polymer (14.17 mg g^{-1}) was >2-fold that of the non-imprinted polymer (6.68 mg g^{-1}) at a 140 mg L^{-1}

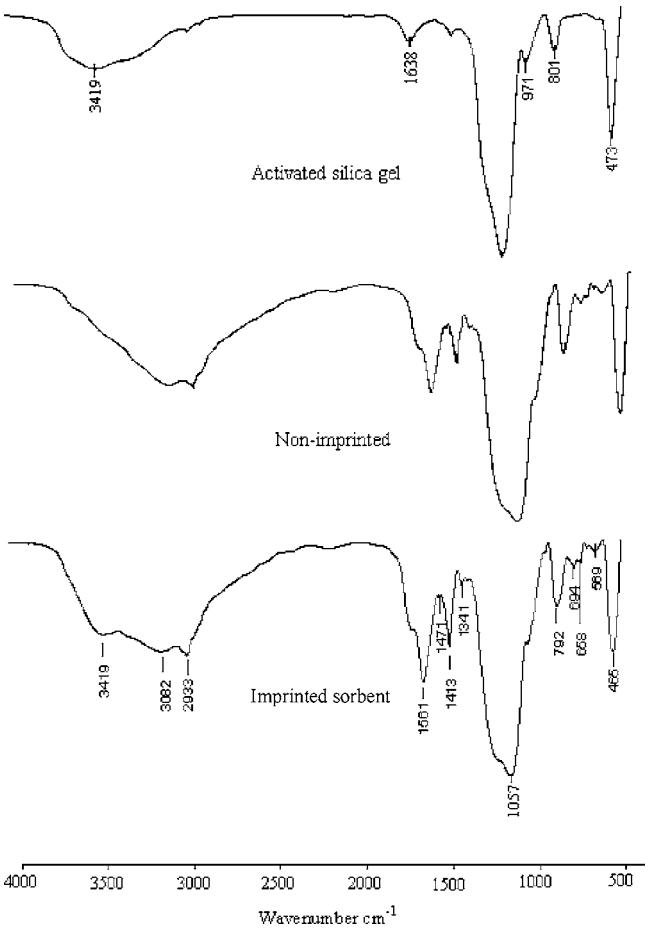

Figure 4. FT-IR spectra of the activated silica gel and non-imprinted and imprinted polymers.

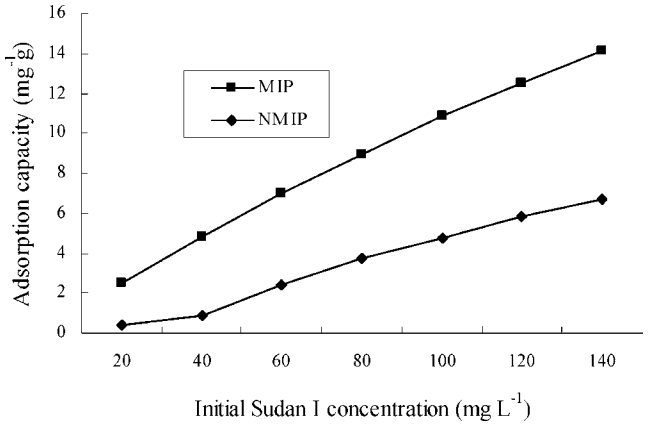

Figure 5. Adsorption isotherms of molecularly imprinted and non-imprinted polymers.

concentration. This indicated that the molecularly imprinted polymer exhibited a strong memory function for Sudan I, and the selective adsorption was obvious.

The Scatchard model was also used for evaluation of the adsorption of imprinted polymer, the equation being (*30*)

$$Q/C = Q/b + Q_{max}/b$$

where C is the initial concentration of the analytes in the solution, Q is the adsorption capacity at adsorption equilibrium, and Q_{max} is the saturated adsorption capacity. From the equation, Q/C versus Q is plotted in **Figure 6**.

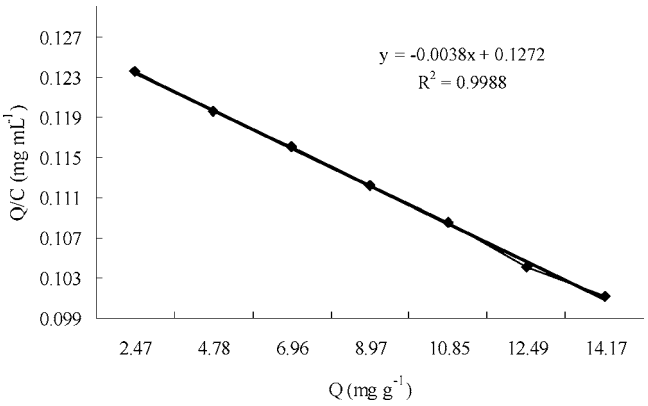

Figure 6. Linearized Scatchard plot of imprinted polymer.

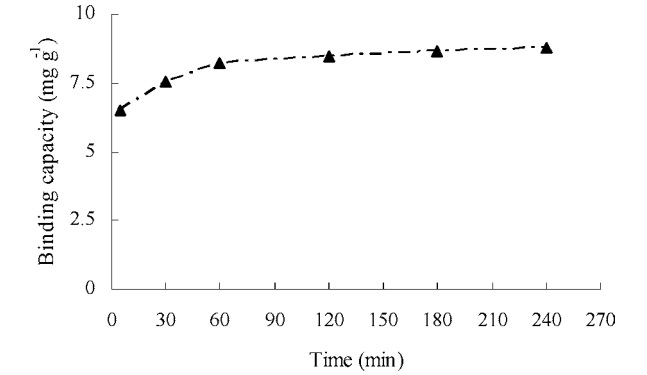

Figure 7. Kinetic uptake plot of imprinted polymer.

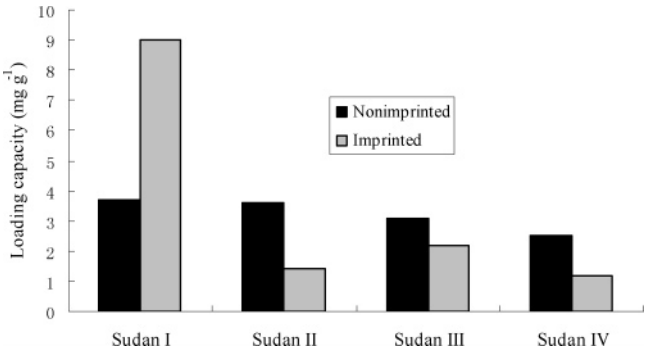

Figure 8. Selective adsorption of Sudan I–IV by the imprinted and non-imprinted polymers.

The results indicated that the adsorption isotherms of Sudan I imprinted polymer toward the template molecules were in good accordance, with linearity and a Scatchard model $R^2 = 0.9988$ in the experimental conditions. The saturated adsorption capacity (Q_{max}) of imprinted sorbent toward Sudan I was 33.47 mg g^{-1} obtained by the linear slope ($-b$). Results from Scatchard analysis also showed that the imprinted polymer had higher binding association constants and more apparent binding sites than non-imprinted sorbent, which demonstrated that the binding affinity of the imprinted polymer was from the specific sites formed by the imprinting effect.

Uptake kinetics of Sudan I by the imprinted functionalized silica gel polymer (80 mg L^{-1} Sudan I onto 20 mg of the imprinted polymer) was also examined and is shown in **Figure 7**. The results indicated that the imprinted polymer had fast uptake kinetics' 72.3% of binding was obtained within a short shaking period of 5 min, and the adsorption equilibrium was

Table 1. Competitive Loading of Sudan I–IV by Imprinted and Non-imprinted Polymer

	adsorption capacity (mg g^{-1})				k_d[a] (mL g^{-1})						
polymer	Sudan I	Sudan II	Sudan III	Sudan IV	Sudan I	Sudan II	Sudan III	Sudan IV	k_1/k_1'	k_2/k_2'	k_3/k_3'
imprinted	2.15	0.25	0.20	0.05	136.9	12.8	10.2	2.51	10.7/ 11.6	13.4/ 14.6	54.5/ 34.7
non-imprinted	0.55	0.60	0.60	0.35	29.1	31.9	31.9	18.1	0.92	0.92	1.57

[a] k_d, distribution coefficient; $k_d = \{(C_i - C_f)/C_f\} \times \{\text{volume of solution (mL)}\}/\{\text{mass of gel (g)}\}$, where C_i and C_f represent the initial and final concentrations. [b] k, selectivity coefficient; $k_1 = k_{\text{SudanI}}/k_{\text{SudanII}}$, $k_2 = k_{\text{SudanI}}/k_{\text{SudanIII}}$, $k_3 = k_{\text{SudanI}}/k_{\text{SudanIV}}$; k', relative selectivity coefficient, $k' = k_{\text{imprinted}}/k_{\text{nonimprinted}}$.

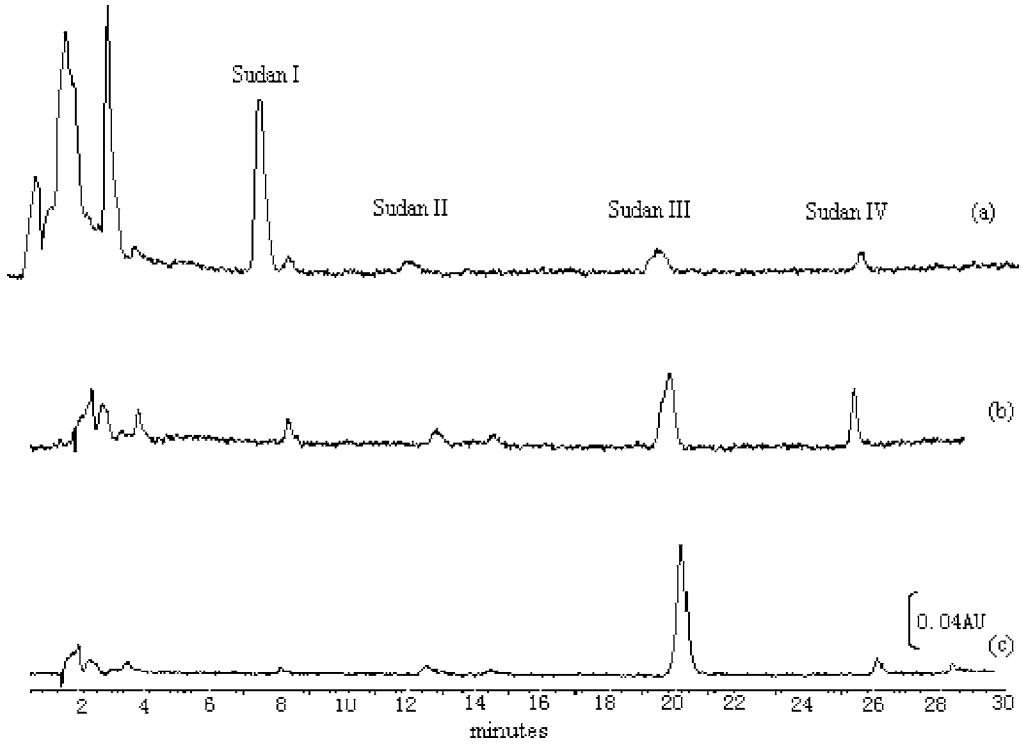

Figure 9. Chromatograms of 50 mL of standard mixture solution of 0.01 μg L^{-1} of Sudan I–IV with on-line solid-phase extraction preconcentration by (**a**) imprinted sorbent, (**b**) non-imprinted sorbent, and (**c**) activated silica.

almost reached within 60 min. If the concentration of Sudan I was lower, the time to saturation would become shorter. The rapid adsorption kinetics of the imprinted sorbent is an obvious advantage for its application in the on-line solid-phase extraction. This means that the surface imprinting greatly facilitates diffusion of the template to the binding sites.

Selective recognition property studies of imprinted polymer and non-imprinted polymer were performed with Sudan I and Sudan II−IV at 80 mg L^{-1}. Results (**Figure 8**) demonstrate that the adsorption capacity of imprinted polymer toward Sudan I was higher than the adsorption capacity toward Sudan II−IV. However, the adsorption capacities of non-imprinted polymer toward Sudan I−IV were almost same.

The competitive selective property of imprinted and non-imprinted polymer toward the mixture of Sudan I and Sudan II−IV at 20 mg L^{-1} was determined by HPLC. It could be evaluated by the distribution coefficient (k_d), selectivity coefficient (k), and relative selectivity coefficient (k') of static adsorption.

Table 1 summarizes the data for uptake capacity; k_d, k, and k' were obtained in the competitive binding experiments. The large k value of the imprinted polymer was an indication of its high selectivity for Sudan I over the related compounds. Comparison of the k values for the imprinted polymer with the corresponding non-imprinted polymer revealed a significant increase in k for Sudan I by imprinting. The k_1 (Sudan I/Sudan II) value of the imprinted polymer (10.7) was 11-fold that of non-imprinted polymer (0.92), whereas the k_2 (Sudan I/Sudan III) value of the imprinted polymer (13.4) was >14-fold that of the non-imprinted polymer (0.92) and the k_3 (Sudan I/Sudan IV) value of the imprinted polymer (54.5) was >34-fold that of the non-imprinted polymer (1.57). This might result from the imprinting effect, the difference of the molecular interactions, and their structures. During the preparation of the imprinted polymer, the template of Sudan I was incorporated into inorganic−organic networks. After removal of Sudan I, the imprinted cavities and specific binding sites of amino groups in a predetermined orientation was formed, whereas the non-imprinted polymer had no such imprinted cavities and specific binding sites. Results also indicated that k_1 and $k_{1'}$ were lower than k_2 and $k_{2'}$ and k_3 and $k_{3'}$, so the imprinted polymer had higher selectivity of Sudan II than of Sudan III and IV because the structure of Sudan II was more closely related to the structure of Sudan I than were those of Sudan III and IV (**Figure 1**).

Factors Affecting the Imprinted Sorbent on the Selective On-Line SPE-HPLC Determination of Sudan I. The applicability of the imprinted sol−gel sorbent for on-line SPE-HPLC determination of trace Sudan I was evaluated. The chemical and flow variables, such as sample acidity, sample loading flow rate, and loading and eluting times, were optimized

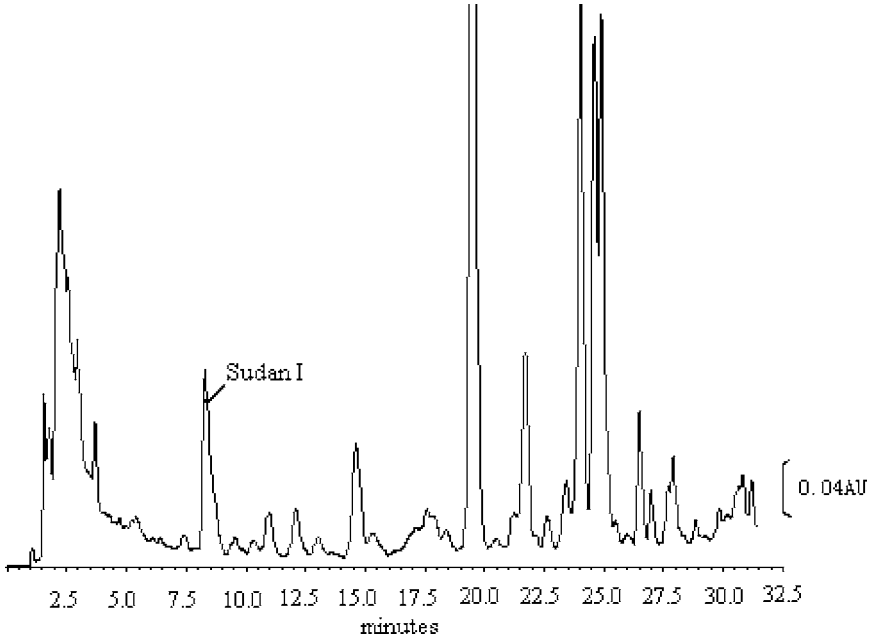

Figure 10. Chromatogram of on-line imprinted sorbent preconcentration coupled with HPLC for 50 mL of chilli powder sample spiked with 0.5 ng g^{-1} of Sudan I at a flow rate of 1.5 mL min^{-1}.

to achieve good sensitivity and precision for the extraction and elution of Sudan I.

The influence of sample pH on the on-line extraction of 0.01 μg L^{-1} Sudan I was tested in the pH range of 3.14−11.00 at a sample flow rate of 1.5 mL min^{-1} for 33 min. The results showed that the maximum chromatographic peak of Sudan I was achieved in the pH range of 5.4−9.0. Outside the optimum pH range, the chromatographic peak area of Sudan I decreased. Therefore, Sudan I could be effectively adsorbed by the imprinted sorbent-packed column in the pH range of 5.4−9.0.

The effect of sample loading flow rate on the on-line solid-phase extraction of 0.01 μg L^{-1} Sudan I was studied. It was found that the chromatographic peak area of Sudan I increased almost linearly as the sample loading flow rate decreased from 1.8 to 0.4 mL min^{-1} for sampling 50 mL, indicating that the kinetics for the adsorption of Sudan I by the imprinted sol−gel sorbent was very fast. The influence of sample loading time on the adsorption of Sudan I was investigated with 0.01 μg L^{-1} Sudan I at a sample flow rate of 1.5 mL min^{-1}. The chromatographic peak areas increased almost linearly as the sample loading time increased up to at least 33 min. Therefore, on the basis of the above results, a 1.5 mL min^{-1} flow rate and 33 min of sample loading time were chosen as the experimental conditions in the following study.

The eluting time required for quantitative desorption of the adsorbed Sudan I when the HPLC injector valve was in the inject position was evaluated to ascertain when the HPLC injector valve should turn to the load position for the next on-line solid-phase extraction during the HPLC separation of the analytes in this cycle. By studying the effect of different desorption times employing mobile phase as eluent, we found that the chromatographic peak of Sudan I increased remarkably as desorption time increased from 0 to 0.5 min, increased slightly as the desorption time increased from 0.5 to 0.8 min, and then leveled off in the range of 0.8−1.5 min. Accordingly, the desorption time of 1.0 min was selected to ensure the complete stripping of the adsorbed Sudan I from the imprinted amino-functionalized silica gel-packed column. Once the adsorbed Sudan I was quantitatively stripped from the sorbent by mobile phase, the HPLC injector valve turned to the load position for the next preconcentration so that the current HPLC separation and the next preconcentration proceeded in parallel. These results showed that Sudan I could be separated completely when the desorption time was fixed on 1.0 min and that the dynamics for the adsorption and desorption of Sudan I by the imprinted sorbent was very fast, making this method suitable for the fast determination of Sudan I in foods. The application lifetime of this SPE column was measured, the results showing that it can be used more than 100 times.

Table 2. Merit of On-line Solid-Phase Extraction by Coupling with HPLC for Trace Determination of Sudan I

enrichment factors	1266
determination limit (S/N = 3) (ng L^{-1})	1.2
peak area precision[a] (n = 5) (%, RSD)	3.66
linear range of the calibration graph (μg L^{-1})	0.005–50
sample consumption (mL)	50

[a] For 0.01 μg L^{-1} Sudan I.

Application and Merit of the Present On-Line SPE-HPLC Using the Developed Imprinted Functionalized Silica Gel Sorbent. The selective adsorptions of Sudan I by the imprinted sorbent, non-imprinted sorbent, and activated silica gel for on-line SPE-HPLC were tested by passing 50 mL of standard aqueous solution containing 0.01 μg L^{-1} Sudan I−IV at a sample flow rate of 1.5 mL min^{-1} (**Figure 9**). Only Sudan I obviously appeared in the chromatogram (**Figure 9a**) after eluting by mobile phase; however, the peaks of Sudan I in chromatograms **b** and **c** were very low, indicating that Sudan I was selectively extracted onto the imprinted sorbent and the selectivity of imprinted sorbent for Sudan I was very high.

The analytical figures of merit for the present on-line solid-phase extraction using the imprinted functionalized silica gel sorbent coupled with HPLC for the determination of trace Sudan I were evaluated under optimal experimental conditions (**Table 2**). With a sample loading flow rate of 1.5 mL min^{-1} for a 33 min extraction, the enrichment factor obtained by the slopes of the linear portion in comparison with the direct injection of 20

Table 3. Recoveries of Sudan I in 2.0000 g of Chilli Powder (Mean ± RSD, $n = 3$)

chilli powder sample	spiked level (ng g^{-1})	recovery of Sudan I (%)
1	0.25	80.31 ± 1.52
2	0.50	87.50 ± 2.71
3	1.00	94.02 ± 1.20

μL standard sample solution was 1266. The detection limit (S/N = 3) was 1.2 ng L^{-1}, the RSD for five rplicate extractions of 0.01 μg L^{-1} Sudan I was 3.66%, and the linear range of the calibration graph was 0.005−50 μg L^{-1}.

To evaluate the usefulness and merit of the imprinted sorbent for on-line SPE-HPLC, chilli powder, which was spiked Sudan I at three levels (0.25, 0.5, and 1.0 ng g^{-1}), from a local market was analyzed. Typical chromatograms of real samples are shown in **Figure 10**. At each concentration, three measurements were performed. The analytical data are shown in **Table 3** , and recoveries ranged from 80.31 to 94.02%.

Conclusion. In this study, a simple molecular imprinting procedure was adopted to synthesize a highly selective Sudan I-imprinted amino-functionalized silica gel sorbent by combining a surface molecular imprinting technique with a sol−gel process. The prepared imprinted material showed good characteristics, such as fast adsorption−desorption dynamics and high affinity and selectivity for Sudan I, making the imprinted polymer very suitable as sorbent for determination of trace Sudan I by the on-line SPE-HPLC. This analytical method can be readily built in the laboratory, and its widespread use will be recommended as a methodology for monitoring the illegal addition of Sudan I in foods.

ABBREVIATIONS USED

HPLC, high-performance liquid chromatography; SPE-HPLC, solid-phase extraction coupled with liquid chromatography; UV, ultraviolet−visible; LC, liquid chromatography; MS, mass spectrometry; Q_{max}, saturated binding capacity; Q, binding capacity; RSD, peak area precision; TEOS, tetraethoxysilicane; APTES, 3-aminopropyltriethoxysilane; DDW, doubly deionized water; SPE, solid-phase extraction; MIP, molecularly imprinted polymer; NMIP, nonmolecularly imprinted polymer.

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