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Molecularly imprinted matrix solid-phase dispersion combined with dispersive liquid–liquid microextraction for the determination of four Sudan dyes in egg yolk

Hongyuan Yan*, Hui Wang, Jindong Qiao, Gengliang Yang

Key Laboratory of Pharmaceutical Quality Control of Hebei Province & College of Pharmacy, Hebei University, Baoding 071002, China

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ABSTRACT

A new kind of aniline–naphthol molecularly imprinted microsphere (MIM) synthesized by aqueous suspension polymerization was applied as a selective sorbent of miniaturized matrix solid-phase dispersion combining with dispersive liquid–liquid microextraction (MSPD–DLLME) for the simultaneous determination of four Sudans in egg yolk samples. The solid sample was directly blended with MIM in MSPD procedure and the eluent of MSPD was used as the dispersive solvent of the followed DLLME for further purification and enrichment of the analytes before HPLC analysis. Good linearity for all the Sudan dyes was ranged from 0.02 $\mu g\,g^{-1}$ to 2.0 $\mu g\,g^{-1}$ ($r^2 \geq 0.9990$) and their recoveries at three spiked levels were ranged from 87.2% to 103.5% with RSD less than 6.1% ($n = 3$). The presented MIM–MSPD–DLLME method combined the advantages of MIM, MSPD and DLLME, and could be applied for the determination of Sudans in complicated food samples.

1. Introduction

Sudan dyes (Sudan I, II, III and IV) are phenyl-azoic derivatives and extensively used as coloring matters in many chemical industries and daily products. Now that they are categorized as class 3 carcinogens and may lead to genotoxic carcinogen and mutagen for human [1–3], Sudan dyes used in foodstuffs are forbidden worldwide according to both the Food Standards Agency (FSA) and the European Union (EU). However, because Sudan dyes can improve the appearance of natural hues, they are still illegally utilized as additives in foods by many merchants, particularly in chilli powders, relishes, chutneys, eggs and ready meals. Therefore, a simple, accurate and practicable method for the identification and quantification of such compounds in foodstuffs is still desired.

Until now, the common methods for the determination of Sudan dyes are mainly liquid chromatography (LC) [4,5], liquid chromatography–mass spectrometry (LC–MS) [6], voltammetry [7,8], electrophoresis [9], chemiluminescence analysis [10] and immunoanalysis [11,12]. Owing to the complexity of sample matrices and low levels of analytes, sample pretreatment and enrichment process become the crucial steps in the analytical procedures. So far, the most widely used sample pretreatment methods are liquid–liquid extraction [13], solid-phase extraction (SPE) [14,15], liquid-phase microextraction [16], cloud point extraction [17], ionic liquids extraction [18] and stir bars microextraction [19]. Although each method has its advantages, most of these procedures suffer from several disadvantages such as large amounts of organic solvent, tedious procedure, or low enrichment factor. Liquid phase microextraction based on hollow fiber technique needed only several microliter organic solvent, but it suffered from relatively low recoveries and poor repeatability, as well as the stir bars microextraction. Recently, Assadi et al. developed a new extraction technique termed as dispersive liquid–liquid microextraction (DLLME) [20,21]. In this method, the appropriate mixture of extraction solvent and dispersive solvent was injected rapidly into an aqueous solution, resulting in a cloudy state consisting of fine droplets of the extraction solvent dispersed in the aqueous phase, which markedly increased the contact surface between phases and reduced extraction time with the increasing enrichment factors [22–24]. The advantages of DLLME were simplicity, rapidity, low cost and high enrichment factors [25–27]. However, all the above methods could not be directly applied for semi-solid and solid samples which must be pretreated into solution to adapt those extraction procedures.

Matrix solid-phase dispersion (MSPD) was one of the most promising techniques for the simultaneous disruption, extraction and clean-up of solid, semi-solid and highly viscous samples [28,29]. It eliminated most of the complications of performing classical LLE and SPE for solid matrixes by direct mechanical blending of sample matrix with an appropriate sorbent and a small

* Corresponding author. Tel.: +86 312 5971107; fax: +86 312 5971107.
E-mail address: yanhy@hbu.edu.cn (H. Yan).

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volume of solvent for washing and elution steps [30,31]. Among them, the sorbent acted both as an abrasive material disrupting sample architecture and as a 'bound' solvent that assisted accomplishing extraction [32]. However, due to the lack of special selectivity of the common sorbents (C_{18}, C_8, silica gel, florisil, alumina, etc.), MSPD was confronted with difficulty of extracting target analytes from complex samples. Therefore, further improving the selectivity of the pretreatment procedures was desired.

Molecular imprinting was an attractive technique for the synthesis of functional polymers having specific molecular recognition properties for a given compound, its analogues, or for a single enantiomer [33–35]. Due to the high selectivity and stability of molecularly imprinted polymers (MIPs), it had been used as a new selective sorbent in SPE and MSPD for extracting organic compounds from complex materials [36]. The application of MIPs allowed the interest analyte to be pre-concentrated and simultaneously removed the interference compounds from the matrix so that selective enrichment and cleanup were achieved, which would lead to a higher accuracy and lower detection limit in the subsequent analysis. In recent years, the MIPs prepared by using one kind of Sudan dyes (most frequently Sudan I) as template had been applied as special sorbents to extract Sudan dyes from food samples [37–39]. However, template leaking was always observed in the actual applications, which affected the results of quantitative analysis.

This work represents the first attempt of using molecularly imprinted microsphere (MIM) as MSPD sorbent to develop a new MIM–MSPD–DLLME–HPLC method for selective extraction and determination of four Sudan dyes in egg yolk samples. The novel MIM synthesized by aqueous suspension polymerization using aniline–naphthol as dummy template showed high affinity to four Sudans and successfully applied as a special sorbent of MSPD–DLLME to improve the selectivity of the pretreatment procedure. The MIM–MSPD–DLLME–HPLC method combined the high selectivity of MIM, excellent clarification of MSPD for complex solid samples and the high enrichment factor coupled with farther purification of DLLME technique, and could be potentially applied for the determination of Sudan dyes in complicated food samples.

2. Experimental

2.1. Chemicals

Sudan I, II, III and IV were obtained from Fuchen Chemical Co. Ltd. (Tianjin, China). Aniline, 2-naphthol, polyvinylpyrrolidone (PVP), tetrachloroethylene, chlorobenzene, chloroform, tetrachloroethane, dichloromethane, dichloroethane, and hexane were obtained from Huaxin Chemical Co. (Baoding, China). Methacryclic acid (MAA), methanol, acetonitrile, ethanol, acetone, acetic acid, and 2,2-azobisisobutyronitrile (AIBN) were purchased from Kermel Chemical Co. Ltd. (Tianjin, China). Ethylene glycoldimethacrylate (EGDMA) was purchased from Sigma–Aldrich (St. Louis, MO, USA). All the other reagents used in the experiment were of the highest grade commercially available. Double deionized water was filtered with 0.45 μm filter membrane before use.

2.2. Instrumentation and conditions

HPLC analysis was performed using a Shimadzu HPLC system equipped with two LC-20AT Solvent Delivery Units, a SUS-20A gradient controller, and a SPD-20A Detector (Shimadzu, Kyoto, Japan). An N-2000 chromatographic workstation (Zheda Zhineng Co. Ltd., Hangzhou, China) was used as a data acquisition system. A 0406-1 centrifuge was obtained from Medical Devices Co. Ltd. (Shanghai, China). The analytical column was purchased from RStech Co. (C_{18}, 5 μm, 150 mm × 4.6 mm I.D., Daejeon, Korea). The mobile phase was methanol–water (98:2, v/v, containing 0.1% methanoic acid) and its flow rate was set at 1.0 mL min^{-1}. The injection volume was 10 μL for all the solutions and the wavelength of UV detector was set at 475 nm.

2.3. Synthesis of the imprinted microspheres

The MIMs were prepared by aqueous suspension polymerization as follows. (I) 3.0 g of polyvinylpyrrolidone was dissolved into 120 mL of water. The solution was poured into a 250 mL flanged reactor flask in a water bath (60 °C) and then was stirred at 500 rpm under a gentle stream (about 60 bubbles per minute). (II) Aniline (0.18 mL), 2-naphthol (0.29 g), MAA (0.5 mL), EGDMA (9.4 mL) and AIBN (200 mg) were dissolved in 20 mL chloroform and sonicated for 5 min to make them fully dissolved. The solution (II) was added dropwise to solution (I). After 24 h polymerization, the solution was filtered and the MIM was washed with methanol–acetic acid (9:1, v/v) to remove the template and monomers. Non-imprinted microsphere (NIM) and Sudan I imprinted-MIM (using Sudan I as template) were prepared and treated in an identical manner.

2.4. Procedure of MIM–MSPD–DLLME

The schematic procedure of the MIM–MSPD–DLLME was shown in Fig. 1. The miniaturized MSPD procedure was achieved by using small amount of sample and proportionately less support or solvent. An aliquot of 0.1 g of the egg yolk sample and 0.2 g of MIM sorbent were placed in a small glass beaker and blended together using a glass bar to obtain complete disruption and dispersion of the sample on the solid support. The homogenized mixture was transferred into an empty cartridge (5 cm × 8 mm I.D., which pre-packed with 50 mg of MIM) and rinsed with 4.0 mL of methanol–water (1:1, v/v), then eluted with 3.0 mL of acetone–acetic acid (95:5, v/v). The eluent collected in a 10 mL conic tube was evaporated to 1.0 mL and mixed with 100 μL of tetrachloroethylene and 5.0 mL of water for further purification and concentration of the analytes by DLLME. The mixture solution was gently shaken and ultrasonicated to form a homogeneous cloudy solution and then the phase separation was performed by a centrifugation at 4000 rpm for 10 min. The sediment phase was evaporated to dryness and re-dissolved in 50 μL of mobile phase for further HPLC analysis.

3. Results and discussion

3.1. Preparation of the imprinted microspheres

Until now, several MIPs had been synthesized by using one kind of Sudan dyes (usually Sudan I) as template and applied as SPE sorbents to extract Sudans from food samples, however, they always suffered from template leaking in real sample application which affected the results of quantitative analysis. Therefore, in order to avoid the effect of template leakage and obtain the imprinted microspheres with special recognition ability to the four Sudan dyes, dummy template was adopted to synthesize MIM. Considering that the Sudan dyes are diazo compounds with the basic structures of benzene, naphthol and azo group, aniline coupled with 2-naphthol was used as the dummy template to synthesize the MIM by aqueous suspension polymerization with MAA as monomer, EGDMA as cross-linker, PVP as dispersant and chloroform as porogen solvent. Due to the imprinting effect of MIM was mainly affected by the molar ratio of template/monomer/cross-linker, different ratios ranged from 1:3:25 to 1:10:40 were investigated and the results revealed that MIM prepared at the molar ratio of 1:4:25 showed satisfactory mechanical strength and special affinity to the four Sudan dyes

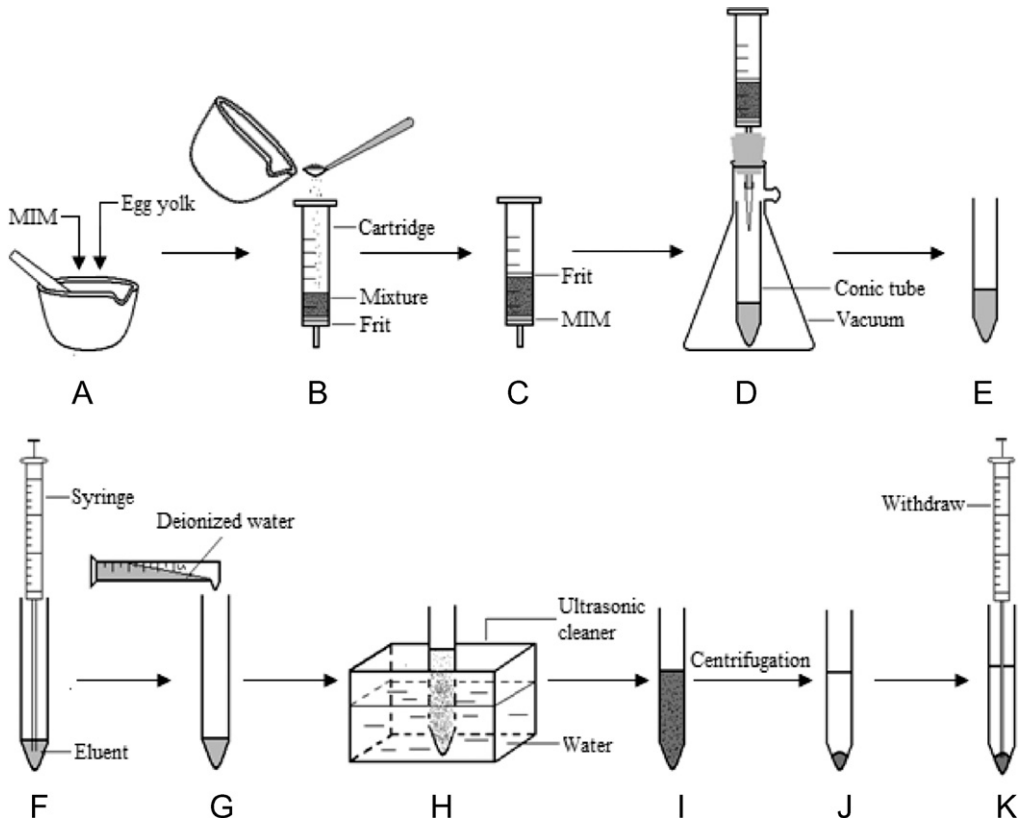


Fig. 1. Schematic procedure of MIM–MSPD–DLLME. (A) Sample-MIM sorbent blending; (B) transfer blend to column; (C) completed MSPD column; (D) washing and elution with a proper solvent using a vacuum pump; (E) eluent to be evaporated; (F) injection of extractant into eluent; (G) injection of deionized water into extractant/dispersant mixture; (H) formation of emulsion with the assistance of ultrasonic cleaner; (I) emulsion of ternary mixture; (J) phase separation by centrifugation; (K) collection of high-density extractant.

(Fig. 2). The volume of porogen solvent also had effect on both the solubility of template–monomer mixture and the morphology of polymers that the small volume of porogen would result in low mechanical strength and large one lead to higher surface area and larger pore size of the MIM. Considering that, 20 mL of chloroform was chosen as the porogen solvent. Moreover, to further improve the recognition of the obtained MIM in aqueous samples, the MIM was prepared using PVP as dispersant in 120 mL of water, which exhibited good mechanical strength and special affinity to analytes.

The adsorption capacity evaluated by dynamic adsorption showed that the adsorption capacity of MIM or NIM towards target analytes increased with the increasing initial concentration. And under the same experimental condition, the MIM offered a higher affinity to the four Sudan dyes than NIM (Fig. 3). Moreover, the MIM showed stronger affinity towards the putative templates (aniline and 2-naphthol) and the four Sudan dyes than the other analytes with the similar hydrophobicity but structurally unrelated to templates, which demonstrated that the binding affinity of the MIM was mainly from the specific sites formed by the imprinting effect.

3.2. Optimization of the MIM–MSPD procedures

One of the outstanding advantages of MSPD is that extraction and clean-up are carried out just in a single step. In MSPD, the sorbent acts both as an abrasive and as a bound solvent that breaks the sample architecture and disperses sample components and further promotes more effective interactions between it and the analytes. In this work, a miniaturized MSPD using smaller sample size and proportionately less dummy-template imprinted MIM sorbent was adopted to reduce solvent consumption and simultaneously avoided the effect of template leaking on quantitative analysis. To optimize the process of miniaturized MIM–MSPD, the main parameters that affected washing and elution steps were investigated based on 0.15 g MIM and 0.10 g eggs yolk sample.

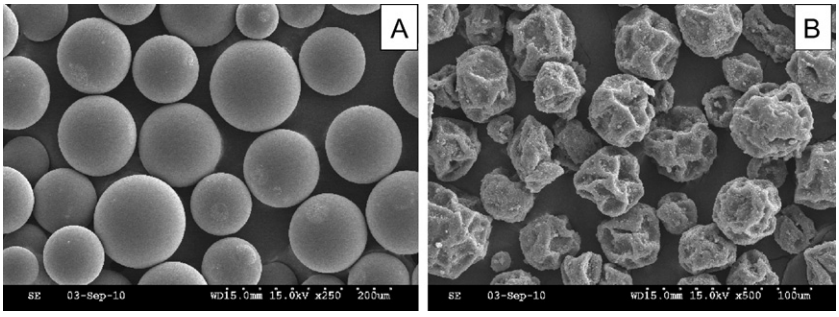


Fig. 2. Scanning electron micrograph of MIMs (ratios of template, monomer and cross-linker: (A) 1:4:25 and (B) 1:8:40).

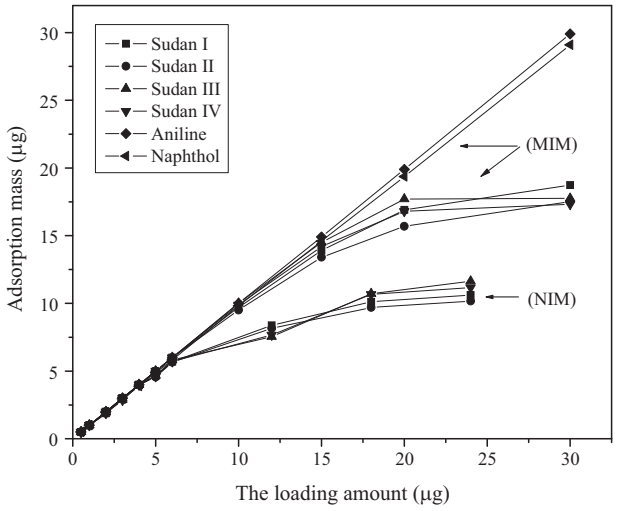


Fig. 3. The adsorption capacity of MIM and NIM.

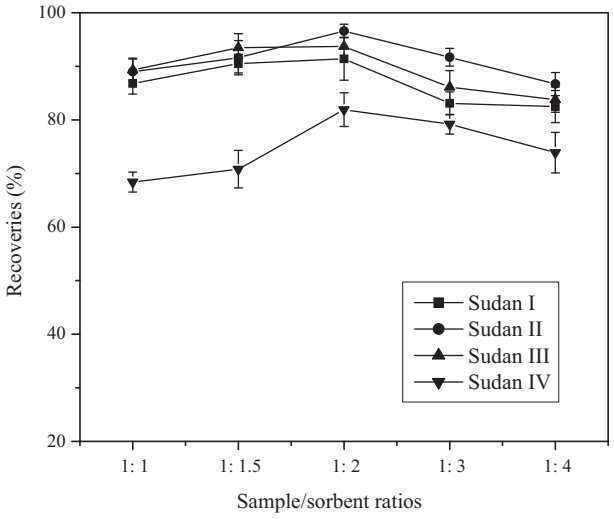
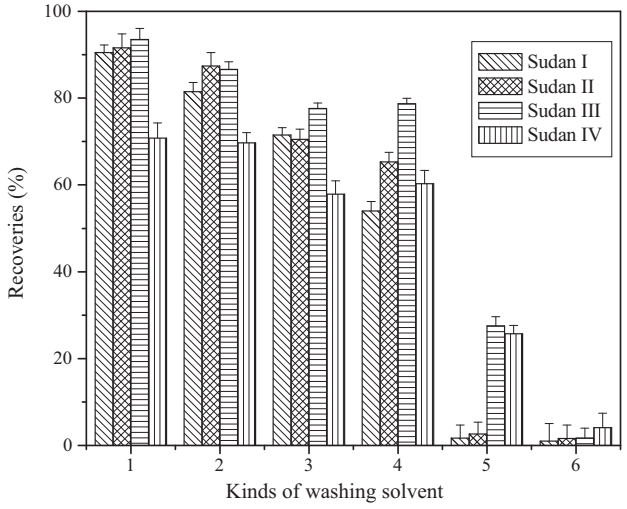


Fig. 5. Effect of sample/sorbent ratios on the recovery of Sudans.

For the washing step, methanol–water (1:1, 2:8, v/v), methanol, acetonitrile–water (1:1, 3:7, v/v) and acetonitrile as washing solutions were compared and the results in Fig. 4 showed that the best recoveries were obtained using methanol–water (1:1, v/v). For the purpose of minimum volume of washing solution able to efficiently rinse the interferences, different volumes of methanol–water (1:1, v/v) ranged from 1.0 to 5.0 mL were investigated and 4.0 mL was found to be the optimum washing volume.

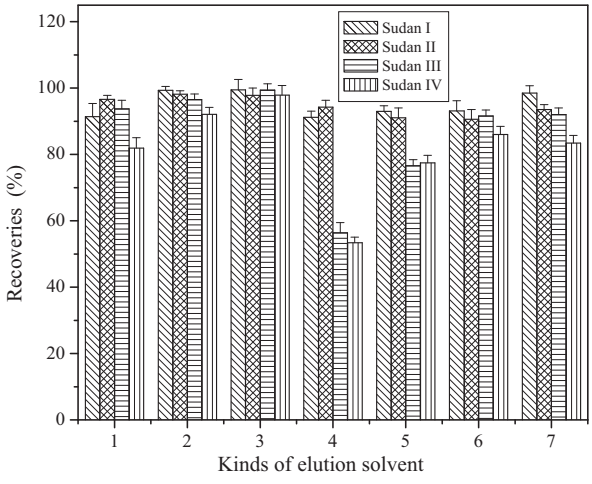
A suitable sample/sorbent ratio could increase the interface area between the analytes and sorbent, and allowed complete adsorption of the sample components to facilitate their transfer into sorbent. Therefore, the ratios of sample/sorbent ranged from 1:1 to 1:4 were evaluated and the results revealed that the ratio of 1:2 provided the best recoveries of Sudans (Fig. 5). Further increasing the proportion of sorbent reduced the recoveries of Sudan dyes because of the strong absorbability of MIM. Moreover, the ratios of sample/sorbent lower than 1:2 led to decreased recoveries and maximized errors which probably generated by the fact that the cartridge packing material was not as homogeneous as required due to the relatively large content of samples. Thereby, 1:2 was applied as the optimized sample/sorbent ratio in the subsequent studies. Moreover, the MIM pre-packed in the bottom of the cartridge acted as MSPD sorbent to further remove interfering matrix components and isolate analytes to perform high recoveries.

The nature of elution solvent was important since the target analytes should be efficiently desorbed while the remaining matrix components should be retained on the cartridge. In this case, a variety of solvents including the mixtures of acetone, acetonitrile, methnaol, ethnaol, dichloromethane, ethyl acetate with acetic acid as elution solvents were evaluated and the results were shown in Fig. 6. Although the best recoveries for four Sudans were obtained using acetonitrile–acetic acid (95:5, v/v) as eluting solvent, the chromatograms of the eluents revealed that interferences were eluted out simultaneously. At the same time, acetone–acetic acid (95:5, v/v) was the alternative elution solvent with cleaner eluents and acceptable recoveries of Sudans. Therefore, different volumes of acetone–acetic acid (1.0, 2.0, 3.0, 4.0 and 5.0 mL) were evaluated and the results revealed that the recoveries of Sudan dyes increased with the increase of elution volume from 1.0 to 3.0 mL and then retained constant even further increasing the volume to 5.0 mL (Fig. 7). Considering the elution efficiency and solvent consump-



(1: Methanol-water(1:1, v/v); 2: Methanol-water(2:8, v/v);
3:Acetonitrile-water(3:7, v/v); 4: Acetonitrile-water(1:1, v/v);
5: Methanol; 6: Acetonitrile)

Fig. 4. Effect of washing solvents on the recovery of Sudans ($n = 3$).



(1: Acetone; 2: Acetone-acetic acid(5%); 3: Acetonitrile-acetic acid(5%)
4: Methanol-acetic acid(5%); 5: Ethanol-acetic acid(5%);
6: Dichloromethane-acetic acid(5%); 7: Ethyl acetate-acetic acid(5%))

Fig. 6. Effect of eluting solvents on the recovery of Sudans.

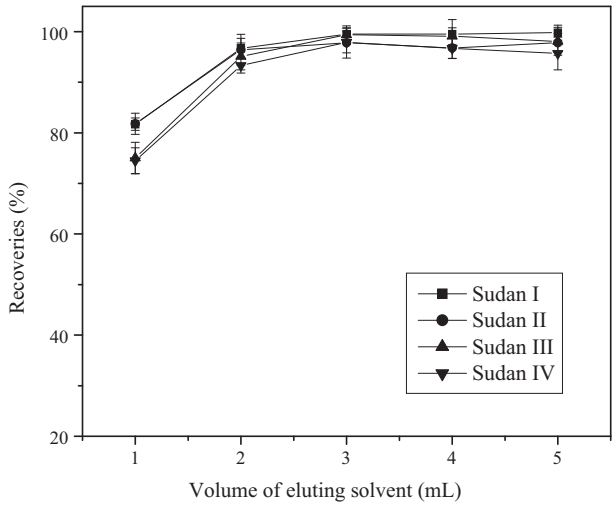
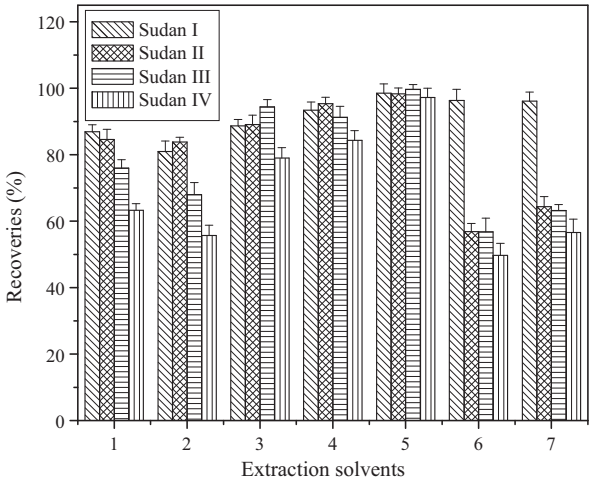


Fig. 7. Effect of the volume of eluting solvent on recovery of Sudans.

tion, 3.0 mL was used as the optimum volume of elution solvent, which was far below the common volume of elution solvent used in ordinary MSPD procedure.

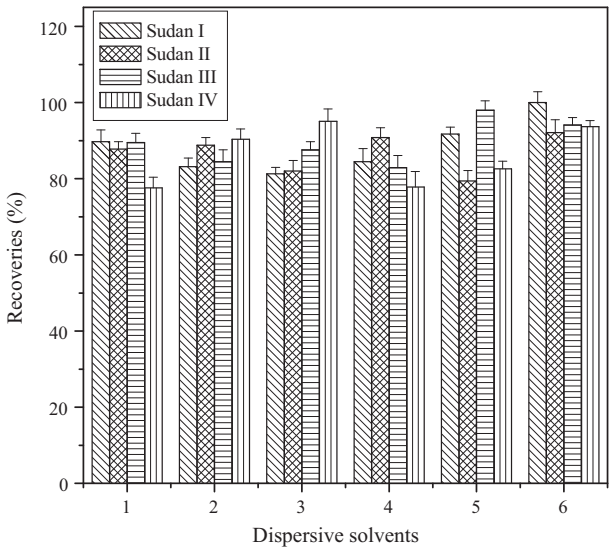
3.3. Optimization of the DLLME procedures

According to the principles of DLLME, the kind of the extraction solvent is an essential consideration, which influences the extraction efficiency greatly. In this work, for further purifying and concentrating the analytes from the eluents of MIM–MSPD, the extraction solvent of the followed DLLME should be selected carefully on the basis of high density, low solubility in water and the high extraction capability to target analytes. Therefore, seven organic solvents (dichlormethane, chloroform, tetrachloroethane, tetrachloroethylene, chlorobenzene, tetrachloromethane, dichloroethane) as extraction solvents were evaluated by applying 100.0 μL of each extraction solvent to the DLLME procedure. The results of Fig. 8 indicated that the best recoveries were achieved by using tetrachloroethylene as extraction solvent. In order to investigate the effect of extraction solvent volume on extraction efficiency, a series of volumes (50, 70, 90, 100, 130, 150 μL) of tetrachloroethylene were investigated and



(1. Dichlormethane 2. Chloroform 3. Dichloroethane 4. Tetrachloroethane 5. Tetrachloroethylene 6. Chlorobenzene 7. Tetrachloromethane)

Fig. 8. Effect of extraction solvents on the recovery of Sudans.



(1: Acetonitrile; 2: Acetonitrile-acetic acid (95:5, v/v); 3: Methanol; 4: Methanol-acetic acid (95:5, v/v); 5: Acetone; 6: Acetone-acetic acid (95:5, v/v))

Fig. 9. Effect of dispersive solvents on the recovery of Sudans.

the results showed that the recoveries of Sudan dyes increased markedly with the increasing volume of tetrachloroethylene from 50 to 100 μL. However, the recoveries of Sudan dyes were almost constant when further increased the volume of tetrachloroethylene to 150 μL, which was due to the completed extraction equilibrium. Therefore, 100 μL of tetrachloroethylene was used as extraction solvent for further works.

For DLLME method, the main criterion for the selection of the dispersive solvent was its miscibility with the organic extraction solvent and the aqueous phase. The addition of dispersive solvent was a crucial step which could disperse the extraction solvent to fine droplets into the aqueous phase and immensely increase the contact surface area for transferring target compounds from sample matrix to extraction solvent. In this MIM–MSPD–DLLME method, an appropriate dispersive solvent of DLLME should also severed as the eluting solvent in MSPD procedure for convenient combination of these two procedures. Therefore, acetonitrile, methanol, acetone and its mixture with acetic acid as dispersive solvents were investigated. According to the results in Fig. 9, acetone–acetic acid (95:5, v/v) was selected as the dispersive solvent of DLLME, which demonstrated the convenient of the MIM–MSPD–DLLME method.

3.4. Features of the MIM–MSPD–DLLME–HPLC method

The developed MIM–MSPD–DLLME–HPLC method was evaluated on the linearity, precision, repeatability, recovery, detection limits, inter-assay and intra-assay deviation under the optimized condition. Calibration curves were constructed using the areas of the chromatographic peaks measured at nine increasing spiked levels, in a range of 0.02–2.0 μg g^{-1}. As shown in Table 1, good linearities were obtained throughout the concentration range for

Table 1
Features of the MIM–MSPD–DLLME–HPLC method.

Analytes	Linear equation	r^2	LOD (ng g^{-1})	RSD (%)
Sudan I	$Y = 8.35 \times 10^1 X + 1.98 \times 10^3$	0.9994	2.4	3.7
Sudan II	$Y = 1.00 \times 10^2 X + 6.64 \times 10^2$	0.9999	2.3	2.9
Sudan III	$Y = 8.01 \times 10^1 X + 1.36 \times 10^3$	0.9990	3.1	3.1
Sudan IV	$Y = 4.94 \times 10^1 X - 1.25 \times 10^2$	0.9991	6.1	6.7

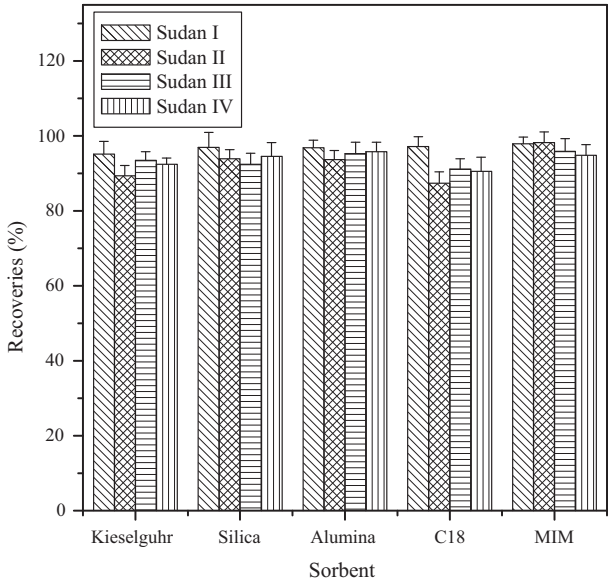


Fig. 10. Comparison of MIM with other sorbents.

the four Sudan dyes with the correlation coefficient (r^2) ≥ 0.9990, and the relative standard deviations (RSDs) was evaluated by performing replicate analyzing the middle spiked level of 0.2 μg g^{-1}. Accuracy and precision of the MIM–MSPD–DLLME–HPLC method were assessed by performing replicate analyses of the spiked samples in five replicates in the same day and consecutive 3 days. The intra-day precision and accuracy of the method evaluated as RSD were ranged from 2.1% to 4.6% and the inter-day reproducibility was less than 7.2%. The limits of detection (LODs) based on signal to noise of 3 were ranged from 2.3 to 6.1 ng g^{-1}. Under the optimum condition, comparing with the direct injection of eluent collected from MSPD procedure, the enrichment factors for Sudan I–IV were 55, 58, 59 and 58 folds, respectively. Moreover, the eluents were cleaner after DLLME, which demonstrated the obvious purification and enrichment of MSPD–DLLME procedure.

To compare the extraction efficiency of MIM with other conventional sorbents, alumina, C_{18}, silica and kieselguhr were also employed in the MSPD procedures according to the previous reports [40–42]. Fig. 10 shows that the recoveries of analytes were all above 80% for the five sorbents under their respective optimized condition. Among them, the highest recoveries (94.9–98.3%) were obtained by MIM. Additionally, Fig. 11 showed that MIM exhibited cleaner chromatograms without interferences than alumina as sorbent (the most commonly used sorbent for Sudans), which intuitively demonstrated the high selectivity and affinity of the MIM towards the target analytes. Additionally, Sudan I imprinted-MIM as sorbents was also evaluated and the obvious template leaking was observed in the MSPD procedure even after washing with huge amounts of organic agent. Therefore, MIM using dummy template is a suitable way to provide the selective sorbents and avoid the effect of template leakage on quantitative analysis.

Moreover, the comparison of the MIM–MSPD–DLLME–HPLC method with other reported methods for determination of Sudan dyes was shown in Table 2. Under the detection system of HPLC–UV, the MIM–MSPD–DLLME procedure provided higher sensitivity than other sensitive detection methods. Moreover, the presented method only needed small sample amount (0.1 g) and little consumption of organic solvent. The potential interferences of egg yolks were also investigated by extracting and analyzing five blank egg yolk samples. No interfering peaks from the sample matrix were observed at the retention times of compounds of interest, which demonstrated the good practicability of the proposed MIM–MSPD–DLLME–HPLC method.

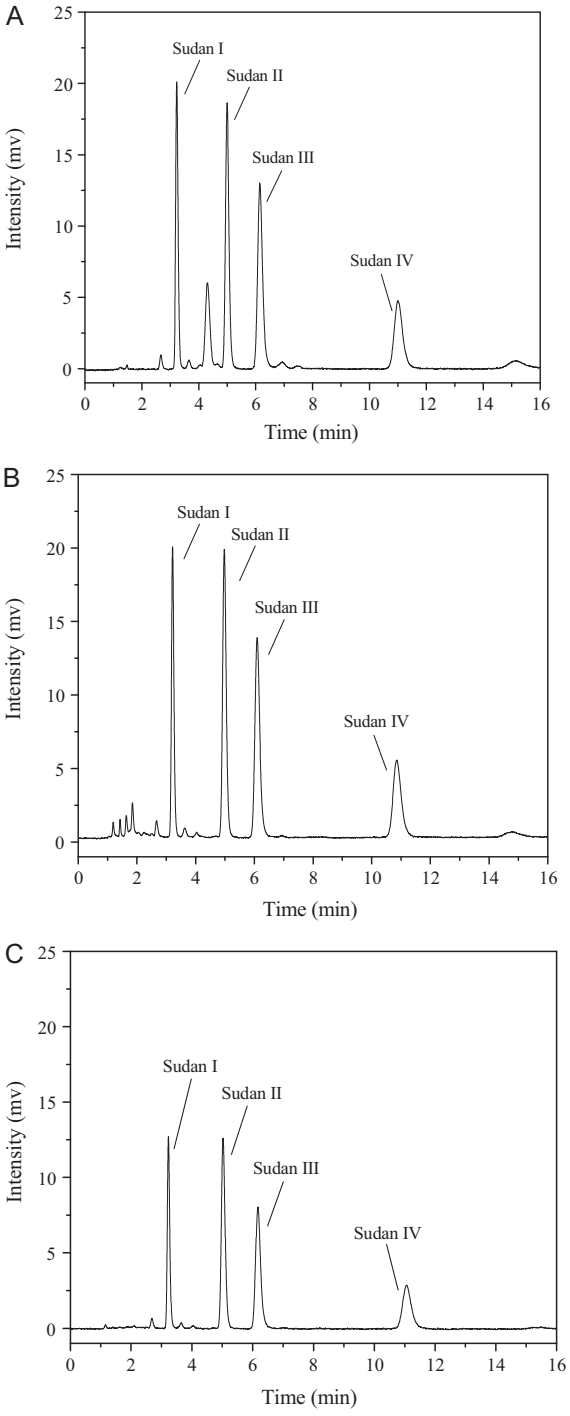


Fig. 11. Chromatograms of the spiked egg yolk samples ((A) Alumina-MSPD; (B) MIM–MSPD; and (C) MIM–MSPD–DLLME; spiked concentration: (A) and (B): 20.0 μg g^{-1}, the eluent was evaporated to dryness and re-dissolved in 1.0 mL of mobile phase; (C) 0.5 μg g^{-1}, the sediment phase was evaporated to dryness and re-dissolved in 50 μL of mobile phase; injection volume: 10.0 μL).

3.5. Analysis of egg yolk samples

In order to validate the MIM–MSPD–DLLME–HPLC method, fifteen egg samples collected from the local markets of Baoding were pretreated under the optimized condition. No residuals of Sudans were observed in all samples, which demonstrated that the mis-

Table 2
Comparison of merits of methods for determination of Sudan I–IV.

Matrix/mass of sample (g)	Sample preparation/volume of solvent (mL)	LOD (ng g^{-1}) I, II, III, IV	Detection	Ref.
Chilli tomato sauce/1.0; Chilli and sauce/1.0	Liquid extraction (LE)/10	4, 3, 5, 11; 11, 3, 9, 24	LC–ESI-MS/MS	[6]
Hot chilli pepper/1.0	LE/65	6, 5, 4, 8	HPLC–CL	[10]
Eggs/10.0	LE-SPE, derivatization/50	4.6, 4.0, 4.8, 4.2	HPLC–UV	[14]
Chilli powder/5.0	LE/100	3.6, 2.4, 17.6, 20.4	LC–ESI-TOF-MS	[43]
Dried chilli and curry/5.0	LE/45	20, 50, 50, 50	LC–APCI–MS	[44]
Hot chilli tomato sauce/1.0	Centrifugation, LE/10	900, 400, 900, 1100	microLC–ESI-Q-TOF–MS	[45]
Chilli powder/1.0; Tomato sauce/1.0	LE/2.0	5, 8, 15, 8	LC–APPI–MS/MS	[46]
Eggs/0.1	MSPD–DLLME/5.1	2.4, 2.3, 3.1, 6.1	HPLC–UV	Present work

Table 3
Recoveries of the MIM–MSPD–DLLME–HPLC method for spiked egg yolks ($n = 3$).

Analytes	Added (ng g^{-1})	Founded (ng g^{-1})	Recovery (%)	RSD (%)
Sudan I	50.0	46.0	92.0	4.0
	100.0	96.2	96.2	4.4
	250.0	245.2	98.1	3.5
Sudan II	50.0	46.1	92.2	6.1
	100.0	103.5	103.5	3.8
	250.0	246.8	98.7	5.8
Sudan III	50.0	43.6	87.2	3.9
	100.0	97.1	97.1	3.6
	250.0	225.0	90.0	4.2
Sudan IV	50.0	46.5	93.0	3.1
	100.0	98.3	98.3	2.1
	250.0	232.9	93.2	4.4

application of the four kinds of Sudans in egg products at local area was not extensive. In the washing fraction of spiked samples, matrix interferences from food matrix were eluted out and no analytes were observed in chromatograms which evaluated the high efficiency and selectivity of the MIM–MSPD. Moreover, the chromatograms of the sediment fractions revealed that the eluents were further purified after the DLLME protocol and no endogenous interferences from the egg yolk matrixes were observed (Fig. 11(C)). To study the effect of sample matrix and the accuracy of the MIM–MSPD–DLLME–HPLC method, recovery experiments were carried out by spiking three different levels of Sudans into egg yolk samples. As seen from Table 3, the average recoveries for all the analytes were in the range of 87.2–103.5% with RSD less than 6.1%, which indicated that the MIM–MSPD–DLLME–HPLC method was reliable and could be used for the determination of Sudans in food samples.

4. Conclusion

This work represents the first attempt of using MIM as selective MSPD sorbent to develop a new MIM–MSPD–DLLME–HPLC method for the selective extraction and determination of four Sudan dyes in egg yolk samples. The new MIM synthesized by suspension polymerization using aniline–naphthol as dummy template showed good affinity to Sudan dyes and was applied as special sorbent of MSPD–DLLME to improve its selectivity. The presented MIM–MSPD–DLLME method combined the advantages of MIM, MSPD and DLLME, and could be potentially applied for the determination of Sudan dyes in complicated food samples.

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