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Preparation of molecularly imprinted polymer with double templates for rapid simultaneous determination of melamine and dicyandiamide in dairy products

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ABSTRACT

In this study, a rapid and accurate determination strategy was established for simultaneous measurement of melamine (MLM) and dicyandiamide (DCD) directly in powdered milk by coupling molecularly imprinted solid-phase extraction (MISPE) with high performance liquid chromatography (HPLC). A novel double-template technique was adopted for preparing SPE packing agent and the obtained double-templated (MLM and DCD) molecularly imprinted polymers (MD-MIPs) was characterized by Fourier-transform infrared spectroscopy and scanning electron microscope (SEM). The molecular recognition ability and the binding capability of the as-prepared polymers towards MLM and DCD were evaluated via static and dynamic binding tests, and it was found that the MD-MIPs showed better affinity and selectivity for both templates compared with single-templated MIPs and non-imprinted polymers (NIPs). An approach based on MISPE and HPLC was then developed and optimized to detect MLM and DCD in powdered milk. The detection limit of the method ($S/N=3$) were 0.13 μg/g for MLM and 0.07 μg/g for DCD, and the relative standard deviation (RSD) of intra-day and inter-day determination for MLM was 3.3% and 4.7%, and 3.5% and 5.9% for DCD. The recoveries in MLM and DCD analysis at three spiked levels were 93.1–100.1% and 75.7–82.5%, respectively, with all RSD less than 5.2%.

1. Introduction

Melamine (MLM) and dicyandiamide (DCD) both belong to nitrogen-rich chemical compounds containing cyanamide as the basic unit. Owing to their high nitrogen content and low price, MLM and DCD were deliberately added to a number of different types of animal and human-food sources to artificially enhance protein concentration. MLM contamination in pet food is associated with renal failure which sickened and killed many pets in early 2007 [1,2], and thousands of Chinese children experienced kidney problems as a result of MLM adulteration of infant formula milk powder in 2008 [3–5]. In September 2012, traces of DCD were found in milk and milk products supplied by manufacturer in New Zealand [6]. While there is no international standard for acceptable levels of DCD in food products and no definite evidence indicating its harm to human body, most of customers are likely to view DCD residues as a contaminant, especially when it is found in food for infants. Therefore, it is crucial to develop a rapid and accurate method to simultaneously measure MLM and DCD in a wide variety of dairy products to ensure the health of customers and the profit of producers.

In consideration of the complexity of real samples and the very small amounts of analytes existing in milk products, it is often necessary to separate and concentrate analytes prior to analysis. Solid-phase extraction (SPE), due to its flexibility, simplicity, high enrichment factor and low consumption of reagent, has been playing an important role in cleaning up and preconcentrating chem/bio-samples. However, lack of selectivity is a serious shortcoming for the conventional SPE process, which influences separation and enrichment efficiency. Fortunately, molecular imprinting, which is known as a technique for the synthesis of polymers with a predetermined selectivity towards the template molecule [7], just compensates for this shortage when it is introduced into SPE for preparing stationary phase. So far, application of molecularly imprinted polymers (MIPs) as SPE sorbent has been massively reported [8–12], and it has developed into a very promising material owing to its prepronounced recognition ability, practicability, reusability, etc [7,13,14].

Recently, several research groups have used molecularly imprinted solid-phase extraction (MISPE) for pretreatment of MLM [15–21] or DCD [22]. One limitation in their work is that the prepared SPE sorbents are expected to serve the assay of only one analyte, either MLM or DCD. Considering that the co-existence

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of both compounds is highly possible in dairy products, it is of great meaning to produce an SPE sorbent able to monitor both molecules simultaneously.

In this regard, we introduced the double-templated MIP technique [23] to synthesize MIPs by involvement of both MLM and DCD as co-templates and the obtained MIPs was named as MD-MIPs. Their morphology and structure information were observed by scanning electron microscope (SEM) and infrared spectroscopy (IR), and the selective recognition ability of MD-MIPs was investigated by adsorption experiments. Furthermore, utilization of MD-MIPs for pretreatment process prior to chromatographic detection was carried out in quantifying MLM and DCD in powdered milk. Selective separation and enrichment of both analytes have been successfully achieved through MISPE, as well as simultaneous determination by the help of high performance liquid chromatography (HPLC).

2. Experimental section

2.1. Materials

Melamine (MLM), dicyandiamide (DCD), cyanuric acid (CYA) and 2-aminopyrimidine (2-AMD) were supplied by Aladdin Co. (Shanghai, China). Their structures are illustrated in Fig. S1. Methacrylic acid (MAA) and 2,2′-azobisisobutyronitrile (AIBN) were obtained from Tianjin Guangfu Chemical Co. (Tianjin, China). Prior to use, MAA was distilled twice under vacuum and AIBN was recrystallized in ethanol. Ethylene glycol dimethacrylate (EGDMA) was purchased from Alfa Aesar Co. (Tianjin, China) and purified by extraction with 10% NaOH aqueous solution, and then dried over anhydrous magnesium sulfate. Powdered milk was purchased from local supermarket. Water was doubly distilled. Solvent for HPLC and SPE were of chromatographic grade. All the other chemicals were of analytical grade and used without further treatment.

2.2. Instrumentation

HPLC experiments were implemented using an Essentia LC-15C system equipped with two LC-15C Solvent Delivery Units, an LC Solution 15C workstation and an SPD-15C UV–vis Detector (Shimadzu, Japan). A KQ3200E ultrasonic cleaner (Kunshan Instrument Co., Jiangsu, China) was set at 40 kHz. Chromatographic separation was performed on a Hypersil NH_2 column (250 mm × 4.6 mm i.d., 5 μm) purchased from Dalian Elite Analytical Instruments Co. (Dalian, China). The mobile phase was acetonitrile–water (3:1, v/v) with a flow rate of 1.0 mL/min. All the measurements were carried out in triplicate. The system suitability of HPLC was tested by assaying standard samples of MLM and DCD at the same concentration (1 μg/mL). The resolution between MLM and DCD is more than 2.0, theoretical plates of both compounds are over 6000, and their tailing factors are less than 1.0. The detailed results are presented in Supporting information (Table S1).

To identify the interaction between template molecules and MAA, a UV-2401 ultraviolet spectrophotometer (Shimadzu, Japan) was employed. Infrared spectra were recorded on a Nicolet EXUS-470 FTIR apparatus (Shimadzu, Japan). Scanning electron microscopy (SEM) images were taken with a JSM-6490LV scanning electron microscope (Tokyo, Japan) using an accelerating voltage of 7 kV.

2.3. Preparation of polymers

The MD-MIPs were synthesized as follows. The templates (MLM, 0.25 mmol and DCD, 0.25 mmol), functional monomer (MAA, 6 mmol) and cross-linker (EGDMA, 30 mmol) were well dissolved in 15 mL of ethanol and 3 mL of water by ultrasonication for 2 min. The solution was stored over night for the formation of a complex of template molecules and monomers. After addition of AIBN (2 wt% relative to the monomers) as initiator, the solution was saturated with dry nitrogen for 5 min and the bottle was placed in a water bath at 55 °C for 24 h. Subsequently, the monolith polymer was crushed, ground, and sieved through a 300 mesh steel sieve. Next the sieved materials were repeatedly suspended in acetone to get rid of small particles. The sediments were further extracted with methanol–acetic acid (80:20, v/v) for 24 h by using a Soxhlet apparatus to remove the templates and then the powders were dried at 50 °C.

As a control, MIPs separately imprinted with MLM and DCD were synthesized in an identical manner and named as M-MIPs and D-MIPs, respectively. Besides, non-imprinted polymers (NIPs) were prepared by the same procedure except for adding MLM and DCD.

2.4. Binding experiments

In static adsorption test, 50.0 mg polymers were placed in a conical flask with stopper containing 5.0 mL DCD or MLM standard solutions which were prepared in acetonitrile–water (3:1, v/v) at the concentrations of 0.001–1.0 mg/mL. After incubation for 24 h at room temperature, the samples were centrifuged, filtered, and then the free concentration of DCD or MLM after adsorption was determined by HPLC–UV. Dynamic binding test was carried out in a similar way except that the initial concentration was constant (MLM at 0.2 mg/mL and DCD at 1.0 mg/mL) and the measurements were taken at different time range (5, 10, 20, 30, 60, 90, 120, 180 and 240 min). The adsorption quantity (Q) was calculated based on the change of the free concentration (C_{free}) and the initial concentration (C_0) of template by Eq. (1), in which V is the volume of the solution and W is the mass of the polymer powder. Meanwhile, maximum binding quantity (Q_{max}) and dissociation constant (K_D) were estimated by processing with Scatchard equation (2).

$$Q = \frac{(C_0 - C_{free}) \times V}{W} \quad (1)$$

$$\frac{Q}{C_{free}} = \frac{Q_{max} - Q}{K_D} \quad (2)$$

2.5. Selectivity of polymers

The selectivity of MD-MIPs was evaluated with MLM, DCD, CYA and 2-AMD. 50.0 mg of MD-MIPs, M-MIPs, D-MIPs and NIPs were separately added to a conical flask containing 5 mL of 6 mmol/L standard solution of each compound. All the mixtures were incubated for 12 h at ambient temperature and then filtered through a 0.22 mm filter membrane. The filtrates of MLM, DCD, CYA and 2-AMD were detected by HPLC–UV at wavelength of 233 nm, 233 nm, 227 nm and 239 nm, respectively.

2.6. Preparation of MD-MIPs and NIPs cartridges

0.2 g MD-MIPs or NIPs particles were packed into an empty SPE cartridge between two polyethylene cribriform plates. Before any use, the cartridges were washed with 3 mL of methanol and conditioned with 3 mL of distilled water. When necessary, the cartridges were cleaned and regenerated by washing with water, methanol–acetic acid (9:1 v/v), methanol, and then dried under vacuum.

2.7. Optimization of MISPE

In order to optimize MISPE procedure, different protocols were attempted during loading, washing, and elution steps. All solutions collected after the three steps were dried with nitrogen at 50 °C. The residue was redissolved with 1.0 mL of mobile phase, filtered through a 0.22 mm filter membrane, and determined by HPLC.

Each extraction was repeated three times and the recovery of analytes were calculated to evaluate the performance.

2.7.1. Optimization of loading solvent

Methanol, ethanol, acetonitrile, acetonitrile–water (3:1, v/v) as the loading solvent were investigated. Successive aliquots (1 mL) of mixture solution containing MLM and DCD at 2 μg/mL in above solvents were loaded onto MISPE cartridge. The solution passing through the cartridge was collected and analyzed by HPLC.

2.7.2. Optimization of washing reagent

To explore the effect of different washing reagents, 2 mL of methanol, methanol–acetonitrile (1:3, v/v), methanol–acetonitrile (1:1, v/v) and methanol–water (3:7, v/v) were adopted.

2.7.3. Optimization of eluting reagent

3 mL typical eluents including methanol–acetic acid (9:1, v/v), methanol–ammonia (9:1, v/v), acetonitrile–acetic acid (9:1, v/v) and acetonitrile–ammonia (9:1, v/v) were tested.

2.8. Detection of powdered milk samples by MISPE–HPLC

2 g powdered milk was weighed into a polypropylene centrifuge tube and spiked with MLM and DCD at different concentrations. After addition of 10.0 mL acetonitrile, the sample was vortexed for 1 min and sonicated for 20 min, followed by centrifugation at 10,000 rpm for 10 min. The supernatant was percolated through MD-MIPs cartridges under the optimal MISPE conditions. Typically, 5.0 mL of sample solution was loaded onto the MIP cartridge at a flow rate of 1.0 mL/min under pressure, and then the cartridges were washed with 2.0 mL acetonitrile–methanol (3:1, v/v) and subsequently eluted with 3.0 mL methanol–ammonia (9:1, v/v). The final eluate was collected and dried with nitrogen at 50 °C. The residue was redissolved into 1.0 mL of mobile phase and analyzed by HPLC.

The linearity of the analytical method was evaluated by a calibration curve at seven concentration levels of DCD or MLM standard solutions, ranging at 0.05–10 μg/mL ($n=5$). Intra-day precision was tested by analysis of spiked powdered milk at 5.0 μg/g of MLM and DCD within a day ($n=5$), while inter-day precision was evaluated via repeated analysis of the same sample each day for five days. In order to study the reproducibility, three MD-MIP cartridges were prepared independently and used for analysis of samples at the same spiked concentration. For assessing the accuracy, the non-spiked samples and the samples spiked with MLM (0.5, 3.5, 5 μg/g) and DCD (0.5, 3.5, 5 μg/g) were measured using the established method ($n=3$). The recoveries were calculated according to the formula: recovery%$=(C_{\text{spike sample}}-C_{\text{non-spiked sample}})/C_{\text{added}}\times 100\%$, in which $C_{\text{spike sample}}$ and $C_{\text{non-spiked sample}}$ were the concentrations of the analyte in spiked sample and non-spiked sample, respectively; C_{added} was the concentration of the standard analyte added. The limit of detection (LOD) was determined using MLM and DCD spiked powdered milk and defined as three times ratio of signal to noise (S/N).

3. Results and discussion

3.1. Synthesis of MD-MIPs

Imprinted polymer was usually synthesized in organic solvents, mainly because the interaction between binding sites and analytes was assumed to be negatively affected by aqueous media [24]. Due to the solubility limitation of MLM in organic solvents, some previous study [17,20,21,25] employed structural analogues of MLM, e.g. cyromazine, as pseudo template to prepare MIPs in organic solvents for MLM analysis. However, the recognition ability of the obtained MIPs is non-predetermined, and the structural difference between the pseudo template and the analyte of interest is very likely to cause impact on the performance of the imprinted material, such as selectivity reduction. On the other side, most of the natural molecular recognition process takes place in aqueous environment. From a practical point of view, synthesis of imprinted polymers which can function effectively in aqueous system is very meaningful. In view of this, polar porogenic solvents such as methanol, dodecanol, acetonitrile, methanol/ethanol–water mixture solution were tried in preparing MIPs. Our results revealed that the most selective imprinted polymers for MLM and DCD were obtained when ethanol–water (5:1, v/v) was applied as porogenic solvent.

As MLM and DCD are both Lewis base, acidic compound MAA was therefore selected to be functional monomer. UV–vis spectrophotometric analysis was used to investigate the interaction between the functional monomer and the template molecules in prepolymerization solution. A series of solutions were prepared containing different molar ratios of MLM, DCD or MLM–DCD (1:1, mol/mol) to MAA in ethanol–water (5:1, v/v). As shown in Fig. 1a, the MLM absorption peaks changed with the increase of MAA concentration, and the maximum absorption wavelength of MLM shifted to longer wavelength (red shift), implying that MLM integrated with MAA. The same situation also appeared for DCD (Fig. 1b), as well as for double templates (Fig. 1c). The above results reflect that MAA is a suitable functional monomer for MLM and DCD. The possible mechanism of red shift is the formation of hydrogen bonds between amino groups of MLM and DCD and carboxyls of MAA [26,27]. Moreover, electrostatic force interaction could be produced between tertiary amine of the templates and hydroxyl groups of MAA. The maximum red shift was achieved when the molar ratio of template molecules and MAA is 1:12, which is also in accord with Yang's work [16] as well. Thus, the molar ratio of MLM–DCD and MAA was set at 1:12 in the following experiments.

3.2. Characterization

The microscopic characteristics of MD-MIPs and NIPs were shown in Fig. 2. The SEM images displayed indiscernible difference in the morphology between the two polymers, both of which illustrated irregular shape with rough surface.

The infrared spectra (Fig. 3) of MLM, DCD, MD-MIPs before and after elution of template were recorded to verify the successful synthesis of the desired products. The broad absorption band at 3450 cm^{-1} (Fig. 3a and c) is indicative of nitro-group N–H stretching vibration of MLM molecules, and the band observed at 2200 cm^{-1} (Fig. 3b and c) corresponds to the stretching vibration of CN bonds attributed to the cyanogroup of DCD molecules. This two characteristic absorption peaks appearing in MD-MIPs before elution of template demonstrated that MLM and DCD were successfully imprinted into the polymers. The disappearance of these characteristic bands after elution implies that the templates have been removed from the MD-MIPs (Fig. 3d).

3.3. Binding assays and Scatchard analysis

The data of the static adsorption experiments (Fig. S2a and b) show that the amounts of MLM and DCD sorbed by polymers increased with the increase of the initial concentration of MLM (Fig. S2a) and DCD (Fig. S2b), and the MD-MIPs displayed higher affinity than NIPs do. It can be observed that the difference of the binding capacity (Q) between MD-MIPs and NIPs became enlarged with increasing the concentration of MLM and DCD until finally both reached equilibrium. The binding capacity for MLM was twice as much as that reported in other work by using single template [16].

High affinity of MD-MIPs is also demonstrated by dynamic adsorption tests. As shown in Fig. S2c and d, MD-MIPs always adsorbed larger amount of MLM and DCD than NIPs do. The time to reach adsorption equilibrium was also found to be faster in DCD

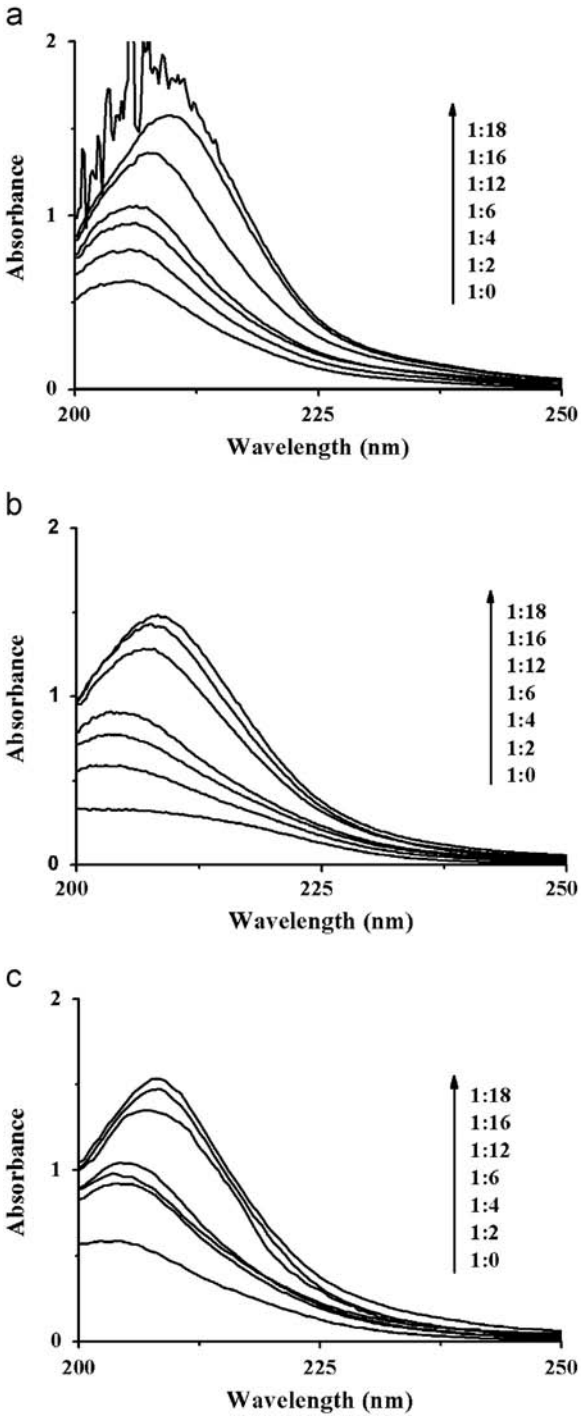

Fig. 1. UV spectra of mixtures of (a) MLM and MAA, (b) DCD and MAA, (c) MLM–DCD and MAA under different molar ratios.

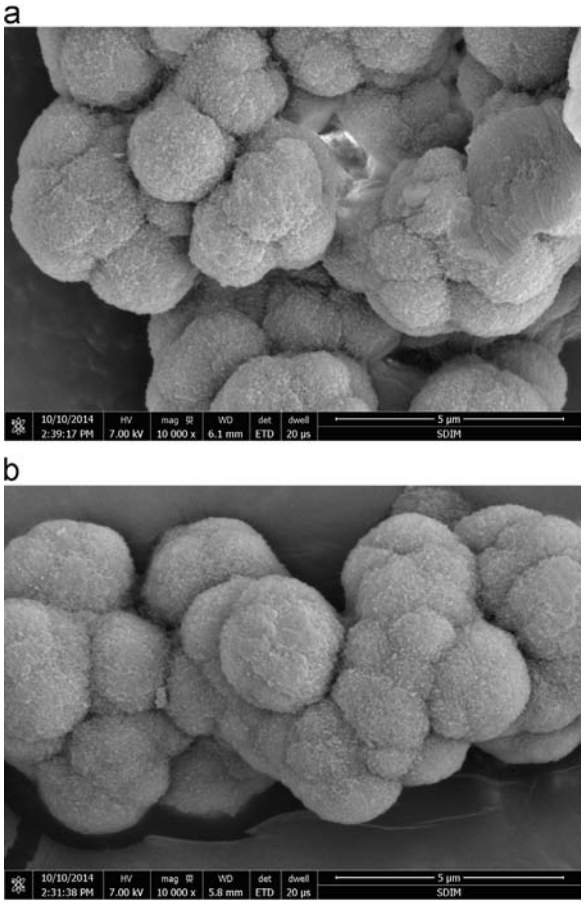

Fig. 2. SEM micrographs of (a) MD-MIPs and (b) NIPs.

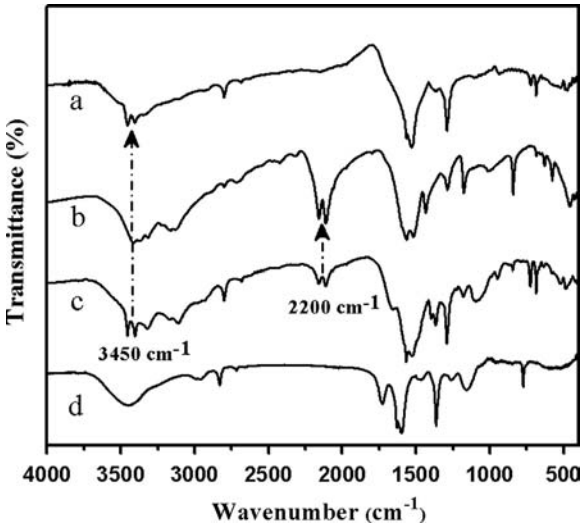

Fig. 3. IR spectra of (a) MLM, (b) DCD, (c) MD-MIPs before and (d) after elution of MLM and DCD.

solution (about 30 min) than that in MLM solution (about 60 min). This might be explained by the larger spatial structure of MLM than that of DCD, which prolongs the process of MLM in adjusting itself to the steric sites for rebinding with MD-MIPs.

As illustrated in Fig. S2e and f, the Scatchard plots of MD-MIPs for MLM and DCD were not linear, which instead could be regarded as a combination of two straight lines with different slopes. As a result, it would be rational to hypothesize that the binding sites can be sorted into two different groups with specific binding properties. The results of Scatchard analysis are listed in Table S2. In MLM solution, the dissociation constant (K_D) and the maximum binding quantity (Q_{max}) of MD-MIPs were 0.02 mg/mL and 1.02 mg/g for high affinity binding sites, and 0.6 mg/mL and 9.26 mg/g for low affinity binding sites. Both values of K_D were higher than the previous reports [20,25]. As for DCD, there also existed high and low affinity binding sites in MD-MIPs, and the values of K_D and Q_{max} were 0.02 mg/mL and 0.28 mg/g for the former and 0.59 mg/mL and 2.58 mg/g for the later.

3.4. Selectivity of the polymers

To evaluate the selective recognition ability of different materials, the amounts of MLM, DCD, CYA and 2-AMD associating with

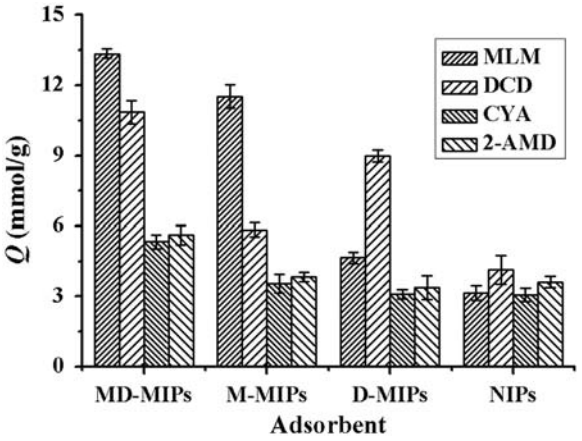

Fig. 4. Binding behavior of different substrates on MD-MIPs and NIPs ($n=3$).

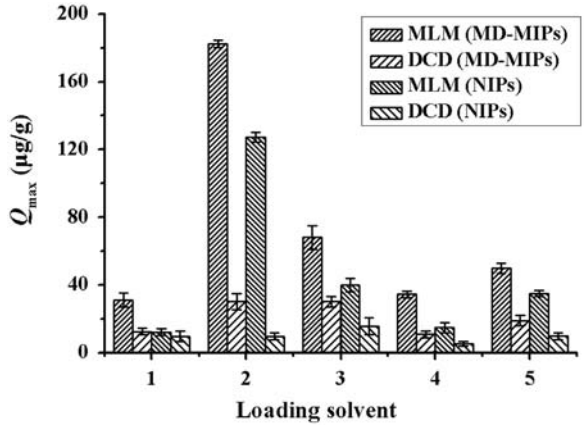

Fig. 5. Effect of loading solvents on the binding capacity of MLM and DCD in MD-MISPE cartridge (1: acetonitrile–water, 3:1, v/v; 2: acetonitrile; 3: ethanol; 4: methanol; 5: water) ($n=3$).

the polymers were compared (Fig. 4). It displayed that M-MIPs and D-MIPs were of greater binding capacity for their corresponding templates compared to structural analogues. It is noteworthy that MD-MIPs adsorbed more MLM and DCD than M-MIPs and D-MIPs did. The participation of MLM and DCD in formation of imprinting cavities into the polymeric network plays a synergistic effect, which can create more transmission channels to facilitate sorption process of template molecules. Consequently, the double template imprinting mode raises the binding capacity.

3.5. Optimization of MISPE procedure

A whole SPE procedure includes packing, conditioning, loading, washing, eluting and regenerating, and the type and volume of solvents for loading, washing and eluting steps should be optimized for a certain analyte in order to enhance specificity and recovery in MISPE procedure.

The role of loading solvent is to extract analyte from real samples as much as possible and meanwhile maintain its affinity in SPE cartridge. In this work, extensively adopted loading solvents [28] including methanol, ethanol, acetonitrile and water were explored. As shown in Fig. 5, the binding capacity of MLM and DCD in the cartridge differed by using different loading solvents, and the maximum binding amount obtained was 182.35 μg/g for MLM and 30.22 μg/g for DCD when acetonitrile was used as the loading solvent.

Second, washing step is a crucial procedure expected to retain the specific interaction between analyte and SPE sorbent, and wash out interferences existing in sample matrix as much as possible. We investigated several washing solvents reported in the relevant references [29,30] and the results are presented in Fig. 6a. It unveils that all the solvents were sufficient to deliver clean extracts and meanwhile maintained affinity for MLM on MD-MIPs. However, as for DCD, it is worth noting that with the increase of methanol in the washing solution, the recovery was decreased. The highest recovery obtained was 99.71% for MLM and 88.25% for DCD with acetonitrile–methanol (3:1, v/v) as washing solvent.

On the basis of the results from washing step and the relevant references [18], the elution step was performed by using methanol or acetonitrile containing acetic acid or ammonia as the main eluent. Fig. 6b reveals that all the eluents led to the recoveries of MLM and DCD more than 75% at the volume of 3 mL. The highest recoveries for MLM and DCD were 100.2% and 98.45%, respectively, when methanol–ammonia (9:1, v/v) solution was employed as eluting solvent. Thereby, 3.0 mL of methanol–ammonia (9:1, v/v) was selected as elution solvent for MISPE.

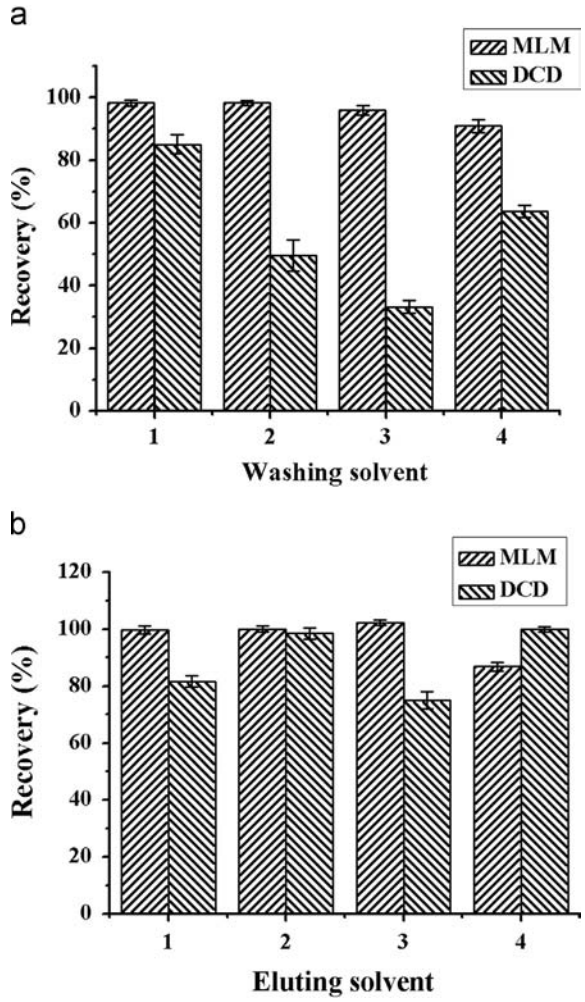

Fig. 6. The optimization of washing and eluting solvents ($n=3$). (a) Effect of washing solvents on the recovery of MLM and DCD (1: acetonitrile–methanol, 3:1, v/v; 2: acetonitrile-methanol, 1:1, v/v; 3: ethanol; 4: ethanol–water, 3:7, v/v); (b) effect of elution solvents on the recovery of MLM and DCD (1: methanol–acetic acid, 9:1, v/v; 2: methanol–ammonia, 9:1, v/v; 3: acetonitrile–acetic acid, 9:1, v/v; 4: acetonitrile–ammonia, 9:1, v/v).

3.6. Determination of MLM and DCD in powdered milk

The performance of the MD-MIPs SPE cartridge in extracting MLM and DCD from powdered milk was studied under the optimized

Table 1
Recovery of MLM and DCD in powdered milk sample detected by MISPE–HPLC ($n=3$).

Analyte	Spiked (μg/g)	Recovery (%)	RSD (%)
MLM	5.0	93.1 ± 2.8	3.1
	3.5	100.1 ± 2.7	2.7
	0.5	96.3 ± 5.0	5.2
DCD	5.0	82.5 ± 3.7	4.5
	3.5	75.7 ± 2.8	3.7
	0.5	79.4 ± 1.9	2.4

Mean value ± S.D.

Table 2
Comparison of the major characteristics of some reported methods used in detecting MLM and DCD.

Analyte	Method	Dynamic range (μg/mL)	LOD (μg/g)	RSD (%)	References
MLM	MISPE–HPLC	0.06–1.26	0.02	7.9	[16]
	MISPE–HPLC	0.24–60	–	< 4	[18]
	HPLC	2–60	0.6	2.9	[31]
	MS	–	0.039	1.4–23.3	[32]
	Electrochemistry	0.0063–0.88	0.00003		[33]
	Fluorescence	0.05–5	0.035–0.11	0.4–1.4	[34]
	This work	0.25–50	0.13	4.7	
DCD	LC–MS	0.0001–0.02	–	0.5–4.9	[35]
	Chemiluminescence	0.05–3	0.003	1.2–2.9	[36]
	GC–MS	0.001–0.05	0.003	–	[37]
	This work	0.25–20	0.07	5.9	

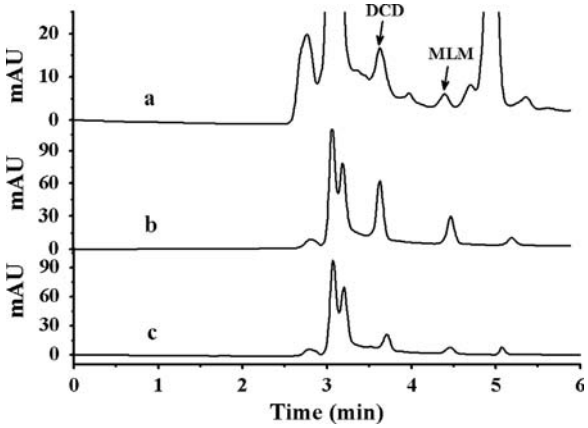

Fig. 7. HPLC chromatograms of milk powder spiked with MLM and DCD at 5 μg/g, (a) before and after extraction with (b) MD-MIPs and (c) NIPs.

protocols achieved in the above work. The calibration curves were in the range of 0.25–50 μg/mL for MLM and 0.25–20 μg/mL for DCD. Their regression equations were $y=3.94\times10^4x+9.97\times10^4$ ($R^2=0.9998$) and $y=9.61\times10^4x+1.36\times10^5$ ($R^2=0.9997$), respectively (x is concentration; y is peak area). The RSD of intra-day and inter-day determination was 3.3% and 4.7% for MLM, and 3.5% and 5.9% for DCD. The RSD for determining MLM and DCD using three MD-MIPs cartridges was 8.7%, indicating good reproducibility of the method. Table 1 demonstrates that when the concentration scope was from 0.5 to 5 μg/g, the average recovery ranged from 93.1 to 100.1% for MLM with RSD less than 5.2%, and from 75.7% to 82.5% for DCD with RSD less than 4.5%. The LOD ($S/N=3$) of the method were 0.13 μg/g for MLM and 0.07 μg/g for DCD. Comparison with other reported methods for measuring MLM and DCD is presented in Table 2. Although the sensitivity of our method is not the highest, the superiority here consists in establishment of simultaneous determination of DCD and MLM in dairy products. Besides, compared with using HPLC alone, our method plays a role in preconcentration and purification, which are proved by a reduced LOD and protection of the chromatographic column (Fig. 7).

Fig. 7a is the HPLC chromatogram of milk powder after addition of MLM and DCD (both 5 μg/g), in which the complexity of the milk powder matrix background was evident. The extraction on MD-MIPs cartridge realized the cleanup of most main interferences in the sample, thus playing a role in protecting the chromatographic column as well as the whole analytical equipments (Fig. 7b). As observed from the chromatograms, after MISPE treatment, the concentration of MLM was high enough to be quantitatively analyzed while it was too low to be quantitated without SPE, suggesting that MLM and DCD of the milk powder sample were purified and enriched effectively.

4. Conclusions

In this work, we synthesized molecularly imprinted polymers with co-participation of MLM and DCD in the imprinting process and applied the as-prepared double-templated MIPs (MD-MIPs) as MISPE sorbent for simultaneous determination of MLM and DCD in powdered milk. The resultant MD-MIPs showed good recognition towards MLM and DCD over the single-templated MIPs and the NIPs. With careful optimization of the whole MISPE procedure, a reliable analytical method based on the MISPE coupled with HPLC was developed for highly selective detection of MLM and DCD in complicated matrix with satisfactory recovery and precision. The proposed method could be a promising alternative for pre-concentration and simultaneous determination of trace amounts of DCD and MLM in dairy products.

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Additional supporting information may be found in the online version of this article at the publisher's web-site.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.talanta.2014.12.032.

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