

Spectrometric Determination of Rhodamine B in Chili Powder After Molecularly Imprinted Solid Phase Extraction

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This paper reports a method using molecularly imprinted polymers that are grafted onto the surface of carboxyl-modified multi-walled carbon nanotubes as the solid-phase extraction adsorbents to detect Rhodamine B in chili powder samples. The polymers were characterized by FTIR and TGA. Various parameters which probably influence efficiency of extraction were optimized. The analytical parameters such as precision, accuracy and linear working range were also determined in optimal experimental conditions. And the proposed method was applied to analysis of Rhodamine B in chili powder samples. The limits of detection and quantification were 2.57 and 8.56 $\mu\text{g/g}$, respectively. The recoveries for analytes were higher than 95% and relative standard deviation values were found to be in the range of 0.83-4.15%. This method was successfully applied for the determination of Rhodamine B.

Key Words : Rhodamine B, Chili powder, Molecularly imprinted polymers, Solid-phase extraction, Multi-walled carbon nanotubes

Introduction

In food industries, colorants are often added to food to enhance its visual aesthetics and promote sales. Rhodamine B (RhB), extensively used for a variety of industrial and scientific applications, is a type of synthetic dye with carcinogenicity.¹ However, RhB has been introduced to food-stuffs gradually due to their colorfastness and low cost.² To ensure food safety of consumer, developed countries have forbidden the use of RhB as colorant in food industry as consequence of the damages on the human health. The US "Colors in Food Regulations" have classified RhB as an illegal colorant and forbidden its use in foodstuffs. But there is still no regulation on the use of RhB in food in some developing country such as Argentine.³ In China, it also has been existed in the list of illegal food additives. Unfortunately, the contamination of RhB in condiments is still occurred under some circumstances, and the monitoring of RhB in real samples has become a concern. Chili powder is an important type of condiments for human being and is widely added in various foods such as pickles. Because the matrix of chili powder sample is very complex, it is necessary to concentrate the analyte and eliminate the matrix effect.

Analytical techniques have been used for determination of RhB, like high pressure liquid chromatography,⁴ capillary electrophoresis,⁵ UV-visible spectrophotometry,⁶ and fluorimetric determination.⁷ Although chromatographic separation enables more sensitive quantitative analysis, it may not be accessible in many laboratories because of the high price of the apparatus and professional operators. UV-visible spectrometry is an attractive method due to its simplicity and lower cost than other apparatus. There are two important

limitations of UV-visible spectrometry to determine food colorants. One is that there are influences of interferent coextracted from samples on the signal of analytes and the other is that lower concentration of analytes probably leads to unsatisfactory quantitative results.⁸ Quantitative UV detection of RhB in real samples requires a sample-preparation technique that can isolate and concentrate target analytes before determination and the technique can also be suitable for routine analysis, with its characteristics of simplicity, rapidity and cheap.

In recent years, several new preconcentration methods including liquid-liquid extraction, liquid-liquid-solid microextraction, solvent-bar microextraction, cloud point extraction have been reported.⁹⁻¹⁶ However, solid phase extraction (SPE) is still recognized as a routine technique to extract analyte from complex matrix and has many advantages including use of small volume of solvent, short extraction time, simple process and easy operation.¹⁷ However, traditional SPE adsorbents, such as C18, have low specificity and selectivity, which result in the coextraction of many other matrix components. It is critical and necessary to develop a new type of adsorbent with high specificity. To obtain better specificity, selectivity and recovery, the application of SPE procedures combined with using molecularly imprinted polymer (MIP) as adsorbent, named molecularly imprinted solid phase extraction (MISPE), has attracted considerable interest recently.¹⁸⁻²¹

Molecular imprinting technique is an attractive approach for the preparation of polymers that possess properties of tailor-made recognition and high affinity. It utilizes template molecules to interact their own recognition sites with complementary functional groups of appropriate monomers and the resulting complexes are then copolymerised with an

excess of cross-linkers in the presence of a free radical initiator. The resultant MIP created a lot of cavities in it with the precise spatial arrangement that are able to selectively recognize target molecules after removal of the template molecules from MIP.²²⁻²⁴

Multi-walled carbon nanotube (MWCNT) was introduced because of their strong interactions, stability under acidic conditions, lack of swelling and large surface area.²⁵ Though a certain amount of corresponding works about grafting MIP polymers onto MWCNT had been reported, the studies of RhB-specific MIP mainly focused on imprinted membrane and imprinted microsphere. In addition, grafting MIP polymers onto MWCNT is still causing the interest of many researchers.²⁶⁻²⁸ Some results had been reported in our previous work.²⁹ Binding ability, SEM and selectivity of structural analogues was studied. In present work, thermal stability, characteristic of MIP by FTIR and selectivity of MIP to food colorants were studied as an extension and follow-up work. The RhB-specific MIP layer which was grafted onto MWCNT was prepared. Then, an analytical method for RhB in chili powder samples was established by using above material as solid phase adsorbent for the separation and preconcentration. Experimental parameters including types of sample extraction solvent, washing solvent and eluent, amount of MIP, flow rate and sample volume were also optimized. The application was successfully and it is potential for this analytical method to use in further investigation of other food samples.

Experimental

Materials. Chili powder used for this study was purchased from a local market in China. RhB was purchased (Aladdin-Reagent Co., China). Methacrylic acid (MAA) and azobisisobutyronitrile (AIBN) were purchased (Sinopharm Chemical Reagent Co., Ltd, China). Carboxyl-modified multi-walled carbon nanotubes (MWCNT-COOH) were obtained from Nanjing (Nanjing XFNANO Materials Tech Co., Ltd, China). Trihydroxymethylpropyl trimethylacrylate (TRIM) was purchased (Sigma-Aldrich, USA). Carmine, amaranth, tartrazine and sunset yellow were purchased (Aladdin-Reagent Co., China). The SPE cartridge was purchased from Waters (Milford, USA). All other reagents were of at least analytical grade and used without any further purification.

Preparation of Molecularly Imprinted Polymer. RhB MIP was prepared as follows: MWCNT-COOH (0.5 g), RhB (1 mmol) and MAA (4 mmol) were weighed into a glass reaction vial and dissolved in acetonitrile (50 mL). The solution was kept stirred for 1 h. Then TRIM (20 mmol) and AIBN (65 mg) were added, the mixture was stirred once again until fully homogenized and was purged with nitrogen for 5 min before being placed in a water bath at 60 °C for 24 h. The resultant polymer was manually sieved by a 100 mesh sieve and washed with a mixture of methanol/acetic acid (9:1, V/V) and methanol in a Soxhlet apparatus to remove the template and nonpolymerized residue. The

obtained polymer particles were subsequently dried and finally stored in a desiccator until used.

The non-imprinted polymers (NIP) for the control experiments were prepared by the same process described above but in the absence of RhB.

Test Procedure. To investigate the applicability of the MIP adsorbent for the extraction of RhB, 250 mg of MIPs were loaded into a 12 × 70 mm empty SPE cartridge and the polymer bed was approximately 20 mm high. The cartridge was preconditioned with 5 mL of acetonitrile prior to each use. Prepared sample solution was passed through the MIP cartridge at a flow rate of 1 mL/min. After washing with 3 mL *n*-hexane to remove interferents, the analytes retained in the cartridge were eluted with 5 mL methanol. The effluent was collected into a test tube and finally analyzed by UV-visible spectrophotometry.

Analysis of Chili Powder Samples. The method was applied to chili powder samples. 0.5 g of chili powder samples were weighed into a conical flask and then mixed with 10 mL of acetonitrile. The samples were extracted by sonication at room temperature for 15 min. After centrifugation at 4000 rpm for 5 min, the obtained supernatant was filtered through a cellulose membrane filter of 0.45 μm pore size. The filter was wash with acetonitrile after sample filtration and the washing fluid was combined with sample solution. The total volume of sample solution was concentrated to the right volume before loading into the cartridge. Then the preconcentration procedure given in the previous section was applied to the final sample solutions. For the recovery experiments, the chili powder was spiked with different amounts of standard RhB before the extraction. The contents of analyte in the samples were determined by UV-visible spectrophotometry. The blanks were prepared in the same way but without sample and standards.

Method Validation. In order to demonstrate the validity of this method, the accuracy and precision were investigated using chili powder samples spiked with a known amount of RhB according to standard curve. Under the optimum conditions, the method validation was performed by repeating the same experiment 6 times. The accuracy and precision were calculated as the recovery and R.S.D. respectively. Limits of detection (LOD) and quantification (LOQ) were determined by analysing blank 10 times.

Results and Discussion

Preparation of Molecularly Imprinted Polymer. The entire process is shown in Figure 1. RhB MIP was prepared on the surface of a type of nano-materials named MWCNT-COOH. Complexes were formed among the interaction of RhB (template), MAA (monomers), and MWCNT by hydrogen bonds. The obtained complexes then were stabilized by polymerization with addition of cross-linker. Eventually, elution of the template leaved cavities with exposed functional groups that were complementary to the target template molecules. The good results of scanning electron microscope obtained in our previous work proved that the poly-

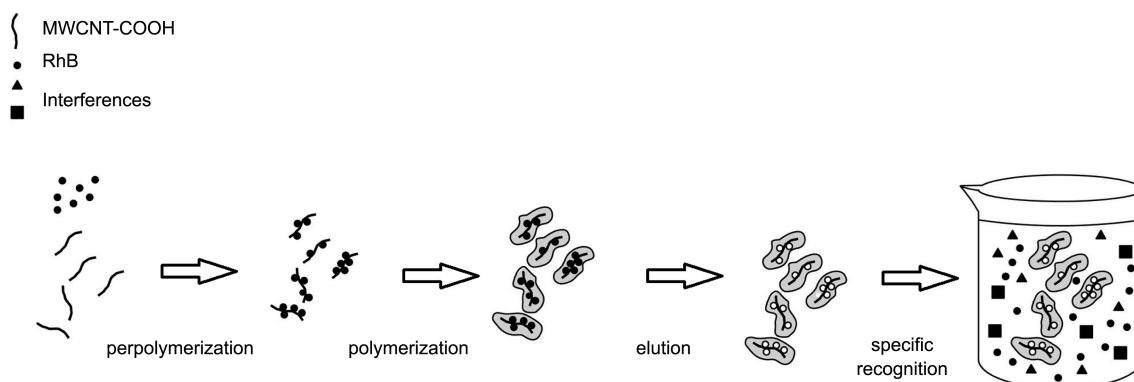


Figure 1. Schematic of imprint process.

mers were grafted onto the surface of MWCNT successfully.²⁹ The highly cross-linked structure of the polymers preserves the skeleton of the cavities after removal of the template and can be used to rebind analytes with great affinity and specificity.

Though RhB MIP have already been reported in the literature, they were mainly synthesized by precipitation polymerization or membrane polymerization.³⁰⁻³² In this study, the RhB-specific MIP layer which was grafted onto MWCNT-COOH was prepared in a novel mode as compared to traditional methods. The grafting technique was adopted because it has been successfully used in many molecular imprinting technology studies and helped generate high affinity binding sites, control the porous properties, and advance the morphology or other structural features of the polymers as reported in Lee's study.³³

Characterization of Molecularly Imprinted Polymer.

The thermo-gravimetric analysis (TGA) curves of the MWCNT-COOH, MIP, and NIP were given in Figure 2. In this study, an amount of analytes were heated from 0 to 730 °C at 10 °C/min under nitrogen at 20 mL/min. From the curves of MWCNT-COOH, we could find that the weight loss of MWCNT-COOH was 4.62% at 730 °C, which indicated that MWCNT-COOH is of a good thermal stability. The curves of MIP and NIP showed similar patterns, which indicated the analogous thermal stability of both polymers. The initial weight loss of them (0-350 °C) were mainly due to the loss of adsorbed water. Both MIP and NIP were almost stable under 350 °C. However, the polymers were

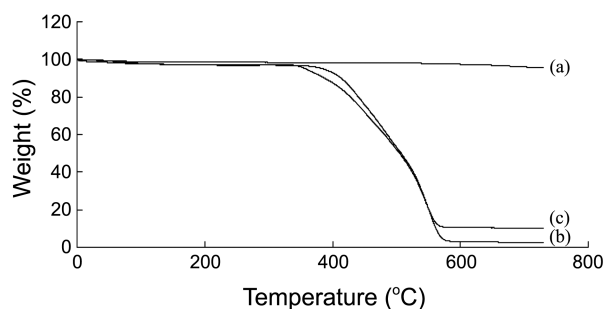


Figure 2. Thermo-gravimetric analysis curves of MWCNT-COOH (a), MIP (b) and NIP (c).

decomposed gradually from 350 to 420 °C and decomposed sharply from 420 to 570 °C. The weight loss of MIP and NIP at 350, 420, and 570 °C was 3.34, 12.09, and 94.71%, and 4.02, 17.51, and 88.88% respectively. The slight difference of thermal stability between MIP and NIP may be attributed to the difference in density after the template molecules in MIP were removed out. The remaining weight could be attributed to the stability of MWCNT-COOH that was embedded into polymers. It indicated that polymers were grafted on the surface of MWCNT-COOH nano-particles successfully and the grafting yield of MIP and NIP was about 94.71% and 88.88%, respectively.

The infrared spectra of MWCNT-COOH and MIP were measured using KBr disks and were shown in Figure 3. In Figure 3(a), the bands at 3421 and 1633 cm^{-1} were attributed to the vibration of OH and C=O bonds in MWCNT-COOH.³⁴ In Figure 3(b), the intensity of C=O bonds became low. Many new absorption bands in a range of 700-1900 cm^{-1} were attributed to the organic groups in MIP at different steps of fabrication. A band at 2973 cm^{-1} was attributed to C-H stretching. The low intensity of band at 1633 cm^{-1} and the new IR bands in Figure 3(b) confirmed that MIP was

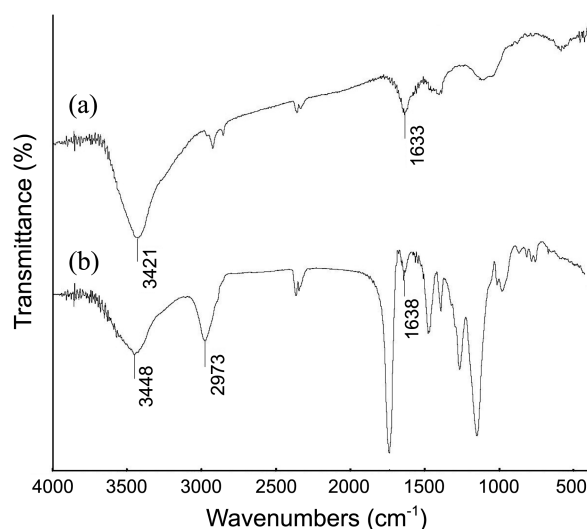


Figure 3. Fourier transform infrared spectra of MWCNT-COOH (a) and MIP (b).

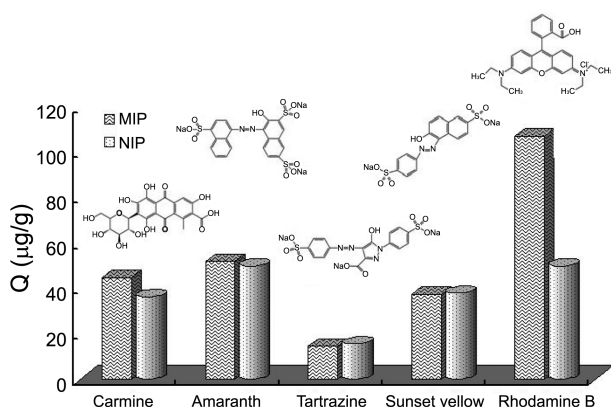


Figure 4. The adsorption selectivity of MIP for different analytes (mMIP = mNIP = 0.05 g, V = 4 mL, t = 6 h, T = 20 °C).

successfully synthesized.

Selective Recognition Study. High selectivity is one of the characteristics of MIP. Several colorants that are usually used in food processing including carmine, amaranth, tartrazine and sunset yellow were mixed with RhB to detect the selectivity of MIP. The amount of analyte bound to the polymers was calculated as adsorption capacity. The results were depicted in Figure 4. It has been found that the MIP exhibited a much larger binding capacity to RhB than to other colorants. This maybe because the cavities in MIP with the precise spatial arrangement that are able to selectively recognize target molecules after removal of the template molecules from MIP. The selectivity and differences between MIP and NIP in the rebinding capacities could reflect the specific selective recognition ability for RhB.

Optimization of the Extraction Conditions. Various parameters which probably influence effect or efficiency of extraction including types of sample extraction solvent, washing solvent and eluent, amount of MIP, flow rate and sample volume were optimized.

Effect of the Types of Sample Extraction Solvent. The type of extraction solvent is an important parameter in this study. In this part, water, ethanol, acetonitrile and acetone with the Et(30) values of 63.1, 51.9, 45.6 and 42.2 were evaluated to find the most suitable sample extraction solvent.³⁵ The results obtained are given in Table 1. Polarity of solvent used for experiment must be able to extract analyte as thorough as possible. In addition, affinity of the solvent to the polymers is also important. Selecting a type of proper solvent is probably helped to form an optimum microenvironment for binding reaction in the process of sample loading. Acetonitrile with maximum recovery was found as the optimal solvent in the entire determination process. This may be because property of acetonitrile was affinity to both RhB and the polymers. Thus, acetonitrile was used as sample extraction solvent in following study until the approving recovery was obtained for the purpose of further optimization.

Effect of the Types of Washing Solvent. Washing step was also important. The type of solvent plays a critical role to remove the interferent effectively from adsorbents where

Table 1. Effect of extraction solvent type on the recovery of RhB (n=3)

Types of sample extraction solvent	Recovery ± SD (%)
water	31.67 ± 1.86
ethanol	40.82 ± 1.86
acetonitrile	68.74 ± 1.77
acetone	11.73 ± 0.41

Table 2. Effect of washing solvent type on the recovery of RhB (n = 3)

Types of washing solvent	Recovery ± SD (%)
<i>n</i> -hexane	92.67 ± 2.15
ethyl acetate	45.51 ± 3.54
acetone	28.62 ± 2.93
ethanol	40.35 ± 2.66
ethanol/water (4:6, V/V)	69.21 ± 1.08

Table 3. Effect of eluent type on the recovery of RhB (n = 3)

Types of eluent	Recovery ± SD (%)
water	24.01 ± 3.78
methanol	90.56 ± 1.47
ethanol	64.26 ± 3.38
acetone	68.31 ± 5.92
Methanol/acetic acid (V/V, 9:1)	114.72 ± 4.62

analyte was leaved only. The MIP washed with a proper solvent before a process of elution can be helpful to maximize the specific interactions between the analytes and binding sites, and to simultaneously decrease non-specific interactions for discarding matrix interference effects. To select an appropriate washing solvent in the MISPE process, solvents with various polarities such as *n*-hexane, ethyl acetate, acetone, ethanol and ethanol/water (4:6, V/V) were tested. The results obtained are given in Table 2. It was found that using *n*-hexane as washing solvent showed higher recovery than the other tested solvents. The recoveries obtained by using the others were all lower than 70%. Possible reason is that RhB molecules which were slightly soluble in them except ethyl acetate could also be washed off the adsorbent along with interferent in the process of cleanup. Thus, *n*-hexane that showed the best result was selected as washing solvent for desorption of interferent in following study.

Effect of Eluent Types. An appropriate eluent should be chosen to ensure the analyte can be completely eluted from MIP cartridge. For this purpose, different types of solvent were also investigated by using water, methanol, ethanol, acetone and methanol/acetic acid (V/V, 9:1), respectively. The results are given in Table 3. In the five types mentioned, especially methanol provided optimal recovery compared with others. In the case of water as the eluent, the recovery was so low that the water could not be selected. Ethanol and acetone could almost achieve the similar recovery that was

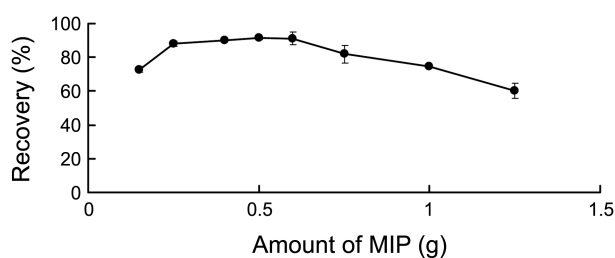


Figure 5. The effect of MIP amount on the recovery of RhB.

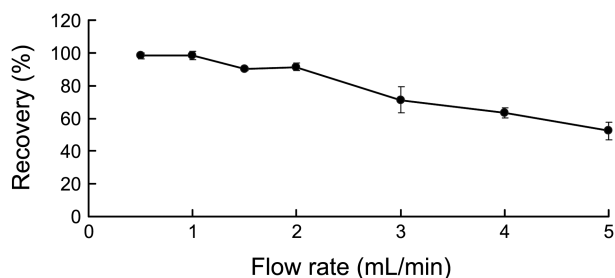


Figure 6. The effect of flow rate on the recovery of RhB.

in a range of 60-70%. This result proved that it was able to desorb the template molecule from the MIP in elution procedure but not work instantly. Generally, methanol/acetic acid (V/V, 9:1) was a familiar solvent which was usually used to deal with resultant MIP right after polymerization to remove the template and nonpolymerized residues.^{36,37} It was the solvent containing methanol and acetic acid employed as an alternative eluent that caused exorbitant recovery, which demonstrated that the solvent was not an adaptive eluent. Finally, methanol was selected as the eluent for desorption and used for the optimization of the other parameters.

Effect of the Amount of MIP. The amount of solid phase in the cartridge is also a main parameter for the recovery of analyte. The effectivity of the MISPE cartridge for concentration of RhB was studied by using different amount of MIP from 0.15 g to 1.25 g. The results given in Figure 5 showed differences in recoveries among the different amounts of cartridge packing. Satisfactory values of recovery (> 90%) was obtained in the range of 0.4 to 0.6 g of MIP. With a lower amount than 0.4 g, the recovery was below 90%. Evidently, a decrease in the recovery of RhB was observed with increasing amount of MIP beyond 0.6 g. For further work, 0.5 g of MIP was selected for subsequent work because it gained the best recovery of 91.50%.

Effect of Flow Rate. The flow rates of sample solution and eluent solutions are two important parameters for the quantitative retention of analytes on the solid phase extraction works. To obtain maximum recovery, the influences of flow rates on the recoveries of RhB were studied in a range of 0.5-5 mL/min with the other conditions kept constant. The results given in Figure 6 showed that recovery values went down slightly at a rate lower than 2 mL/min and dropped rapidly as the rate was faster than that. The faster flow rate resulted in incompletely adsorption of analytes in

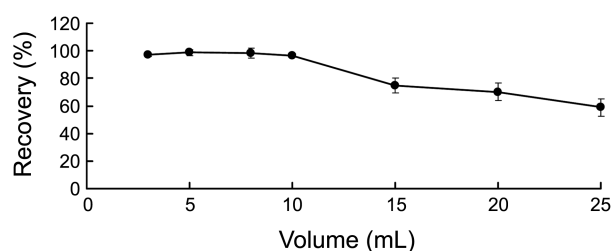


Figure 7. The effect of sample volume on the recovery of RhB.

the process of sample loading, which affected the accuracy of results. Quantitative recoveries (> 95%) were obtained as the flow rate was in a range of 0.5-1 mL/min. In consideration of time saving, 1 mL/min was optimal flow rate that was selected for further use in the following experiment. The recovery could reach to 98.54% at the flow rate of 1 mL/min.

Effect of Sample Volume. Sample volume used in solid-phase extraction is another important condition that must be considered to ensure reliable, reproducible analytical results. In order to investigate the effect of sample volume on the recovery, different volume of sample solution (3-25 mL) containing 8 μg of RhB respectively were loaded at the rate of 1 mL/min. The results are given in Figure 7. It was found that the recoveries were above 95% in the sample volume ranging from 3 to 10 mL. The values decreased gradually with increasing volume above 10 mL. In this study, 5.0 mL was selected as the optimal sample volume in the MISPE procedure because it gained the best recovery of 98.77%.

Analysis of Real Samples and Validity of the Method. In order to evaluate the performance of the MISPE method, several analytical characteristics, such as linearity, limits of detection and quantification, accuracy and precision were evaluated under the optimized conditions.

The calibration curve was linear in the range of 0-12 $\mu\text{g}/\text{mL}$ for RhB-methanol solution. The regression equation was $Y = 0.0888X - 0.0108$, where Y was absorbance and X was the concentration of RhB ($\mu\text{g}/\text{mL}$). The regression coefficient was $R^2 = 0.9991$. The limits of detection and quantification, calculated as $3\sigma/m$ and $10\sigma/m$ at 90% confidence level (σ was the standard deviation of 10 blank measurements and m was the slope of the calibration line), were 2.57 and 8.56 $\mu\text{g}/\text{g}$, respectively.

The MISPE column is examined by using standard addition method to evaluate the accuracy and precision of the method. In the experiment, 0.5 g chili powder samples were

Table 4. Analytical performance of MIP in spiked dried chili powder analyzed by MISPE

Sample	Added (mg/kg)	Found ^a (mg/kg)	Recovery \pm SD (%)	RSD (n = 6, %)
	0	2.52 \pm 0.10	-	-
Chili	12	14.40 \pm 0.25	99.01 \pm 4.11	4.15
Powder	60	61.06 \pm 0.34	97.60 \pm 1.15	1.18
	108	109.36 \pm 0.44	98.94 \pm 0.82	0.83

^aAverage \pm SD

spiked with three amounts of the investigated RhB and processed by MISPE procedure. The results are shown in Table 4. It indicated that the method had good recoveries from 98.94% to 99.01%. For all tested samples, the relative standard deviation (RSD) was in the range of 0.83-4.15%, which was found under 5.0%. The accuracy and precision of the method were acceptable.

Reusability. The reusability is one of important advantages of the novel adsorbents. To examine the long-term stability of the MIP, the adsorption-desorption cycle of RhB was repeated 20 times by using the same adsorbent. 0.5 g chili powder samples were spiked with 8 μ g RhB and processed by MISPE procedure. The reusability was assessed by monitoring the change in recoveries. The results showed that there was no remarkable reduction in the adsorption capacity of the MIP adsorbents. It is indicated that repeated use of the MIP was feasible.

Conclusions

In this work, a simple, low cost and time saving molecularly imprinted solid phase extraction protocol was developed for the determination of RhB in chili powder. Molecular imprinting technology was adopted to synthesize a highly selective RhB imprinted polymer that was grafted onto the surface of MWCNT-COOH. The results of thermo-gravimetric analysis indicated that the polymers exhibited a good thermal stability (< 350 °C). The MISPE column showed good stability under the optimum conditions and could be used for more than 20 cycles with only slight loss in its specific adsorption behavior. Although some features of MIP such as hydrophilicity still need to improve, results of quantitative recovery, satisfactory accuracy and precision are used to demonstrate that MIP has great potential in the application as adsorbents for the determination of RhB in real samples like chili powder.

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