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Poly(*N*-isopropylacrylamide-*co*-*N*,*N*′-methylene bisacrylamide) monolithic column embedded with γ-alumina nanoparticles microextraction coupled with high-performance liquid chromatography for the determination of synthetic food dyes in soft drink samples

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ABSTRACT

The present work proposes a study of the synthesis of poly(*N*-isopropylacrylamide-*co*-*N*,*N*′-methylene bisacrylamide) monolithic column embedded with γ-alumina nanoparticles and its applications to the extraction of synthetic food dyes in soft drink samples. The monolithic column was synthesized inside fused silica capillaries using thermally initiated free-radical polymerization with *N*-isopropylacrylamide (NIPAAm) as the monomer, *N*,*N*′-methylene bisacrylamide (MBAAm) as the cross-linker, dimethylsulfoxide (DMSO) and dodecanol as the porogen. γ-Alumina nanoparticles were introduced to prevent the swelling of the organic polymer and enhance the loading capacity. In order to obtain optimum experimental conditions, sample pH, sample flow rate, sample volume, eluent flow rate were investigated. Under the optimum conditions, we obtained acceptable linearities, low limits of detection, and good intra-day/inter-day relative standard deviations. When applied to the determination of four synthetic food dyes (Tartrazine, Sunset Yellow, Allura Red, and Azorubine) in soft drink samples, satisfactory recoveries were obtained in the range of 90.4–109.2%.

1. Introduction

Food dyes are one of the most important groups of food additives. Natural dyes are usually preferable to be used but they suffer from the disadvantages of being unstable and easily undergoing degradation. Therefore, synthetic food dyes are widely used in food products. Synthetic food dyes are mainly aniline compounds made of benzene, toluene, and naphthalene, some of which are harmful to human organism. For this reason, many countries make strict rules to limit the type, scope and dosage of synthetic food dyes [1].

Chromatographic and spectrometric approaches have been employed for the determination of synthetic food dyes [2–6], among which HPLC with DAD or MS detectors is most frequently used. Other techniques such as electroanalytical methods and capillary electrophoresis have also been reported for this goal [7–9]. Due to the complex matrix effect and the low level concentration of synthetic food dyes in food samples, direct determinations are often difficult. Hence, the pretreatment and concentration step is inevitable before the determination of synthetic food dyes to enhance the sensitivity and selectivity. Various methods have been developed for the preconcentration of synthetic food dyes. Liquid–liquid extraction (LLE) [10] and solid-phase extraction (SPE) [4,11] are conventional methods to separate and concentrate synthetic food dyes because of its simplicity, speed, and wide scope. However, the drawbacks of LLE and SPE are well known of being laborious, complex and time-consuming. Moreover, LLE requires considerable amounts of toxic organic solvents which makes it environmentally unfriendly and unhealthy to human body. For this reason, many other techniques have been developed for the preconcentration of synthetic food dyes, including ultrasounds assisted solvent extraction [12], solid-phase microextraction (SPME) [13], etc.

SPME, first developed in 1990 by Arthur and Pawliszyn [14], is a solvent-free extraction technique which incorporates sample pretreatment, concentration and sample introduction in a single procedure. Nevertheless, SPME fiber is fragile and has limited lifetime, and the sample carry-over is also a problem. Polymer monolith microextraction (PMME) is introduced as a kind of SPME technique based on the use of the polymer capillary monolithic column, which has shown several attractive features including frit-free construction, biocompatibility, easy preparation with good control of porosity and diverse surface chemistry. Furthermore, the

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convective mass transfer procedure and low pressure-drop offered by the monolithic porous structure facilitate the extraction process. However, polymer capillary monolithic columns suffer from swelling when exposed to different organic mobile phases, resulting in the weak mechanical stability [15]. Recently, functionalization of monoliths with nanoparticles has been rapidly developed which features a very high surface-to-volume ratio and specific chemistry [16–21]. Latex nanoparticles coated monolithic stationary phases were first introduced by Haddad et al. for ion-exchange chromatography [16,17]. Gold nanoparticles modified polymer monoliths were recently developed and used for the separation of peptides [18–20]. Divinylbenzene-based porous monoliths containing encapsulated titanium dioxide and/or zirconium dioxide nanoparticles for selective extraction of phosphopeptides from tryptic digests were also reported [21]. In our previous work, a PMME-HPLC-MS method based on polymer monolithic columns incorporated with graphene nanosheets was developed and used for the enrichment of glucocorticoids [22].

Polyacrylamide-based monolithic columns for PMME have been successfully synthesized for the determination of different kinds of analytes, such as drugs [23,24] and environmental pollutants [25]. γ-Alumina nanoparticles are porous inorganic material with adjustable pore dimensions (10–20 nm), which has advantages of the uniformity of particle size, large surface area, high porosity, strong ability of adsorption, and excellent dispersibility in many organic solvents. The objective of this study is to exploit the potential of a novel PMME method using a poly(*N*-isopropylacrylamide-*co*-*N*,*N*′-methylene bisacrylamide) monolithic column embedded with γ-alumina nanoparticles (hereafter abbreviated as poly(NIPAAm-*co*-MBAAm)-Al_2O_3 monolithic column). The monolithic column was prepared by one step in situ polymerization procedure and combined with HPLC for the determination of synthetic food dyes. Four synthetic food dyes, Tartrazine, Sunset Yellow, Allura Red, Azorubine, were chosen as representatives. Several important parameters affecting the extraction efficiency such as sample pH, sample flow rate, sample volume, and eluent flow rate were optimized. As far as we know, it is the first time that poly(NIPAAm-*co*-MBAAm)-Al_2O_3 monolithic column is applied to the determination of the target analytes.

2. Experimental

2.1. Chemicals and materials

NIPAAm and MBAAm were purchased from TCI (Tokyo, Japan) and Sigma-Aldrich (USA), respectively. γ-Alumina nanoparticles, PEG (M_n=20,000), and 3-(trimethoxysilyl) propylmethacrylate were purchased from Aladdin Reagent Co., Ltd., China. Dodecanol, dimethylsulfoxide, acetone, and azobisisobutyronitrile (AIBN) were obtained from Tianjin Chemical Plant (Tianjin, China). NIPAAm and AIBN were purified by recrystallization from *n*-hexane and ethanol, respectively, followed by being dried in vacuum at room temperature. All other reagents were used as received.

HPLC grade methanol and acetonitrile were from Fisher Scientific (New Jersey, USA). Tartrazine, Sunset Yellow, and Allura Red were obtained from Aladdin Reagent Co., Ltd., China. Azorubine was purchased from Dr. Ehrenstorfer GmbH (Germany). The structures of Tartrazine, Sunset Yellow, Allura Red, Azorubine were shown in Fig. 1. The stock standard solutions of 1000 μg mL^{-1} of synthetic food dyes were prepared in double distilled water (DDW). The stock standard solutions and diluted standard solutions were stored in dark glass volumetric flasks in the dark at 4 °C. All solvents and solutions for HPLC analysis were filtered through a Millipore filter (pore size 0.45 μm). Fused silica capillaries (530 μm, i.d.) were purchased from Yongnian Optical Conductive Fiber Plant (Handan, China). DDW was used for all the experiments.

2.2. Instrument and analytical conditions

Chromatographic analysis was performed on an Agilent 1100 liquid chromatography system, equipped with a quaternary pump and degasser, a thermostated autosampler (4 °C) and column compartment (35 °C), a multiple wavelength detector, and Chem-Station software. A reverse phase Agilent Zorbax Eclipse XDB-C18 column (4.6 mm × 250 mm, 5 μm) was employed for the chromatographic separation. A phenomenex C18 security guard column (4.0 mm × 3.0 mm) from Phenomenex, Torrance, CA was used to protect the column. The mobile phase consisted of A and B (A:B=55:45), where A represented 0.1 mol L^{-1} aqueous ammonium acetate solution, B represented a mixture of methanol:acetonitrile (80:20, *V*/*V*). A was filtered by vacuum through a membrane filter with a pore diameter 0.45 μm. The flow rate of eluent was always kept constant at 0.8 mL min^{-1} and the injection volume was set at 5 μL. All experiments were carried out at room temperature. The diode-array detector was programmed to monitor the synthetic food dyes at a range of 350–800 nm. The detection and determination of each substance was employed at the appropriate absorbance wavelengths, i.e., 427 nm for Tartrazine, 490 nm for Sunset Yellow, 507 nm for Allura Red, and 520 nm for Azorubine.

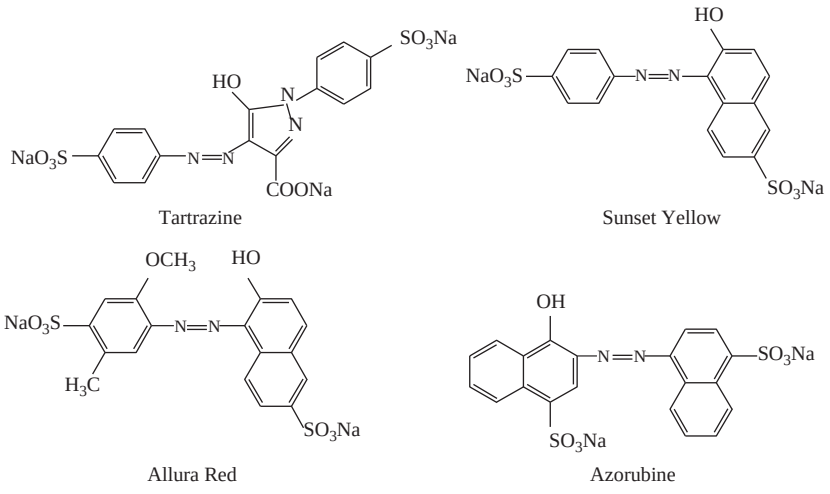

Fig. 1. Molecular structures of the target synthetic food dyes. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

For pH measurements, a pH-3C digital pH meter (Shanghai Rex Instruments Factory, China) was employed. The Milli-Q SP system (Millipore, Milford, MA, USA) was used to prepare DDW. An LSP01-1A programmable syringe pump (Baoding Longer Precision Pump Co., Ltd., Hebei, China) was used for pushing solutions.

2.3. Synthesis of poly(NIPAAm-co-MBAAm)-Al_2O_3 monolithic column

The fused silica capillary (20 cm × 0.53 mm, i.d.) was washed subsequently with acetone, 0.1 mol L^{-1} NaOH, DDW, 0.1 mol L^{-1} HCl, DDW, and acetone each for 30 min. Before polymerization, the inner wall of the fused silica capillary was modified with 3-(trimethoxysilyl) propylmethacrylate (50% in acetone, *V*/*V*) to enable covalent attachment of the monolith. After sealing the two ends of the capillary with silicon rubber, the reaction was allowed to perform at 50 °C for 14 h. The capillary was then washed with acetone and purged with N_2 for 1 h. In preliminary experiments, γ-alumina nanoparticles were proved to be dispersed uniformly in binary porogens, DMSO and dodecanol. The pre-polymerization mixture solution containing 60.0 mg NIPAAm, 140.0 mg MBAAm, 70.0 mg PEG, 2.0 mg AIBN, mixtures of DMSO (682.0 mg) and dodecanol (357.0 mg) containing 70.0 mg γ-alumina nanoparticles was sonicated to obtain a homogeneous solution and then purged with N_2 for 10 min to remove O_2. The reaction was initiated at 70 °C for 24 h. After the polymerization was complete, the capillary was washed with methanol to remove unreacted component and porogenic solvent for 24 h.

2.4. Sample preparation

All soft drink samples were purchased from local supermarkets. 1 mL soft drink sample was diluted with 0.02 mol L^{-1} phosphate buffered solution (PBS) (pH=4.0) in a dark volumetric flask. The sample solutions were degassed in ultrasonic bath for 15 min and filtered through 0.45 μm disposable syringe filters. The filtrate was collected in dark volumetric flask of 10 mL at 4 °C.

2.5. Polymer monolith microextraction procedure

The PMME apparatus included a regular plastic syringe (5 mL), a plastic pinhead (one part of the whole syringe) and the poly(NIPAAm-*co*-MBAAm)-Al_2O_3 monolithic column tube (530 μm, i.d. × 2 cm) [26]. The syringe barrel was coupled seamlessly to one end of the pinhead, while the metallic needles on the other end of the pinhead were removed and replaced by a 2 cm monolithic capillary tube (cut from the prepared monolithic capillary) with adhesive. The PMME procedure consisted of four successive steps, preconditioning, sample loading, washing, and desorption. For preconditioning, 0.2 mL methanol was introduced into the syringe and pushed to pass through the monolithic capillary at a speed of 0.05 mL min^{-1}, and then 0.5 mL NaH_2PO_4 (pH 4.0) was expelled at a flow rate of 0.15 mL min^{-1}. After that, 0.8 mL sample solution was injected at 0.03 mL min^{-1} in the same way. In the washing step, 0.2 mL NaH_2PO_4 (pH 4.0) was expelled to flow through the capillary at a flow rate of 0.15 mL min^{-1}. In order to avoid the contamination of the eluate, the residual solution in the pinhead and the monolithic capillary was removed with a clean syringe. In the desorption step, 0.5% ammonia solution/methanol (1:1, *V*/*V*) was injected to the monolithic capillary at 0.05 mL min^{-1} for 2 min and the eluate was collected into a vial for HPLC determinations.

3. Results and discussion

3.1. Preparation of poly(NIPAAm-co-MBAAm)-Al2O3 monolithic column

An optimized polymerization mixture should afford a monolithic column which has good homogeneity and permeability. PEG was employed as the polymer modifier in the synthesis procedure, indicating that it could effectively prevent the monolithic bed from being inconsecutive and bubbles resulted from the shrinkage of polymer [27]. The polymer modifier/(monomer+-cross-linker) weight ratio was 35/100 [23].

The method for the preparation of poly(NIPAAm-*co*-MBAAm)-Al_2O_3 monolithic column was shown in Section 2.3. In order to optimize the dosage of γ-alumina nanoparticles, different amounts of γ-alumina nanoparticles (30.0 mg, 50.0 mg, 70.0 mg, 80.0 mg, 90.0 mg) were admixed in the constant amount of polymerization mixtures containing 60.0 mg monomer, 140.0 mg cross-linker, 1039.0 mg porogens, and 70.0 mg PEG. It was observed that the column mixed with 70.0 mg γ-alumina nanoparticles had best permeability, while the columns which were mixed with 30.0 mg, 50.0 mg, 80.0 mg, and 90.0 mg had very high back pressure. A possible reason may be that the amount of less than 70.0 mg γ-alumina nanoparticles could not improve the swelling of the monolithic column effectively, while the amount of more than 70.0 mg γ-alumina nanoparticles was so large that it plugged the flow-through pores.

The morphology of poly(NIPAAm-*co*-MBAAm) and poly(NIPAAm-*co*-MBAAm)-Al_2O_3 monolithic columns was investigated by SEM analysis. Results were presented in Fig. 2. The micrographs clearly demonstrated that the size of the microglobules and the pores in poly(NIPAAm-*co*-MBAAm)-Al_2O_3 monolithic column was smaller than those in poly(NIPAAm-*co*-MBAAm) monolithic column, implying that γ-alumina nanoparticles increased the mechanical stability of porous polymer monolithic column effectively.

3.2. Optimization of PMME conditions

The experimental conditions were optimized with 1.0 μg mL^{-1} Tartrazine, Sunset Yellow, Allura Red, and Azorubine. Several experimental parameters affecting the extraction efficiency including

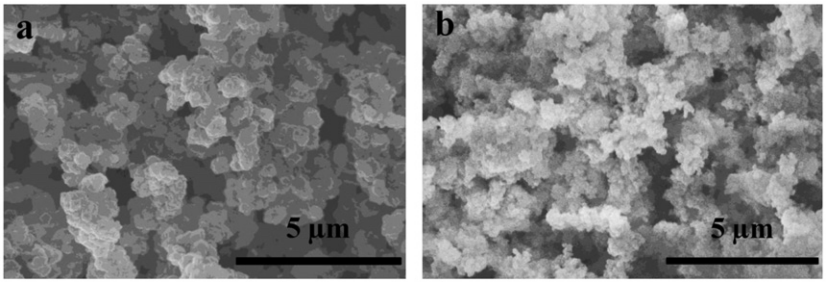

Fig. 2. SEM micrographs of (a) poly(NIPAAm-*co*-MBAAm) monolithic column and (b) poly(NIPAAm-*co*-MBAAm)-Al_2O_3 monolithic column.

sample pH, sample flow rate, sample volume, and eluent flow rate were investigated for the purpose of obtaining the best efficiency of the PMME method. The peak area of analytes as the HPLC response was used to evaluate the extraction efficiency under various conditions.

Sample pH is a significant parameter affecting quantitative adsorption and recovery of the analytes. It always influences the interactions between the analytes and the extraction material. In order to evaluate the effect of sample pH, the standard solutions were loaded onto the poly(NIPAAm-*co*-MBAAm)-Al_2O_3 monolithic column after pH adjustment using H_3PO_4 or NaOH solutions. The effect of sample pH was investigated in the range of 2.0–13.0. As shown in Fig. 3, the peak area first increased but then decreased after reaching a highest value. This may be explained with the interaction between the analytes and the monolithic column. Electrostatic repulsion and hydrogen bonding contribute to the interaction [24,28,29]. There are two kinds of hydrogen bonding in the system. One is formed by the interaction between the amide group of the monolithic column and the phenolic group and/or the sulfo group of synthetic food dyes. The other is due to the interaction between the phenolic group and/or the sulfo group of synthetic food dyes and the hydroxyl group of γ-alumina nanoparticles embedded in the polymer monolithic column, which is formed when the γ-alumina nanoparticles are exposed to protic solvents. When pH is too low, the electrostatic repulsion increases due to the protonation of the analytes and the monolithic column embedded with γ-alumina nanoparticles. The decrease of the peak area at high pH is because of the decrease of the hydrogen bonding interaction. As a consequence, pH of 4.0 was selected as the optimized sample pH in the present work.

The flow rate of the sample solution can not only affects the recoveries of the analytes, but also controls the time of analysis. The sample flow rate was investigated in the range of 0.01–0.07 mL min^{-1}. Fig. 4 showed the effect of sample flow rate on the extraction efficiency, indicating that a decrease of the extraction efficiency occurs when increasing the flow rate. In the view of shorter extraction time, 0.03 mL min^{-1} was chosen in the present work.

In order to investigate the effect of sample volume on the extraction efficiency, the sample volumes within the range of 0.1–1.6 mL at a constant sample flow rate of 0.03 mL min^{-1} were investigated in the present work. As shown in Fig. 5, the peak area increases with increasing sample volumes for all the analytes. The extraction equilibrium was not reached until the sample volume is 1.6 mL, indicating that the poly(NIPAAm-*co*-MBAAm)-Al_2O_3 monolithic column has remarkable extraction capacity for the analytes. Considering the analytical time, 0.8 mL was selected as the optimized sample volume.

In order to achieve an accurate quantitative analysis of synthetic food dyes, it is necessary to find a suitable eluent with an optimum eluent flow rate. 0.1 mL 0.5% ammonia solution/methanol (1:1, *V*/*V*) was selected as the eluent, by which more than 98% extracted analytes were eluted. The effect of eluent flow rate in the range of 0.01–0.07 mL min^{-1} on the peak area was investigated. As shown in Fig. 6, the peak area increases in the beginning and decreases at higher eluent flow rates for four analytes. Therefore, 0.05 mL min^{-1} was optimized as the eluent flow rate.

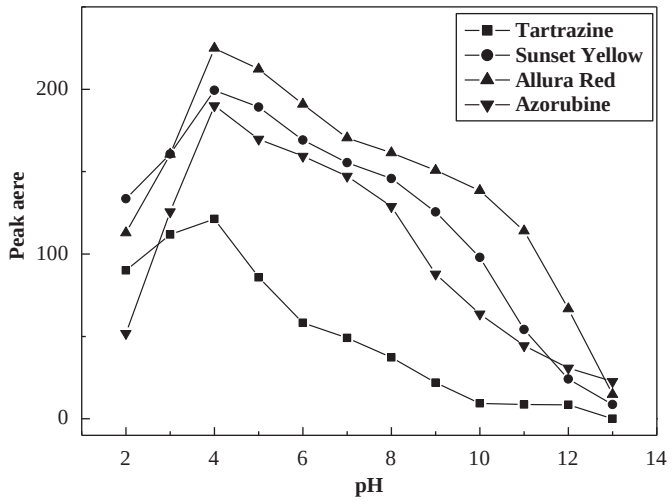

Fig. 3. Effect of sample pH on the PMME method. Synthetic food dyes concentration=1.0 μg mL^{-1}, sample flow rate=0.03 mL min^{-1}, sample volume=0.8 mL, eluent flow rate=0.05 mL min^{-1}, extraction conditions and HPLC conditions were outlined in Section 2.

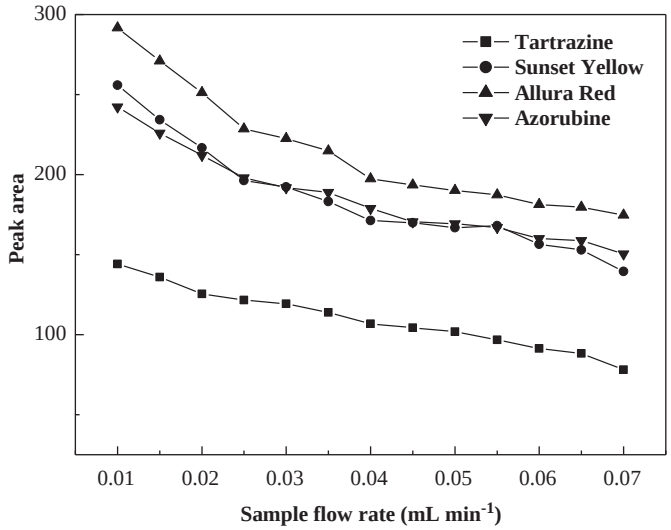

Fig. 4. Effect of sample flow rate on the PMME method. Synthetic food dyes concentration=1.0 μg mL^{-1}, sample pH 4.0, sample volume=0.8 mL, eluent flow rate=0.05 mL min^{-1}, extraction conditions and HPLC conditions were outlined in Section 2.

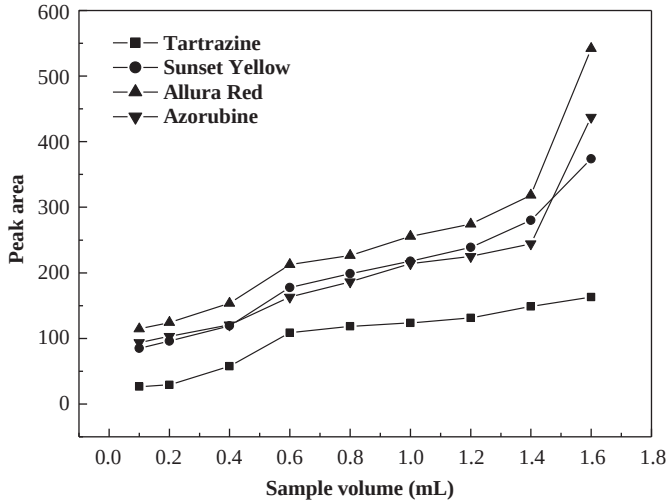

Fig. 5. Effect of sample volume on the PMME method. Synthetic food dyes concentration=1.0 μg mL^{-1}, sample pH 4.0, sample flow rate=0.03 mL min^{-1}, eluent flow rate=0.05 mL min^{-1}, extraction conditions and HPLC conditions were outlined in Section 2.

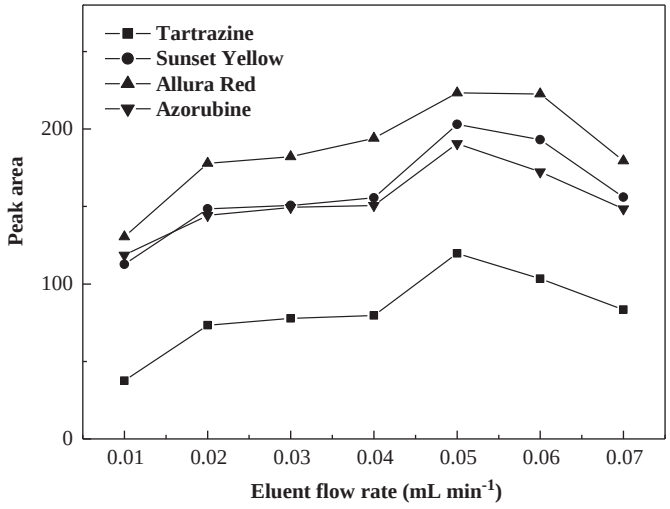

Fig. 6. Effect of eluent flow rate on the PMME method. Synthetic food dyes concentration = 1.0 μg mL^{-1}, sample pH 4.0, sample flow rate = 0.03 mL min^{-1}, sample volume = 0.8 mL, extraction conditions and HPLC conditions were outlined in Section 2.

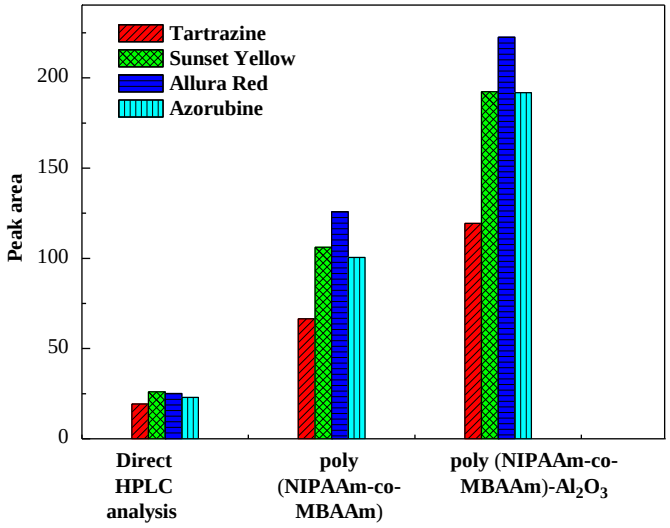

Fig. 7. Comparison of the analytical performance of poly(NIPAAm-*co*-MBAAm)-Al_2O_3 monolith with poly(NIPAAm-*co*-MBAAm) monolith and direct HPLC analysis for four synthetic food dyes. Synthetic food dyes concentration for PMME and direct HPLC analysis = 1.0 μg mL^{-1}. PMME conditions and HPLC conditions were outlined in Section 2.

Table 1
Features of the PMME method[a].

Analytes	Linear range (ng mL^{-1})	r	LOD (ng mL^{-1})	LOQ (ng mL^{-1})	RSD	
					Intra-day (%)	Inter-day (%)
Tartrazine	50–5,000	0.9996	9.9	33.0	4.3	6.7
SunsetYellow	50–10,000	0.9991	9.5	31.6	3.7	9.8
Allura Red	50–10,000	0.9999	9.3	30.9	5.5	7.8
Azorubine	50–10,000	0.9999	11.5	38.2	6.1	5.6

[a] RSD values were calculated for 1.0 μg mL^{-1} synthetic food dyes (n = 5).

3.3. Features of the PMME method

The PMME method was validated under the above optimum experimental conditions with respect to extraction efficiency, linear range, precision, limits of detection (LOD), and limits of quantification (LOQ). Results were shown in Table 1. The regression coefficients (r) values ranged from 0.9991 to 0.9999. LODs and LOQs, determined as S/N ratios of 3 and 10, varied in the range of 9.3–11.5 ng mL^{-1} and 30.9–38.2 ng mL^{-1}, respectively, indicating that the present method had low LOD and LOQ values. The enrichment factors of Tartrazine, Sunset Yellow, Allura Red, and Azorubine, calculated by the ratio of the slope of calibration curves with/without employing the PMME method, were determined as 9.4, 12.2, 12.1, and 11.8, respectively. The reproducibility of the method was evaluated by the intra-day and inter-day precisions. The intra-day relative standard deviations (RSDs) were obtained in five replicates for all the target analytes. The inter-day precision data was determined by the analysis of all the target analytes in five consecutive days. The intra-day and inter-day RSDs were in the range of 3.7–6.1% and 5.6–9.8%, respectively. Hence, the developed method for synthetic food dyes is precise, sensitive, and low consumption in sample and organic solvent.

The analytical performance after the enrichment using poly(-NIPAAm-*co*-MBAAm)-Al_2O_3 monolithic column has been compared with that using poly(NIPAAm-*co*-MBAAm) monolithic column under the optimal experimental conditions. Results were shown in Fig. 7. Direct HPLC analysis results were also illustrated in Fig. 7, indicating that the peak areas were observably improved after the preconcentration using poly(NIPAAm-*co*-MBAAm)-Al_2O_3 and poly(NIPAAm-*co*-MBAAm) monolithic columns. Poly(NIPAAm-*co*-MBAAm)-Al_2O_3 monolithic column manifests better enrichment capacity than poly(NIPAAm-*co*-MBAAm) monolithic column, demonstrating its prominent enrichment ability for synthetic food dyes. This implies that γ-alumina nanoparticles can not only improve the permeability, but also take part in extraction mechanism of the target analytes. As mentioned above, it may be because of the interaction between the hydroxyl group of γ-alumina nanoparticles and the phenolic group and/or the sulfo group of synthetic food dyes.

In addition, the present method was evaluated by comparing to some reported methods for the determination of synthetic food dyes. It was illustrated that the LOD values were comparable to those obtained with other preconcentration strategies, e.g., SPE-HPLC (0.027–0.051 μg mL^{-1}) [4], LLE-LC-MS (0.01 μg mL^{-1}) [10], and SPE-CE (0.05–0.40 μg mL^{-1}) [11], or those without the preconcentration step, e.g., spectrophotometric method (0.04–0.08 μg mL^{-1} [5] and 0.021–0.860 μg mL^{-1} [6]). Furthermore, other preconcentration methods may consume a large amount of organic solution and need complex operations. For instance, in the SPE method [4], 15 mL of ammonia solution/ethanol was used to elute the SPE column, while only 0.1 mL of ammonia solution/methanol was needed in the present PMME method. Moreover, the eluate in the SPE method needed to be neutralized, evaporated to dryness, and redissolved, while the eluate could be directly used for HPLC analysis in this method.

3.4. Reproducibility of preparation for monolithic capillary and stability in use

The reproducibility for the preparation of an extraction medium is an important factor ensuring the robustness and the practicability of monolithic capillary. To investigate the reproducibility of the column preparation process of intra-batch and inter-batch, RSDs of the extracted amounts for analytes were calculated. The intra-batch RSDs for Tartrazine, Sunset Yellow, Allura Red, and Azorubine were 0.7%, 1.5%, 4.3%, and 1.6%, respectively, calculated by five extractions of 1.0 μg mL^{-1} synthetic food dyes using five columns synthesized in one batch.

Table 2
Recovery values (%) for soft drink samples ($n=3$)[a].

Samples	Analytes	Found ($\mu g\,mL^{-1}$)	Recovery mean (%) ± SD	
			Level 1	Level 2
Carbonated beverage A	Tartrazine	3.30 ± 0.01	98.4 ± 3.8	96.4 ± 4.6
	Sunset Yellow	nd	106.6 ± 2.3	92.3 ± 4.8
	Allura Red	1.60 ± 0.03	100.5 ± 3.0	100.4 ± 0.1
	Azorubine	nd	97.3 ± 2.8	108.4 ± 3.1
Carbonated beverage B	Tartrazine	10.31 ± 0.01	96.3 ± 1.4	94.9 ± 6.3
	Sunset Yellow	17.10 ± 0.05	93.4 ± 0.7	103.0 ± 3.7
	Allura Red	nd	103.9 ± 1.2	102.7 ± 1.8
	Azorubine	nd	104.1 ± 2.1	100.2 ± 3.4
Carbonated beverage C	Tartrazine	2.51 ± 0.02	97.3 ± 0.5	104.0 ± 1.7
	Sunset Yellow	nd	106.4 ± 0.9	100.7 ± 2.5
	Allura Red	nd	107.1 ± 2.3	107. 6 ± 3.7
	Azorubine	nd	105.6 ± 1.6	102.5 ± 4.1
Carbonated beverage D	Tartrazine	4.32 ± 0.03	104.1 ± 0.7	98.9 ± 3.0
	Sunset Yellow	nd	98.0 ± 1.9	104.9 ± 3.7
	Allura Red	nd	105.8 ± 3.8	107.9 ± 3.3
	Azorubine	nd	93.3 ± 2.1	103.7 ± 4.1
Carbonated beverage E	Tartrazine	nd	93.8 ± 4.2	90.4 ± 2.7
	Sunset Yellow	27.75 ± 0.13	97.3 ± 3.3	96.7 ± 4.5
	Allura Red	nd	100.1 ± 2.6	107.1 ± 3.1
	Azorubine	nd	92.6 ± 4.4	98.8 ± 4.7
Carbonated beverage F	Tartrazine	1.20 ± 0.01	108.7 ± 2.6	95.6 ± 3.5
	Sunset Yellow	nd	104.7 ± 1.4	92.3 ± 4.8
	Allura Red	nd	109.2 ± 0.7	100.4 ± 3.7
	Azorubine	nd	107.8 ± 1.5	108.4 ± 3.1
Carbonated beverage G	Tartrazine	4.65 ± 0.02	99.3 ± 1.2	101.6 ± 0.7
	Sunset Yellow	13.33 ± 0.07	101.5 ± 0.8	104.9 ± 0.9
	Allura Red	nd	102.0 ± 1.7	103.2 ± 3.7
	Azorubine	nd	106.8 ± 1.2	102.5 ± 2.3
Fruit juice A	Tartrazine	nd	103.4 ± 1.6	103.9 ± 0.9
	Sunset Yellow	2.42 ± 0.09	105.6 ± 2.7	104.2 ± 2.5
	Allura Red	nd	93.5 ± 1.3	104.5 ± 1.9
	Azorubine	nd	101.4 ± 0.8	102.9 ± 2.3
Fruit juice B	Tartrazine	1.30 ± 0.01	98.1 ± 4.4	101.5 ± 2.7
	Sunset Yellow	1.40 ± 0.02	100.4 ± 3.3	98.7 ± 1.7
	Allura Red	nd	94.2 ± 1.6	96.7 ± 1.2
	Azorubine	nd	97.5 ± 3.1	104.2 ± 1.9
Fruit juice C	Tartrazine	nd	96.6 ± 2.7	93.7 ± 3.4
	Sunset Yellow	nd	97.4 ± 1.5	104.1 ± 2.8
	Allura Red	nd	99.2 ± 3.6	103.5 ± 3.3
	Azorubine	0.80 ± 0.14	104.1 ± 1.8	99.5 ± 0.1

nd: not detected. Level 1 and Level 2 were shown in Fig. 8.

[a] Extraction conditions and HPLC conditions were outlined in Section 2.

The inter-batch RSDs were 1.2%, 1.7%, 3.4%, and 5.1%, respectively, calculated by five extractions of 1.0 $\mu g\,mL^{-1}$ mixture sample solution using five columns synthesized in five batches. Results demonstrated good reproducibilities of monolithic capillary preparation. In order to examine the stability of the columns, the extraction efficiencies were confirmed within continuous 70 days of operation. As a result, no apparent extraction efficiency decrease was found, indicating that the multiple use of the monolithic column is practicable.

3.5. Analysis of real samples

Under the optimized conditions, the PMME method was applied to the determination of Tartrazine, Sunset Yellow, Allura Red, and Azorubine in several soft drinks (seven carbonated beverage samples and three fruit juice samples) from local supermarkets. All the real samples were spiked with the four synthetic food dyes standard solutions at two concentration levels to assess the matrix effects, Level 1 (1.0 $\mu g\,mL^{-1}$ for each synthesized food dye) and Level 2 (2.0 $\mu g\,mL^{-1}$ for each synthesized food dye). Unspiked samples were also analyzed. All samples obtained were analyzed in three replicates. The recoveries were calculated and listed in Table 2. Typical chromatograms of unspiked and spiked samples were shown in Fig. 8. Satisfactory recoveries were obtained in the range of 90.4–109.2%, exhibiting that the present method is effective for the determination of Tartrazine, Sunset Yellow, Allura Red, and Azorubine in soft drink samples.

4. Conclusions

A poly(NIPAAm-*co*-MBAAm) monolithic column embedded with γ-alumina nanoparticles was successfully synthesized and introduced to the PMME-HPLC system as the extraction medium. Thanks to γ-alumina nanoparticles dispersed in binary porogens (DMSO and dodecanol) and polymermodifier (PEG), we obtained the column bed with good permeability and high loading capacity. Furthermore, the study manifested good synthesis reproducibility and extraction stability of the monolithic column. The column was successfully applied to the extraction of Tartrazine, Sunset Yellow, Allura Red, Azorubine in several soft drink samples. Effects of sample pH, sample flow rate, sample volume, and eluent flow rate were investigated to obtain the optimum experimental conditions. Validation parameters of the present method, including LODs, LOQs, and precisions, are satisfactory for the determination of the target analytes. Based on the present work, the method was proved to

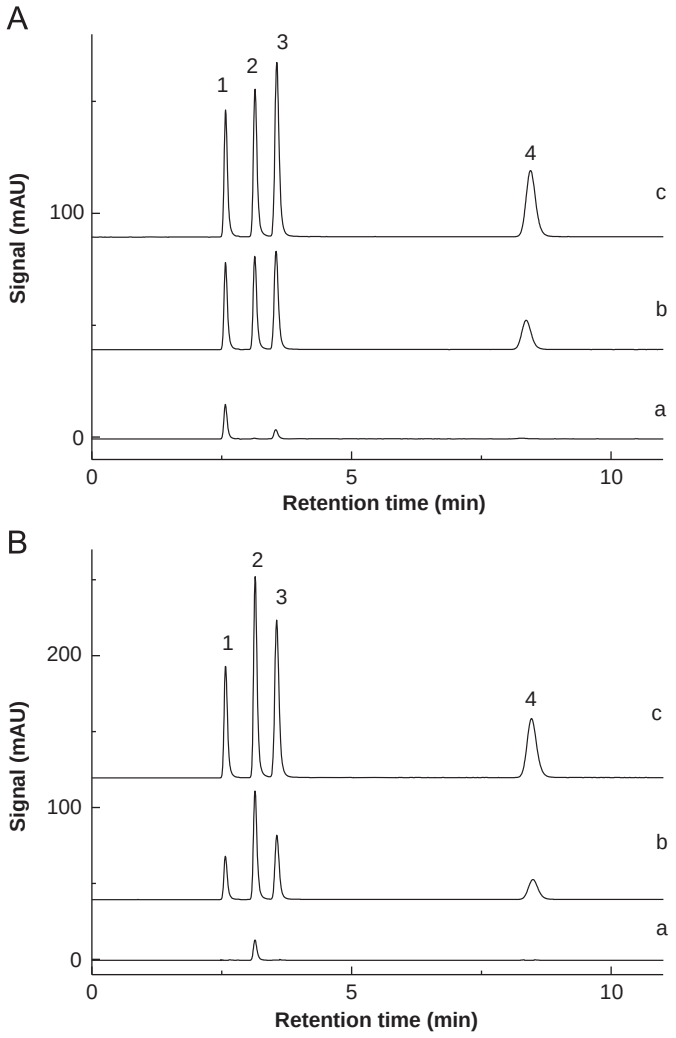

Fig. 8. Chromatograms of soft drink samples obtained by PMME procedures. (A) Carbonated beverage A; (B) Fruit juice A. (a) Unspiked soft drink sample, (b) Soft drink sample spiked with Level 1, (c) Soft drink sample spiked with Level 2. The samples were spiked at 1 μg mL^{-1} (Level 1); 2 μg mL^{-1} (Level 2) (Tartrazine, Sunset Yellow, Allura Red, Azorubine). Peak: 1–Tartrazine, 2–Sunset Yellow, 3–Allura Red, 4–Azorubine. HPLC conditions were outlined in Section 2. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

be simple, rapid, sensitive, and selective for the determination of synthetic food dyes in soft drink samples.

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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.2012.10.065.

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