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Analytical Methods

Molecularly imprinted solid-phase extraction coupled to liquid chromatography for determination of Sudan dyes in preserved beancurds

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ABSTRACT

New molecularly imprinted microspheres synthesized by suspension polymerisation using phenylamine and naphthol as mimic template were successfully applied as selective sorbents for the solid-phase extraction used for the simultaneous determination of four Sudan dyes from preserved beancurd products. The obtained imprinted microspheres showed good recognition and selectivity to the four Sudan dyes in aqueous solution and the affinity could be easily controlled by adjusting the property of the solution. Under the selected experimental condition, the recoveries of the Sudan dyes in preserved beancurds at three spiked levels were ranged between 90.2–104.5% with the relative standard deviation of less than 6.8%. The limit of detection (LOD) and limit of quantification (LOQ) based on a signal-to-noise of 3 and 10 were in the range of 0.005–0.009 μg g^{-1} and 0.015–0.030 μg g^{-1}, respectively. Comparing with alumina and C_{18}-based extraction, the selectivity and repeatability of molecularly imprinted solid-phase extraction (MISPE) were obviously improved. This method could be potentially applied for the determination of Sudan dyes in complicated food samples.

1. Introduction

The Sudan dyes used in this rationale were as follows: Sudan I (1-[(2,4-dimethylphenyl)azo]-2-naphthalenol), Sudan II (1-(phenylazo)-2-naphthol), Sudan III (1-(4-phenylazophenylazo)-2-naphthol), and Sudan IV (o-tolyazo-o-tolyazo-betanaphthol). The Sudan dyes are a family of azo-aromatic compounds that are widely used as colouring agents in chemical industries for oils, plastics, waxes, petrol, printing floor polishing, and spirit varnishing, etc. These dyes are categorised as class 3 carcinogens by the International Agency for Research on Cancer (IARC) and considered as a possible genotoxic carcinogen and mutagen to human (Rebane, Leito, Yurchenko, & Herodes, 2010; Xu, Heinze, Paine, Cerniglia, & Chen, 2010). Unfortunately, as the Sudan dyes prolong the appearance of natural red hues in some food additives, they are still illegally utilised by some merchants, for the sales of chilli powders, relishes, chutneys, seasonings, sauces, and ready meals. (Calbiani et al., 2004; He et al., 2007; Pardo, Yusàa, Leóna, & Pastor, 2009). Therefore, with respect to the illegal uses of Sudan dyes, it is imperative to develop a fast and sensitive analytical method for identification and quantification of such compounds in foodstuffs.

Several analytical methods such as liquid chromatography (López-Jiménez, Rubio, & Pérez-Bendito, 2010), voltammetric techniques (Lin, Li, & Wu, 2008), capillary electrophoresis (Mejia, Ding, Mora, & Garcia, 2007), immunoanalysis (Wang et al., 2009), and chemiluminescence analysis (Zhang, Zhang, & Sun, 2006) have been developed for the determination of Sudan dyes in different foodstuffs. Although these methods had been successfully applied for the analysis of Sudan dyes residues in food products at trace levels, they suffered from tedious procedure, time consumption or high cost. So a simple and effective sample pretreatment procedure is still desired. Until now, the most widely used sample pretreatment techniques are liquid–liquid extraction (Zhang et al., 2006) and solid-phase extraction (SPE) (Xu, Wang, Fang, Song, & Zhang, 2010). Recently, some new extraction methods, such as liquid phase microextraction (Arce, Nozal, Simonet, Valcárcel, & Ríos, 2009), pressurised liquid extraction (Pardo et al., 2009), dual solvent-stir bars microextraction (Yu, Liu, Lan, & Hu, 2008), ionic liquids extraction (Fan et al., 2009), and matrix solid-phase dispersion (Hou, Li, Cao, Zhang, & Wu, 2010) have been developed. Although each methods above has its advantage, but they all suffer from low selectivity, especially for low levels of analytes in complex samples.

Molecular imprinting is a rapidly developing technique for the preparation of polymers having specific molecular recognition properties (Mahony, Nolan, Smyth, & Mizaikoff, 2005). In recent years, molecularly imprinted polymers (MIPs) have appeared as new selective sorbents for SPE of organic compounds in complex materials (Qiao, Sun, Yan, & Row, 2006). The potential value of MISPE lies in the ability of selectively isolating specific compounds or their structural analogues from a complex matrix. The application of these synthetic polymers as sorbents allow the analytes of interest to be preconcentrated while simultaneously removing

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interfering compounds from the matrix so that selective enrichment and cleanup are obtained, resulting in a higher accuracy and a lower detection limit in the subsequent analysis (Zhang, Zhang, Hu, & Yao, 2010). Another advantage of MISPE is that it can be reused many times without losing its high selectivity. Consequently, this method has been widely used in biological, pharmaceutical, and environmental analysis. Recently, several works about extraction and determination of Sudan dyes by MIPs using Sudan I as template had been reported (Puoci et al., 2005; Zhao, Zhao, Liu, & Zhang, 2010). However, the template leaking was often observed in its real applications, which affected the results of quantitative analysis.

The objective of this study was to synthesise specific imprinted microspheres by suspension polymerisation using phenylamine and naphthol as mimic template to overcome the drawbacks of template leakage and to use this material as special SPE sorbent for the selective extraction and quantitative determination of four kinds of Sudan dyes in preserved beancurd products. The factors affecting the extraction and separation of the analytes were discussed in detail and the applicability of this method was evaluated. The proposed method could be applied for the pretreatment and determination of Sudan dyes in complicated food samples.

2. Materials and methods

2.1. Chemicals and materials

Sudan I, II, III, and IV were obtained from Fuchen Chemical Co. Ltd. (Tianjin, China) and their molecular structures are shown in Fig. 1. Phenylamine, 2-naphthol, polyvinylpyrrolidone (PVP), chloroform, dichloroethane, and hexane were obtained from Huaxin Chemical Reagent Co. (Baoding, China). Methacryclic acid (MAA) and 2,2-azobisisobutyronitrile (AIBN) were purchased from Kermel Chemical Reagents Co. (Tianjin, China). Ethylene glycoldimethacrylate (EGDMA) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Acetone, methanol, acetic acid, and methanoic acid were purchased from Huadong Chemical Reagent Co. (Tianjin, China). Double deionized water was filtered with 0.45 μm filter membrane before use.

2.2. Apparatus

HPLC analysis was performed using a LC-20A system equipped with two LC-20AT Solvent Delivery Units, a SUS-20A gradient controller, and a SPD-20A UV–VIS Detector (Shimadzu, Kyoto, Japan). An N-2000 data workstation (Zheda Zhineng Co. Ltd., Hangzhou, China) was used as a data acquisition system. The analytical column (150 mm × 4.6 mm I.D., C_{18}, 5 μm) was purchased from RStech Co., Korea. The mobile phase was methanol–methanoic acid (99.7:0.3, v/v) and its flow rate was set at 1.0 mL min^{-1}. The detection wavelength of the detector was set at 475 nm. All of the glassware for preparation of the samples and standard solutions were washed with deionized water and acetone and then dried at room temperature.

2.3. Synthesis of the imprinted microspheres

The imprinted microspheres were prepared by suspension polymerisation as follows: phenylamine (0.18 mL), 2-naphthol (0.29 g), MAA (0.5 mL) were dissolved in chloroform (20 mL) and EGDMA (9.4 mL), AIBN (200 mg) were added into the mixture solution and sonicated for 5.0 min to make it fully dissolved. Three grams of polyvinylpyrrolidone was dissolved into 120 mL of water by stirring at 60 °C in a 250 mL flanged reactor flask and then the organic mixture was admitted into the flask at 500 rpm under a gentle stream. Finally, the polymerisation took place in a water bath at 60 °C for 24 h. After polymerisation, the solution was filtered and the imprinted microspheres were washed with methanol–acetic acid (9:1, v/v) to remove templates and monomers. Non-imprinted microspheres (NIP) was prepared and treated in an identical manner.

2.4. The procedure of MISPE

Five hundred milligrams of dry imprinted microspheres were packed into empty polypropylene cartridges (60 mm × 10 mm) with two glass–wool frits at each end. The cartridges were pretreated with 5.0 mL of chloroform and methanol, followed by loading 2.0 mL of sample solution. Then the SPE cartridges were washed by 3.0 mL of water–methanol (8:2, v/v) and eluted by 5.0 mL of chloroform–acetic acid (95:5, v/v). The eluents were evaporated to dryness under vacuum at 25 °C, and the residues were reconstituted with mobile phase to 0.5 mL for subsequent HPLC analysis.

2.5. Adsorption capacity of the MIP, NIP, C18, and alumina

To investigate the adsorption capacity of the molecularly imprinted microspheres (500 mg packing), four kinds of Sudan dyes were dissolved into methanol to make the concentrations of 2.0 μg mL^{-1}. The MISPE cartridges were conditioned with 5.0 mL of methanol and followed by loading of various amounts of (0.5–6 mL) sample solution. Then it was washed by 3.0 mL of water–methanol (8:2, v/v) and eluted by 5.0 mL of chloroform–acetic acid (95:5, v/v). The eluents were evaporated to dryness under vacuum

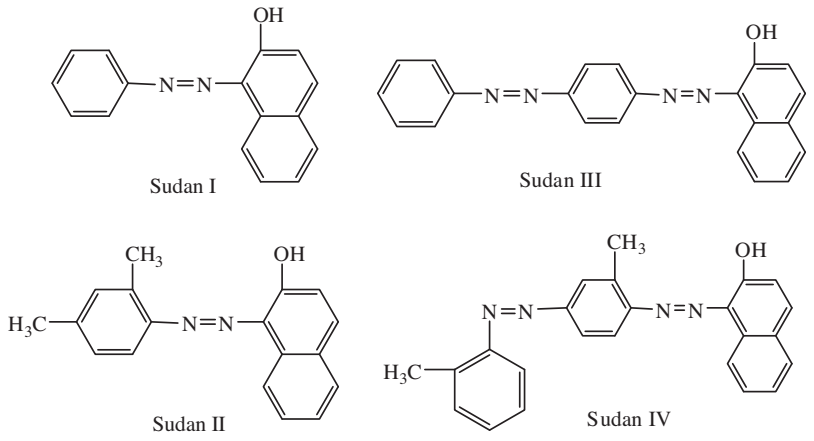

Fig. 1. The chemical structures of the four Sudan dyes.