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Analytical Methods

Crown ether microfunctionalized carbon nanotubes for dispersive micro-solid-phase extraction of sudan dyes and their metabolites

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ABSTRACT

In this study, dispersive micro solid phase extraction (DMSPE) combined with ultrahigh-performance liquid chromatography coupled with quadrupole time-of-flight tandem mass spectrometry method was established to extract and determine sudan dyes and their metabolites in real samples. The crown ether microfunctionalized multi-walled carbon nanotubes (MWCNTs) was applied as the sorbent in DMSPE procedure. Several experimental parameters that can effect the extraction performance of the DMSPE method were investigated separately by a univariate method. The validation data showed that the limits of detection were in the range of 0.084–13.13 μg/kg, the mean recoveries were ranged from 80.15 to 103.58% for six samples. Compared with other published methods, the proposed method was more effective, more time-saving and more eco-friendly. Finally, the developed method was successfully applied to enrich and detect sudan dyes and their metabolites in real samples.

1. Introduction

Carbon nanotubes (CNTs), the important one-dimensional tubular carbon materials, have become one of the most attractive nanomaterials due to their unique structure and outstanding properties such as high surface-to-volume ratio, highly porous nature, hollow structures, high mechanical strength and stiffness (Mittal, Dhand, Rhee, Park, & Lee, 2015). Recently, CNTs were considered as promising materials in adsorption technology for the use as adsorbent since they can interact with lots of analytes through hydrophobic interaction, electrostatic forces, dipole–dipole interaction, hydrogen bond, and π-π stacking (Popov, 2004). However, the raw CNTs are hard to disperse in solvents due to their strong tendency to spontaneously agglomerate as a result of the Van der Waals forces between the nanotubes which limit their applications. Therefore, various functionalization methods for improving the properties and dispersibility of CNTs have been developed. Modified CNTs can be prepared trough covalent and non-covalent modifications (Meng, Fu, & Lu, 2009). The covalent functionalization is commonly performed through an oxidation step by using strong acids and ultra-sonication pretreatments, these treatments may introduce considerable defects and damage to the side walls which can destroy the perfect structure and physical properties of CNTs (Meng et al., 2009; Rossell et al., 2013). Whereas, the non-covalent functionalization is carried out by coating CNTs with polymers or surfactant molecules using various adsorption forces (e.g. van der Waals force, electrostatic forces, hydrogen bonds, and π-stacking interactions) (Lefrant et al., 2004; Nakayama-Ratchford, Bangsaruntip, & Sun, 2007; Yu, Huang, Liu, & Tang, 2008). Besides, the non-covalent functionalization can be achieved under mild reaction conditions so the structure and physical properties of CNTs remain unaltered (Meng et al., 2009). At present, several non-covalent modified CNTs had been prepared such as congo red (Jagusiak et al., 2017), gemini surfactant (Dobies et al., 2017), Ce $(H_2O)_3[15\text{-}MC_{Cu(II)Phalaha}\text{-}5]C_{13}$ (Katkova et al., 2017), β-cyclodextrin (Na et al., 2006), 1-aminoethyl-3-methylimidazolium bromide (Zhou et al., 2009) and carboxymethyl cellulose (Han, Liu, Zhao, Cai, & Zhao, 2017) functionalized CNTs. However, these non-covalent functionalization methods needed a large number of functional materials and long functionalization time, and the operation was often complicated. Therefore, it is necessary to develop an easy operating, time-saving and effective non-covalent functionalization method.

Dispersive solid-phase extraction (DSPE), first introduced by Anastassiades and co-workers (Anastassiades, Lehotay, Stajnbaher, & Schenck, 2003), is a sample pretreatment technique with various advantages such as simplicity, high enrichment factor, high extraction efficiency, high selectivity, short time requirement and consumption of small volumes of organic solvent (Basheer, Alnedhary, Rao, & Lee,

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2009; Pardasani, Kanaujia, Purohit, Shrivastava, & Dubey, 2011). Dispersive micro-solid phase extraction (DMSPE) was a miniature model of DSPE by using micro amounts of the adsorbed phase, which was reported by Tsai and his coworker (Tsai, Chuang, et al., 2009; Tsai, Huang, Huang, Hsue, & Chuang, 2009). In DMSPE process, sorbent material is directly added to a sample solution, the adsorption is achieved within extremely short time due to the large surface area between sorbent and aqueous phase (Furusawa, 2011). Then, the sorbent material is isolated by centrifugation or filtration, the target analytes are eluted by an appropriate desorption solvent. Compared with DSPE, DMSPE has various advantages: shorter time requirement, simpler operation, smaller quantity of organic solvent and sorbent. To choose an appropriate adsorbent material is a key point of DMSPE technique, since it can significantly impact the selectivity and the enrichment efficiency for target analytes. At present, various materials had been used as adsorbent in DMSPE, such as microcrystalline cellulose (Cao, Peng, Du, Zhang, & Xu, 2017), graphitized multi-walled carbon nanotubes (Wang et al., 2015), mesoporous carbon (Yahaya et al., 2015), Iron oxide functionalized graphene oxide (Kazemi et al., 2016), and nanoparticles (Khezeli, & Daneshfar, 2017). However, until now, there are no reports on crown ether coated multi-walled carbon nanotubes (MWCNTs) as a sorbent material for DMSPE process. Particularly, it is a meaningful to develop functionalized materials as a sorbent for the determination of colorants, due to the illegal use of dyes is considered to be unsafe for public health.

Since natural dyes in foods are unstable and altered rapidly during food processing and storage, synthetic dyes with various advantage such as low cost, easily availability and excellent stability are widely used in food products to enhancing the aesthetical appeal of foods (Vachirapatama, Mahajaroensiri, & Visessanguan, 2008). The synthetic azo dyes, such as the commonly used Sudan dyes, have been widely used as coloring agents in many chemical industries and daily products, like coloring agents in plastics, waxes, petrol, shoe polishes, and printing inks. The International Agency for Research on Cancer (IARC) classified Sudan dyes as category 3 carcinogen to humans, and the metabolites of sudan dyes (*ortho*-aminoazotoluole and 4-aminoazobenzene) have been classified as category 2 by IARC (Rebane, Leito, Yurchenko, & Herodes, 2010). Thus, Sudan dyes have been forbidden as feed additives in any national and international food regulation act (Piątkowska, Jedziniak, Olejnik, Żmudzki, & Posyniak, 2018). To ensure the health of consumers, a reliable, sensitive and high-efficiency analytical method for the determination and quantitation of sudan dyes and their metabolites in food products is urgently required. In the literature, sonication combined with liquid chromatography-tandem mass spectrometry (LC-MS/MS) (Tsai, Kuo, & Shih, 2015), blended with homogenizer combined with gel permeation chromatography-liquid chromatography-tandem mass spectrometry interfaced with electrospray ionization (GPC-LC-ESI-MS/MS) (Sun, Wang, & Ai, 2007), liquid–liquid extraction combined with LC-MS/MS (Piątkowska, Jedziniak, & Żmudzki, 2014), ultrasound-assisted extraction (UAE) combined with LC-MS/MS (Chen et al., 2013), pressurised liquid extraction combined with liquid chromatography with electrospray ionization-tandem mass spectrometry (LC-ESI-MS/MS) (Pardo, Yusà, León, & Pastor, 2009), shake and oscillation combined with liquid chromatography/time-of-flight mass spectrometry (LC-TOF-MS) (Cui et al., 2010), liquid–liquid extraction combined with ultra performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) (Schummer, Sassel, Bonenberger, & Moris, 2013), on-line SPE combined with ultrahigh performance liquid chromatography ultraviolet–visible detection (UHPLC-UV) (Khalikova, Šatínský, Šmidrkalová, & Solich, 2014) and molecularly imprinted solid-phase extraction (MISPE) combined with high performance liquid chromatography ultraviolet–visible detection (HPLC-UV) (Qiao, Geng, He, Wu, & Pan, 2011) have been used in the extraction and determination of dyes in food products. However, these methods all have some shortcomings such as complicated operation, long time requirement and large quantity of organic solvents. Therefore, it is necessary to develop a simple, time-saving and sensitive methods to extract, identify and quantify the illegal dyes in complex matrices.

In this study, the crown ether modified MWCNTs prepared by microfunctionalization process was applied as sorbent in DMSPE procedure to preconcentrate the sudan dyes and their metabolites in salted duck egg yolk and tomato sauce. Utilizing non-covalent to coat the crown ether onto the MWCNTs surface lead to minimization of the surface tension realized mainly by van der Waals and π–CH interactions between the surface of MWCNTs and modified the wettability and surface charge, which result in efficient dispersibility of MWCNTs in water. UHPLC-Q-TOF/MS was used to identify and quantify the target analytes. Six compounds including p-aminoazobenzene, sudan orange G, o-aminoazotoluene, sudan II, sudan III and sudan red 7B were selected as target analytes to evaluate the extraction performance of this method. Several parameters that can influence the performance of the method including the type and concentration of sorbent, agitation time and the type of elution solvents were investigated and optimized. The validation of the method was performed in terms of linearity, precision, limits of detection and quantification, reproducibility, and accuracy. Finally, the developed method was successfully used to preconcentrate and identify sudan dyes and their metabolites in real samples.

2. Experimental

2.1. Reagents and materials

Benzo-15-crown-5, 18-Crown-6, 15-Crown-5 and 2-(Hydroxymethyl)-12-crown-4 were provided by Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). The MWCNTs (1–3 nm ID, 3–20 nm OD, 0.1–10 μm long) was purchased from Alfa Aesar China (Tianjin, China). Chromatography-grade methanol, ethanol, acetonitrile and formic acid were supplied by Tedia Company Inc. (Fairfield, US). HPLC grade ethyl acetate was provided by Tianjin Siyou Fine Chemical Co., Ltd. (Tianjin, China). Chloroform (analytical grade) was obtained from Quzhou Juhua Reagent Co. Ltd (Quzhou, China). The pure water used in the this study was supplied by Hangzhou Wahaha Group Co., Ltd. (Hangzhou, China). The 0.22 μm disposable nylon membranes were purchased from Jinteng Laboratory Equipment Co., Ltd. (Tianjin, China). Standards of p-aminoazobenzene, sudan orange G, o-aminoazotoluene, sudan II, sudan III and sudan red 7B were obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China), their structures were shown in Fig. S1 (see Supplementary material). The standard stock solutions were prepared by dissolving each analyte in chromatographic grade ethanol at a concentration of 100 μg/mL. The working solutions were prepared by diluting the stock solutions with ethanol. Samples of salted duck egg yolk, tomato sauce, chili sauce, hot pot sauce, protein and ham sausage were purchased from a local supermarket (Hangzhou, China).

2.2. Instrumental conditions

The analytes were detected and identified on an Agilent 1290 UHPLC system coupled with a Q-TOF mass spectrometry (Agilent Technologies, Santa Clara, CA). The chromatographic separation was carried out on an Agilent SB-C_{18} column (1.8 μm, 50 mm × 4.6 mm i.d.) maintained at 25 °C. The mobile phase consisted of 0.1% formic acid in water (A) and methanol (B), the elution gradient was as follows: 0–2 min, 20–40% B; 2–3 min, 40–70% B; 3–4 min, 70–70% B; 4–10 min, 70–100% B; 10–11 min, 100–100% B. The flow rate was maintained at 0.4 mL/min, and the injection volume was set at 2 μL. A 6530 Q-TOF MS equipped with a ESI source was used for identification of target analytes by operating in the positive ion mode. The mass spectrometric data was collected from *m/z* 100 to 1000. The source parameters were as follows: capillary voltage, 3500 V; drying gas temperature, 350 °C; nebulizer pressure, 35 psi; drying gas flow, 12 L/min; fragmentor