Development of Fe$_3$O$_4$-modified-carboxylic MWCNTs for extraction and determination of pigments in beverage

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Abstract. A nanomaterial comprising Fe$_3$O$_4$-modified-carboxylic multi-walled carbon nanotubes (Fe$_3$O$_4$-MWCNTs-COOH) was prepared by a co-precipitation method. Combined with HPLC-photodiode array detector (DAD), Fe$_3$O$_4$-MWCNTs-COOH was used to determine pigments in beverage. Some important parameters that could influence extraction efficiency were optimized. The results indicated that Fe$_3$O$_4$-MWCNTs-COOH combined with HPLC-DAD is a promising solid-phase extraction material for the sample pretreatment in the determination of pigments.

1. Introduction

Edible pigment is a broad category of the most common food additives, which can improve the sense of food and stimulate consumers’ purchasing desire. Many foods suffer from oxidation, fading and other phenomena in the process of manufacturing, transportation or storage, which seriously affecting their appearances. Therefore, it is essential to add the pigments to regulate and control this problem [1]. Many countries have made corresponding regulations and strict provisions on the amount and type of synthetic pigments added to food [2]. Up to now, many analytical techniques have been developed for the determination of the edible pigments, such as high performance liquid chromatography (HPLC) [3], SPE combined UV-visible spectrophotometry [4], liquid chromatography/tandem mass spectrometry (LC-MS) [5] and so on.

In this study, Fe$_3$O$_4$ magnetic nanoparticles (MNPs) were prepared by the classical liquid phase synthesis-chemical precipitation method, and the carboxyl modified multi-walled carbon nanotubes (MWCNTs) were magnetically functionalized with the MNPs [6]. The obtained magnetic material was used to adsorb pigment molecules in the beverage samples. After desorption, HPLC-PAD was used for qualitative and quantitative analysis of the sample solution.

2. Experimental methods

2.1. Reagents and materials

Ferric chloride was purchased from Tianjin No.3 Chemical Reagent Factory (Tianjin, China). Ferrous chloride was purchased from Fuchen Chemical Reagents Factory (Tianjin, China). Ammonium hydroxide was provided by Xi’an SanPu Chemical Reagent Co., Ltd. (Shaanxi, China). Acetic acid was bought from Tianjin Beilian Fine Chemicals Development Co., Ltd. (Tianjin, China). All the above reagents are of analytical grade purity. Acetonitrile and methanol (HPLC grade) were supplied by Guanghua SciTech Co., Ltd. (Guangzhou, China). Carboxylated MWCNTs (o.d., 30-50 nm; -COOH content, 1.06 wt%; length, ~20 μm; purity >95%) were purchased from Chengdu Organic
Chemical Co., Ltd. (Chengdu, China). The beverage (orange Fenda) was obtained from Shaanxi Taigu Coca Cola Beverage Co., Ltd. (Shaanxi, China). Ultrapure water was prepared by an ultra-purification system. Food additives Sunset yellow and Tartrazine were from Shanghai Dyestuff Research Institute Co., Ltd. (Shanghai, China).

2.2. Instrumentation
The HPLC analysis was performed in a WATERS Series (WATERS Technologies, Milford, MA) LC system, which was equipped with an e2695 Alliance Quaternary Pump, a 2998 Photodiode-Array Detector (PAD), an Alliance Col Heater column oven and an automatic sampler. The system was controlled by an Empower 2 Personal Single System. Chromatographic separation was performed with an Innvoal ODS-2C18 reverse separation column (5 μm, 250 mm × 4.6 mm) (Tianjin Agela Technologies Co., Ltd., Tianjin, China). Transmission electron microscopy (TEM) images were taken on a Hitachi H-7650 transmission electron microscope (Hitachi, Japan). The PALL ultrapure water systems were supplied by ELGA Lab Water Instrument Co., Ltd. (Bucks, UK).

2.3. Preparation of Fe₃O₄ Nanoparticles
Firstly, 0.1257 g ferric chloride hexahydrate and 0.0470 g ferrous chloride tetrahydrate were accurately weighed into a 250 mL three-necked flask containing 80 mL of deoxygenated water followed by continuously stirring to dissolution. When the temperature of the solution reached 80°C, dropwise added 60 ml of 5% ammonium hydroxide with intensive stirring for 60 min. The oxygen must be prevented from the entering system during the whole reaction process. After the reaction was completed, Fe₃O₄ deposition was separated by an external magnetic field and washed with deoxygenated water until the pH value was nearly neutral. After static settlement, the supernatant was decanted. The target product Fe₃O₄ nanoparticles were obtained after dried at room temperature. The optimized chemical coprecipitation method was used to prepare magnetic multi-walled carbon nanotubes.

2.4. Preparation of Magnetic Multi-walled Carbon Nanotubes
Firstly, 0.1257 g ferric chloride hexahydrate and 0.0470 g ferrous chloride tetrahydrate were added into a 250 mL three-necked flask containing 80 mL of deoxygenated water, and were continuously stirred to dissolution. Then 0.2041 g MWCNTs-COOH was added to the above solution. When the temperature of the solution reached 80°C, dropwise added 60 ml of 5% ammonium hydroxide with intensive stirring for 60 min. Oxygen must be prevented from entering the system during the whole reaction process. After the reaction was completed, the sediment Fe₃O₄-MWCNTs-COOH was separated by an external magnetic field, washed with deoxygenated water, and the pH value was detected to be about 7 after static duration. By this time, the supernatant was poured out, dried and ground at normal temperature to obtain the magnetically functionalized multi-walled carbon nanotube.

2.5. Experimental Process
Pigment extraction was completed in four steps: extraction, separation, desorption and analysis.

Extraction. Firstly, 20 mg of magnetic MWCNTs-COOH solid powder were accurately measured as the adsorbent, and then 4 mL of beverage sample solution were added and shaken for about 30 s. The magnetic carbon nanotube particles were uniformly dispersed in the solvent. After being shaken gently for 25 min and then kept undisturbed for a period of time to achieve the balance of target analyte between magnetic multi-walled carbon nanotubes and the sample solution.

Separation and Desorption. A strong magnet was placed at the bottom of the micro centrifuge tube. Then magnetic MWCNTs-COOH and the sample solution were separated by an external magnetic field, and the supernatant was sucked off by a syringe. A proper amount of desorbent was added into the micro centrifuge tube for desorption. Then the desorption solution was collected.

HPLC analysis. The conditions of HPLC were set as follows: column temperature 30°C, mobile phase (methanol: water = 40:60), flow rate 1.0 ml/min⁻¹, wavelength 450 nm, injected volume 20 μL.
3. Results and discussions

3.1. TEM characterization
Transmission electron microscope (TEM) was used to characterize Fe₃O₄ nanoparticles, carboxylated multi-walled carbon nanotubes and carboxylated multi-walled carbon nanotubes modified by magnetic nanoparticles. Figure 1a showed that the Fe₃O₄ nanoparticles were spherical particles. It was observed that the carboxylated multi-walled carbon nanotubes were tubular substances with small diameter and long length, as shown in Figure 2b and 2c. The particle size distribution of Fe₃O₄ nanoparticles could be observed between 10 nm and 15 nm.

![Figure 1. TEM image of Fe₃O₄ nanoparticles (a), carboxylated multi-walled carbon nanotubes (b) and carboxylated multi-walled carbon nanotubes modified by magnetic nanoparticles (c).](image)

3.2. VSM characterization
The VSM hysteresis loops of pure Fe₃O₄ nanoparticles and magnetic carboxylated multi-walled carbon nanotubes were illustrated in Figure 2. The maximum saturation magnetization (Ms) of Fe₃O₄ nanoparticles and magnetic carboxylated multi-walled carbon nanotubes were 67.5 emu/g and 7.5 emu/g respectively, which proved that the carboxylated carbon nanotubes modified with Fe₃O₄ nanoparticles had certain paramagnetism and could be separated quickly by the external magnetic field. The maximum saturation magnetization of magnetic multi-walled carbon nanotubes was much lower than that of pure Fe₃O₄ nanoparticles, because the content of Fe₃O₄ nanoparticles attached to the surface of the modified multi-walled carbon nanotubes was very few. Consequently, this might reduce the magnetic characteristics of Fe₃O₄, but do not affect the magnetic utilization of the whole compound.

![Figure 2. VSM magnetization curves of pure Fe₃O₄ and Fe₃O₄-MWCNTs-COOH sorbents.](image)

3.3. Optimization of adsorption condition
In order to optimize the conditions for extraction of pigment from beverages by the magnetic material,
this study investigated the extraction efficiency under different conditions.

Effect of adsorbent dosage The amount of magnetic MWCNTs would have a greater impact on the extraction effect. Carboxylic radicals which generated after ionization of carboxylated MWCNTs could enhance the adsorption with pigment molecules in beverages by forming hydrogen bonds. Figure 3a showed the adsorption effect when the different amounts of adsorbent were used. When 10 mg adsorbent was used, the adsorption effect was the best.

Effect of extraction time Theoretically, the longer the adsorption time was, the more pigments would be obtained during the final desorption. However, the best adsorption time was 25 min (Figure 3b). It was likely because that after reaching the adsorption equilibrium, part of the adsorbed pigments were redissolve into the solution. Or the adsorbent did not have specificity for this substance. With the increase of adsorption time, other substances were adsorbed in large quantities, resulting in a decrease in the extraction content of the pigment.

Effect of desorption agent types After the adsorption, four commonly used organic solvents-methanol, acetonitrile, methanol/acetic acid (9:1) and acetonitrile/formic acid (9:1) were selected as the desorption agents. As shown in Figure 3c, methanol had the best desorption effect.

Effect of desorption agent dosage, time and number of desorption times Four dosages were investigated for the same desorbent. The result showed the amount of 2 mL desorbent had the best desorption effect (Figure 3d). Then, the desorption time was evaluated. It was clearly observed that the peak area was the largest when the desorption time was 20 min (Figure 3e). However, with the increase of desorption time, some of the desorbed pigments would be adsorbed again, which would lead to a decrease of adsorbed pigment contents. Three levels of desorption times were optimized. The result showed that almost all the pigments adsorbed on the magnetic MWCNTs can be desorbed at the first time (Figure 3f).

Determination of pigment content in beverages Under the optimal conditions, a series of analytical parameters were optimized, including linear range and correlation coefficients. The linear range of the calibration curve was 0-30 μg/mL both for Tartrazine and Sunset yellow; the correlation coefficients ($r^2$) were 0.9922 and 0.9190, respectively; the regression equation of Sunset yellow is $y=4615.5x+12985$; the curve regression equation of Tartrazine is $y=13010x+29754$. The results indicated that the developed analytical method was reliable.

The national standard of China (GB 2760-2007, "Hygienic Standard for the Use of Food Additives")
stipulates that the maximum usage limits of Tartrazine and Sunset yellow in food are 0.05 and 0.025 g/kg [7], respectively. The experimental result (Figure 4) showed that, the contents of two synthetic pigments in Fenda beverage are 0.0973 and 0.022 mg/kg, respectively, which are not beyond the national standard.

Figure 4. HPLC chromatogram of the extraction process: A-beverage sample without extraction; B-injected sample solution after extraction and desorption. a-Tartrazine; b-Sunset yellow.

4. Conclusion
In the present study, Fe$_3$O$_4$-MWCNTs-COOH was successfully developed as an ideal magnetic nanomaterial for solid phase extraction in beverage analysis. Under the optimized condition, the sorbents exhibited satisfactory affinity to target analyte. This proposed method also has several advantages, such as rapidity, simplicity, ease-of-use, and environmentally friendly. Therefore, Fe$_3$O$_4$-MWCNTs-COOH sorbents are potential materials for solid phase extraction in the sample pretreatment of pigment analysis in beverage sample.

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