Title:

PEGylation of magnetic multi-walled carbon nanotubes for enhanced selectivity of
dispersive solid phase extraction

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Abstract

Carbon nanotubes (CNTs) possess large potential as extraction absorbents in solid phase extraction. They have been widely applied in biomedicine research, while very rare application in natural product chemistry has been reported. In this work, methoxypolyethylene glycol amine (mPEG-NH₂) is covalently coupled to CNTs-magnetic nanoparticles (CNTs-MNP) to prepare a novel magnetic nanocomposite (PEG-CNTs-MNP) for use as dispersive solid-phase extraction (DSPE) absorbent. The average particle size was 86 nm, and the saturation magnetization was 52.30 emu/g. This nanocomposite exhibits excellent dispersibility in aqueous systems, high selectivity and fast binding kinetics when used for extraction of Z-ligustilide, the characteristic bioactive compound from two popular Asian herbal plants, *R. chuanxiong* and *R. ligusticum*. HPLC quantification of Z-ligustilide extracted from the standard sample solution showed a high recovery of 98.9%, and the extraction rate from the extracts of the above two herbs are both around 70.0%. To our knowledge, this is the first report on using PEG-CNTs-MNP as DSPE nanosorbents for selective extraction of natural products. This nano-material has promising application in isolation and enrichment of targeted components from complex matrices.

Keywords: Magnetic carbon nanotubes; PEGylation; Dispersive solid-phase extraction; Z-ligustilide; Herbal extracts

1. Introduction

Identification of the active constituents in herbal medicines and quality control of the herbs are critical for their preventive and therapeutic applications [1]. Many methods have been developed to isolate active compounds from natural resources. However, owing to the structural similarities and sometimes instability of the active constituents, it is important to find new approaches that are simpler, faster and more selective to separate the targeted compound from complex matrix in herbal decoction. In recent years, dispersive solid-phase extraction (DSPE) has been demonstrated to be a powerful technique to pre-concentrate analytes at trace levels in complex matrix due to its convenience and high selectivity in analytical processes [2-5]. *Rhizoma chuanxiong* and *R. ligusticum* are two well known medicinal plants which have been widely used in traditional Chinese medicines. Z-ligustilide is a major active component in the volatile oils of these plants [6, 7], which exhibits a wide range of pharmacological activities including vasorelaxation [8], analgesic [9], neuroprotection [10], anti-inflammatory activity [11, 12]. Owing to its availability and distinctive fragrance, Z-ligustilide has usually been chosen as chemical marker for the quality control of the related herbs [13-16].

The dihydrobenzene structure of Z-ligustilide is chemically active so that it can easily undergo oxidation, dimerization, isomerization, or rapid decomposition at relative high temperature. Sample handling is critical when analyzing and purifying this thermolabile compound [17]. Up to date, there have been many reports on analysis of Z-ligustilide, including HPLC/MS, GC/MS [6, 18], CCC/GC [19] and

HSCCC/preparative-LC [17]. These methods usually involved solid phase extraction. Since the extraction procedure exhibited low extraction specificity, hence matrix effects were persistent. Therefore, more specific sample preparation method is necessary for the separation and analysis of this important natural product.

Over the past several years, multi-walled carbon nanotubes (MCNTs) have attracted tremendous attention in various fields including biology and biomedicine due to their excellent mechanical, thermal, as well as electrical properties [20-25]. The nanoscopic dimensions, rigid platform and extremely large surface areas of MCNTs allow efficient loading of a variety of molecules, conferring them with great potential as extraction absorbents in solid phase extraction (SPE). Especially, the compounds possessing aromatic structure can be adsorbed onto the polyaromatic surface of MCNTs via π - π stacking interactions [26]. On the other hand, if using only carbon nanotubes in the SPE, time-consuming centrifugation is needed for the separation of the analytes and carbon nanotubes from the complex matrix. As magnetic separation technology has drawn wide attention in separation science [27], the MCNTs coupled with magnetic Fe_3O_4 nanoparticles (CNTs-MNP) will afford an excellent SPE absorbent. Recently, functionalized carbon nanotubes were used for pre-concentration of small molecules from human plasma [26, 28-29], drug delivery [30, 31], and medical bioimaging [32-34]. We recently found CNTs-MNP had good extraction ability for Z-ligustilide in *Angelica sinensis* [35], but the CNTs-MNP showed poor solubility and stability in aqueous solutions. In magnetic SPE, it is important for nanosorbents to disperse homogeneously in the sample solution for the quantification

of the target compounds. Introduction of hydrophilic moieties onto the CNTs' hydrophobic surface might be a solution to improve the solubility and biocompatibility of CNTs-MNP. Polyethylene glycol (PEG) compounds have been widely used in non-covalent modification of carbon nanotubes [26, 36-39]. Unfortunately, previous study showed that non-covalently adsorbed PEGs coatings decreased CNTs' dispersion stability and adsorption specificity for the analytes [40]. On the contrary, covalent functionalization of CNTs with amine-terminated PEG has been reported to be a promising and rational approach for the stable dispersion in various media [41-43]. Thus, the material combining the magnetic nature and the stability of the covalent PEG-CNT will result in an excellent absorbent for the DSPE of the target moleculars.

In this work, we prepared a novel PEG functionalized CNTs-MNP nanoparticles (PEG-CNTs-MNP) for the separation of *Z*-ligustilide from *R. chuanxiong* and *R. ligusticum* extracts. PEG-CNTs-MNP was characterized by transmission electron microscopy (TEM), vibrating sample magnetometer (VSM), cyclic voltammetry (CV) and differential pulse voltammetry (DPV). Dynamic light scattering (DLS) was used to evidence its excellent solubility and stable dispersion in water. The adsorption, separation, storage stability and regeneration properties of PEG-CNTs-MNP were evaluated by equilibrium binding assay. Furthermore, *Z*-ligustilide in herbal extracts has been extracted by DSPE with this material. To the best of our knowledge, this is the first attempt to synthesize a PEG functionalized CNTs-MNP for the enrichment and isolation of bioactive compounds from herbal extracts.

2. Experimental section

2.1. Materials

R. chuanxiong and *R. ligusticum* were obtained from Chengdu traditional Chinese medicinal plants market (Chengdu, China). MWCNTs with 20–40 nm in diameter were purchased from Shenzhen Nanotech Port Co., Ltd. (Shenzhen, China). Ferric chloride ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$), ethylene glycol, sodium acetate anhydrous, trisodium citrate were purchased from Chengdu Tianhua Chemical Technology Co., Ltd. (Chengdu, China). 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC·HCl), N-hydroxysuccinimide (NHS), Methoxy polyethylene glycol amine were purchased from Sigma-Aldrich Chemistry Co., Ltd. (United States). Z-ligustilide was supplied by Chengdu Must Bio-Technology Co., Ltd (Chengdu, China). Acetonitrile and methanol used for HPLC was of chromatographic grade (JT Baker, USA). Ultrapure water was produced by a Milli-Q (18.2 M Ω) system (Millipore, Bedford, MA, USA) was used in all the experiments. Other chemicals and solvents were of analytical grade.

2.2. Characterization of the nanocomposite.

The morphologies and sizes of magnetic nanoparticles were carried out by JEM-100CX high resolution transmission electron microscope (JEOL CO., Japan), SZ-100 dynamic light scattering (HORIBA CO., Japan), respectively. Magnetic properties were observed by a vibrating sample magnetometer (VSM) (MPMS-XL-7,

USA) at room temperature. Cyclic voltammetry (CV) and differential pulse voltammetry (DPV) characterization was performed with an Electrochemical Workstation (Gaoss Union EC550, China).

2.3. Preparation of PEG functionalized CNTs-MNP

2.3.1 Synthesis of oxidization of MWCNTs

Four hundred milligram of pristine multi-walled carbon nanotubes (MWCNTs) were dispersed in 50 mL of concentrated nitric acid and stirred under magnetic field at 60 °C for 7 h to obtain the oxidized CNTs. The oxidized CNTs were washed with copious amount of distilled water for several times until the pH value reached to neutral and dried by vacuum freeze dryer for further use.

2.3.2 Preparation of CNTs-MNP

Forty milliliters of ethylene glycol solution containing 200 mg of oxidized CNTs and 900 mg of $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ was constantly stirring for 2 h. Subsequently, 3.6 g of sodium acetate, 0.15 g of trisodium citrate, and 1.0 g of PEG2000 were added with stirring for 1 h. The mixture was sealed in a Teflon-lined stainless steel autoclave and maintained at 200 °C for 10 h. The CNTs-MNP were rinsed with water followed by ethanol for five times with the help of a permanent magnet and dried at 0 °C for 8 h.

2.3.3 Preparation of PEG-CNTs-MNP

The carboxyl groups at CNTs-MNP were firstly activated using EDC/NHS according to the method previously reported [43]. In brief, 15 mg of EDC·HCl and 21 mg of NHS were dissolved in 10 mL of MES buffer (50 mM, pH 6.5), to which 80 mg

of CNTs-MNP were added. The reaction was carried out at a water bath at 0 °C for 30 min to obtain an active ester. The CNTs-MNP with the active ester were collected by an external magnet and re-dispersed in 10 mL of PBS buffer (50 mM, pH 7.0) containing methoxy polyethylene glycol amine (2 kDa MW, mPEG-NH₂), and then shaken at room temperature for 12 h. The PEG-CNTs-MNP was magnetically separated and washed several times with pure water and finally re-dispersed in 8 mL of PBS buffer and stored at 4 °C before use.

2.4. HPLC analysis

Chromatographic analysis was performed by a Shimadzu HPLC system equipped with two LC-20AD pumps and UV-1800 spectrophotometer (Shimadzu, Kyoto, Japan). A Tianhe kromasil reversed-phase column (C18, 250 mm×4.6 mm, 5 µm particle) with a guard column (C18, 4.0 mm× 3.0 mm, 5 µm particle) at constant temperature (35 °C) and a detection wavelength (280 nm and 330 nm) was used for separation. Gradient elution of A: water with 0.1% formic acid and B: methanol, starting from 40–100% B in 23 min and 100% B for 23-28 min. Flow rate was set at 0.8 mL/min. HPLC-MS/MS measurements were obtained by a Thermo-Quest Finnigan LCQ^{DECA} system equipped with an electrospray ionization source (Thermo-Quest LC/MS Division). The mass spectrometry was operated in the positive mode and the detector parameters were set as follows: spray voltage was 4.5 kV; lens voltage was 18 V; capillary voltage was -13 V and capillary temperature was 300 °C; nebulizer sheath gas N₂ and nebulizer auxiliary gas N₂ flow rates of 80 and

20 units, respectively.

2.5 . Detection of Z-ligustilide in medicinal plants

2.5.1 Sample preparation

One gram of air-dried powdered *R. chuanxiong* and *R. ligusticum*, were extracted respectively with 40 mL of methanol by ultra sonication at room temperature for 30 min. The filtrate was evaporated to dryness and then dissolved in 40 mL of 50% methanol (named S₀) and stored in the dark at 4 °C until further tests.

2.5.2 DSPE of Z-ligustilide from extracts of *R. chuanxiong* and *R. ligusticum*

According to our previously reported method [44], DSPE based on PEG-CNTs-MNP coupled with HPLC-UV was developed to quantitative analyze Z-ligustilide in two medicinal plants (*R. chuanxiong* and *R. ligusticum*). Twenty miligram of PEG-CNTs-MNP was added to the Eppendorf tube containing 1 mL of S₀ solution. The tube was shaken vigorously for 15 min using a vortex oscillator. After that, the PEG-CNTs-MNP were separated by placing on an external magnet for 1 min, and washed three times using 3 × 1 mL distilled water to remove non-specific binding compounds. Finally, PEG-CNTs-MNP was eluted with 1 mL of acetonitrile to release the enriched compound. The eluent was stored as S₅. The S₀ and S₅ were then analyzed by HPLC-DAD and HPLC-MS/MS. In the mean time, CNTs-MNP was used to extract Z-ligustilide from the two extracts with the same procedure in order to compare the extraction ability of the two types of absorbent.

2.5.3 Secondary DSPE of Z-ligustilide

To further investigate the extraction specificity of PEG-CNTs-MNP, we established secondary DSPE method. Briefly, CNTs-MNP absorbents were dispersed into the *R. chuanxiong* and *R. ligusticum* extracts (S_0) to incubate 15 min, respectively, and then was washed with pure water, finally was eluted with acetonitrile. The obtained eluents (E_0) were actually identical to S_5 as mentioned above, which were subjected to DSPE with PEG-CNTs-MNP. Ten milligram of PEG-CNTs-MNP adsorbent was added to E_0 to run a new round of dispersive solid phase extraction to obtain E_5 . In the mean time, the E_0 was extracted with CNTs-MNP to compare the extraction specificity with that of PEG-CNTs-MNP.

3. Results and discussion

3.1 Preparation and functionalization of CNTs-MNP nanoparticles

PEG-CNTs-MNP was synthesized from MWCNTs following the route illustrated in Scheme 1. The CNTs-MNP was firstly prepared via a hydrothermal reaction [45]. Then, PEG and CNTs-MNP coupling was achieved by activating the carboxyl groups of the MWCNTs with EDC/NHS followed by covalently cross-linking them to the primary amino groups of mPEG-NH₂. The surface charges of the PEG-CNTs-MNP and CNTs-MNP were compared. The zeta-potential of CNTs-MNP and PEG-CNTs-MNP were -51 mV, -42 mV, respectively, indicating that the mPEG-NH₂ had been successfully covalently conjugated with CNTs-MNP. During the PEGylation procedure, the -NH₂ groups of PEG were covalently bond to the carboxyl groups at CNTs' surface. As a result, the surface charge of PEG-CNTs-MNP was significantly

decreased compared to CNTs-MNP, leading to the above change of zeta-potential.

3.2. Characterizations of PEG-CNTs-MNP nanoparticles

3.2.1 Morphological characterization

The transmission electron micrographs of CNTs-MNP and PEG-CNTs-MNP were shown in Figure 1. The length of the nanotubes ranged from dozens of nanometers to several micrometers, with the outer diameter of 30-40 nm and the wall thickness of about 10 nm. The nanotubes penetrated the Fe₃O₄ nanoparticles with diameters about 200 nm to form a rosary structure. The outside of the PEG-CNTs-MNP was a grayish coating layer, which was possibly ascribable to the mPEG-NH₂ attached onto the surface of CNTs-MNP. Because water dispersibility of nano-adsorbents is important in magnetic SPE, dynamic light scattering (DSL) was employed to characterize the dispersion and size of the nanocomposites. The average particle sizes for CNTs-MNP and PEG-CNTs-MNP were 266 and 86 nm. The results demonstrated that after surface modification by mPEG-NH₂, the new synthesized nanocomposites acquired dramatically improved water dispersibility.

3.2.2 Magnetic properties

Magnetic property is crucial for magnetic nanoparticles when used in magnetic separation. The magnetic properties of CNTs-MNP and PEG-CNTs-MNP were measured by VSM at room temperature. The magnetization curves (Fig. 2A) showed the saturation magnetization of CNTs-MNP and PEG-CNTs-MNP was 54.86 and 52.30 emu/g, respectively. The existence of mPEG-NH₂ on the surface of CNTs-MNP

contributes to the decrease of the saturation magnetization. Figure 2A also showed that both nanoparticles have no magnetic hysteresis, revealing their superparamagnetic character. Fig. 2B shows the dispersion ability of CNTs-MNP and PEG-CNTs-MNP in water for 5min. The former could not be dispersed well in water, while the later exhibited excellent water dispersibility which making it ideal nano-adsorbents in complex matrix analyses. Fig. 2C showed the dispersion and separation process of PEG-CNTs-MNP in the absence and presence of an external magnet, respectively. Owing to the superparamagnetism, the particles were attracted to the wall in a short time (about 5 s) by an external magnetic field, which could facilitate the manipulation of the nanoparticles in the practical applications.

3.2.3 CV and DPV characterization

Electrochemical methods were utilized to characterize the synthesized PEG-CNTs-MNP. The cyclic voltammograms (CV) and differential pulse voltammetry (DPV) curves of the nanoparticles in PBS (pH 7.0) containing 5 mM FeCN($K_3Fe(CN)_6$) and 0.1 M KCl are shown in Fig.3. A pair of stable and well defined redox peak can be observed in Figure 3A (CVs). The cathodic and anodic peak potentials were at 142 mV and 289 mV, respectively, which corresponded to the reversibility of the redox peak of $[Fe(CN)_6]^{4-/3-}$. Both CNTs-MNP and PEG-CNTs-MNP exhibited the same redox peak potential indicating they had no electrical activity. Compared to the redox peaks $[Fe(CN)_6]^{4-/3-}$ of curve a, the oxidation current of curve b enhance, indicating that CNTs-MNP have good

conductivity that can increase the electron transmission of $[\text{Fe}(\text{CN})_6]^{4-/3-}$. The peak current in curve slightly decreased compared to that of curve b, which was ascribable to the decreased electron-transfer efficiency of $[\text{Fe}(\text{CN})_6]^{4-/3-}$ due to the attachment of mPEG-NH₂ onto the surface of CNTs-MNP. In addition, DPV was employed to characterize the functionalization of CNTs-MNP nanoparticles. Due to the higher sensitivity of DPV than CVs, the peak currents in Figure 3B (DPV) were higher than those in Figure 3A. Similarly, the peak current of curve (c) diminished with the subsequent modifications of mPEG-NH₂ on the surface of CNTs-MNP for curve (b) and they were found to be higher than that of curve (a).

3.3. Storage stability and regeneration of PEG-CNTs-MNP nanoparticles

To test the stability of PEG-CNTs-MNP against long term storage, the nanoparticles were stored in refrigerator at 4 °C for 50 days. In brief, we took 10 mg of PEG-CNTs-MNP to extract Z-ligustilide at day 5, 10, 20, 30 and 50, respectively.

The recoveries of Z-ligustilide extracted by PEG-CNTs-MNP during this period were compared with those by CNTs-MNP as depicted in Fig. 4A. The recoveries with CNTs-MNP were all found to be above 100%, while those from PEG-CNTs-MNP were slightly lower than 100% except for the first ten days. It is noted that the recovery curve of the PEG-CNTs-MNP is more stable than the CNTs-MNP, suggesting that the storage stability of PEG-CNTs-MNP was better than that of CNTs-MNP.

The regeneration of the adsorbent is crucial for the DSPE's economics. The

reusability of PEG-CNTs-MNP was evidenced by ten rounds of usages in comparison with CNTs-MNP (Fig. 4B). An equivalent amount of Z-ligustilide was adopted during every test and the absorbent was washed with pure water three times before being reused in the next round. It is found that the recoveries for both adsorbents maintained above 96% after ten rounds' of usage, and the changes between every two rounds for PEG-CNTs-MNP was smaller than CNTs-MNP, suggesting a better reusability of the former. It could be explained by the fact that covalent immobilization of mPEG-NH₂ on the CNTs-MNP surface enhances the water-dispersibility and homogeneousness. This excellent reusability could significantly reduce the operation cost in practical applications.

3.4. Absorption kinetics of PEG-CNTs-MNP nanoparticles

The calibration curve was prepared by measuring five different concentrations of Z-ligustilide (i.e. 0.625, 1.25, 2.50, 5.00 and 7.50 µg/mL). Each standard solution was injected into the HPLC-DAD system for analysis in triplicate. The regression equation was $y = 17857x - 1661.5$ ($R = 0.9998$), where y was the peak area, x was the concentration (µg/mL), and R was the correlation coefficient. In this work, we used recovery of Z-ligustilide to evaluate the efficiency of DSPE method with PEG-CNTs-MNP [46]. Recovery (R) was calculated according to the equation: $R = ((C_0 \times V_0) / (C_s \times V_s)) \times 100 \%$. Where C_0 was the concentration (ug/mL) of Z-ligustilide in the S_5 (V_0 was 1 mL), and C_s was the initial concentration (ug/mL) of Z-ligustilide in the S_0 whose volume (V_s) was 1 mL.

The absorption kinetics of PEG-CNTs-MNP nanoparticles is one of the key factors for fast separation in practical application, and was investigated with Z-ligustilide solution at different time intervals. As shown in Figure 5, PEG-CNTs-MNP had fast adsorption kinetics within approximately 30 s, which was 95% of the total equilibrium recovery. The recovery slowly increased with the prolonged binding time. After adsorption for 3 min, the recovery flattened out, showing no significant increase from 3 to 5 min. The adsorption kinetics of CNTs-MNP nanoparticles was also examined. In comparison, the recovery of Z-ligustilide on CNTs-MNP remained slightly low from 30 s to 2 min. This fact further demonstrated the rapid extraction dynamics of PEG-CNTs-MNP, which can be homogeneously dispersed into the sample solution due to the CNTs' surface modification of mPEG-NH₂, and thus may speed up the interaction between the adsorbent and the analytes.

3.5. Sample analysis

The PEG-CNTs-MNP based DSPE coupled with HPLC-UV method was applied to extract Z-ligustilide present in the extracts of *R. chuanxiong* and *R. ligusticum*. Considering the complicated matrix in traditional Chinese medicine (TCM) and low concentration of Z-ligustilide, the absorption time of 3 min was selected so as to achieve sufficient adsorption. The PEG-CNTs-MNP (10 mg) was directly dispersed into the solutions of *R. chuanxiong* and *R. ligusticum* extract, respectively. After mixing for 3 min, the PEG-CNTs-MNP were magnetically separated, and then washed

three times with pure water to remove the impurities. Finally, the sample enriched with the nanoabsorbents was eluated using ethanol (S_5) to be analyzed by HPLC. Figure 6 showed HPLC chromatograms of *R. chuanxiong* (A) and *R. ligusticum* (B) extract (curve b), extracted by CNTs-MNP (curve c) and PEG-CNTs-MNP (curve d). Curve d revealed that only Z-ligustilide was extracted from both extracts by the PEG-CNTs-MNP, and almost all the other chemical compounds present in the two extracts were successfully removed after the DSPE process. There were obvious impurities in curve c, indicating that the CNTs-MNP was not as specific as PEG-CNTs-MNP, which was ascribable to its higher hydrophobicity causing nonspecific binding [47]. As shown in Figure 6A, two compounds (**1** and **2**) were found in S_5 and were identified by HPLC-MS/MS (Figure 7).

Compound **1** had a molecular weight of 192 (m/z 193, $[M + H]^+$), exhibiting abundant $[M + H]^+$ ion as well as a fragment ion $[M + H - H_2O]^+$ in positive ion mode and weak $[M - H]^-$ ion in negative ion mode mass spectrometry, which were consistent with those of phthalides [48]. In the MS/MS spectrum (Figure 7A), characteristic fragment ions were observed at m/z 175 by loss of H_2O and at m/z 147 by successive loss of CO via ring-opening, corresponding to the fragmentation of α,β -unsaturated lactone. The other ions such as m/z 119 $[147 - C_2H_4]^+$, 105 $[147 - C_3H_6]^+$, 91 $[147 - C_4H_8]^+$, and 78 $[147 - C_5H_9]^+$ were obtained by subsequent loss of different alkyl groups from m/z 147 through ring arrangement. Therefore, this compound was identified as senkyunolide A. Compound **2** was found to have a molecular weight of 190 (m/z 191, $[M + H]^+$), and the characteristic fragment ions at

m/z 173 and 145 in MS/MS (Figure 7B) indicated that it was phthalides. Compound **2** was identified as *Z*-ligustilide, which was the dominant constituent in the volatile oil of *R. chuanxiong*. Their chemical structures were also confirmed by the retention time with the authentic samples as shown in Figure 7.

It is worth noting that these two compounds have very similar chemical structures. Both can be extracted by CNTs-MNP from the *R. chuanxiong* extract. However, only *Z*-ligustilide is extracted by PEG-CNTs-MNP. These results indicate that PEGylation of CNTs-MNP may alter the nano interfaces, and thus affect binding selectivity [47]. The most important alteration is the introduction of a large number of hydroxyl groups which increase the hydrophilicity of the material, reducing undesirable nonspecific hydrophobic interactions with analytes. The second change is the decrease in the number of carboxyl group on the negatively charged CNTs-MNP surface, diminishing the surface charge. These alterations enhance the π - π stacking between the nanosorbent and the analyte. In this case, *Z*-ligustilide possesses better planarity than senkyunolide A as it has three double bonds that are in cross conjugation to the lactone ring, while the latter has only two conjugated double bonds.

As shown in Figure 8, *Z*-ligustilide was the major component in the mixture solution (E0), and when CNTs-MNP nanoparticles was used to re-extract E0, there still existed several components due to nonspecific adsorption. Interestingly, only *Z*-ligustilide was extracted when using PEG-CNTs-MNP nanoparticles to re-extract E0. These results demonstrated that PEG-CNTs-MNP had excellent selectivity as well as enrichment ability. Furthermore, because of the good magnetic propertie of

PEG-CNTs-MNP, rapid and efficient separation was achieved. This merit is especially propitious to detect and separate analytes with very similar chemical structures.

In the scale-up experiment, 23.0 mg and 26.3 mg of Z-ligustilide were extracted from 4 mL of reserved solution of *R. chuanxiong* extract containing 36.0 mg of the target compound by CNTs-MNP and PEG-CNTs-MNP, respectively, which represent the extraction rate of 63.9% and 73.1% for the two absorbents. The purities of Z-ligustilide extracted were as high as 85.0% and 91.7% compared to that of 6.1% in the crude extract. On the other hand, 8.7 mg and 11.4 mg Z-ligustilide were extracted from 4 mL of reserved solution of *R. ligusticum* crude extract containing 16.0 mg of the target compound by CNTs-MNP and PEG-CNTs-MNP, respectively, which represent an extraction rate of 54.4% and 71.3% for the two absorbents. The purities of Z-ligustilide were 81.5% and 88.9% compared to that of 4.9% in the crude extract. These results indicate that PEG-CNTs-MNP is a much better dispersive solid phase extraction absorbent than the non PEGylated magnetic carbon nanotubes for Z-ligustilide enrichment from herbal crude extracts. In addition, in this scale up experiment, we evaluated the binding capacity of the two absorbents and found that every 10.0 mg of PEG-CNTs-MNP can bind 2.0 mg of Z-ligustilide, while the non PEGylated one can bind 1.6 mg of Z-ligustilide.

4. Conclusions

In this work, novel PEG-CNTs-MNP nanocomposites with excellent water dispersibility and stability were prepared by covalent coupling of mPEG-NH₂ to

carboxylated CNTs. The PEG-CNTs-MNP used as DSPE absorbent exhibited preferable extraction characteristics such as short absorption equilibrium time, high binding capacity and extraction selectivity. The specific recognition towards Z-ligustilide, an important herbal ingredient, has never been achieved before. The nanocomposites also possess a satisfactory superparamagnetism, leading to convenient and highly efficient extraction. This work also provides an in-depth understanding of the interaction mechanism of PEG-CNTs-MNP with small molecules, and shows promising application for the analysis of interesting compounds in physiological fluids.

Acknowledgements

Financial support from the National Science Foundation of China (No. 81173536), the State Key Laboratory of Phytochemistry and Plant Resources in West China (No. P2015-KF12), and the West Light Foundation of the Chinese Academy of Sciences is gratefully acknowledged.

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Figure Captions

Scheme 1. Procedure of preparing PEG-CNTs-MNP and their application as DSPE adsorbents for extracting Z-ligustilide.

Figure 1. TEM images of CNTs-MNP and PEG-CNTs-MNP.

Figure 2. (A) VSM curves of CNTs-MNP and PEG-CNTs-MNP, (B) Dispersion of CNTs-MNP and PEG-CNTs-MNP in water, and (C) Magnetic separation of PEG-CNTs-MNP.

Figure 3. Cyclic voltammograms (A) and Differential pulse voltammetry (B) of the electrode at different stages: (a) bare screen printed carbon electrode, (b) CNTs-MNP, (c) PEG-CNTs-MNP. Carbon rings served as the working and counter electrodes, Ag/AgCl reference electrode.

Figure 4. Storage stability (A) and reusability (B) of CNTs-MNP and PEG-CNTs-MNP.

Figure 5. Adsorption kinetics curves of Z-ligustilide of PEG-CNTs-MNP and CNTs-MNP

Figure 6. HPLC chromatograms of DSPE process of *R. chuanxiong* (A) and *R. ligusticum* (B): (a) is for the standard solution of Z-ligustilide, (b) is for the plant

597 extract (S_0), (c) is for the S_5 of each plant extracted with CNTs-MNP, and (d) is for the
598 S_5 of each plant extracted with PEG-CNTs-MNP.

599 Figure 7. Positive ion CID mass spectra of the two compounds: (A) MS/MS and
600 chemical structures of senkyunolide A; (B) MS/MS and chemical structures of
601 Z-ligustilide.

602 Figure 8. HPLC chromatograms of the DSPE process of E_0 of *R. chuanxiong* (A) and
603 *R. ligusticum* (B): (a) is for the E_0 of the two plants; (b) is for E_5 extracted with
604 CNTs-MNP; and (c) is for the E_5 extracted with PEG-CNTs-MNP.

CNTs-MNP

PEGylation CNTs-MNP