Synthesis, characterization and application of magnetic carbon nanotubes for the simultaneous extraction and high performance liquid chromatographic determination of codeine and morphine in human urine, blood serum, opium and tablet samples

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ABSTRACT

In the present research, magnetic carbon nanotubes were synthesized and applied as nanoadsorbent for the simultaneous solid phase extraction of codeine and morphine prior to their determination via high performance liquid chromatography. The morphology of the synthesized nanoadsorbent was investigated using Fourier transform infrared spectroscopy and field emission scanning electron microscopy. Under the optimum conditions, codeine and morphine were linear in the concentration range of $0.003–1.0\,\mu g\,mL^{-1}$. Also, the detection limits of $0.9\,ng\,mL^{-1}$ and $0.8\,ng\,mL^{-1}$ were achieved for codeine and morphine, respectively. The intra-day and inter-day relative standard deviations were 6.8% and 7.8% for codeine and 4.8% and 8.5% for morphine, respectively. Finally, the suggested method was successfully applied in human urine, blood serum, opium, opium residues and tablet samples with satisfactory results.

1. Introduction

Codeine and morphine are the main alkaloids in poppy seeds that have pharmacological activities [1]. The role of morphine as an analgesic in the treatment of agonizing pain is well recognized [2]. It is also a metabolite of the heroine, which is produced after initial metabolism to acetylmorphine. Codeine or 3-methylmorphine, a common cough suppressant, is widely used to relieve moderate pain [3]. On the other hand, these drugs have high potential for abuse which may lead to severe psychological and physical dependence. Also, excessive use of these phenolic compounds causes toxic symptoms [4]. So, a fast and simple method for the analysis of codeine and morphine in various samples is required. Until now, different analytical techniques have been performed for the determination of these two compounds such as electrochemical methods [5,6], liquid chromatography [7,8], gas chromatography [9] and electrophoresis [10,11]. Due to the matrix interferences in real samples, a separation or preconcentration step is necessary to improve sensitivity and selectivity of their determination.

Among the traditional extraction methods, magnetic solid phase extraction (MSPE) has attracted great attention because of its significant advantages such as great selectivity, wide variety of sample matrices, high recoveries, good reproducibility, amenable to automation, fast sorbent separation and low solvent volumes [12]. It has been used for a lot of applications in medicine, diagnostics, analytical chemistry and environmental technology [13–17]. In this new version of extraction technique, Fe_3O_4-based materials, as adsorbents can well disperse into sample solution by a mechanical stirrer or an ultrasonic bath. Therefore, the contact area between the analytes and the adsorbent are sufficient to ensure a fast mass transfer. After separation, the magnetic adsorbents have been easily isolated from the medium with the help of an external magnet.

Carbon nanotubes (CNTs) are characterized by outstanding physicochemical properties such as small size, low density, excellent thermal and chemical stability, π-π electrostatic stacking and high electrical conductivity [18,19]. Furthermore, the large specific surface area makes CNTs possess great potential as adsorbents for the extraction of many kinds of organic and inorganic analytes [20–23]. The introduction of Fe_3O_4 nanoparticles into the carbon

nanotubes system will produce magnetic nanocomposites which combine the separation convenience of magnetic materials and high adsorption capacity of CNTs [24,25].

In the present study, magnetic carbon nanotubes (MCNTs) nanocomposites have been synthesized and used as adsorbent for simultaneous preconcentration of codeine and morphine in a batch extraction procedure. Afterward, the extracted analytes were determined using high performance liquid chromatography-ultraviolet detection (HPLC-UV).

2. Experimental

2.1. Standards and reagents

Codeine phosphate and Morphine sulfate were purchased from Sigma-Aldrich Chemical Company (St. Louis, MO, USA). Acetonitrile, methanol and ethanol (HPLC grade) were prepared from Merck (Darmstadt, Germany). Individual stock solutions ($1000 \, mg \, L^{-1}$) of codeine and morphine were prepared in methanol. A standard mixture of two analytes was prepared at a final concentration of $100 \, mg \, L^{-1}$ in methanol and working solutions were prepared in distilled water from mixture solution. Multi-walled carbon nanotubes (MWCNTs) with an average outer diameter of 5–20 nm, length of 1–10 mm, number of walls 3–15 and surface area of $350 \, m^2 \, g^{-1}$ were supplied from PlasmaChem GmbH (Berlin, Germany). Ferric chloride hexahydrate ($FeCl_3.6H_2O$), ferrous sulfate heptahydrate ($FeSO_4.7H_2O$), potassium dihydrogen phosphate, phosphoric acid, nitric acid and sodium hydroxide were of analytical grade and were prepared from Merck.

2.2. Instrumentation

High performance liquid chromatography system (Agilent, Palo Alto, CA, USA) equipped with a ZORBAX SB-C$_{18}$ (4.6×250 mm) column, a UV detector and a Chemstation software was applied for the determination of codeine and morphine. An aqueous mobile phase composed of $0.02 \, mol \, L^{-1}$ potassium dihydrogen phosphate, adjusted to pH = 3 with phosphoric acid, and methanol (85:15, v/v) with a flow rate of $1.0 \, mL \, min^{-1}$ were also used. The effluent was monitored at 240 nm to omit the effluent absorption effect. The field emission-scanning electron microscope (FE-SEM) (model AIS 2100, Seron Technology, Gyeonggi-do, South Korea) and Fourier transform infrared (FT-IR) spectrometer (Tensor 27, Bruker, Ettlingen, Germany) were applied for morphological studies of the synthesized nanoadsorbent. To stir the sample solution, a RH B-KT/C (IKA, Staufen, Germany) magnetic stirrer was employed. The pH measurements were carried out with a 827 pH-meter (Metrohm, Herisau, Switzerland) supplied with a combined glass-calomel electrode. An ultrasound wave bath (Model Tecna 6, Taranto, Italy) was used to agitate the samples.

2.3. Synthesis of magnetic nanocomposites

At the first, the MWCNTs were purified by heating at 300 °C for 40 min to remove amorphous carbon, graphitic nanoparticles and catalyst impurities. Then they were oxidized by refluxing in concentrated nitric acid at 60 °C for 5 h [26]. Subsequently, the mixture was filtered. The filtered MWCNTs was rinsed and this process was continued until the output deionized water has a pH more than acidic range and finally dried at 80 °C throughout night to achieve oxidized MWCNTs. In order to prepare the magnetic nanocomposites, 2.98 g ferrous sulfate heptahydrate and 1.54 g ferric chloride hexahydrate dissolved in 200 mL deionized water in a beaker. Then 1.0 g oxidized MWCNTs was added into solution and the mixture was stirred and sonicated vigorously for 20 min under N$_2$ atmosphere at 75 °C. After that, 30 mL NaOH aqueous solution (2.0 mol

L^{-1}) was added to mixture dropwise and the pH of the final mixture was adjusted at 10 ± 0.2. To prevent critical oxidation, N$_2$ atmosphere was used throughout the reaction. Finally, this suspension stirred with a mechanical stirrer for 4 h at room temperature. The brown precipitates were then collected by a strong magnet with the power of 6000 G, washed with distilled water and dried under vacuum at 70 °C for 5 h. This synthesized nanoadsorbent will apply for simultaneous preconcentration and extraction of codeine and morphine in sample solutions and other different real samples.

2.4. General procedure

Adsorption experiments were performed in 50 mL glass beaker containing 20 mL aqueous solution of codeine and morphine (pH = 9) and 0.06 g MCNTs nanocomposite. The mixture was stirred vigorously for 15 min and after that, nanoadsorbent was gathered, meanwhile the supernatant was decanted. To ensure the removal of impurities and are not faced with additional chromatographic peaks, adsorbent was washed with a small amount of distilled water and transferred to a glass tube for desorption of analytes.

In desorption process, 1.0 mL of a 1:1 mixture of methanol-acetonitrile was added to the tube containing nanoadsorbent and stirred for 6 min. Then, nanoadsorbent was controlled by the magnet and desorption solvent was transferred into a clean and thoroughly dry sample container.

Finally, the solvent was evaporated under a gentle stream of nitrogen, the dried residue was redissolved in $100 \, \mu L$ methanol and then $10 \, \mu L$ of it was injected to HPLC for analysis.

3. Results and discussion

3.1. Characterization of the synthesized nanoadsorbent

The composition and morphology of the nanocomposite were characterized by Fourier transform infrared spectrometry and field emission-scanning electron microscopy.

The FT-IR spectra of the oxidized MWCNTs (Fig. 1a) and the MCNTs nanocomposite (Fig. 1b) were recorded by using KBr pellet method as follows: A small amount of powder sample (about 0.1–2% of the KBr amount, or just enough to cover the tip of spatula) mixed with the KBr powder. Subsequently mixture was grinded for 5 min. If the sample is in large crystals, the sample should be grinded separately before adding KBr. Just enough amount of sample was used to cover bottom in pellet die. It was placed in press and pressed at 5000–10000 psi. The pressed disc should be nearly clear if properly made. If it is translucent, regrind and repress.

As can be seen from the mentioned figures, that acid treatment introduces various functional groups on the surface of MWCNTs. The peaks at $3420.22 \, cm^{-1}$, $1712.66 \, cm^{-1}$ and $1401.44 \, cm^{-1}$ in the spectrum of oxidized MWCNTs can be assigned to O—H bond stretching vibration, C=O bond stretching vibration and O—H bond bending vibration, respectively. Moreover, the symmetric and asymmetric C—H bond stretching vibration at $2925.12 \, cm^{-1}$, $2854.13 \, cm^{-1}$ and C=C bond at $1636.56 \, cm^{-1}$ can be observed in the oxidized MWCNTs. In the FT-IR spectrum of magnetic nanocomposite, a sharp peak at $556.38 \, cm^{-1}$ for Fe–O approved magnetization of oxidized MWCNTs.

The FE-SEM images of the oxidized MWCNTs and the synthesized nanocomposites were depicted in Figs. 2 and 3, respectively.

3.2. Optimization of extraction conditions

To evaluate the feasibility of the synthesized nanoadsorbent for the extraction of the target analytes, several experimental factors affecting the extraction recovery including the type and volume