Analytical Biochemistry 542 (2018) 76–83

Contents lists available at ScienceDirect

Analytical Biochemistry

journal homepage: www.elsevier.com/locate/yabio

Hydrophilic modified magnetic multi-walled carbon nanotube for dispersive solid/liquid phase microextraction of sunitinib in human samples

Sara Hooshmand, Zarrin Es'haghi*

Department of Chemistry, Payame Noor University, Tehran, P.O. Box 19395-4697, Iran

ARTICLE INFO

Keywords:
Magnetic-MWCNT nanoparticles
Dispersive solid–liquid phase microextraction
Sunitinib
Honey
Human samples

ABSTRACT

In this paper, a novel approach for the efficient microextraction and determination of anticancer drug, sunitinib from human samples is described. We synthesized a new nanocomposite; honey coated magnetic multi-walled carbon nanotubes (Honey@magnetic-CNTs). This nanocomposite retains the magnetic properties of individual magnetic nanoparticles (MNPs) and can be effectively separated under an external magnetic field. The synthesized nanoparticles were characterized by FT-IR, VSM, EDAX and XRD and TEM studies. The spherical particles obtained before and after the functionalization had sizes of 14 nm and 16 nm, respectively. The method is based on Honey@magnetic-CNTs assisted dispersive solid-liquid phase microextraction for determination and analysis of the drug. The influences of experimental parameters were investigated. Under optimal conditions, the applied nanocomposite showed good performance, high sensitivity and fast extraction of the analyte from biological samples. In linearity test, the regression correlation coefficient was obtained more than 99% for analyte of interest and linear dynamic range for the proposed method was from 5 to 5000 ng mL^{-1}. Method detection and quantification limits were 1.58 ng mL^{-1} and 5.28 ng mL^{-1}, respectively. Relative standard deviation was 3.15%.

Introduction

Sunitinib (marketed as Sutent) is a novel small molecularly targeted anticancer drug that inhibits several tyrosine kinases. It has shown survival benefits in advanced renal cell carcinomas as well as in advanced hepatocellular carcinomas and gastrointestinal stromal tumors, respectively. It was approved by the FDA for the treatment of renal cell carcinoma (RCC). Chemical structure of sunitinib is shown in Fig. 1.

Sunitinib is soluble in acidic aqueous solutions (25 mg mL^{-1} at pH 1.2–6.8). Solubility of the drug rapidly decreases at pH greater than 6.8. For this reason, sunitinib is classified as a low soluble compound according to the Biopharmaceutics Classification System (BCS). It was also the first cancer drug simultaneously approved for two different indications [1]. The chemical analysis of drug residues is currently performed using methods mainly based on analytical separation techniques involving the pretreatment techniques. Considering sunitinib as a new potential antitumor drug, a sensitive and selective bioanalytical method is required for its pre-concentration and determination studies. Some papers were published on sunitinib determination and its behavior in different environments. A simple high performance thin layer chromatography (HPTLC) has been developed and validated for determination of sunitinib and possible impurities. The samples were applied in forms of bands on an aluminum TLC plate pre-coated with silica gel and were separated using dichloromethane, methanol, toluene, ammonia solution as the mobile phase [2,3]. In another study, sunitinib determination was performed under different operational conditions to find the most suitable method. Reverse phase HPLC determination of sunitinib using UV detector, was performed with its isomerisation study, method development and validation [4]. The purpose of another study was to develop a simple and sensitive high-performance liquid chromatographic method with UV-Visible detection for quantification of sunitinib concentrations in human plasma [5]. However there is no report on using honey-modified magnetized CNT nanosorbents in determination of sunitinib. Carbon nanotubes (CNTs) have been considered as an important area of research due to their unique and tunable surface plasmon resonance (SPR) and their applications in biomedical science including drug delivery, tissue/tumor imaging, photothermal therapy and immune chromatographic identification of pathogens in clinical specimens [6]. CNTs have also been the focus of wide research in recent years due to their excellent mechanical, thermal and electrical properties. As a result of their nanoscale dimensions and high surface area, CNTs could also be considered as efficient templates for the assembly of nanoparticles on their surface. Various compounds and structures used in decoration of CNTs can

* Corresponding author.
E-mail address: eshaghi@pnu.ac.ir (Z. Es'haghi).

https://doi.org/10.1016/j.ab.2017.11.019
Received 15 August 2017; Received in revised form 23 November 2017; Accepted 25 November 2017

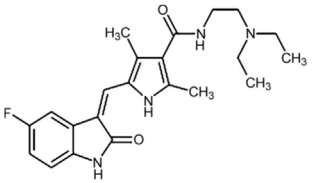

Fig. 1. Chemical structure of sunitinib.

increase their surface functionality and other properties, such as their electrical and magnetic characteristics. In recent reports, the attachment of various inorganic nanoparticles to either external or internal surface of the CNT cavity, through several experimental methods has been described. In this context, it is important to note that the size control of CNTs is of primary importance for the purpose of tailoring their physical and chemical properties [7]. CNTs have widely been studied for their great potential applications in various fields from nanotechnology to biomedicine. Preparation of magnetic CNTs presents new opportunities in nanobiotechnology and biomedical applications. Some preparation techniques have been developed during the last few years to obtain magnetic CNTs such as grafting nanotubes with magnetic ferro-fluidics, attachment of magnetic nanoparticles to CNTs or their polymeric coating. These strategies provide novel multi-purpose systems that can be used in the biomedical field. The chemical combination of magnetic nanoparticles and CNTs follows various strategies including encapsulation of magnetic molecules inside the carbon nanotubes or grafting/decorating CNTs on their surface using bioconjugation chemistry or electrochemical deposition. The strategies of attaching the synthesized nanoparticles have been achieved through covalent bonds, electrostatic interactions, π–π stacking, and hydrophobic interactions. Many other strategies have been developed in the last few years and we discuss encapsulation of magnetic molecules inside the carbon nanotubes in this work [8]. Eco-friendly and cost-effective procedures for the synthesis of nanoparticles are of interest to chemists specially to find green methods of inorganic material synthesis [9,10]. A comprehensive report on the role of microorganisms and plants in the synthesis of nanoparticles has been reviewed by Mohanpuria et al. [11,12]. However, there is no report on the synthesis of nanoparticles using natural honey -the food of Gods-which was mankind's only sweetener for centuries. The major constituents of it are fructose and glucose, it also contains amino acids that help build up calcium in the body [13–17]. Also, it contains ingredients that can function as anti-oxidants which play a vital role in the prevention of cancer. Though the biomodification of some nanoparticles has been carried out by several groups of scientists, but there is no report on the use of natural honey for the synthesis and modification of multi-walled carbon nanotube nanoparticles. Here, there is a report on a green modification of magnetized CNT nanoparticles using honey as both a stabilizer and a reducing and capping agent [18,19].

Ecological hazards for CNTs have been extensively reported, however most studies suggest that surface modification reduces clearance time in mammals. In this study we have covered the CNT surface with honey, which is completely harmless. In this honey mediated nanoparticles, honey acts as a stabilizer agent and notably functions as a precursor in the surface of CNT nanoparticle. So, this method does not produce toxic byproducts. Honey contains proteins (0.1–0.5%) and is also rich in vitamins such as C and B1 and B2 complex vitamins including riboflavin, nicotinic acid, B6, and pantothenic acid. Consequently, the entry of the remains of this adsorbent into nature not only does not damage the environment but is also a source of nutrition for microorganisms. So, this absorbent is a green nanocomposite.

Using the synthesized nanosorbent in the modified dispersive liquid-liquid microextraction (DLLME) technique, dispersive solid-liquid phase microextraction (DSLME), we could overcome many of the limitations of the original DLLME method. Dispersive liquid-liquid microextraction (DLLME) was invented in 2006 by Assadi et al. based on a ternary component solvents system where the dispersion of fine droplets of the extraction solvent in the aqueous phase is possible [20]. DLLME developed soon and researchers draw attention to it like other techniques, however, DLLME also has its drawbacks, mainly result from the extraction solvent. In DLLME, extraction solvent should be heavier than water (i.e., carbon tetrachloride, chlorobenzene, chloroform, tetrachloroethylene and carbon disulfide). Thus, there are a limited number of solvents available for DLLME which are mostly inefficient for numerous analytes. To overcome these drawbacks, the extraction solvent has been replaced by solid phase consisting of nanoparticles in DSLME. Compared to DLLME method, performance of DSLME method is more facile as well as the extraction procedure which is really short. Magnetic solid phase microextraction diminishes the use of additional steps such as centrifugation which decreases the handling of the sample. Liquid-liquid extraction (LLE) and solid phase extraction (SPE) are conventional sample pretreatment techniques for drugs of abuse analysis in biological samples. However, they are rather effortful, time consuming and use large amounts of toxic solvents. Thus, solventless sample preparation techniques such as liquid-phase microextraction (LPME), supercritical fluid extraction (SFE) and solid phase microextraction had already been offered for the analysis of drugs of abuse. Stir bar sorptive extraction (SBSE) a developed form of SPME is a kind of novel and solvent-free sample pretreatment technique with high concentration factor, good reproducibility and high sensitivity. Other literatures about microextraction for pre-treating samples with complex matrix were also studied [21–24]. Compared to most conventional methods, this technique requires very little sample solution and toxic and expensive organic solvents. It is a reliable pretreatment method for the fast trace analysis in many complicated biological and environmental matrices. The method shows flexibility, simplicity and more convenient handling with satisfactory LOD and LOQ. Excellent clean-up of the drug in human matrices as complicated matrices, good linearity and reasonable relative recovery can also be achieved. The aim of the present study was to develop a sensitive microextraction method that can facilitate the rapid determination of sunitinib in human plasma, urine, hair and nail samples using analytical chromatographic separation and sample preparation procedure.

Experimental

Reagents

The drug, sunitinib malate chemically designated as (Z)-N-[2-(diethylamino) ethyl]-5-[(5-fluoro-2-oxo-1,2-dihydro-3H-indol-3-ylidene) methyl]-2,4-dimethyl-1H-pyrrole-3carboxamide(S)-2-hydrosuccinate, was obtained from Pfizer (New York City, United States). Organic solvents and analytical reagents were obtained from Merck (Darmstadt, Germany). All reagents used were of analytical grade and all solutions were prepared with double-distilled water. Stock drug solution (1000 mg L^{-1}) was prepared by dissolving 5 mg sunitinib in methanol and diluting to 5 mL. The samples were stored below 4 °C prior to use.

Instruments

High-performance liquid chromatographic analyzer (Agilent 1200 series) was used for separation at ambient temperature. High-performance liquid chromatographic analyzer was Agilent Technologies 1200 Series. A perfectsil targetODS column (250 × 4.6 mm id, particle size 5 μm) was used for separation at ambient temperature (25 ± 0.5 °C). The column was equilibrated with the mobile phase (flow rate of 1.0 mL min^{-1}), water (0.1% v/v formic acid)/acetonitrile (40:60 v/v %). The injection volume was 20 μL and the detection wavelength was 220 nm and run time was 10 min. A UV-Vis Spectrophotometer, model T80 (PG Instruments, UK), was used for recording absorbance spectra. The X-ray diffraction (XRD) analysis was performed with SIEMENS,