Journal of Chromatography A, 1274 (2013) 44–53

Contents lists available at SciVerse ScienceDirect

Journal of Chromatography A

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

# Development of novel molecularly imprinted magnetic solid-phase extraction materials based on magnetic carbon nanotubes and their application for the determination of gatifloxacin in serum samples coupled with high performance liquid chromatography

Deli Xiao[a], Pierre Dramou[a], Nanqian Xiong[b], Hua He[a,c,*], Hui Li[a], Danhua Yuan[a], Hao Dai[a]

[a] *Department of Analytical Chemistry, China Pharmaceutical University, Nanjing 210009, China*
[b] *School of Chemistry and Life Science, Guizhou Normal College, Guizhou 550003, China*
[c] *Key Laboratory of Drug Quality Control and Pharmacovigilance, Ministry of Education, China Pharmaceutical University, Nanjing 210009, China*

ARTICLE INFO

*Article history:*
Received 9 September 2012
Received in revised form
29 November 2012
Accepted 5 December 2012
Available online 13 December 2012

*Keywords:*
Molecularly imprinted polymers
Magnetic carbon nanotubes
Magnetic solid phase extraction
Gatifloxacin
Serum samples

ABSTRACT

A novel composite imprinted material, on the basis of magnetic carbon nanotubes (MCNTs)-incorporated layer using gatifloxacin as a template, methacrylic acid as a functional monomer, and ethylene glycol dimethacrylate as a cross-linker, was successfully synthesized by a surface imprinting technique. Adsorption dynamics and a Scatchard adsorption model were employed to evaluate the adsorption process. The results showed that magnetic carbon nanotubes molecularly imprinted polymers (MCNTs@MIP) displayed a rapid dynamic adsorption and a high adsorption capacity of 192.7 μg/mg toward GTFX. Applied MCNTs@MIP as a sorbent, a magnetic solid phase extraction method coupled with high performance liquid chromatography (MSPE-HPLC) was developed for the determination of GTFX in serum samples. The recoveries from 79.1 ± 4.8% to 85.3 ± 4.2% were obtained. MCNTs@MIP can not only be collected and separated fast by external magnetic field but also have high surface-to-volume ratio, outstanding mechanical properties and specific recognition toward template molecule. In addition, the MCNTs@MIP could be regenerated, which could be used for five cycles with lost of less than 7.8% of its recovery on average. These analytical results of serum samples display that the proposed method based on MCNTs@MIP is applicable for fast and selective extraction of therapeutic agents from biological fluids.

## 1. Introduction

The fluoroquinolones, which comprise a relatively large and constantly expanding group of substances, have emerged as one of the most important class of antibiotics [1]. Gatifloxacin (GTFX), (±)-1-cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-7-(3-methyl-1-piperazinyl)-4-oxo-3-quinoline carboxylic acid (Fig. S1), is an effective antibiotic of the fourth generation fluoroquinolone family with expanded activity against gram-positive bacteria and atypical pathogens by inhibiting the bacterial enzymes DNA gyrase and topoisomerase IV [2]. Although GTFX has been widely used as an effective antibiotic, its bioavailability is relatively low when used in some special physiological environments such as trans-corneal, trans-dermal and trans-lymphatic systems which need a long residence time and prolonged high drug concentration, a large amount of GTFX eventually enters into blood through the mucosa absorption, which may cause side effects like dyspnea, arrhythmia and pathoglycemia [3], therefore, the determination procedure of GTFX in biological samples is important for protecting human health.

Several methods have been described for the determination of GTFX in biological samples, including spectrophotometry [4], spectrofluorimetry [5], polarography [6], voltammetry [7], chemiluminescence [8] and high performance liquid chromatography (HPLC) [1,9–12]. The method based on HPLC is the universal method of the quantitative determination of GTFX. However, these methods are unsatisfactory because of the extremely low GTFX concentration and interference of complex matrix in serum sample, several isolation and enrichment processes were required and the analytical procedure was always time-consuming and solvent-depending [5,11]. Thus, to utilize a solid adsorbent with specific recognition and time-saving property for serum sample preparation would be a feasible way to solve those aforementioned problems.

Owing to the chemical, mechanical and thermal stability together with high selectivity for template molecules, molecularly imprinted polymers (MIPs) have been utilized for a wide variety

---

* Corresponding author at: China Pharmaceutical University, 24 Tongjia Lane, Nanjing 210009, Jiangsu Province, China. Tel.: +86 025 83271505; fax: +86 025 83271505.
*E-mail addresses:* dochehua@163.com, jcb315@163.com (H. He).

0021-9673/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.chroma.2012.12.011

of applications, including chromatography [13], protein separation [14], solid-phase extraction [15], drug-controlled release [2,16] and sensor devices [17,18]. The imprinting technique is a well-established and simple technique for synthesizing materials with specific molecular recognition properties [19–21]. Although the bulk MIP prepared by conventional methods exhibits high selectivity [22,23], some disadvantages were suffered, such as the heterogeneous distribution of the binding sites, embedding of most binding sites, and poor site accessibility for template molecule [24]. To resolve these problems, scientists have made efforts to prepare core–shell structural MIP. By preparing the MIP film on a solid–support substrate, the surface-imprinting technique provides an alternative way to improve mass transfer and reduce permanent entrapment of the template. In previous investigations, $SiO_2$ [25,26], $TiO_2$ [27] and $Fe_3O_4$ [28–31] have been widely used in the surface-imprinting process.

Recently, in order to avoid leakages and fragility of traditional support materials, special attentions have been directed to combine magnetic nanoparticles (MNPs) with nanosized materials such as carbon nanotubes (CNTs) for magnetic carbon nanotubes (MCNTs) preparation. Various MCNT synthetic methods have been described in the literatures. Correa-Duarte et al. have synthesized MCNTs through a layer-by-layer assembly approach [32]. Georgakilas et al. have used pyrene as interlinker for the attachment of capped magnetic nanoparticles on the surface of CNTs [32,33]. Gao et al. have prepared MCNTs via the electrostatic attraction between CNTs and magnetic nanoparticles [34]. Jia et al. have combined magnetite beads with CNTs based on a hydrothermal technique [35]. We prepared MCNTs by a simple solvothermal process, which can easily alter the size (100–350 nm), location and denseness of $Fe_3O_4$ beads fixed on CNTs as well as the MCNT structure via controlling the reaction parameters [36].

To the best of our knowledge, although some researches studied MIP using fluoroquinolone as template [37–39], there is no report of the preparation of molecularly imprinted polymers on the surface of magnetic carbon nanotubes (MCNTs@MIP) and application for magnetic solid phase extraction. When MCNTs are encapsulated inside of MIP, the resulting MCNTs@MIP can be easily collected and separated by an external magnetic field without additional centrifugation or filtration [40,41], which makes separation easier and faster. Besides, MCNTs@MIP could have outstanding mechanical properties of CNTs. Moreover, MCNTs@MIP can not only selectively recognize the template molecules in complex matrix but also possess more imprinted cavities within the polymer network due to the high surface-to-volume ratio of MCNTs [42]. Therefore, this work focused on a simple and effective method to synthesize novel MIP based on magnetic carbon nanotubes and the application in magnetic solid phase extraction. The adsorption kinetics, static adsorption and selective recognition of MCNTs@MIP were investigated in detail. And then MCNTs@MIP were used in magnetic solid phase extraction of GTFX in serum samples.

## 2. Experimental

### 2.1. Materials

Ferric chloride hexahydrate ($FeCl_3{\cdot}6H_2O$), sodium acrylate ($CH_2{=}CHCOONa$, Na acrylate), sodium acetate ($CH_3COONa$, NaOAc), ethylene glycol (EG), diethylene glycol (DEG), methacrylic acid (MAA), ethylene glycol dimethacrylate (EGDMA), polyvinylpyrrolidone (PVP) and azobisisbutyronitrile (AIBN) were obtained from Aladdin. Gatifloxacin (GTFX) and pazufloxacin (PZFX) were purchased from Sinopharm Chemical Reagent Co. Ltd., China. Carbon nanotubes with outer diameter 10–20 nm and length 5–15 μm were purchased from Shenzhen Nanotechnologies Port Co. Ltd., China.

### 2.2. Instruments and HPLC analysis

UV–vis absorption was characterized by UV1800 UV-Vis spectrophotometer (Shimadzu Corporation, Japan). The morphology of the as-synthesized nanoparticles was studied using a S-3000 scanning electron microscopy (SEM, Hitachi Corporation, Japan) and a FEI Tecnai G2 F20 transmission electron microscope (TEM). The surface groups on the as-synthesized nanoparticles were measured with a 8400 s FT-IR spectrometer (Shimadzu Corporation, Japan). Phase identification was done by the X-ray powder diffraction (XRD) pattern, using X' TRA X-ray diffractometer with Cu Kα irradiation at $\gamma = 0.1541$ nm.

The HPLC analyses were performed on a Shimadzu LC-20AT HPLC system including a binary pump and a diode array detector (Shimadzu, Kyoto, Japan). A Shimadzu VP-ODS C18 (5 μm particle size, 250 mm × 4.6 mm) analytical column was used for analyte separation. The mobile phase was a mixture of triethylamine phosphate (1%, pH 4.30)–acetonitrile (13/87, v/v) delivered at a flow rate of 1.0 mL/min. The injection volume was 20 μL, and the column effluent was monitored at 325 nm.

### 2.3. Synthesis of MCNTs

As shown in Fig. 1, carboxylation of CNTs (c-CNTs) was carried out by blending the CNTs (0.5 g) with 100 mL of sulfuric acid and nitric acid mixture (mole ratio: 3/1). This solution was dispersed by ultrasonication for about 2 h and refluxed under magnetic stirring at 80 °C for 12 h. Then, the dispersed solution was filtered and the particles obtained were washed to neutrality, and dried in vacuum at 65 °C overnight.

The preparation of MCNTs was carried out according to our previous work [36]. c-CNTs (0.4 g), $FeCl_3{\cdot}6H_2O$ (2.4 g, 9 mmol), Na acrylate (3.4 g) and NaOAc (3.4 g) were added into a mixture of ethylene glycol (EG, 22.5 mL) and diethylene glycol (DEG, 22.5 mL) under ultrasonication for about 1 h. The homogenous black solution obtained was transferred to a Teflon-lined stainless-steel autoclave and sealed to heat at 200 °C. After reaction for 10 h, the autoclave was cooled to room temperature. The MCNTs obtained were washed several times with ethanol and water, and then dried in vacuum at 65 °C for 10 h. In parallel, a reference was prepared by the same protocol with magnetic nanoparticles (MNPs), but without adding c-CNTs.

### 2.4. Synthesis of MCNTs@MIP

In a typical procedure, MCNTs@MIP were synthesized as follows: the GTFX (1.0 mmol) was dissolved in 10 mL of DMSO, and then 4.0 mmol of MAA was added into it. This mixture was stirred for 30 min for preparation of the preassembly solution. The MCNTs (1.0 g) were mixed with 4 mL of dimethylsulfoxide (DMSO) and ultrasounded for 5 min. Then 20 mmol of EGDMA and the preassembly solution were added into the mixture of MCNTs in DMSO. This mixture was subjected to ultrasound for 30 min for the preparation of the prepolymerization solution. The PVP (0.4 g) used as dispersant was dissolved into 100 mL of DMSO:water (9:1, v/v) in a three-necked round-bottomed flask. The mixture was stirred at 300 rpm and purged with nitrogen gas to displace oxygen while the temperature increased to 60 °C. The prepolymerization solution was added into the three-necked flask, and then 0.1 g of AIBN was also added into it. The reaction was allowed to proceed at 60 °C for 12 h. After the polymerization, the polymers were separated and washed with methanol:acetic acid (6:4, v/v) several times under ultrasound until the template molecule could not be detected by