Applied Surface Science 292 (2014) 378–386

Contents lists available at ScienceDirect

Applied Surface Science

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

Optimization and evaluation of chelerythrine nanoparticles composed of magnetic multiwalled carbon nanotubes by response surface methodology

Yong Huang[a,1], Yulin Yuan[b,1], Zhide Zhou[a,1], Jintao Liang[a], Zhencheng Chen[a], Guiyin Li[a,*]

[a] School of Life and Environmental Sciences, Guilin University of Electronic Technology, Guilin, Guangxi 541014, China
[b] Department of Clinical Laboratory, the People's Hospital of Guangxi Zhuang Autonomous Region, Nanning, Guangxi 530021, China

ARTICLE INFO

Article history:
Received 12 September 2013
Received in revised form 8 November 2013
Accepted 26 November 2013
Available online 6 December 2013

Keywords:
Magnetic carbon nanotubes
Response surface methodology
Drug delivery
Optimization
Chelerythrine

ABSTRACT

In this study, a new chelerythrine nanomaterial targeted drug delivery system (Fe_3O_4/MWNTs-CHE) was designed with chelerythrine (CHE) as model of antitumor drug and magnetic multiwalled carbon nanotubes (Fe_3O_4/MWNTs) nanocomposites as drug carrier. The process and formulation variables of Fe_3O_4/MWNTs-CHE were optimized using response surface methodology (RSM) with a three-level, three-factor Box–Behnken design (BBD). Mathematical equations and response surface plots were used to relate the dependent and independent variables. The experimental results were fitted into second-order response surface model. When Fe_3O_4/MWNTs:CHE ratio was 20.6:1, CHE concentration was 172.0 μg/mL, temperature was 34.5 °C, the drug loading content and entrapment efficiency were 3.04 ± 0.17% and 63.68 ± 2.36%, respectively. The optimized Fe_3O_4/MWNTs-CHE nanoparticles were characterized by scanning electron microscopy (SEM), Zeta potential, *in vitro* drug release and MTT assays. The *in vitro* CHE drug release behavior from Fe_3O_4/MWNTs-CHE displayed a biphasic drug release pattern and followed Korsmeyer–Peppas model with Fickian diffusion mechanism for drug release. The results from MTT assays suggested that the Fe_3O_4/MWNTs-CHE could effectively inhibit the proliferation of human hepatoma cells (HepG2), which displayed time or concentration-dependent manner. All these preliminary studies were expected to provide a theoretical basis and offer new methods for preparation efficient magnetic targeted drug delivery systems.

1. Introduction

With the rapid development of nanotechnology, carbon nanotubes (CNTs), including multi-walled carbon nanotubes (MWNTs) and single-walled carbon nanotubes (SWNTs), have been explored for potential uses in biology and medicine field [1–3]. Their small size, high surface area, inert chemical composition, and unique physical properties have made them extensively investigated for transport of DNA, nucleic acids, drugs, and a variety of other potential therapeutics [4,5]. However, the further applications of CNTs in the field of nanomedicine were limited due to their insolubility, biodegradation and off-target toxicity [2]. Surface functionalization aids CNTs to become biocompatible, improving their solubility in physiological solutions and selective binding to biotargets [6,7]. Among them, functionalization of CNTs with magnetic Fe_3O_4 nanoparticles has been suggested to be of great importance owing to their biocompatibility and potential applications in nanomedicine such as biological labeling, drug targeting, MRI contrast agents [8–11]. These multifunctional magnetic nanocomposites are capable of carrying drug molecules via exo- and endohedral functionalization and are steerable by an external magnetic field with the presence of magnetic nanoparticles in the nanotube core. Nowadays, CNTs based delivery systems have shown promise for *in vivo* cancer treatment including delivery of several conventional chemotherapeutic drugs and small interfering RNA (siRNA) [12,13]. Functionalized CNTs have plenty of inner spaces where the incorporation of drugs is possible, and on the tube walls of CNTs, drugs can be physically adsorbed. Furthermore, the edges of the tube holes have oxidized functional groups where covalent attachment of chemicals is possible. Yang et al. [14] presented a novel magnetic lymphatic-targeting gemcitabine delivery system using functionalized multiwalled carbon nanotubes (mMWNTs-GEM). Administration of mMWNTs-GEM resulted in obvious regression and inhibition of lymph node involvement in pancreatic cancer model under magnetic field. Wang et al. [15] prepared magnetic multiwall carbon nanotubes (MMWCNTs) by a simple solvothermal process which were used

* Corresponding author. Tel.: +86 773 2217609, fax: +86 773 2217609.
E-mail address: liguiyin01@163.com (G. Li).
[1] These authors contributed equally to this work.

0169-4332/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.apsusc.2013.11.148

to load an anticancer molecule, epirubicin hydrochloride. The MMWCNTs were suitable to be applied to a magnetic targeted drug delivery system due to its magnetic properties, high adsorption surfaces, and excellent adsorption capacities.

Chelerythrine(CHE), a quarternary benzo[c]phenanthridinium alkaloids derived from the greater celandine plant and other poppy-fumaria species, exhibits various bioactivities such as antimicrobial, anti-inflammatory, antitumor and antiplaque effect [16,17]. In the last few years, CHE has also shown great activity as a specific cytotoxic agent in many tumors, such as pancreatic cancer, gastric cancer, prostate cancer, malignant melanoma, hepatocellular carcinoma and so on [18–20]. In order to improve the therapeutic index and reduce the side effects, good delivery strategy need to be devised to enhance delivery of drug to a tumor organ.

In previous research we have prepared functionalized multi-walled carbon nanotubes decorated with magnetic nanoparticles (Fe_3O_4/MWNTs) using a very facile, green and efficient hydrothermal process [21]. The saturated magnetization of the synthesized Fe_3O_4/MWNTs nanocomposites, with diameters ranging from 15 nm to 30 nm, reached 55.833 emu/g and showed the characteristics of superparamagnetism. Herein, we designed a novel magnetic CNTs based targeting chelerythrine delivery system using functionalized Fe_3O_4/MWNTs as carrier and chelerythrine as an anticancer drug. The process and formulation variables were optimized using response surface methodology (RSM) with a three-level, three-factor Box–Behnken design (BBD) to find out the most suitable components ratio for the optimized formulation. The structure and interactions of the obtained Fe_3O_4/MWNTs-CHE targeted nanoparticles were characterized by Zeta potential and SEM. To evaluate performances of the resulting Fe_3O_4/MWNTs-CHE delivery system, drug release behavior and cytotoxicity *in vitro* were also studied.

2. Material and methods

2.1. Materials

MWNTs with a purity of more than 95% and an outer diameter of 40–60 nm were provided by Shenzhen Nanotech Port Co., Ltd. Ferrous sulfates heptahydrate ($FeSO_4{\cdot}7H_2O$) H_2O_2 and polyethylene glycol (PEG-20000) were purchased from Tianjin No.3 Chemical Plant. Chelerythrine chloride of purity 98% was purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China) Ltd. MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] were obtained from Beyotime Institute of Biotechnology (Jiangsu, China). DMEM medium (Dulbecco's Modification of Eagle's Medium), trypsin and fetal bovine serum were obtained from Hyclone Corporation. All other reagents and solvents were of analytical grade and were used without further purification. The water used in the study was prepared using a Milli-Q Water Purification System (Milli-Pore, Bedford, MA, USA).

2.2. Preparation of Fe_3O_4/MWNTs nanocomposites

The Fe_3O_4/MWNTs nanocomposites were prepared by hydrothermal method using $FeSO_4{\cdot}7H_2O$ as the iron source, H_2O_2 as the oxidant and acid treated MWNTs as starting reagents according to the reported method [21]. In a typical procedure, 200 mg HNO_3-treated MWNTs was first dispersed in 15 mL deionized water by sonication for 15 min. Then, 4.5 mmol $FeSO_4{\cdot}7H_2O$ (1.251 g) was dissolved into the above solution and 5 mL polyethylene glycol solution (50 g L^{-1}) was added. The solution was achieved at 30 °C under vigorous stirring by adding 15 mL of diluted ammonia (2.5%) was added. During the reaction process, the pH was maintained at about 10. Afterwards, 0.135 mL 30% H_2O_2 was added slowly into the solution and the reaction mixture was stirred for 10 min to obtain a homogeneous solution. After that, the as-formed slurry was transferred into a 50 mL Teflon-lined stainless steel autoclave and was heated at 160 °C for 5 h in a furnace. After cooling down to room temperature, the Fe_3O_4/MWNTs nanocomposites were recovered from the reaction mixture by a permanent magnet with a surface magnetization of 6000 G, and washed three times with water and ethanol, dried at 80 °C for 12 h under vacuum.

2.3. Preparation and optimization of CHE loaded magnetic Fe_3O_4/MWNTs nanocomposites by RSM

Magnetic Fe_3O_4/MWNTs nanoparticles (40 mg) were redispersed in 20 mL of PBS (0.2 mol/L, pH 7.4) containing 1 mmol/L EDC and 5 mmol/L NHS, and incubated for 4 h at room temperature. The nanoparticles were separated by magnetic decantation and then washed three times with deionized water. Then, 20.0 mL CHE solution (150 μg/mL) was added to the particles. The resulting suspensions were subsequently incubated at 25 °C for 4 h, which proved to be a sufficient period to reach equilibrium. At the end of incubation, the Fe_3O_4/MWNTs-CHE were collected by magnetic separation and washed twice with deionized water, and finally dried in a vacuum oven at 60 °C.

The above Fe_3O_4/MWNTs-CHE preparation procedure was optimized by a three-level, three-factor Box–Behnken design (BBD), with Fe_3O_4/MWNTs:CHE ratio (X_1), CHE concentration (X_2) and temperature (X_3) as the independent variables. The levels of those independent variables were based on preliminary trials. The drug loading content (Y_1) and encapsulation efficiency (Y_2) were used as response variables. The dependent and independent variables selected are shown in Table 1 along with their low, medium, and high levels. A total of 17 experiments was designed and performed in random order.

The non-linear quadratic model generated by the design is shown as follows:

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3 + b_{11}X_{12} + b_{22}X_{22} + b_{33}X_{32} \quad (1)$$

where Y denotes the measured response (dependant variable) associated with each factor-level combination; X_1, X_2, and X_3 are the independent variables. The coefficients of the polynomial equation are represented by b_0 (intercept), b_1, b_2, and b_3 (linear effects), b_{11}, b_{22}, and b_{33} (quadratic effects), and b_{12}, b_{13}, and b_{23} (interaction effects). The terms X_iX_i and X_i^2 (i = 1, 2 or 3) represent the interaction and quadratic terms, respectively [22].

Design-Expert Version 7.0 software was applied for generating and evaluating the statistical experimental design. The quality of the fitted model was expressed by the coefficient of determination R^2, and its statistical significance was determined by F-test. After generating the polynomial equations relating to the dependent and independent variables, optimization was performed using a desirability function to obtain the levels of X_1, X_2, and X_3, which maximized Y_1 and Y_2.

2.4. Determination of drug loading content and encapsulation efficiency

During the above Fe_3O_4/MWNTs-CHE preparation procedure, all the clear supernatants were collected, and the concentration of the unbound CHE was assayed by a UV–vis spectrophotometer (756CRT, Shanghai Precision Scientific Instrument Co., Ltd., China) at the wavelength of 270 nm. The encapsulation efficiency and drug loading content of CHE loaded magnetic Fe_3O_4/MWNTs nanoparticles were determined according to the decrease in initial CHE solution. All the experiments were carried out in triplicate. The