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Hydrophobic lapatinib encapsulated dextran-chitosan nanoparticles using a toxic solvent free method: fabrication, release property & in vitro anti-cancer activity

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ABSTRACT

Dextran sulfate-chitosan (DS-CS) nanoparticles, which possesses properties such as nontoxicity, biocompatibility and biodegradability have been employed as drug carriers in cancer therapy. In this study, DS-CS nanoparticles were synthesized and their sizes were controlled by a modification of the divalent cations cross-linkers (Ca^{2+}, Zn^{2+} or Mg^{2+}). Based on the optimized processing parameters, lapatinib encapsulated nanoparticles were developed and characterized by Dynamics Light Scattering (DLS) measurements, Fourier Transform Infrared Spectroscopy (FT-IR) and Scanning Electron Microscopy (SEM). Calcium chloride ($CaCl_2$) facilitated the formation of bare (100.3 ± 0.80 nm) and drug-loaded nanoparticles (134.3 ± 1.3 nm) with narrow size distributions being the best cross-linker. The surface potential of drug-loaded nanoparticles was -16.8 ± 0.47 mV and its entrapment and loading efficiency were $76.74 \pm 1.73\%$ and $47.36 \pm 1.27\%$, respectively. Cellular internalization of nanoparticles was observed by fluorescence microscopy and MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) assay was used to determine cytotoxicity of bare and drug-loaded nanoparticles in comparison to the free drug lapatinib. The MTT assay showed that drug-loaded nanoparticles had comparable anticancer activity to free drug within a duration of 48 h. The aforementioned results showed that the DS-CS nanoparticles were able to entrap, protect and release the hydrophobic drug, lapatinib in a controlled pattern and could further serve as a suitable drug carrier for cancer therapy.

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1. Introduction

Lapatinib is a small tyrosine kinase inhibitor chemical molecule composed of two members of the epidermal growth factor receptor family (EGFR family) - HER1 (EGFR) and HER2 [1,2]. Over expression and/or amplification of HER1 and HER2 were reported in different types of cancer, specifically in some breast cancers [3,4]. Lapatinib has been approved by the US food and drug administration (FDA) for the treatment of HER2-positive metastatic breast cancer [2,5]. From a therapeutic point of view, virtually all chemical drugs have high levels of toxicity and no specificity in patients, and have the potential of causing various side effects [6]. Encapsulation of drugs within nontoxic and biocompatible nanoparticles for controlled and sustained release to the target (due to tumor accumulation and enhanced permeability and retention (EPR) effect) using nanotechnology is one of the strategies for cancer therapy [7,8]. More than 99% of free lapatinib binds to albumin and alpha-1 acid glycoprotein [9]. As a result, it is imperative to protect lapatinib from plasma protein adsorption by encapsulating it inside a drug carrier that will make it possible for lapatinib remain in the blood circulation and eventually get to its target sites [9].

Nano-carriers have been used to encapsulate and deliver drugs in either passive or active ways, making it possible for the limitations associated with bare drugs such as poor stability, insolubility in water, side effects and low selectivity to be surmounted [10,11]. Biopolymers, particularly carbohydrates have superior properties such as nontoxicity, biocompatibility and biodegradability. Among the carbohydrates, dextran sulfate and chitosan have received much attention over the years with regard to nanoparticulate systems [12–16]. In addition, the negative charge of dextran has the potential of reducing cell toxicity caused by the high positive charge of chitosan. Chitosan is a deacetylated chitin derivative biopolymer in which the reactive amino groups can provide condition for electrostatic interactions [17]. Chitosan's excellent physical, chemical and biological properties have made it an auspicious

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candidate for vast applications specifically in controlled delivery systems [17–19].

The dextran sulfate-chitosan (DS-CS) nanoparticles are formed by electrostatic interaction between sulfate groups of dextran sulfate and amino groups of chitosan. This is one of the simplest methods of synthesizing nanoparticles without using external cross-linking agents [20]. The stable DS-CS nanoparticles are highly tunable in terms of the particles' zeta potential, which can be achieved by modification of the polymers' ratio and pH [20,21]. Hydrophobic drugs can be loaded into the hydrophilic DS-CS nanoparticles; furthermore, the drugs could be diffused into the target area in the body. This will help to circumvent the use of toxic solubilizing agents like DMSO (dimethyl sulfoxide) that is usually required for the formulation of hydrophobic drugs [22–24].

The aim was to develop a biocompatible polysaccharide delivery system for the hydrophobic lapatinib with improved delivery mechanism in a passive manner using a toxic solvent free method. In order to achieve this goal, DS-CS nanoparticles (with size optimized to 100.3 ± 0.80 nm) were synthesized using Ca, Zn or Mg cation cross-linkers, and characterized employing DLS, FTIR and SEM techniques. Furthermore, lapatinib was encapsulated inside the DS-CS nanoparticles. Cytotoxicity, as well as entrapment and loading efficiency (%), and release behavior of drug-loaded DS-CS nanoparticles were studied. The present study demonstrated the features of lapatinib encapsulated in polysaccharide polymeric nanoparticles and the potential of this particulate system in carrying hydrophobic drugs.

2. Materials and methods

2.1. Materials

Dextran sulfate sodium salt (DS) was purchased from Molekula Ltd (No. 38417956). FITC-Dextran sulfate sodium was from TdB (Cat. No. FDSS500). Low molecular weight chitosan (CS) from Sigma–Aldrich (Cat. No. 448869) was purified and used. Lapatinib Ditosylate (Tykerb, Tyverb, GW-572016) was purchased from BioVision, Inc. (925.46 MW). Acetonitrile (HPLC-grade), Calcium chloride, zinc sulfate, magnesium chloride and all other reagents were from Merck (Germany) as analytical grade. RPMI 1640 culture medium was purchased from Invitrogen (22400-089, Carlsbad, CA, USA). Trypsin-EDTA (T4049), Dulbecco's Phosphate Buffered Saline (D8662), Fetal Bovine Serum (FBS, F2442) and MTT salt ([3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide]) (M5655) were from Sigma.

2.2. Purification of chitosan

Chitosan purification was performed based on Nasti's work [25] with some modifications as described in our previous study [26]. Briefly, 5 g of CS was dissolved in 500 mL of acetic acid solution of 2% w/v in double distilled water and stirred overnight. Then the solution was boiled for 15 min to denature protein contaminant and centrifuged at 4500 rpm for 10 min to pelletize the aggregation. The supernatant was collected and passed through filters with 0.4 μm pore size. CS was collected by adding 1 N sodium hydroxide and increasing the solution pH to 9. The CS sediment was washed twice by centrifugation and dispersing in pure water. In final step the solution was freeze-dried and stored at 4 °C.

2.3. Nanoparticle preparation

2.3.1. DS-CS nanoparticles

CS solution of defined volume [0.1% (w/v) in 0.2% (v/v) acetic acid solution] was added dropwise to DS solution [0.1% (w/v) in distilled water] in a ratio of 1:3 (w/w) 15 min after addition of cross-linker (e.g. $CaCl_2$) (1 M) under magnetic stirring at room temperature. 30 mins' stirring with a speed of 550 rpm was applied for the final solution, followed by being centrifuged for 15 min with a speed of 5000 rpm. Finally the supernatant was sonicated for 20 min.

2.3.2. DS-lapatinib-CS nanoparticles

Two different protocols were used to prepare drug loaded nanoparticles. In first protocol drug was mixed with CS solution in different weight ratio to make mixing solution, followed by being added to DS-calcium chloride solution under magnetic stirring. In second protocol various amount of drug was added to DS solution under magnetic stirring. After 10 min calcium chloride solution was added and the following steps were repeated as mentioned before. One protocol with better entrapment efficiency was chosen for the rest of experiments.

The synthesis process of the lapatinib-loaded nanoparticles and their storage were done in the absence of light.

2.4. Experimental design

Model was built and the effect of each parameter was evaluated by applying both mathematical and statistical techniques in response surface methodology (RSM). The aim was to optimize independent variables to achieve best outcome. In this study, Box-Behnken design (BBD) as one of the most frequently RSM design with three factors, each one in three levels, was used to optimize nanoparticles' synthesis in case of size. The ratio of dextran sulfate to chitosan (X_1), the ratio of calcium chloride to dextran sulfate (X_2) and initial concentration of dextran in reaction (X_3) as three independent factors were chosen according to previous reports [20,27,28] and described in Table 1.

The generation and evaluation of the statistical experimental design was done by using Design Expert (STAT-EASE, 7.0.0, Minneapolis, MN) software. The analysis of variance (ANOVA) was applied to determine if the parameter was significant.

2.5. Characterization of nanoparticles

The mean size and zeta potential of particles were determined by Dynamics Light Scattering (DLS) measurements using Malvern Zetasizer ZS series and Scattering Particle Size Analyzer (Malvern Co., UK). Scanning electron microscopy (SEM) was applied to investigate the morphological features of nanoparticles as well as evaluation of size, PDI (polydispersity index) and aggregation of the particles. To this end, nanoparticle samples were coated by gold under vacuum, and then studied by a FE-SEM (Hitachi S-4160).

FTIR spectra were recorded in the region 4000–400 cm^{-1} to investigate the polymer's interaction as well as polymers and drug interaction using Nicolet IR100 FT-IR Spectrometer. Powder of samples were mixed with pure potassium bromide (KBr) and compacted by manual tablet press.

2.6. Entrapment and loading efficiency

The entrapment efficiency of lapatinib within the nanoparticles was evaluated. For aiming that a defined volume of each prepared drug loaded nanoparticles was lyophilized and then re-dispersed in a defined volume of DMSO, the suspension was centrifuged at 20,000 rpm for 20 min and the supernatant was collected. Amount of free drug within supernatant was determined by using reverse-phase HPLC (AKTA purifier) with a UV detector measuring absorbance at 268 nm and C18 column. The mobile phase was composed of double distilled water and acetonitrile in a gradient of 70:30 to 50:50 (v/v) with a flow rate of 1 mL/min.

Table 1
Variables used in Box-Behnken experimental design.

Independent variables	Symbol	Levels		
		−1	0	1
DS/CS ratio (w/w)	X_1	2	3	4
Ca/DS ratio (w/w)	X_2	20	27.5	35
DS concentration (mg/mL)	X_3	0.016	0.020	0.024

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